

November 1, 2019

Mr. Rick Butler
South Treatment Plant
1200 Monster Road SW
RNM-NR-0100
Renton, WA 98057

Dear Rick:

Enclosed please find our report on NPDES biomonitoring tests conducted with secondary effluent collected October 1 to October 7, 2019 at the King County Vashon Treatment Plant.

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

Chronic Toxicity Tests

Test Organism	Growth IC25 ^a (% Effluent)	Growth NOEC ^b (% Effluent)	Difference in Growth from Control (Control vs ACEC ^c)
Topsmelt (<i>Atherinops affinis</i>)	>100	100	Not Significantly Different ($\alpha = 0.05$)
Mysid Shrimp (<i>Mysidopsis bahia</i>)	>100	100	Not Significantly Different ($\alpha = 0.05$)

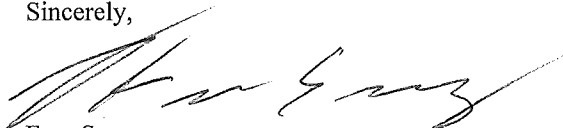
^a Concentration of effluent inhibiting growth by 25%

^b No Observed Effect Concentration

^c Acute Critical Effluent Concentration (1.12% effluent)

If you would like additional information, please call me at (206) 477-7117.

Sincerely,



Fran Sweeney
Aquatic Toxicology Supervisor
King County Environmental Laboratory

Enclosures

cc: Jeff Lafer: KSC-NR-0503
Erin McCabe: LAB-NR-0100

**NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM
BIOLOGICAL MONITORING REPORT FOR THE
VASHON TREATMENT PLANT
9621 SW 171 STREET
VASHON, WA 98070**

**PERMIT NO: WA0022527
PROJECT NO: 421488**

4th Quarter 2019 Chronic Toxicity Tests

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

Test Numbers: 9312, 9311

Test Date: October 2, 2019

Report Date: November 1, 2019

METHODS

Sample

Three samples of unchlorinated secondary effluent from the Vashon Treatment Plant were collected on ice by flow-paced compositing over 24-hour periods on Days 0, 2 and 5 of testing. The samples were delivered to the King County Environmental Laboratory (KCEL) on Days 0, 2 and 5 in one or two 20-L glass jars containing approximately 16 – 17 liters each. Samples from the two jars were combined and mixed for use in testing. The chronic tests were initiated within 7 hours after sampling on Day 0. The unused samples were stored in the dark in a $4 \pm 2^{\circ}\text{C}$ refrigerator.

Day/Time of Collection		Day 0	Day 2**	Day 5
		10-1-19 / 0647 h to 10-2-19 / 0647 h	10-3-19 / 0637 h to 10-4-19 / 0637 h	10-6-19 / 0610 h to 10-7-19 / 0610 h
Delivered to KCEL		10-2-19 / 0847 h	10-4-19 / 0850 h	10-7-19 / 0903 h
Log-in number		L73401-1	L73401-5	L73401-6
At Plant	pH	7.58	7.57	7.60
	Temp. °C	2.2	2.5	2.8
	TRC, mg/L*	0.03	0.04	0.02
At KCEL	pH**	7.61	7.58/7.64	7.60
	Temp. °C	2.0	1.8/1.7	1.9
	DO, mg/L**	10.8	10.3/10.4	10.5

*Measurement by field kit

**2-20L Carboys received, therefore 2 data points recorded for each parameter (pH, DO, Temp).

Chemical characteristics of the samples are listed below:

Parameter	Day 0	Day 2	Day 5	Units
Total NH ₃ -N	0.0296	0.0388	0.0502	mg/L
Conductivity	630	656	693	µmhos/cm
Total Alkalinity	175	177	178	mg/L as CaCO ₃
Total Hardness	105	107	108	"

CONTROL WATER

The control (dilution) water used for the chronic tests with *A. affinis* and *M. bahia* was artificial seawater prepared by adding 35.7 g of Hawaiian Marine Mix (HMM) brand salts and 0.20 g of NaHCO₃ per L of Millipore Super Q deionized water (DW) and mixing until dissolved to obtain a salinity of 30‰. The artificial seawater was then aerated for ≥ 2 hours and filtered to 0.45 µm before use.

CHRONIC TESTS

Topsmelt - *Atherinops affinis*

The topsmelt chronic toxicity test (#9312) was conducted as outlined in Chapman *et al.* (1995). The larvae were received from Aquatic Biosystems as 9 days old (9/22/19 hatch). Upon receipt the temperature was 19.4°C, with salinity of 28 ppt to 32 ppt (multiple bags of organisms). The larvae were placed into three 1.5-L crystallizing dishes and acclimated for 24 hours at 20°C in an environmental chamber with light aeration. During acclimation, the larvae were fed 4 mL live *Artemia* nauplii per dish 2 times a day. Following acclimation, the larvae were loaded directly into the test chambers with a nylon screen. At test initiation the topsmelt larvae were 10 days old.

The effluent sample was warmed to approximately 20°C and the salinity adjusted to 30 ppt by adding 35.7 g of HMM salts and 0.20 g of NaHCO₃ per liter of sample, after which the sample was diluted with HMM artificial seawater to the concentrations listed below. Five replicates of five fish each were tested at each concentration, including the HMM-only control. Test chambers were 600-mL beakers containing 200 mL of test solution. Assignment of the larvae to the test chambers was random, as was placement of the test chambers in the environmental chamber. The test was incubated for 7 days at 20 ± 1.0°C on a 16:8 h light:dark cycle. Solutions were renewed daily (75%), and larvae were fed newly-hatched *Artemia* nauplii two times per day (1 drop per test chamber for each feeding). Survival and water quality measurements were recorded every 24 hours at solution renewal and can be found on the photocopied pages from the laboratory notebook in the “Bench Sheets” section of this report. Temperature was measured daily by digital thermometer in one replicate of each concentration and in replicates at six shelf positions (4 outer corner + 2 center), as well as recorded at 15-minute intervals using an Onset Tidbit data logger placed in a beaker of water among the test beakers. At the end of the test, surviving animals were inactivated in ice water, rinsed, placed into tared aluminum foil weigh pans (hand-made) and dried at 60°C for 19 hours. Each replicate (one rep across treatments) was processed by a single analyst. After the pans cooled in a desiccator, dry weight was measured to the nearest 0.01 mg to determine growth.

Test #	Start Date/ Time	End Date/ Time	Effluent Concentrations (%)	Larvae Age	# Reps/ Trtmt	# Orgs/ Rep
9312 (Topsmelt)	10-2-19 / 1350 h	10-9-19/ 1340 h	0, 0.15 ^a , 1.12 ^b , 12.5, 25, 50, 100	10 days	5	5

^a CCEC (Chronic Critical Effluent Concentration)

^b ACEC (Acute Critical Effluent Concentration)

Mysid Shrimp - *Mysidopsis bahia*

The mysid shrimp chronic toxicity test (#9311) was conducted as outlined in US EPA (2002). The mysid juveniles were received from Aquatic Biosystems as 7 days old and were 7 days old at test initiation. Upon receipt the temperature was 18.3 °C and the salinity was 24 ppt. The larvae were transferred to three 1.5-L crystallizing dishes and gradually (over a period of 2 hours) brought to the test temperature of 26 ± 1°C in a water bath with light aeration. During acclimation, the larvae were fed 4 mL live *Artemia* nauplii per dish. Following acclimation, the larvae were loaded directly into the test chambers with a nylon screen.

The effluent sample was warmed to approximately 25-26°C and the salinity adjusted to 30 ppt by adding 35.7 g of HMM salts and 0.20 g of NaHCO₃ per liter of sample, after which the sample was diluted with HMM artificial seawater to the concentrations listed below. Eight replicates of five mysids each were tested at each concentration, including the HMM-only control. Test chambers were 400-mL beakers containing 250 mL of test solution. Assignment of the larvae to the test chambers was random, as was placement of the test chambers in the water bath. The test was incubated for 7 days at 26 ± 1.0°C on a 16:8 h light:dark cycle. Solutions were renewed daily (80%), and larvae were fed newly-hatched *Artemia* nauplii two times per day (2 drops per test chamber for each feeding). Survival and water quality measurements were recorded every 24 hours at solution renewal and can be found on the photocopied pages from the laboratory notebook in the “Bench Sheets” section of this report. Temperature was measured daily by digital thermometer in one replicate of each concentration and in replicates at six water bath positions (4 outer corner + 2 center), as well as recorded at 15-minute intervals using an Onset Tidbit data logger placed in a beaker of water among the test beakers. At the end of the test, surviving animals were rinsed with iced RO water and dried at 60°C for 20 hours in tared aluminum weigh boats. After the pans cooled in a desiccator, dry weight was measured to the nearest 0.01 mg.

Test #	Start Date/ Time	End Date/ Time	Effluent Concentrations (%)	Mysid Age	# Reps/ Trtmt	# Orgs/ Rep
9311 (Mysid)	10-2-19 / 1410 h	10-9-19 / 1300 h	0, 0.15 ^a , 1.12 ^b , 12.5, 25, 50, 100	7 days	8	5

^a CCEC (Chronic Critical Effluent Concentration)

^b ACEC (Acute Critical Effluent Concentration)

QUALITY CONTROL

Copper sulfate was used as a reference toxicant in chronic toxicity tests with topsmelt and *Mysidopsis*. 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. Throughout the reference toxicant test with topsmelt (#9324), temperature, pH and dissolved oxygen measurements remained within acceptable limits (Chapman *et al.*, 1995). Topsmelt control survival and mean control weight met acceptability criteria (Chapman *et al.*, 1995), and the LC50 for survival was within control limits. For the reference toxicant test with mysids (#9325), temperature, pH and dissolved oxygen values remained within acceptable limits during the test (US EPA 2002). In addition, the test met acceptability criteria regarding control survival and mean control weight; however, the IC25 endpoint exceeded the current upper control limits (US EPA 2002).

Endpoint data for the reference toxicant tests is summarized in the following table:

Test #:	9324	9325
	Topsmelt	Mysid
Control Survival (%)	100	90
Criteria	≥ 80	≥ 80
Acceptable?	Yes	Yes
Control Growth (mg/ind)	1.116	0.211
Criteria	≥ 0.85	≥ 0.20
Acceptable?	Yes	Yes
Survival LC50 (µg/L)	203.7	
LC50 Control Limits	55.2 – 471.0	
Growth IC25 (µg/L)	151.2	221.9
IC25 Control Limits	43.5 – 341.8	48.6 – 178.5
Acceptable?	Yes	*

*IC25 (growth) is above upper control limit (± 2 SD)

Water Quality Measurements

Water quality parameters and methods are listed in the following table:

Parameter	Method
Water Quality Tests	APHA (1992); US EPA (1991).
Temperature	Disense Traceable digital thermistor thermometer with calibration and USB probe (#900 80-09) and Onset, Tidbit (v2) UTBI-001 Temperature Logger.
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 (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).
Salinity	Temperature compensated refractometer (KCEL #438)
Pesticides and PCB's	Continuous liquid extraction method (US EPA Method #608; KCEL #733)
Organic Analysis	Continuous liquid extraction method for BNA's (US EPA Method #625; KCEL #731)
Volatile Organics	Purge and trap method (US EPA Method #624; KCEL #732)
Total Metals	ICP-MS for Cd Ref. Tox. (US EPA Method #200.8; KCEL #618); ICP for Cd, Cr, Cu, Ni, Pb and Zn (US EPA Method #200.7; KCEL #612)

RESULTS

CHRONIC TESTS

Topsmelt – *Atherinops affinis*

Average weights per fish (based on the number of surviving fish) at the end of the 7-day chronic test with topsmelt are listed in the following table:

% Sample	GROWTH (Mean Weight per Fish in mg at 7 Days)						# Fish Tested	Survival %
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Mean		
0	1.174	1.480	0.896	0.820**	1.392	1.152	23**	96
0.15 ^a	0.984	0.544	1.232	1.194	1.274	1.046	25	92
1.12 ^b	1.118	1.006	1.230	0.902	1.238	1.099	25	96
12.5	1.030	0.738	0.858	0.844	1.288	0.952	25	92
25	1.200	1.034	1.320	0.928	1.312	1.159	25	92
50	0.638	1.190	1.004	1.322	1.018	1.034	25	84
100	1.108	1.174	2.380	1.350	1.136	1.429	25	100

^a CCEC

^b ACEC

** Beaker tipped over Day 5, 2 organisms lost. (n=3)

The NOEC for growth and survival was 100%. Growth in the ACEC of 1.12 % effluent was not significantly different from the control ($p < 0.05$; 1-tailed homogenous t-test). The IC25 was >100% effluent. The growth CSPS for the ACEC was found to be 4.6 %, which is below the maximum allowable difference of 39%. The unionized ammonia level in 100% effluent reached a maximum of 0.004 mg NH₃-N/L during the 7-day test.

Mysid Shrimp – *Mysidopsis bahia*

Average weights per mysid larva (based on the number of surviving mysids) at the end of the 7-day chronic test are listed in the following table:

% Sample	GROWTH (Mean Weight per Organism in mg at 7 Days)									#Mysids Tested	Survival %
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Mean		
0	0.290	0.222	0.310	0.198	0.242	0.290	0.266	0.200	0.252	40	95
0.15 ^a	0.222	0.278	0.294	0.286	0.266	0.238	0.220	0.290	0.262	40	95
1.12 ^b	0.264	0.252	0.372	0.290	0.298	0.245**	0.252	0.304	0.285	39**	100
12.5	0.208	0.260	0.186	0.216	0.212	0.262	0.278	0.302	0.241	40	90
25	0.240	0.308	0.236	0.208	0.206	0.286	0.228	0.232	0.243	40	95
50	0.102	0.290	0.280	0.292	0.218	0.188	0.336	0.242	0.244	40	88
100	0.332	0.288	0.252	0.204	0.334	0.330	0.276	0.312	0.291	40	100

^a CCEC

^b ACEC

** One live organism lost in renewal Day 5. (n=4)

The NOEC for growth and survival was 100%. Growth in the ACEC of 1.12 % effluent was not significantly different from the control ($p < 0.05$; 1-tailed homogenous t-test). The IC25 was >100% effluent. The growth CSPS for the ACEC was found to be 0 %, which is below the maximum allowable difference of 39%. The unionized ammonia level in 100% effluent reached a maximum of 0.004 mg NH₃-N/L during the 7-day test.

QUALITY CONTROL

Salinity, pH and temperature remained within acceptable limits throughout the chronic tests (Chapman *et al.*, 1995; US EPA, 2002). Water quality data recorded during testing is shown on the photocopied pages from the laboratory notebook in the "Bench Sheets" section of this report. As shown below, both tests met acceptability criteria regarding control performance, including survival and growth (Chapman *et al.*, 1995; US EPA, 2002).

Test #:	9312	9311
	Topsmelt	Mysid
Control Survival (%)	96	95
Criteria	≥ 80	≥ 80
Acceptable?	Yes	Yes
Control Growth (mg/ind)	1.152	0.252
Criteria	≥ 0.85	≥ 0.20
Acceptable?	Yes	Yes
Control vs. ACEC	Non-sig	Non-sig
Survival NOEC	100 %	100 %
Growth NOEC	100 %	100 %
IC25	> 100 %	> 100 %
PMSD for Growth	39.7 %	21.7 %
Criteria	< 50 (ref tox)	12-30
Acceptable?	Yes	Yes

Protocol Deviations

As noted under Quality Control, the mysid reference toxicant IC25 exceeded the current upper control limit (mean of most recent 20 IC25 \pm 2SD). All other QA/QC was acceptable, and no toxicity was observed in the effluent sample. Therefore, we do not believe this has an overall impact on the effluent test data quality.

Minor temperature excursion of 21.2 C of was observed during water exchanges on day 4 of the topsmelt reference toxicant test.

Tested By:

King County Department of Natural Resources & Parks
Water and Land Resources Division
Environmental Laboratory
322 West Ewing Street
Seattle WA 98119
(206) 477-7117

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

REFERENCES

- APHA. 1992.** Standard Methods for the Examination of Water and Wastewater. 18th Edition. American Public Health Association, American Water works Association, Water Pollution Control Association, Washington D.C.
- Chapman, G., D. Denton and J. Lazorchak. 1995.** Short-term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms. 1st Edition. EPA/600/R-95-136.
- Shapiro, S. 1990.** How to test normality and other distributional assumptions. Volume 3, revised. American Society for Quality Control, Milwaukee, WI.
- Sokal, R. and F. Rohlf. 1981.** Biometry: the principles and practice of statistics in biological research. 2nd Edition. W.H. Freeman and Company, New York, NY.
- US EPA. 2002.** Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. 3rd Edition. EPA-821-R-02-014, October, 2002. US Environmental Protection Agency, Office of Water (4303T), Washington, DC.
- 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.** Lab guidance and whole effluent toxicity test review criteria. Publication no. WQ-R-95-80. WA State Department of Ecology, Water Quality Program, Olympia, WA.

Effluent Tests:

Bench Sheets, Calculations, and Statistics

VASHON PLANT NPDES ANALYSIS (10/2019 #9311, 9312)

TOPSMELT #9312

Survival

- 1) Survival NOEC = >100% effluent
- 2) SD for survival in one or more concentration was < 0.2, so use Mean Dry Biomass for growth analyses (WA DOE 2016)

Growth

- 1) Use Mean Dry Biomass values
- 2) t-Test 2-Sample (Control vs ACEC):
Non-significant with $\alpha > 0.05$
- 3) Linear Interpolation: Include all concentrations; Mean Dry Biomass data
IC25 = >100% effluent
- 4) Dunnett's Multiple Comparison Test (normal, homogeneous, equal #reps)
Not significant at ACEC
NOEC_{growth} = >100% effluent
PMSD = 39.7 %
- 5) t-Test (Control vs ACEC):
Non-significant with $\alpha > 0.05$

$$\begin{aligned}\text{CSPS} &= [(W_{\text{CTRL}} - W_{\text{ACEC}}) / W_{\text{CTRL}}] \times 100 \% \\ \text{Growth} &= [(1.152 - 1.099) / 1.152] \times 100 \% \\ &= 4.6 \% (< \text{max allowable of } 39\%; \therefore \text{passes criterion})\end{aligned}$$

MYSID #9311

Survival

- Survival NOEC = >100% effluent
SD for survival in all concentrations was < 0.2, so use Mean Dry Biomass for growth analyses (WA DOE 2016)

Growth

- 1) Use Mean Dry Biomass values
- 2) t-Test 2-Sample (Control vs ACEC):
Non-significant with $\alpha > 0.05$
- 3) Linear Interpolation: Include all concentrations; Mean Dry Biomass data
IC25 = >100% effluent
- 4) Dunnett's Multiple Comparison Test (normal, homogeneous, equal #reps)
Not significant at ACEC
NOEC_{growth} = >100% effluent
PMSD = 21.7 %
- 5) t-Test (Control vs ACEC):
Non-significant with $\alpha > 0.05$

$$\begin{aligned}\text{CSPS} &= [(W_{\text{CTRL}} - W_{\text{ACEC}}) / W_{\text{CTRL}}] \times 100 \% \\ \text{Growth} &= [(0.252 - 0.262) / 0.252] \times 100 \% \\ &= (-3.921) \% (< \text{max allowable of } 39\%; \therefore \text{passes criterion})\end{aligned}$$

Unionized Ammonia in Water Calculations

Organism	Test #	pH _{max}	°C _{max}	T (°K)	Sal _{max} (ppt)	I	pKa ^(S,298)	pKa ^(S,T)	Total Ammonia (mg/L)	NH ₃ -N, (mg/L)
Mysid	9311	8.463	26.1	299.3	30	0.616	9.317	9.281	0.0296	0.0039
Topsmilet	9312	8.512	19.9	293.1	31	0.638	9.320	9.477	0.0388	0.0038

$$I = [(19.9273)(\text{Sal})] / [1000 - (1.005109)(\text{Sal})]$$
$$\text{pKa}^{(S,298)} = 9.2406 + (0.12375 \times I)$$
$$\text{pKa}^{(S,T)} = (2729.69/T) + (\text{pKa}^{(S,298)} - 9.1345) - (7.1 \times 10^{-5} T)$$
$$\text{NH}_3\text{-N} = \text{Tot Amm} / [1 + 10^{(\text{pKa} - \text{pH})}]$$

Data_{max} from 100% effluent test concentration.

King County Environmental Lab Analytical Report

Project: 421488						Project: 421488						Project: 421488								
Locator: VS_EFF						Locator: VS_EFF						Locator: VS_EFF								
Descrpt: VASHON TP/FINAL EF						Descrpt: VASHON TP/FINAL EF						Descrpt: VASHON TP/FINAL EF								
Sample: L73401-4						Sample: L73401-5						Sample: L73401-6								
Matrix: LC EFFLUENT						Matrix: LC EFFLUENT						Matrix: LC EFFLUENT								
ColDate: 10/1/19 8:47						ColDate: 10/3/19 6:37						ColDate: 10/6/19 6:10								
ClientLoc: AquaTox Test #'s 9311, 9312						ClientLoc: 2 ISCO 3710 samplers at Effluent Ch						ClientLoc: 1 ISCO 3710 sampler at Effluent Cha								
WET Weight Basis						WET Weight Basis						WET Weight Basis								
Parameters		Value	Qual	MDL	RDL	Units	Value		Qual	MDL	RDL	Units	Value		Qual	MDL	RDL	Units		
CV KEROUEL & AMINOT 1997		0.0296	SH	0.002	0.01	mg/L	0.0388		SH	0.002	0.01	mg/L	0.0502		SH	0.002	0.01	mg/L		
Ammonia Nitrogen							177							178						
CV SM2320-B							177							178						
Total Alkalinity		175		1	5	mg CaCO3/L	177			1	5	mg CaCO3/L	178			1	5	mg CaCO3/L		
MT EPA 200.7 (MOD)							177							178						
Calcium, Total, ICP		24100		100	250	ug/L	24000			100	250	ug/L	23800			100	250	ug/L		
Magnesium, Total, ICP		10900		30	150	ug/L	11500			30	150	ug/L	11700			30	150	ug/L		
MT EPA 200.7 (MOD)*SM2340B							11500							11700						
Hardness, Calc		105		0.37	1.24	mg CaCO3/L	107			0.37	1.24	mg CaCO3/L	108			0.37	1.24	mg CaCO3/L		

Vashon NPDES Characterization (1033898)
***Atherinopsis affinis* – 7-day Chronic Test**

Test#: 9312
 Test Date: 10/2/19

ORGANISMS (Hold 24 hours before testing)

Received from ABS via Red EX as 9 days old (Hatch date: 9-22-19). Arrived at KCEL at 0945 h on 10-1-19 in 2 double plastic bags. 6 dead removed. At Arrival: pH 7.177/7.132. D.O. 9.6/10.9 mg/L, Temp 19.4, 19.4 °C, Salinity 28/32 ppt. Placed in 3 1.5L crystallizing dishes. Fed 4 mL *Artemia* nauplii/dish at 1110 h. Acclimation: Placed in 20°C EC at 1050 h with light aeration. Replaced 50 % with HMM.

Fed 4 mL *Artemia*/dish at 1110 h on 10-1 Analyst JA
 Fed 4 mL *Artemia*/dish at 1700 h on 10-1 Analyst JA
 Fed 4 mL *Artemia*/dish at 0745 h on 10-2 Analyst EY

DILUTION WATER/SAMPLE

- Hawaiian Marine Mix (HMM) #HW-1016 Synthetic Seawater:** Prep by adding 35.7 g HMM artificial sea salts (Lot# —, Rec'd 3/11/19, Opened 10/4/19.) + 0.2 g NaHCO₃ in 1L MilliQ. Sal 30ppt, 0.45 µm filtered. Aerate > 2h before use.
- Vashon Final Effluent:** Salinity adjusted to 30 ppt by adding 71.4 g HMM sea salts + 0.40 g NaHCO₃/2L effluent after warming.
- AAC Sample#:** 73401-1; **Wkpg #:** 46167050

Sample Data	Day 0			Day 2			Day 5		
Sample #:	<u>73401-1</u>			<u>73401-5</u>			<u>73401-6</u>		
Collect Date:	<u>10-1-19 to 10-2-19</u>			<u>10-3-19 to 10-4-19</u>			<u>10-6-19 to 10-7-19</u>		
Collect Time:	<u>0647 h to 0647h</u>			<u>0637 h to 0637h</u>			<u>0610 h to 0610 h</u>		
Auto Sample Set:	<u>225 mL 20 min</u>			<u>225 mL 20 min</u>			<u>225 mL 20 min</u>		
Est. Flow/#Samples:	<u>mgd / 73</u>			<u>mgd / 73</u>			<u>mgd / 73</u>		
Delv'd to KCEL:	<u>0647 h on 10-2-19</u>			<u>0850 h on 10-4-19</u>			<u>0903 h on 10-7-19</u>		
By:	<u>D.R.</u>			<u>D.R.</u>			<u>DR</u>		
Container:	<u>1 5 gal Glass</u>			<u>2 5 gal Glass</u>			<u>1 5 gal Glass</u>		
Vol. (L):	<u>29.5 L ± 15.5 L</u>			<u>± 17 x 2 L</u>			<u>16.5 L</u>		
At Plant:	<u>7.58</u>	<u>2.229</u>	<u>0.03</u>	<u>7.57</u>	<u>2.5</u>	<u>0.04</u>	<u>7.60</u>	<u>2.787</u>	<u>0.02</u>
pH, Temp, TRC		°C	mg/L		°C	mg/L		°C	mg/L
At KCEL:	<u>7.614</u>	<u>2.0</u>	<u>10.8</u>	<u>7.594</u>	<u>1.8</u>	<u>10.3</u>	<u>7.601</u>	<u>1.9</u>	<u>10.5</u>
pH, Temp, D.O.		°C	mg/L	<u>7.637</u>	<u>1.7</u>	<u>10.4</u>		°C	mg/L
					°C				
Storage:	<u>In dark at 4 ± 2°C</u>			<u>In dark at 4 ± 2°C</u>			<u>In dark at 4 ± 2°C</u>		

Vashon NPDES Characterization (1033898)
***Atherinopsis affinis* - 7-day Chronic Test**

Test#: 9312
 Test Date: 10/2/19

DILUTIONS

Code	% Sample	mL Sample	Decant (mL)
White	100	2000	1000
Red	50	≤ 2000 w/HMM	1000
Orange	25	≤ 2000 w/HMM	1000
Yellow	12.5	≤ 2000 w/HMM	1000
Green	1.12 (ACEC)	11.2 mL ≤ 1000 w/HMM	1000
Gray	0.15 (CCEC)	1.5 mL ≤ 1000 w/HMM	1000
Blue	0	0	1000 mL HMM only

PROCEDURE

- Pour 2L of effluent into a 4-L beaker.
- Bring to 20°C and measure pH, D.O. in unsalted effluent.
- Adjust salinity to 30 ppt with HMM and measure pH, D.O. and salinity of salted effluent.
- Prepare solutions as above in 2-L graduated cylinder; decant to 1-L flasks and bring to 20°C.
- Pour 200 mL of each treatment to each of five (5) 600-mL beakers (A→E)/trtmt.
- Place beakers randomly in EC # 8556 and bring solutions to 20°C. Setup at 1300 1450^h h.
- Measure D.O. and Temp in 0h (New) soln's.
- Add 5 larvae/beaker:
 - Warm to 20 °C, 15 ml HMM in 35 1-oz plastic cups.
 - Add 5 larvae/cup, randomize cups.
 - Just before loading test beakers draw down to ~5 ml in cups.
 - Add one randomly chosen cup/beaker.
- Start test at 131450 h on 10/2/19. Start counts verified by JS & _____. Place Tidbit temp recorder (SN 10680548) in beaker w/DW into EC # 8556, test(R)shelf.
- Sample for 0h water quality with unsalted effluent: ✓ Analyst: Gy
- Feed larvae 1 drop *Artemia* nauplii/beaker 2x/day.
- Renew solutions daily:
 - Remove 150 mL (≈ 75%) of old solution from each beaker by decant or bulb and pipet; also remove waste and excess feed.
 - Replace ≤ 200 mL with new solution (20°C) by pouring down side of beaker.
 - Record # alive, and remove dead larvae; count larvae before and after renewal.
- Measure D.O. in 0h (New) and 24h (Old) solutions and pH and Salinity in 24 h (old) solutions daily in each treatment. Measure Temp daily in 1 rep/trtmt and in 6 positions (4 corner + 2 center) just prior to renewal. Feed larvae 1-2 drop of *Artemia* nauplii/beaker 4x/day (2 drops last feeding of day).
- End test at 1340 h on 10-9-19. Process by rep (1 analyst/rep):
 Rep A EF Rep B FS Rep C EF Rep D JA Rep E FS

Vashon NPDES Characterization (1033898)
***Atherinopsis affinis* – 7-day Chronic Test**

Test#: 9312
 Test Date: 10/2/19

15. Inactivate larvae in ice water in net. Rinse larvae onto screen with ice water and place larvae into tared aluminum foil weigh pans.

- Into 60°C oven at 1503 h on 10-9-19.
- Into desiccator at 1000 h on 10-10-19.
- Weigh at 1650 h on 10-10-19 by YB with Mettler XP105 balance.

HMM SEA SALT BATCHES

Day	Batch	Prep Date	Sal (ppt)	pH	D.O. (mg/L)	Analyst
0	2 / 3	9-30/10-1	30/30	7.924/7.793	7.2/7.1	JA
1	1	9-30	30	7.964	7.0	Gy
2	4	10-1	30	7.837	7.1	Gy
3	4 / 6	10-1/10-2	30/30	7.837/8.222	7.1/6.9	EF
4	8	10-3	30	7.641	6.7	Gy
5	11	10-4	30	7.897	6.9	FL
6	13	10/6	30	7.757	6.8	Gy

EFFLUENT SALINITY ADJUSTMENT

Effluent Before Salting Up

Day→	0	1	2	3	4	5	6
pH	7.611	7.708	7.673	7.547	7.793	7.628	7.710
D.O. (mg/L)	10.2	10.0	9.5	9.8	9.7	9.7	9.9
Analyst:	Gy	Gy	RL	EF	Gy	Gy	EF

Effluent After Salting Up

Day→	0	1	2	3	4	5	6
pH	8.496	8.550	8.597	8.565	8.472	8.493	8.529
D.O. (mg/L)	7.9	7.8	7.4	7.6	7.7	7.7	7.5
Sal. (ppt)	30	30	30	30	30	30	30
Analyst:	Gy	Gy	RL	EF	Gy	Gy	EF

Vashon NPDES Characterization (1033898)
Atherinopsis affinis – 7-day Chronic Test

Test#: 9312
 Test Date: 10/2/19

MEASUREMENTS

Day	Temperature (°C) SN: 170786325 (24-Hour Solutions)							Analyst
	Blue	Gray	Green	Yellow	Orange	Red	White	
0	20.9	20.8	20.8	20.5	20.7	20.7	20.8	FS
1	20.0	19.8	19.9	20.0	20.0	20.0	20.0	FS
2	20.1	20.3	20.2	20.0	20.1	20.1	19.9	Gy
3	19.9	19.9	19.7	19.7	19.5	19.7	19.7	FS
4	20.5	19.9	20.1	19.9	19.8	20.1	20.1	Gy
5	19.2	19.9	19.8	19.9	19.0	19.9	19.7	GH
6	19.7	19.9	19.7	19.9	19.7	19.7	19.1	EF
7	20.2	19.5	19.9	20.0	19.5	19.6	19.9	Gy

Temperature, °C in 6 Positions (4 Corner + 2 Center)

Code	Rep	0d	1d	2d	3d	4d	5d	6d	7d
White	C	20.9	20.0	20.2	19.9	20.2	19.7	19.1	19.6
Orange	C	20.9	20.0	20.1	19.9	20.0	19.0	19.7	19.9
Gray	E	20.9	20.0	20.2	19.7	20.2	19.9	19.4	19.8
Green	W	20.7	20.0	20.4	19.7	20.0	19.8	19.7	19.7
Blue	C	20.7	19.5	19.2	19.5	19.3	19.2	19.0	19.1
Blue	E	20.6	19.5	20.1	19.7	19.6	19.8	19.7	19.7

FS FS

Sample #	Sample Type	Day Sampled	T. Hard. (mg/L as CaCO ₃)	T. Alk. (mg/L as CaCO ₃)	T. NH ₄ ⁺ (mg/L)	NO ₂ -N (mg/L)	COND. (µmhos/cm)
73416 -1	HMM	0		117			
73401 -4	EFFL	0	105	175	0.0296	—	630
-5	EFFL	2	107	177	0.0388	—	656
-6	EFFL	5	108	178	0.0502	—	693
						Analyst:	JA

Feeding Schedule (Time, h) (1 drop/beaker)

Day	1 st	2 nd	Analyst
0	13:45	1750	YB / JA
1	0920	1800	EF / JA
2	1125	1830	Gy / JA
3	0930	1850	FS / JA
4	1030	1915	Gy / JA
5	1125	1710	YB / JA
6	0950	1700	Gy / JA

Vashon NPDES Characterization (1033898)
Atherinopsis affinis - 7-day Chronic Test

Test#: 9312
 Test Date: 10/2/19

Chemistry

Day	pH (24-Hour Solutions)							Analyst
	Blue	Gray	Green	Yellow	Orange	Red	White	
1	7.907	8.108	8.101	8.057	8.147	8.300	8.451	GY
2	7.980	8.004	7.948	8.080	8.152	8.279	8.419	JA
3	7.993	8.004	7.968	8.091	8.221	8.360	8.572	GY
4	7.896	7.989	7.993	8.036	8.121	8.273	8.449	JS/RR
5	7.859	7.867	7.895	7.986	8.025	8.241	8.445	GY
6	7.901	7.955	7.921	8.050	8.131	8.279	8.422	JS
7	7.932	7.961	7.976	8.061	8.136	8.304	8.435	JA

Day	Salinity (ppt) (24-Hour Solutions)							Analyst
	Blue	Gray	Green	Yellow	Orange	Red	White	
1	30	30	30	30	30	30	31	GY
2	30	30	30	30	30	30-31	30	JA
3	30	30	30	31	31	31	31	GY
4	31	30	30	31	31	31	31	JS/RR
5	30	30	30	30	30	30	31	GY
6	30	30	30	30	30	30	30	JS
7	30	30	30	31	30	31	30	JA

Day	D.O. (mg/L); 0-Hour & 24-Hour Solutions								Analyst
	Blue		Gray		Green		Yellow		
	0h	24h	0h	24h	0h	24h	0h	24h	
0	7.2		7.2		7.3		7.3		GY
1	7.0	6.5	6.7	6.9	6.7	7.0	7.0	6.6	GY
2	7.0	6.7	7.1	6.8	7.1	6.7	7.1	6.5	JA
3	7.0	6.6	7.0	6.6	7.0	6.5	7.0	6.6	GY
4	7.2	6.2	7.3	7.1	7.3	7.0	7.3	6.8	JS/RR
5	7.0	6.6	7.0	6.5	7.1	6.6	7.1	6.5	JS
6	7.1	6.9	7.2	6.9	7.2	6.8	7.3	6.7	JS
7		6.7		6.9		6.9		6.7	JA

	D.O. (mg/L); 0-Hour & 24-Hour Solutions								
Day	Orange		Red		White				Analyst
	0h	24h	0h	24h	0h	24h			
0	7.3		7.3		7.2				GY
1	7.1	6.5	7.2	6.5	7.4	6.5	6.2		GY
2	7.1	6.5	7.1	6.5	7.2	6.4			JA
3	7.0	6.6	7.1	6.7	7.2	6.6			GY
4	7.2	6.6	7.3	6.6	7.4	6.5			JS/RR
5	7.1	6.6	7.2	6.6	7.6	6.6			JS
6	7.1	6.5	7.2	6.8	7.4	6.6			JS
7		6.8		6.8		6.9			JA

Vashon NPDES Characterization (1033898)
Atherinopsis affinis - 7-day Chronic Test

Test#: 9312
 Test Date: 10/2/19

Trtmt	Cumulative Daily Survival (# Alive/Rep) at 7d N = 5/Rep						Tot # Surv	% Surv*	Analyst
	Day	A	B	C	D	E			
Blue (0%)	1	5	5	5	5	5	1		FS
	2	5	5	5	5	5	Adjustment for Rep P.		GY
	3	5	5	5	5	5			FS
	4	5	5	5	5	5	23	96	GY
	5	5	5	5	3*	5	45		GH
	6	5	5	5	2	5	22	88	
	7	5	5	5	2	5			
Gray (0.15%) CCEC	1	5	5	5	5	5			FS
	2	5	4	5	5	5			GY
	3	5	4	5	5	5			FS
	4	5	4	5	5	5			GY
	5	5	4	5	5	5			FS
	6	5	3	5	5	5	23	92	GY
	7	5	3	5	5	5			
Green (1.12%) ACEC	1	5	5	5	5	5			FS
	2	5	5	5	5	5			GY
	3	5	5	5	5	5			FS
	4	5	5	5	4	5			GY
	5	5	5	5	4	5			GH
	6	5	5	5	4	5	24	96	EF
	7	5	5	5	4	5			
Yellow (12.5%)	1	5	5	5	4	5			FS
	2	5	5	5	4	5			FS
	3	5	5	5	4	5			FS
	4	5	5	5	4	5			GY
	5	5	5	5	4	5			FS
	6	5	4	5	4	5	23	92	GY
	7	5	4	5	4	5			
Orange (25%)	1	5	5	5	5	5			FS
	2	5	5	5	5	5			GY
	3	5	5	5	4	5			FS
	4	5	5	5	4	5			GY
	5	5	5	5	4	5			GH
	6	5	5	5	4	4	23	92	EF
	7	5	5	5	4	4			
Red (50%)	1	4	5	5	5	5			FS
	2	4	5	4	5	5			FS
	3	4	5	4	5	5			FS
	4	3	5	4	5	4			GY
	5	3	5	4	5	4			FS
	6	3	5	4	5	4	17	84	GY
	7	3	5	4	5	4			

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* knocked over

Vashon NPDES Characterization (1033898)
Atherinopsis affinis - 7-day Chronic Test

Test#: 9312
 Test Date: 10/2/19

(Cumulative Survival, Cont'd)

Trtmt	Cumulative Survival (# Alive/Rep) at 7d N = 5/Rep						Tot # Surv	% Surv*	Ana
	Day	A	B	C	D	E			
White (100%)	1	5	5	5	5	5			
	2	5	5	5	5	5			
	3	5	5	5	5	5			
	4	5	5	5	5	5			
	5	5	5	5	5	5			
	6	5	5	5	5	5			
	7	5	5	5	5	5	25	100	

s = stressed

*Pass if control survival $\geq 80\%$

Growth (Dry Weight/fish at 7 Days)

Trtmt	Dry Weight per Fish at 7 Days						Mean W
	Pan#	A	B	C	D	E	
Blue (0%)	Pan#	1	2	3	4	5	
	T.wt (mg)	85.64	92.00	87.01	81.67	87.82	
	Tare wt (mg)	79.77	84.60	82.53	79.21	80.86	
	Net wt (mg)	5.87	7.4	4.48	2.46	6.96	
	N	5	5	5	5 *	5	$\bar{X} = 1.1$
	mg/fish	1.174	1.480	0.896	0.492	1.392	$\bar{X} = 1.1$
Gray (0.15%) CCEC	Pan#	6	7	8	9	10	
	T.wt (mg)	82.16	82.33	82.28	82.61	99.29	
	Tare wt (mg)	77.24	79.61	76.12	76.64	92.92	
	Net wt (mg)	4.92	2.72	6.16	5.97	6.37	
	N	5	5	5	5	5	$\bar{X} = 1.04$
	mg/fish	0.984	0.544	1.232	1.194	1.274	$\bar{X} = 1.0$
Green (1.12%) ACEC	Pan#	11	12	13	14	15	
	T.wt (mg)	100.62	89.22	93.70	88.73	90.76	
	Tare wt (mg)	95.03	84.19	87.55	84.22	84.57	
	Net wt (mg)	5.59	5.03	6.15	4.51	6.19	
	N	5	5	5	5	5	
	mg/fish	1.118	1.006	1.230	0.902	1.238	$\bar{X} = 1.0$
Yellow (12.5%)	Pan#	16	17	18	19	20	
	T.wt (mg)	84.19	89.29	90.44	85.96	86.46	
	Tare wt (mg)	79.04	85.60	86.15	81.74	80.02	
	Net wt (mg)	5.15	3.69	4.29	4.22	6.44	
	N	5	5	5	5	5	
	mg/fish	1.030	0.738	0.858	0.844	1.288	$\bar{X} = 0.9$
Orange (25%)	Pan#	21	22	23	24	25	
	T.wt (mg)	90.79	88.57	77.70	90.29	91.15	
	Tare wt (mg)	84.79	83.40	71.10	85.65	84.59	
	Net wt (mg)	6.0	5.17	6.6	4.64	6.56	
	N	5	5	5	5	5	
	mg/fish	1.200	1.034	1.320	0.928	1.312	$\bar{X} = 1.15$

* Adjusted for lost organs N=3

From Day 5.

mg/fish = 0.82

FS

Vashon NPDES Characterization (1033898)
Atherinopsis affinis - 7-day Chronic Test

Test#: 9312
 Test Date: 10/2/19

(Growth, Cont'd)

Trtmt	Dry Weight per Fish at 7 Days						
		A	B	C	D	E	Mean Wt**
Red (50%)	Pan#	26	27	28	29	30	
	T.wt (mg)	89.56	91.48	90.45	87.05	100.30	
	Tare wt (mg)	86.37	85.53	85.43	80.44	95.21	
	Net wt (mg)	3.19	5.95	5.02	6.61	5.09	
	N	5	5	5	5	5	
	mg/fish	0.1038	1.190	1.004	1.322	1.018	$\bar{X} = 1.034$
White (100%)	Pan#	31	32	*33 96.95	34	35	
	T.wt (mg)	87.32	82.88	*27.20	89.84	88.65	
	Tare wt (mg)	81.78	77.01	85.05	83.09	82.97	
	Net wt (mg)	5.54	5.87	11.9	6.75	5.68	
	N	5	5	5	5	5	
	mg/fish	1.108	1.174	2.380	1.350	1.136	$\bar{X} = 1.429$

**Pass if mean control weight ≥ 0.85 mg/ind

*noticeable salt deposits in this pan.

Blank Tare Pans			
Pan #:	36	37	38
Pan Wt (mg) Before:	81.11	77.51	83.06
After:	81.10	77.52	83.04

Random Number Assignment

Code	Rep	Random #	Code	Rep	Random #	Code	Rep	Random #
Blue	A	20 21	Yellow	A	5 32	White	A	31 24
	B	25 31		B	18 30		B	8 3
	C	32 9		C	23 20		C	19 1
	D	7 13		D	14 7		D	4 6
	E	6 35		E	26 12		E	22 25
Gray	A	34 29	Orange	A	35 18			
	B	28 33		B	25 8			
	C	9 20		C	29 28			
	D	3 34		D	11 10			
	E	10 23		E	27 17			
Green	A	4 15	Red	A	30 11			
	B	15 14		B	16 10			
	C	12 4		C	2 6			
	D	17 2		D	24 22			
	E	33 27		E	13 19			

NOTES:

* 85 chgd placement
 Glassware rinse with hot tap and DW before use.

CETIS Analytical Report

Report Date: 28 Oct-19 15:09 (p 1 of 5)
Test Code/ID: 9312AACVA / 05-7820-2634

Pacific Topsmelt 7-d Survival and Growth Test

King County Metro Services, WQ Lab

Analysis ID: 00-6403-0259	Endpoint: 7d Survival Rate	CETIS Version: CETISv1.9.5
Analyzed: 21 Oct-19 14:38	Analysis: Nonparametric-Control vs Treatments	Status Level: 1
Batch ID: 19-9592-2114	Test Type: Growth-Survival (7d)	Analyst: LS
Start Date: 02 Oct-19 13:50	Protocol: EPA/600/R-95/136 (1995)	Diluent: Deionized Water
Ending Date: 09 Oct-19 13:40	Species: Atherinops affinis	Brine: Hawaiian Marine Mix
Test Length: 7d	Taxon: Actinopterygii	Source: Aquatic Biosystems, CO Age: 10d
Sample ID: 04-0045-4694	Code: L73401-2	Project: Effluent Characterization (Biannual)
Sample Date: 02 Oct-19 06:47	Material: POTW Effluent	Source: Vashon Permit WA002252-7 (WA0022
Receipt Date: 02 Oct-19 08:47	CAS (PC):	Station:
Sample Age: 7h (2 °C)	Client: Vashon Island Treatment Plant	

Chronic NPDES characterization (Vashon TP)

Vashon effluent DAY 0: L73401-1 coll. 10/1/19-10/2/19 0647h-0647h; rec'd at KCEL 10/2/19 at 0847h in 1 5-gal glass jar; at plant pH = 7.58, T= 2.2oC, TRC = 0.03mg/L; at KCEL pH=7.61, T=2.0oC, DO= 10.8 mg/L; DAY2: L73401-5 coll. 10/3/19-10/4/19 0637h-0637h, rec'd at KCEL at 0850h in 2 glass 5gal glass jars, at plant: pH=7.57, T=2.5oC, TRC= 0.04mg/L; at lab pH = 7.58/7.64, T= 1.8/1.7oC, DO = 10.3/10.4mg/L; DAY 5: L73401-6 coll. 10/6/19-10/7/19 0610h-0610h, rec'd at KCEL at 0903hh in 1 glass 5gal glass jar, at plant: pH=7.60, T=2.8oC, TRC= 0.02mg/L; at lab pH = 7.60, T=1.9oC, DO=10.5mg/L;

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	C > T	100	>100	n/a	1	20.26%

Steel Many-One Rank Sum Test

Control	vs	Conc-%	Test Stat	Critical	Ties	DF	P-Type	P-Value	Decision(α:5%)
Dilution Water		0.15	27	16	1	8	CDF	0.8267	Non-Significant Effect
		1.12	28	16	1	8	CDF	0.8838	Non-Significant Effect
		12.5	26	16	1	8	CDF	0.7547	Non-Significant Effect
		25	26	16	1	8	CDF	0.7547	Non-Significant Effect
		50	23	16	1	8	CDF	0.4756	Non-Significant Effect
		100	30	16	1	8	CDF	0.9557	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	Lower	Upper	Overlap	Decision
Control Resp	0.9333	0.8	>>	Yes	Passes Criteria
PMSD	0.2026	<<	0.25	No	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0967564	0.0161261	6	0.727	0.6316	Non-Significant Effect
Error	0.621084	0.0221816	28			
Total	0.71784		34			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	2.306	3.528	0.0619	Equal Variances
	Mod Levene Equality of Variance Test	0.5399	3.812	0.7720	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.855	0.9146	3.0E-04	Non-Normal Distribution

7d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	0.9333	0.7482	1.0000	1.0000	0.6667	1.0000	0.0667	15.97%	0.00%
0.15		5	0.9200	0.6979	1.0000	1.0000	0.6000	1.0000	0.0800	19.44%	1.43%
1.12		5	0.9600	0.8489	1.0000	1.0000	0.8000	1.0000	0.0400	9.32%	-2.86%
12.5		5	0.9200	0.7840	1.0000	1.0000	0.8000	1.0000	0.0490	11.91%	1.43%
25		5	0.9200	0.7840	1.0000	1.0000	0.8000	1.0000	0.0490	11.91%	1.43%
50		5	0.8400	0.6322	1.0000	0.8000	0.6000	1.0000	0.0748	19.92%	10.00%
100		5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-7.14%

CETIS Analytical Report

Report Date: 28 Oct-19 15:09 (p 2 of 5)
Test Code/ID: 9312AACVA / 05-7820-2634

Pacific Topsmelt 7-d Survival and Growth Test

King County Metro Services, WQ Lab

Analysis ID: 00-6403-0259 Endpoint: 7d Survival Rate CETIS Version: CETISv1.9.5
Analyzed: 21 Oct-19 14:38 Analysis: Nonparametric-Control vs Treatments Status Level: 1

Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	1.267	1.051	1.484	1.345	0.9553	1.345	0.07799	13.76%	0.00%
0.15		5	1.253	0.9984	1.508	1.345	0.8861	1.345	0.09184	16.38%	1.09%
1.12		5	1.298	1.165	1.43	1.345	1.107	1.345	0.04763	8.21%	-2.40%
12.5		5	1.25	1.088	1.412	1.345	1.107	1.345	0.05833	10.43%	1.36%
25		5	1.25	1.088	1.412	1.345	1.107	1.345	0.05833	10.43%	1.36%
50		5	1.158	0.9183	1.398	1.107	0.8861	1.345	0.08639	16.68%	8.61%
100		5	1.345	1.345	1.346	1.345	1.345	1.345	0	0.00%	-6.15%

7d Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.0000	1.0000	1.0000	0.6667	1.0000
0.15		1.0000	0.6000	1.0000	1.0000	1.0000
1.12		1.0000	1.0000	1.0000	0.8000	1.0000
12.5		1.0000	0.8000	1.0000	0.8000	1.0000
25		1.0000	1.0000	1.0000	0.8000	0.8000
50		0.6000	1.0000	0.8000	1.0000	0.8000
100		1.0000	1.0000	1.0000	1.0000	1.0000

Angular (Corrected) Transformed Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.345	1.345	1.345	0.9553	1.345
0.15		1.345	0.8861	1.345	1.345	1.345
1.12		1.345	1.345	1.345	1.107	1.345
12.5		1.345	1.107	1.345	1.107	1.345
25		1.345	1.345	1.345	1.107	1.107
50		0.8861	1.345	1.107	1.345	1.107
100		1.345	1.345	1.345	1.345	1.345

7d Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	5/5	5/5	5/5	2/3	5/5
0.15		5/5	3/5	5/5	5/5	5/5
1.12		5/5	5/5	5/5	4/5	5/5
12.5		5/5	4/5	5/5	4/5	5/5
25		5/5	5/5	5/5	4/5	4/5
50		3/5	5/5	4/5	5/5	4/5
100		5/5	5/5	5/5	5/5	5/5

CETIS Analytical Report

Report Date: 28 Oct-19 15:09 (p 3 of 5)
Test Code/ID: 9312AACVA / 05-7820-2634

Pacific Topsmelt 7-d Survival and Growth Test

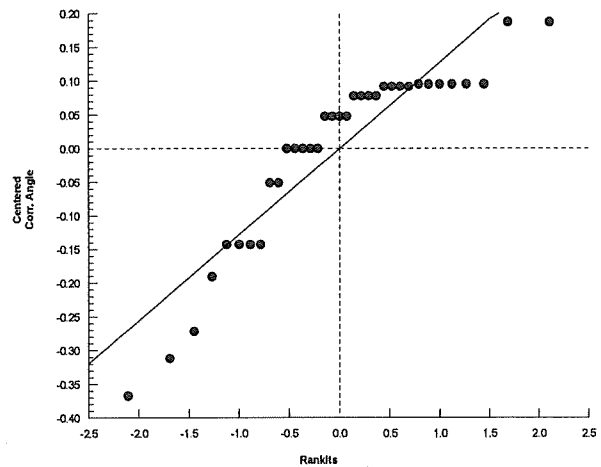
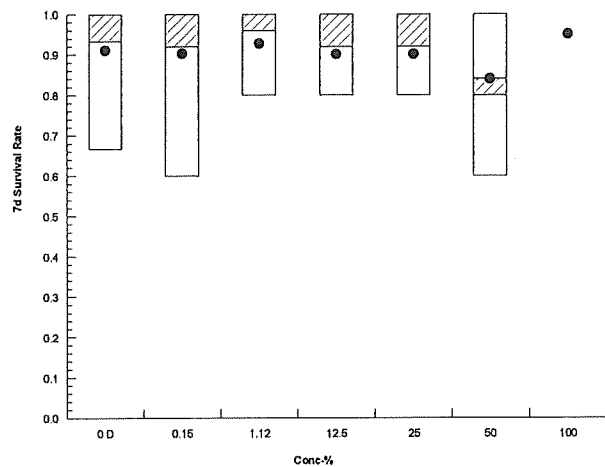
King County Metro Services, WQ Lab

Analysis ID: 00-6403-0259
Analyzed: 21 Oct-19 14:38

Endpoint: 7d Survival Rate
Analysis: Nonparametric-Control vs Treatments

CETIS Version: CETISv1.9.5
Status Level: 1

Graphics



CETIS Analytical Report

Report Date: 28 Oct-19 15:09 (p 4 of 5)
 Test Code/ID: 9312AACVA / 05-7820-2634

Pacific Topsmelt 7-d Survival and Growth Test

King County Metro Services, WQ Lab

Analysis ID: 15-1785-5173	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.9.5
Analyzed: 21 Oct-19 14:41	Analysis: Parametric-Control vs Treatments	Status Level: 1
Batch ID: 19-9592-2114	Test Type: Growth-Survival (7d)	Analyst: LS
Start Date: 02 Oct-19 13:50	Protocol: EPA/600/R-95/136 (1995)	Diluent: Deionized Water
Ending Date: 09 Oct-19 13:40	Species: Atherinops affinis	Brine: Hawaiian Marine Mix
Test Length: 7d	Taxon: Actinopterygii	Source: Aquatic Biosystems, CO Age: 10d
Sample ID: 04-0045-4694	Code: L73401-2	Project: Effluent Characterization (Biannual)
Sample Date: 02 Oct-19 06:47	Material: POTW Effluent	Source: Vashon Permit WA002252-7 (WA0022)
Receipt Date: 02 Oct-19 08:47	CAS (PC):	Station:
Sample Age: 7h (2 °C)	Client: Vashon Island Treatment Plant	

Chronic NPDES characterization (Vashon TP)

Vashon effluent DAY 0: L73401-1 coll. 10/1/19-10/2/19 0647h-0647h; rec'd at KCEL 10/2/19 at 0847h in 1 5-gal glass jar; at plant pH = 7.58, T= 2.2oC, TRC = 0.03mg/L; at KCEL pH=7.61, T=2.0oC, DO= 10.8 mg/L; DAY2: L73401-5 coll. 10/3/19-10/4/19 0637h-0637h, rec'd at KCEL at 0850h in 2 glass 5gal glass jars, at plant: pH=7.57, T=2.5oC, TRC= 0.04mg/L; at lab pH = 7.58/7.64, T= 1.8/1.7oC, DO = 10.3/10.4mg/L; DAY 5: L73401-6 coll. 10/6/19-10/7/19 0610h-0610h, rec'd at KCEL at 0903hh in 1 glass 5gal glass jar, at plant: pH=7.60, T=2.8oC, TRC= 0.02mg/L; at lab pH = 7.60, T=1.9oC, DO=10.5mg/L;

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	100	>100	n/a	1	39.68%

Dunnett Multiple Comparison Test

Control	vs	Conc-%	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Dilution Water		0.15	0.5623	2.407	0.457	8	CDF	0.6534	Non-Significant Effect
		1.12	0.2822	2.407	0.457	8	CDF	0.7670	Non-Significant Effect
		12.5	1.057	2.407	0.457	8	CDF	0.4261	Non-Significant Effect
		25	-0.0337	2.407	0.457	8	CDF	0.8661	Non-Significant Effect
		50	0.6213	2.407	0.457	8	CDF	0.6272	Non-Significant Effect
		100	-1.459	2.407	0.457	8	CDF	0.9971	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	Lower	Upper	Overlap	Decision
Control Resp	1.152	0.85	>>	Yes	Passes Criteria
PMSD	0.3968	<<	0.5	No	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.699693	0.116616	6	1.293	0.2925	Non-Significant Effect
Error	2.52514	0.0901834	28			
Total	3.22483		34			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	8.77	16.81	0.1869	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9341	0.9146	0.0371	Normal Distribution

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	1.152	0.7896	1.515	1.174	0.82	1.48	0.1307	25.35%	0.00%
0.15		5	1.046	0.6709	1.42	1.194	0.544	1.274	0.135	28.86%	9.27%
1.12		5	1.099	0.9185	1.279	1.118	0.902	1.238	0.06492	13.21%	4.65%
12.5		5	0.9516	0.6844	1.219	0.858	0.738	1.288	0.09625	22.62%	17.42%
25		5	1.159	0.9437	1.374	1.2	0.928	1.32	0.07746	14.95%	-0.56%
50		5	1.034	0.7147	1.354	1.018	0.638	1.322	0.1152	24.89%	10.24%
100		5	1.43	0.7596	2.1	1.174	1.108	2.38	0.2413	37.74%	-24.05%

CETIS Analytical Report

Report Date: 28 Oct-19 15:09 (p 5 of 5)
Test Code/ID: 9312AACVA / 05-7820-2634

Pacific Topsmelt 7-d Survival and Growth Test

King County Metro Services, WQ Lab

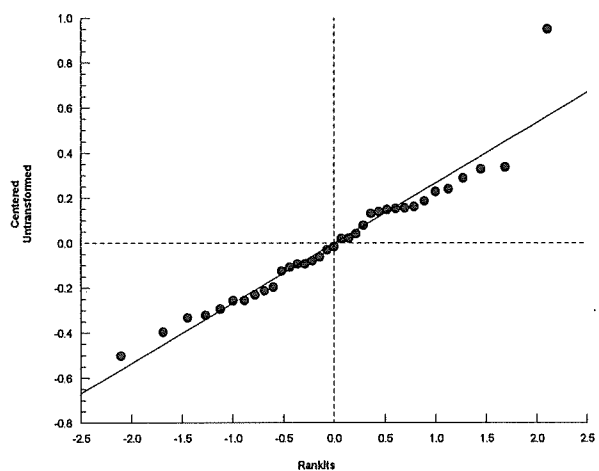
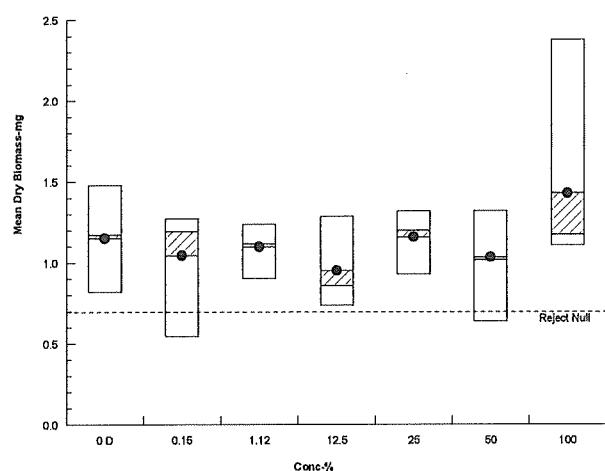
Analysis ID: 15-1785-5173 Endpoint: Mean Dry Biomass-mg
Analyzed: 21 Oct-19 14:41 Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.9.5
Status Level: 1

Mean Dry Biomass-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.174	1.48	0.896	0.82	1.392
0.15		0.984	0.544	1.232	1.194	1.274
1.12		1.118	1.006	1.23	0.902	1.238
12.5		1.03	0.738	0.858	0.844	1.288
25		1.2	1.034	1.32	0.928	1.312
50		0.638	1.19	1.004	1.322	1.018
100		1.108	1.174	2.38	1.35	1.136

Graphics



CETIS Analytical Report

Report Date: 28 Oct-19 15:09 (p 1 of 4)

Test Code/ID: 9312AACVA / 05-7820-2634

Pacific Topsmelt 7-d Survival and Growth Test

King County Metro Services, WQ Lab

Analysis ID: 01-9960-2751	Endpoint: 7d Survival Rate	CETIS Version: CETISv1.9.5
Analyzed: 21 Oct-19 14:39	Analysis: Linear Interpolation (ICPIN)	Status Level: 1
Batch ID: 19-9592-2114	Test Type: Growth-Survival (7d)	Analyst: LS
Start Date: 02 Oct-19 13:50	Protocol: EPA/600/R-95/136 (1995)	Diluent: Deionized Water
Ending Date: 09 Oct-19 13:40	Species: Atherinops affinis	Brine: Hawaiian Marine Mix
Test Length: 7d	Taxon: Actinopterygii	Source: Aquatic Biosystems, CO Age: 10d
Sample ID: 04-0045-4694	Code: L73401-2	Project: Effluent Characterization (Biannual)
Sample Date: 02 Oct-19 06:47	Material: POTW Effluent	Source: Vashon Permit WA002252-7 (WA0022
Receipt Date: 02 Oct-19 08:47	CAS (PC):	Station:
Sample Age: 7h (2 °C)	Client: Vashon Island Treatment Plant	

Chronic NPDES characterization (Vashon TP)

Vashon effluent DAY 0: L73401-1 coll. 10/1/19-10/2/19 0647h-0647h; rec'd at KCEL 10/2/19 at 0847h in 1 5-gal glass jar; at plant pH = 7.58, T= 2.2oC, TRC = 0.03mg/L; at KCEL pH=7.61, T=2.0oC, DO= 10.8 mg/L; DAY2: L73401-5 coll. 10/3/19-10/4/19 0637h-0637h, rec'd at KCEL at 0850h in 2 glass 5gal glass jars, at plant: pH=7.57, T=2.5oC, TRC= 0.04mg/L; at lab pH = 7.58/7.64, T= 1.8/1.7oC, DO = 10.3/10.4mg/L; DAY 5: L73401-6 coll. 10/6/19-10/7/19 0610h-0610h, rec'd at KCEL at 0903hh in 1 glass 5gal glass jar, at plant: pH=7.60, T=2.8oC, TRC= 0.02mg/L; at lab pH = 7.60, T=1.9oC, DO=10.5mg/L;

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1272662	200	Yes	Two-Point Interpolation

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits		Overlap	Decision
Control Resp	0.9333	Lower	Upper	Yes	Passes Criteria

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC50	>100	n/a	n/a	<1	n/a	n/a

7d Survival Rate Summary

			Calculated Variate(A/B)							Isotonic Variate	
Conc-%	Code	Count	Mean	Min	Max	Std Dev	CV%	%Effect	A/B	Mean	%Effect
0	D	5	0.9333	0.6667	1.0000	0.1491	15.97%	0.0%	22/23	0.9378	0.0%
0.15		5	0.9200	0.6000	1.0000	0.1789	19.44%	1.43%	23/25	0.9378	0.0%
1.12		5	0.9600	0.8000	1.0000	0.0894	9.32%	-2.86%	24/25	0.9378	0.0%
12.5		5	0.9200	0.8000	1.0000	0.1095	11.91%	1.43%	23/25	0.92	1.9%
25		5	0.9200	0.8000	1.0000	0.1095	11.91%	1.43%	23/25	0.92	1.9%
50		5	0.8400	0.6000	1.0000	0.1673	19.92%	10.0%	21/25	0.92	1.9%
100		5	1.0000	1.0000	1.0000	0.0000	0.00%	-7.14%	25/25	0.92	1.9%

7d Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.0000	1.0000	1.0000	0.6667	1.0000
0.15		1.0000	0.6000	1.0000	1.0000	1.0000
1.12		1.0000	1.0000	1.0000	0.8000	1.0000
12.5		1.0000	0.8000	1.0000	0.8000	1.0000
25		1.0000	1.0000	1.0000	0.8000	0.8000
50		0.6000	1.0000	0.8000	1.0000	0.8000
100		1.0000	1.0000	1.0000	1.0000	1.0000

CETIS Analytical Report

Report Date: 28 Oct-19 15:09 (p 2 of 4)
Test Code/ID: 9312AACVA / 05-7820-2634

Pacific Topsmelt 7-d Survival and Growth Test

King County Metro Services, WQ Lab

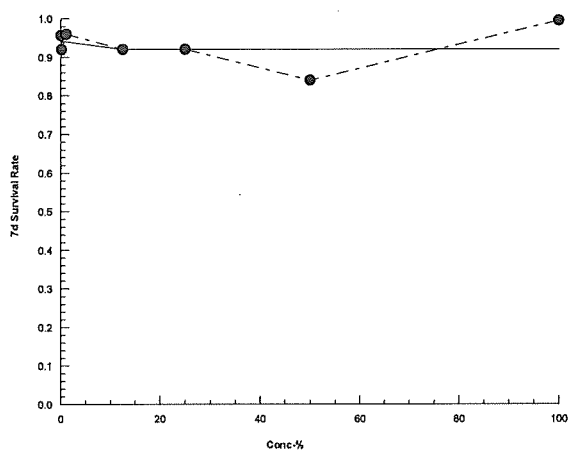
Analysis ID: 01-9960-2751 Endpoint: 7d Survival Rate
Analyzed: 21 Oct-19 14:39 Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.9.5
Status Level: 1

7d Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	5/5	5/5	5/5	2/3	5/5
0.15		5/5	3/5	5/5	5/5	5/5
1.12		5/5	5/5	5/5	4/5	5/5
12.5		5/5	4/5	5/5	4/5	5/5
25		5/5	5/5	5/5	4/5	4/5
50		3/5	5/5	4/5	5/5	4/5
100		5/5	5/5	5/5	5/5	5/5

Graphics



CETIS Analytical Report

Report Date: 28 Oct-19 15:09 (p 3 of 4)

Test Code/ID: 9312AACVA / 05-7820-2634

Pacific Topsmelt 7-d Survival and Growth Test

King County Metro Services, WQ Lab

Analysis ID: 04-2301-0967	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.9.5
Analyzed: 21 Oct-19 14:41	Analysis: Linear Interpolation (ICPIN)	Status Level: 1
Batch ID: 19-9592-2114	Test Type: Growth-Survival (7d)	Analyst: LS
Start Date: 02 Oct-19 13:50	Protocol: EPA/600/R-95/136 (1995)	Diluent: Deionized Water
Ending Date: 09 Oct-19 13:40	Species: Atherinops affinis	Brine: Hawaiian Marine Mix
Test Length: 7d	Taxon: Actinopterygii	Source: Aquatic Biosystems, CO Age: 10d
Sample ID: 04-0045-4694	Code: L73401-2	Project: Effluent Characterization (Biannual)
Sample Date: 02 Oct-19 06:47	Material: POTW Effluent	Source: Vashon Permit WA002252-7 (WA0022
Receipt Date: 02 Oct-19 08:47	CAS (PC):	Station:
Sample Age: 7h (2 °C)	Client: Vashon Island Treatment Plant	

Chronic NPDES characterization (Vashon TP)

Vashon effluent DAY 0: L73401-1 coll. 10/1/19-10/2/19 0647h-0647h; rec'd at KCEL 10/2/19 at 0847h in 1 5-gal glass jar; at plant pH = 7.58, T= 2.2oC, TRC = 0.03mg/L; at KCEL pH=7.61, T=2.0oC, DO= 10.8 mg/L; DAY2: L73401-5 coll. 10/3/19-10/4/19 0637h-0637h, rec'd at KCEL at 0850h in 2 glass 5gal glass jars, at plant: pH=7.57, T=2.5oC, TRC= 0.04mg/L; at lab pH = 7.58/7.64, T= 1.8/1.7oC, DO = 10.3/10.4mg/L; DAY 5: L73401-6 coll. 10/6/19-10/7/19 0610h-0610h, rec'd at KCEL at 0903hh in 1 glass 5gal glass jar, at plant: pH=7.60, T=2.8oC, TRC= 0.02mg/L; at lab pH = 7.60, T=1.9oC, DO=10.5mg/L;

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1996877	200	Yes	Two-Point Interpolation

Test Acceptability Criteria

TAC Limits					
Attribute	Test Stat	Lower	Upper	Overlap	Decision
Control Resp	1.152	0.85	>>	Yes	Passes Criteria

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC25	>100	n/a	n/a	<1	n/a	n/a

Mean Dry Biomass-mg Summary

			Calculated Variate							Isotonic Variate	
Conc-%	Code	Count	Mean	Min	Max	Std Dev	CV%	%Effect		Mean	%Effect
0	D	5	1.152	0.82	1.48	0.2922	25.35%	0.0%		1.152	0.0%
0.15		5	1.046	0.544	1.274	0.3018	28.86%	9.27%		1.12	2.83%
1.12		5	1.099	0.902	1.238	0.1452	13.21%	4.65%		1.12	2.83%
12.5		5	0.9516	0.738	1.288	0.2152	22.62%	17.42%		1.12	2.83%
25		5	1.159	0.928	1.32	0.1732	14.95%	-0.56%		1.12	2.83%
50		5	1.034	0.638	1.322	0.2575	24.89%	10.24%		1.12	2.83%
100		5	1.43	1.108	2.38	0.5396	37.74%	-24.05%		1.12	2.83%

Mean Dry Biomass-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.174	1.48	0.896	0.82	1.392
0.15		0.984	0.544	1.232	1.194	1.274
1.12		1.118	1.006	1.23	0.902	1.238
12.5		1.03	0.738	0.858	0.844	1.288
25		1.2	1.034	1.32	0.928	1.312
50		0.638	1.19	1.004	1.322	1.018
100		1.108	1.174	2.38	1.35	1.136

CETIS Analytical Report

Report Date: 28 Oct-19 15:09 (p 4 of 4)
Test Code/ID: 9312AACVA / 05-7820-2634

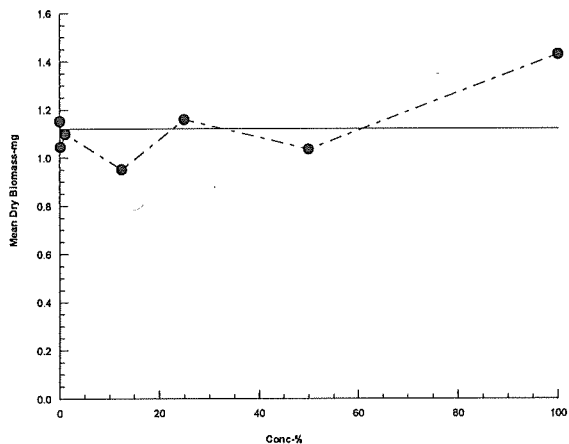
Pacific Topsmelt 7-d Survival and Growth Test

King County Metro Services, WQ Lab

Analysis ID: 04-2301-0967 Endpoint: Mean Dry Biomass-mg
Analyzed: 21 Oct-19 14:41 Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.9.5
Status Level: 1

Graphics



CETIS Summary Report

Report Date: 28 Oct-19 15:09 (p 1 of 2)

Test Code/ID: 9312AACVA / 05-7820-2634

Pacific Topsmelt 7-d Survival and Growth Test

King County Metro Services, WQ Lab

Batch ID: 19-9592-2114	Test Type: Growth-Survival (7d)	Analyst: LS
Start Date: 02 Oct-19 13:50	Protocol: EPA/600/R-95/136 (1995)	Diluent: Deionized Water
Ending Date: 09 Oct-19 13:40	Species: Atherinops affinis	Brine: Hawaiian Marine Mix
Test Length: 7d	Taxon: Actinopterygii	Source: Aquatic Biosystems, CO Age: 10d
Sample ID: 04-0045-4694	Code: L73401-2	Project: Effluent Characterization (Biannual)
Sample Date: 02 Oct-19 06:47	Material: POTW Effluent	Source: Vashon Permit WA002252-7 (WA0022
Receipt Date: 02 Oct-19 08:47	CAS (PC):	Station:
Sample Age: 7h (2 °C)	Client: Vashon Island Treatment Plant	

Chronic NPDES characterization (Vashon TP)

Vashon effluent DAY 0: L73401-1 coll. 10/1/19-10/2/19 0647h-0647h; rec'd at KCEL 10/2/19 at 0847h in 1 5-gal glass jar; at plant pH = 7.58, T= 2.2oC, TRC = 0.03mg/L; at KCEL pH=7.61, T=2.0oC, DO= 10.8 mg/L; DAY2: L73401-5 coll. 10/3/19-10/4/19 0637h-0637h, rec'd at KCEL at 0850h in 2 glass 5gal glass jars, at plant: pH=7.57, T=2.5oC, TRC= 0.04mg/L; at lab pH = 7.58/7.64, T= 1.8/1.7oC, DO = 10.3/10.4mg/L; DAY 5: L73401-6 coll. 10/6/19-10/7/19 0610h-0610h, rec'd at KCEL at 0903hh in 1 glass 5gal glass jar, at plant: pH=7.60, T=2.8oC, TRC= 0.02mg/L; at lab pH = 7.60, T=1.9oC, DO=10.5mg/L;

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	TU	PMSD	S
00-6403-0259	7d Survival Rate	Steel Many-One Rank Sum Test		100	>100	n/a	1	20.3%	1
15-1785-5173	Mean Dry Biomass-mg	Dunnett Multiple Comparison Test		100	>100	n/a	1	39.7%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	%	95% LCL	95% UCL	TU	S
01-9960-2751	7d Survival Rate	Linear Interpolation (ICPIN)		EC50	>100	n/a	n/a	<1	1
04-2301-0967	Mean Dry Biomass-mg	Linear Interpolation (ICPIN)		IC25	>100	n/a	n/a	<1	1

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
00-6403-0259	7d Survival Rate	Control Resp	0.9333	0.8	>>	Yes	Passes Criteria
01-9960-2751	7d Survival Rate	Control Resp	0.9333	0.8	>>	Yes	Passes Criteria
04-2301-0967	Mean Dry Biomass-mg	Control Resp	1.152	0.85	>>	Yes	Passes Criteria
15-1785-5173	Mean Dry Biomass-mg	Control Resp	1.152	0.85	>>	Yes	Passes Criteria
00-6403-0259	7d Survival Rate	PMSD	0.2026	<<	0.25	No	Passes Criteria
15-1785-5173	Mean Dry Biomass-mg	PMSD	0.3968	<<	0.5	No	Passes Criteria

7d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	5	0.9333	0.7482	1.0000	0.6667	1.0000	0.0667	0.1491	15.97%	0.00%
0.15		5	0.9200	0.6979	1.0000	0.6000	1.0000	0.0800	0.1789	19.44%	1.43%
1.12		5	0.9600	0.8489	1.0000	0.8000	1.0000	0.0400	0.0894	9.32%	-2.86%
12.5		5	0.9200	0.7840	1.0000	0.8000	1.0000	0.0490	0.1095	11.91%	1.43%
25		5	0.9200	0.7840	1.0000	0.8000	1.0000	0.0490	0.1095	11.91%	1.43%
50		5	0.8400	0.6322	1.0000	0.6000	1.0000	0.0748	0.1673	19.92%	10.00%
100		5	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-7.14%

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	5	1.152	0.7896	1.515	0.82	1.48	0.1307	0.2922	25.35%	0.00%
0.15		5	1.046	0.6709	1.42	0.544	1.274	0.135	0.3018	28.86%	9.27%
1.12		5	1.099	0.9185	1.279	0.902	1.238	0.06492	0.1452	13.21%	4.65%
12.5		5	0.9516	0.6844	1.219	0.738	1.288	0.09625	0.2152	22.62%	17.42%
25		5	1.159	0.9437	1.374	0.928	1.32	0.07746	0.1732	14.95%	-0.56%
50		5	1.034	0.7147	1.354	0.638	1.322	0.1152	0.2575	24.89%	10.24%
100		5	1.43	0.7596	2.1	1.108	2.38	0.2413	0.5396	37.74%	-24.05%

CETIS Summary Report

Report Date: 28 Oct-19 15:09 (p 2 of 2)

Test Code/ID: 9312AACVA / 05-7820-2634

Pacific Topsmelt 7-d Survival and Growth Test

King County Metro Services, WQ Lab

7d Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.0000	1.0000	1.0000	0.6667	1.0000
0.15		1.0000	0.6000	1.0000	1.0000	1.0000
1.12		1.0000	1.0000	1.0000	0.8000	1.0000
12.5		1.0000	0.8000	1.0000	0.8000	1.0000
25		1.0000	1.0000	1.0000	0.8000	0.8000
50		0.6000	1.0000	0.8000	1.0000	0.8000
100		1.0000	1.0000	1.0000	1.0000	1.0000

Mean Dry Biomass-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.174	1.48	0.896	0.82	1.392
0.15		0.984	0.544	1.232	1.194	1.274
1.12		1.118	1.006	1.23	0.902	1.238
12.5		1.03	0.738	0.858	0.844	1.288
25		1.2	1.034	1.32	0.928	1.312
50		0.638	1.19	1.004	1.322	1.018
100		1.108	1.174	2.38	1.35	1.136

7d Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	5/5	5/5	5/5	2/3	5/5
0.15		5/5	3/5	5/5	5/5	5/5
1.12		5/5	5/5	5/5	4/5	5/5
12.5		5/5	4/5	5/5	4/5	5/5
25		5/5	5/5	5/5	4/5	4/5
50		3/5	5/5	4/5	5/5	4/5
100		5/5	5/5	5/5	5/5	5/5

King County Environmental Laboratory Lab Review Report

Reported: 28-Oct-19 15:12 ~ Data Source: ELD

Listtype / Method: AQTOPSMELT-CHRONIC / EPA600/R-95/136
Run ID / Workgroup: R238570 / WG167050

CollectDate	Tspan	Project	Mat	Locator	Sample	Parameter	Value	Units	Qual	Mdl	Rdl	Textvalue
2019/10/01 08:47:00		421488	LC	VS_EFF	L73401-2	Survival NOEC Growth NOEC	100 100	%sample %sample				t-Test: Control vs ACEC not significant; p > 0.05
						Growth IC25 Survival LC50		%sample %sample	TA TA			Indeterminate (>100% effluent) Indeterminate (>highest concentration tested)
						Test Number Date Analyzed Prep Date		none none				9312 02-OCT-19 13:50 02-OCT-19 13:00

No products missing

Vashon NPDES Characterization (1033898),
***Mysidopsis bahia* 7-Day Chronic Renewal Test**

Test#: 9311
 Test Date: 10/2/19

ORGANISMS

Mysids received from ABS via FedEx as 7 days old (Hatch Date: 9/25/19) Arrived at 1020 h on 10-2-19.
 At Arrival: Salinity 24 ppt, Temp 18.3 °C; pH 7.424 ^{7.347} ^{9a}; D.O. 11.3 mg/L. Shipped in 1 double plastic bag. 3 dead removed.
 Acclimation: Placed into 3 1.5L cryst dishes. Into 20 °C waterbath at 1038 h. Fed 4 mL *Artemia* nauplii/dish at 1043 h. Diluted 1/3 w/30ppt HMM
 At 1125 h incr WB temp to 22.5 °C
1205 h " " " 23.5 °C
1240 h " " " 25.5 °C
 h " " " °C

SAMPLE/DILUTION WATER

- Hawaiian Marine Mix (HMM) #HW-1016 Synthetic Seawater:** 35.7 g HMM artificial sea salts (Lot # —, Rec'd 3/11/19, Opened 10/4/19) + 0.2 g NaHCO₃ in 1L MilliQ Water. Sal 30ppt, 0.45 µm filtered. Aerate > 2h before use.
- Vashon Final Effluent:** Salinity adjusted to 30 ppt by adding 71.4 g HMM sea salts + 0.40 g NaHCO₃/2L effluent after warming. For sample information see Test # 9312 Day 0, 2, 5
- LIMS MYC Sample #:** 73401-3; Wkgrp # 446167051

DILUTIONS

Code	% Sample	Sample (mL)	HMM (mL)
Blue	0	0	2000 (HMM-only)
Gray	0.15 (CCEC)	3	≤ 2000
Green	1.12 (ACEC)	22.4	≤ 2000
Yellow	12.5	250	≤ 2000
Orange	25	500	≤ 2000
Red	50	1000	≤ 2000
White	100	2000	0

PROCEDURE

- Pour effluent into each of 2 (two) 4-L beakers.
- Bring to 26°C and measure pH, D.O. of unsalted effluent.
- Adjust salinity to 30 ppt with HMM; measure pH, D.O., salinity of salted effluent.
- Prep solutions as above in 2000-mL "Class A" graduated cylinder; decant to 2 L glass flasks and bring to 26°C in waterbath. On Day 0, go to Step 5 w/o warming.
- Pour 250 mL each trtmt to each of (8) 400-mL beakers (Reps A-H)/trtmt.
- Place beakers randomly in waterbath # A and bring solutions to 26°C. Setup at — h.
- Add 5 mysids/beaker directly into solutions with polyscreen; rinse screen with DW between beakers.

Vashon NPDES Characterization (1033898),
***Mysidopsis bahia* 7-Day Chronic Renewal Test**

Test#: 9311
 Test Date: 10/2/19

8. Start test at 1400 h on 10-2-19. Counts verified PR & —. Take 0h chemical samples ✓ Acidify legit Analyst Gy. Place HOBO/Tidbit temp recorder (SN 26067701) in beaker w/DW in water bath.
9. Feed mysids 2 drops *Artemia* nauplii/beaker 2x/day.
10. Renew solutions daily:
 - a. Remove approximately 200 mL test solution + waste + excess food by decanting and/or pipet+bulb.
 - b. Replace \leq 250 mL with fresh solution (26°C) by pouring down side of beaker.
 - c. Count larvae before and after renewal.
11. Record #live and remove dead mysids daily at renewal. Measure Temp daily in 1 rep/trtmt and in 6 positions (4 corner + 2 center) just prior to renewal. Measure D.O. daily in 0h (new) and 24h (old) solns. Measure pH and Salinity in 24h (old) solns daily.
12. End test at 1300 h on 10-9-19. Measure temp in 1 rep/trtmt & in 6 beakers (4 corner + 2 center). Measure pH, DO and salinity. Record survival. Rinse larvae with ice water onto screen and place into tared weigh pans. Process by rep (1 analyst/rep):

Rep	Analyst	Rep	Analyst
A	JA	E	EF
B	PS	F	ES
C	Gy	G	Gy
D	FF	H	JA

Into 60°C oven at 1407 h on 10-9-19. Into desiccator at 1000 h on 10-10-19. Weigh at 1600 h on 10-10-19 by PS with Mettler XP105 balance.

FEEDING SCHEDULE (Time, h) (2 drops/beaker)

Day	1	2	Analyst
0	1438	1750	JA / JA
1	0920	1800	EF / JA
2	1245	1830	Gy / JA
3	0930	1845	EF / JA
4	1040	1915	PS / JA
5	1130	1705	PS / JA
6	0855	1655	Gy / JA

NOTES

Glassware rinses w/hot tap & DW before use.

Effluent After Salting Up

Vashon NPDES Characterization (1033898),
Mysidopsis bahia 7-Day Chronic Renewal Test

Test#: 9311
Test Date: 10/2/19

MEASUREMENTS

Day	Temperature (°C) SN: 170780325 (24-Hour Solutions)							Analyst
	Blue	Gray	Green	Yellow	Orange	Red	White	
0	26.1	26.1	26.1	26.1	26.1	26.1	26.1	GY
1	26.0	26.1	26.2	26.0	26.0	25.9	26.1	GY
2	26.1	26.2	26.1	26.1	26.0	26.1	25.9	ER
3	26.0	26.1	26.1	26.0	26.0	26.0	25.9	ER
4	26.2	26.2	25.9	26.0	26.1	26.1	25.7	RR
5	26.1	26.1	26.1	26.1	26.1	26.1	25.9	ER
6	26.1	26.1	26.1	26.1	26.1	26.1	26.1	GY
7	26.2	26.2	26.1	25.9	26.1	25.9	26.1	GY

Code	Rep	Temperature, °C in 6 Positions (4 Corner + 2 Center)							
		0d	1d	2d	3d	4d	5d	6d	7d
Gray	F	26.1	26.1	26.2	26.2	26.2	26.1	26.2	26.1
Gray	H	26.1	26.2	26.2	26.1	26.2	26.2	26.2	26.1
Green	H	26.0	26.1	26.1	26.1	25.9	26.1	26.1	26.0
Yellow	A	25.9	26.0	26.1	26.0	26.0	26.1	26.1	26.0
Yellow	D	26.0	26.1	26.0	26.0	26.0	26.1	26.1	26.1
White	A	25.8	25.7	25.9	25.9	25.7	25.9	25.9	25.8

Day	pH (24-Hour Solutions)							Analyst:
	Blue	Gray	Green	Yellow	Orange	Red	White	
1	7.871	7.898	7.908	8.042	8.170	8.332	8.463	GY
2	7.839	7.843	7.909	7.922	8.091	8.238	8.390	JA
3	7.869	7.870	7.921	8.056	8.289	8.306	8.440	GY
4	7.809	7.778	7.874	8.000	8.137	8.298	8.447	JA/RR
5	7.850	7.773	7.738	7.950	8.061	8.209	8.367	GY
6	7.951	7.915	7.908	8.076	8.140	8.287	8.425	JA
7	7.740	7.866	7.926	8.031	8.111	8.273	8.456	JA

Day	Salinity (ppt) (24-Hour Solutions)							Analyst
	Blue	Gray	Green	Yellow	Orange	Red	White	
1	30	30	30	30	30	30	30	GY
2	31	30	30	30	30	30	30	JA
3	30	30	30	30	30	30	30	GY
4	30	30	30	30	30	30	30	JA/RR
5	30	30	30	30	30	30	30	GY
6	30	30	30	30	30	30	30	JA
7	31	32	31	32	32	32	31	JA

Vashon NPDES Characterization (1033898),
Mysidopsis bahia 7-Day Chronic Renewal Test

Test#: 9311
Test Date: 10/2/19

Day	D.O. (mg/L); 0-Hour & 24-Hour Solutions								Analyst
	Blue		Gray		Green		Yellow		
	0h	24h	0h	24h	0h	24h	0h	24h	
0	7.0		7.1		7.1		6.9		GY
1	6.2	5.5	6.9	5.4	6.9	5.2	6.8	5.4	GY
2	6.9	5.1	6.9	5.2	6.9	5.6	6.9	4.8	JA
3	6.7	4.6	6.7	4.7	6.7	4.9	6.7	5.2	GY
4	6.8	5.3	6.9	5.0	7.1	5.7	7.1	5.4	GS/RR
5	6.9	5.0	6.8	4.6	6.9	4.9	6.8	5.2	GY
6	6.9	6.0	7.1	5.7	7.0	5.9	7.0	6.0	GS
7	7.0	6.1		5.9		6.0		6.1	JA

Day	Orange		Red		White		Analyst
	0h	24h	0h	24h	0h	24h	
0	6.9		6.9		6.9		GY
1	6.8	5.2	6.6	5.2	6.5	5.0	GY
2	6.9	5.1	6.7	4.9	6.8	4.8	JA
3	6.7	4.7	6.6	4.8	6.8	4.8	GY
4	7.1	5.6	7.1	5.2	7.0	5.2	GS/RR
5	6.8	5.1	6.8	4.7	6.8	4.8	GY
6	7.0	5.8	7.1	5.7	7.0	5.5	GS
7		6.0		6.0		6.0	JA

Random Number Positions								
Code	Rep	Random #	Code	Rep	Random #	Code	Rep	Random #
Blue	A	55 53	Yellow	A	44 21	White	A	18 10
	B	18 13		B	10 38		B	58 4
	C	22 2		C	21 40		C	27 40
	D	30 42		D	38 56		D	48 37
	E	4 24		E	18 55		E	19 20
	F	52 52		F	4 18		F	14 17
	G	38 7		G	44 28		G	42 45
	H	30 11		H	47 23		H	30 26
Gray	A	31 33	Orange	A	29 32			
	B	25 54		B	20 51			
	C	24 16		C	20 30			
	D	9 47		D	39 48			
	E	8 22		E	52 44			
	F	46 25		F	43 19			
	G	23 6		G	22 14			
	H	12 27		H	7 50			
Green	A	16 15	Red	A	2 36			
	B	17 31		B	40 12			
	C	41 41		C	34 35			
	D	6 3		D	59 34			
	E	5 29		E	3 8			
	F	45 43		F	56 39			
	G	1 5		G	37 9			
	H	28 1		H	36 49			

* GS chgd placement

Vashon NPDES Characterization (1033898),
Mysidopsis bahia 7-Day Chronic Renewal Test

Test#: 9311
Test Date: 10/2/19

Trtmt	Cumulative Survival (# Alive/Rep) at 7 Days									Tot #Surv	Mean %Surv*	Analyst
	Day	A	B	C	D	E	F	G	H			
Blue (0%)	1	5	4 ^c	5	5	5	5	5	5			GY
	2	5	4	5	5	5	5	5	5			EF
	3	5	4	5	5	5	5	5	5			EF
	4	5	4	5	5	5	5	5	5			RR
	5	5	4	5	5	5	5	5	4 ^c			EF
	6	5	4	5	5	5	5	5	4			GY
	7	5	4	5	5	5	5	5	4			
Gray (0.15%) CCEC	1	4 ^c	5	5	5	5	5	5	5			EF
	2	4	5	5	5	5	5	5	5			FS
	3	4	5	5	5	5	5	5	5			GY
	4	4	5	5	5	5	5 ¹⁵	5	5			JS
	5	4	5	5	5	5	4	5	5			GY
	6	4	5	5	5	5	4	5	5			EF
	7	4	5	5	5	5	4	5	5			
Green (1.12%) ACEC	1	5	5	5	5	5	5	5	5			EF
	2	5	5	5	5	5	5	5	5			FS
	3	5	5	5	5	5	5	5	5			EF
	4	5	5	5	5	5	5	5	5			RR
	5	5	5	5	5	5	5 ¹⁴	5	5			JA
	6	5	5	5	5	5	4	5	5			GY
	7	5	5	5	5	5	4	5	5			
Yellow (12.5%)	1	5	5	4 ^c	5	5	5	5	5			GY
	2	5	5	4	5	5	5	5	5			EF
	3	5	5	4	5	5	5	5	5			GY
	4	5	5	4	5	5	5	5	5			JS
	5	5	5	4	5	5	5	5	5			EF
	6	4 ⁺	5	4	5	4	5	5	5			EF
	7	4	5	3	5	4	5	5	5			
Orange (25%)	1	5	5	5	5	5	5	5	5			EF
	2	5	5	5	5	5	5	5	5			FS
	3	5	5	5	5	5	5	5	5			EF
	4	5	5	5	5	4	5	5	5			RR
	5	5	5	5	5	4	5	5	5			GY
	6	5	5	5	5	4	5	5	5			GY
	7	4	5	5	5	4	5	5	5			
Red (50%)	1	5	5	5	5	4 ^c	5	5	4 ^c			GY
	2	4 ^c	5	5	5	4	5	5	4			EF
	3	4	5	5	5	4	5	5	4			GY
	4	3	5	5	5	4	5	5	4			JS
	5	3	5	5	5	4	5	5	4			EF
	6	3	5	5	5	4	5	5	4			
	7	3	5	5	5	4	4	5	4			
White (100%)	1	5	5	5	5	5	5	5	5			EF
	2	5	5	5	5	5	5	5	5			FS
	3	5	5	5	5	5	5	5	5			EF
	4	5	5	5	5	5	5	5	5			RR
	5	5	5	5	5	5	5	5	5			JA
	6	5	5	5	5	5	5	5	5			GY
	7	5	5	5	5	5	4	5	5			

s = stressed

*Pass if mean control survival $\geq 80\%$

5FS

Vashon NPDES Characterization (1033898),
Mysidopsis bahia 7-Day Chronic Renewal Test

Test#: 9311

Test Date: 10/2/19

Dry Weight (mg) per Mysid at 7 Days

Trtmt		A	B	C	D	E	F	G	H	Mean**
	Pan#	1	2	3	4	5	6	7	8	
Blue (0%)	Tot. Wt (mg)	85.16	90.52	89.96	83.35	81.93	95.18	87.17	76.49	
	Tare wt (mg)	83.71	89.41	88.41	82.36	80.72	93.34	85.84	75.49	
	Net wt (mg)	1.45	1.11	1.55	0.99	1.21	1.45	1.33	1.00	
	N	5	5	5	5	5	5	5	5	
	Wt (mg/org)	0.290	0.222	0.310	0.198	0.242	0.290	0.266	0.200	0.252
	Pan#	9	10	11	12	13	14	15	16	
Gray (0.15%) CCEC	Tot. Wt (mg)	76.45	90.03	85.46	86.64	85.60	84.22	84.96	80.81	
	Tare wt (mg)	75.34	88.64	83.99	85.21	84.27	83.03	83.86	79.36	
	Net wt (mg)	1.11	1.39	1.47	1.43	1.33	1.19	1.10	1.45	
	N	5	5	5	5	5	5	5	5	
	X (mg/org)	0.222	0.278	0.294	0.286	0.266	0.238	0.220	0.290	0.262
	Pan#	17	18	19	20	21	22	23	24	Adjust
Green (1.12%) ACEC	Tot. Wt (mg)	81.59	78.94	86.61	86.14	94.14	74.23	76.14	81.30	M=4
	Tare wt (mg)	80.27	77.68	84.75	84.69	92.65	73.25	74.88	79.78	*.295
	Net wt (mg)	1.32	1.26	1.86	1.45	1.49	0.98	1.26	1.52	0.285
	N	5	5	5	5	5	84	5	5	PS
	X (mg/org)	0.264	0.252	0.372	0.290	0.298	0.196	0.252	0.304	0.279
	Pan#	25	26	27	28	29	30	31	32	
Yellow (12.5%)	Tot. Wt (mg)	79.42	76.21	75.92	78.16	82.66	84.44	81.03	89.75	
	Tare wt (mg)	78.39	74.91	74.99	77.08	81.60	83.13	79.64	88.24	
	Net wt (mg)	1.04	1.30	0.93	1.08	1.06	1.31	1.39	1.51	
	N	5	5	5	5	5	5	5	5	
	X (mg/org)	0.208	0.260	0.186	0.216	0.212	0.262	0.278	0.302	0.241
	Pan#	33	34	35	36	37	38	39	40	
Orange (25%)	Tot. Wt (mg)	79.42	78.13	81.24	85.02	96.42	83.34	81.02	77.70	
	Tare wt (mg)	78.22	76.59	80.06	84.58	95.39	81.91	79.88	76.54	
	Net wt (mg)	1.20	1.54	1.18	1.04	1.03	1.43	1.14	1.16	
	N	5	5	5	5	5	5	5	5	
	X (mg/org)	0.240	0.308	0.236	0.208	0.206	0.286	0.228	0.232	0.243
	Pan#	41	42	43	44	45	46	47	48	
Red (50%)	Tot. Wt (mg)	75.23	92.30	79.53	82.51	83.30	83.83	78.26	76.99	
	Tare wt (mg)	74.72	90.85	78.13	81.05	82.21	82.89	76.58	75.78	
	Net wt (mg)	0.51	1.45	1.40	1.46	1.09	0.94	1.68	1.21	
	N	5	5	5	5	5	5	5	5	
	X, mg	0.102	0.290	0.280	0.292	0.218	0.188	0.336	0.242	0.244
	Pan#	49	50	51	52	53	54	55	56	
White (100%)	Tot. Wt (mg)	82.49	98.90	98.40	80.09	86.77	83.21	84.63	88.96	
	Tare wt (mg)	80.83	74.46	97.14	79.07	85.10	81.56	83.25	87.40	
	Net wt (mg)	1.66	1.44	1.26	1.02	1.67	1.65	1.38	1.56	
	N	5	5	5	5	5	5	5	5	
	X (mg/org)	0.332	0.288	0.252	0.204	0.334	0.330	0.276	0.312	0.291

**Pass if mean control weight ≥ 0.2 mg/ind

Blank Tare Pan Weights (mg)

Pan #:	57	58	59
Before:	72.51	83.14	77.70
After:	72.53	83.09	77.70

CETIS Analytical Report

Report Date: 28 Oct-19 14:32 (p 1 of 5)
Test Code/ID: 9311MYCVA / 05-1403-5673

Mysidopsis 7-d Survival, Growth and Fecundity Test

King County Metro Services, WQ Lab

Analysis ID: 01-1020-7541	Endpoint: 7d Survival Rate	CETIS Version: CETISv1.9.5
Analyzed: 28 Oct-19 14:31	Analysis: Nonparametric-Control vs Treatments	Status Level: 1
Batch ID: 20-8839-6101	Test Type: Growth-Survival (7d)	Analyst:
Start Date: 02 Oct-19 14:10	Protocol: EPA/821/R-02-014 (2002)	Diluent: Deionized Water
Ending Date: 09 Oct-19 13:00	Species: Mysidopsis bahia	Brine: Hawaiian Marine Mix
Test Length: 6d 23h	Taxon: Malacostraca	Source: Aquatic Biosystems, CO Age: 7d
Sample ID: 03-0724-9154	Code: L73401-3	Project: Effluent Characterization (Biannual)
Sample Date: 02 Oct-19 06:47	Material: POTW Effluent	Source: Vashon Permit WA002252-7 (WA0022
Receipt Date: 02 Oct-19 08:47	CAS (PC):	Station:
Sample Age: 7h (2 °C)	Client: Vashon Island Treatment Plant	

Vashon effluent DAY 0: L73401-1 coll. 10/1/19-10/2/19 0647h-0647h; rec'd at KCEL 10/2/19 at 0847h in 1 5-gal glass jar; at plant pH = 7.58, T= 2.2oC, TRC = 0.03mg/L; at KCEL pH=7.61, T=2.0oC, DO= 10.8 mg/L; DAY2: L73401-5 coll. 10/3/19-10/4/19 0637h-0637h, rec'd at KCEL at 0850h in 2 glass 5gal glass jars, at plant: pH=7.57, T=2.5oC, TRC= 0.04mg/L; at lab pH = 7.58/7.64, T= 1.8/1.7oC, DO = 10.3/10.4mg/L; DAY 5: L73401-6 coll. 10/6/19-10/7/19 0610h-0610h, rec'd at KCEL at 0903hh in 1 glass 5gal glass jar, at plant: pH=7.60, T=2.8oC, TRC= 0.02mg/L; at lab pH = 7.60, T=1.9oC, DO=10.5mg/L;

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	C > T	100	>100	n/a	1	12.54%

Steel Many-One Rank Sum Test

Control	vs	Conc-%	Test Stat	Critical	Ties	DF	P-Type	P-Value	Decision(α:5%)
Dilution Water		0.15	68	46	2	14	CDF	0.8571	Non-Significant Effect
		1.12	76	46	1	14	CDF	0.9817	Non-Significant Effect
		12.5	63	46	2	14	CDF	0.6681	Non-Significant Effect
		25	68	46	2	14	CDF	0.8571	Non-Significant Effect
		50	59	46	2	14	CDF	0.4732	Non-Significant Effect
		100	76	46	1	14	CDF	0.9817	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.139755	0.0232925	6	1.68	0.1460	Non-Significant Effect
Error	0.679552	0.0138684	49			
Total	0.819307		55			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	9.46	3.195	6.8E-07	Unequal Variances
	Mod Levene Equality of Variance Test	2.223	3.195	0.0564	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.8693	0.9426	2.1E-05	Non-Normal Distribution

7d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	8	0.9500	0.8726	1.0000	1.0000	0.8000	1.0000	0.0327	9.75%	0.00%
0.15		8	0.9500	0.8726	1.0000	1.0000	0.8000	1.0000	0.0327	9.75%	0.00%
1.12		8	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-5.26%
12.5		8	0.9000	0.7736	1.0000	1.0000	0.6000	1.0000	0.0535	16.80%	5.26%
25		8	0.9500	0.8726	1.0000	1.0000	0.8000	1.0000	0.0327	9.75%	0.00%
50		8	0.8750	0.7506	0.9994	0.9000	0.6000	1.0000	0.0526	17.01%	7.89%
100		8	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-5.26%

CETIS Analytical Report

Report Date: 28 Oct-19 14:32 (p 2 of 5)
Test Code/ID: 9311MYCVA / 05-1403-5673

Mysidopsis 7-d Survival, Growth and Fecundity Test

King County Metro Services, WQ Lab

Analysis ID: 01-1020-7541 Endpoint: 7d Survival Rate CETIS Version: CETISv1.9.5
Analyzed: 28 Oct-19 14:31 Analysis: Nonparametric-Control vs Treatments Status Level: 1

Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	8	1.286	1.194	1.378	1.345	1.107	1.345	0.03897	8.57%	0.00%
0.15		8	1.286	1.194	1.378	1.345	1.107	1.345	0.03897	8.57%	0.00%
1.12		8	1.342	1.334	1.35	1.345	1.318	1.345	0.003395	0.72%	-4.37%
12.5		8	1.228	1.082	1.375	1.345	0.8861	1.345	0.06195	14.26%	4.46%
25		8	1.286	1.194	1.378	1.345	1.107	1.345	0.03897	8.57%	0.00%
50		8	1.199	1.054	1.343	1.226	0.8861	1.345	0.06107	14.41%	6.78%
100		8	1.345	1.345	1.345	1.345	1.345	1.345	0	0.00%	-4.63%

7d Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	1.0000	0.8000	1.0000	1.0000	1.0000	1.0000	1.0000	0.8000
0.15		0.8000	1.0000	1.0000	1.0000	1.0000	0.8000	1.0000	1.0000
1.12		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
12.5		0.8000	1.0000	0.6000	1.0000	0.8000	1.0000	1.0000	1.0000
25		0.8000	1.0000	1.0000	1.0000	0.8000	1.0000	1.0000	1.0000
50		0.6000	1.0000	1.0000	1.0000	0.8000	0.8000	1.0000	0.8000
100		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Angular (Corrected) Transformed Detail

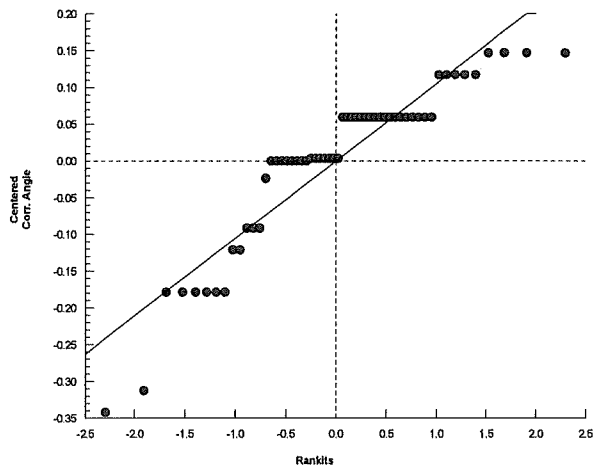
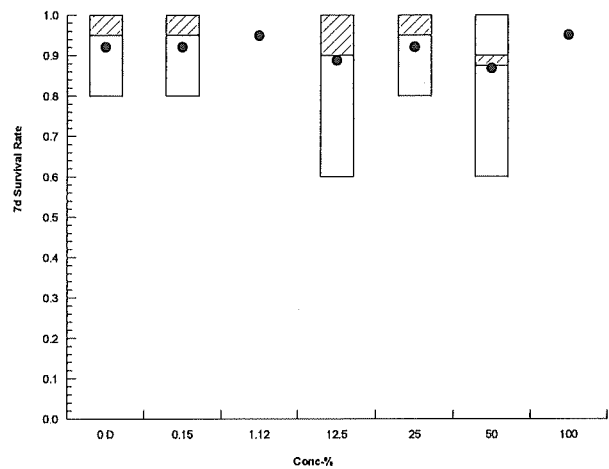
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	1.345	1.107	1.345	1.345	1.345	1.345	1.345	1.107
0.15		1.107	1.345	1.345	1.345	1.345	1.107	1.345	1.345
1.12		1.345	1.345	1.345	1.345	1.345	1.318	1.345	1.345
12.5		1.107	1.345	0.8861	1.345	1.107	1.345	1.345	1.345
25		1.107	1.345	1.345	1.345	1.107	1.345	1.345	1.345
50		0.8861	1.345	1.345	1.345	1.107	1.107	1.345	1.107
100		1.345	1.345	1.345	1.345	1.345	1.345	1.345	1.345

7d Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	5/5	4/5	5/5	5/5	5/5	5/5	5/5	4/5
0.15		4/5	5/5	5/5	5/5	5/5	4/5	5/5	5/5
1.12		5/5	5/5	5/5	5/5	5/5	4/4	5/5	5/5
12.5		4/5	5/5	3/5	5/5	4/5	5/5	5/5	5/5
25		4/5	5/5	5/5	5/5	4/5	5/5	5/5	5/5
50		3/5	5/5	5/5	5/5	4/5	4/5	5/5	4/5
100		5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

Mysidopsis 7-d Survival, Growth and Fecundity Test			King County Metro Services, WQ Lab	
Analysis ID: 01-1020-7541	Endpoint: 7d Survival Rate	CETIS Version: CETISv1.9.5		
Analyzed: 28 Oct-19 14:31	Analysis: Nonparametric-Control vs Treatments	Status Level: 1		

Graphics



CETIS Analytical Report

Report Date: 28 Oct-19 14:32 (p 4 of 5)
Test Code/ID: 9311MYCVA / 05-1403-5673

Mysidopsis 7-d Survival, Growth and Fecundity Test

King County Metro Services, WQ Lab

Analysis ID: 13-3007-1578	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.9.5
Analyzed: 28 Oct-19 14:32	Analysis: Parametric-Control vs Treatments	Status Level: 1
Batch ID: 20-8839-6101	Test Type: Growth-Survival (7d)	Analyst:
Start Date: 02 Oct-19 14:10	Protocol: EPA/821/R-02-014 (2002)	Diluent: Deionized Water
Ending Date: 09 Oct-19 13:00	Species: Mysidopsis bahia	Brine: Hawaiian Marine Mix
Test Length: 6d 23h	Taxon: Malacostraca	Source: Aquatic Biosystems, CO Age: 7d
Sample ID: 03-0724-9154	Code: L73401-3	Project: Effluent Characterization (Biannual)
Sample Date: 02 Oct-19 06:47	Material: POTW Effluent	Source: Vashon Permit WA002252-7 (WA0022
Receipt Date: 02 Oct-19 08:47	CAS (PC):	Station:
Sample Age: 7h (2 °C)	Client: Vashon Island Treatment Plant	

Vashon effluent DAY 0: L73401-1 coll. 10/1/19-10/2/19 0647h-0647h; rec'd at KCEL 10/2/19 at 0847h in 1 5-gal glass jar; at plant pH = 7.58, T= 2.2oC, TRC = 0.03mg/L; at KCEL pH=7.61, T=2.0oC, DO= 10.8 mg/L; DAY2: L73401-5 coll. 10/3/19-10/4/19 0637h-0637h, rec'd at KCEL at 0850h in 2 glass 5gal glass jars, at plant: pH=7.57, T=2.5oC, TRC= 0.04mg/L; at lab pH = 7.58/7.64, T= 1.8/1.7oC, DO = 10.3/10.4mg/L; DAY 5: L73401-6 coll. 10/6/19-10/7/19 0610h-0610h, rec'd at KCEL at 0903hh in 1 glass 5gal glass jar, at plant: pH=7.60, T=2.8oC, TRC= 0.02mg/L; at lab pH = 7.60, T=1.9oC, DO=10.5mg/L;

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	100	>100	n/a	1	21.68%

Dunnett Multiple Comparison Test

Control	vs	Conc-%	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Dilution Water		0.15	-0.4094	2.357	0.055	14	CDF	0.9407	Non-Significant Effect
		1.12	-1.395	2.357	0.055	14	CDF	0.9968	Non-Significant Effect
		12.5	0.5064	2.357	0.055	14	CDF	0.6770	Non-Significant Effect
		25	0.3987	2.357	0.055	14	CDF	0.7218	Non-Significant Effect
		50	0.3771	2.357	0.055	14	CDF	0.7304	Non-Significant Effect
		100	-1.67	2.357	0.055	14	CDF	0.9988	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.020563	0.0034272	6	1.591	0.1697	Non-Significant Effect
Error	0.105521	0.0021535	49			
Total	0.126084		55			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	6.898	16.81	0.3304	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9726	0.9426	0.2311	Normal Distribution

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	8	0.2522	0.2161	0.2884	0.254	0.198	0.31	0.01528	17.13%	0.00%
0.15		8	0.2618	0.2361	0.2874	0.272	0.22	0.294	0.01085	11.73%	-3.77%
1.12		8	0.2846	0.2495	0.3197	0.277	0.245	0.372	0.01485	14.75%	-12.83%
12.5		8	0.2405	0.2067	0.2743	0.238	0.186	0.302	0.01431	16.83%	4.66%
25		8	0.243	0.2129	0.2731	0.234	0.206	0.308	0.01273	14.81%	3.67%
50		8	0.2435	0.1818	0.3052	0.261	0.102	0.336	0.0261	30.32%	3.47%
100		8	0.291	0.2525	0.3295	0.3	0.204	0.334	0.01627	15.81%	-15.36%

Mean Dry Biomass-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	0.29	0.222	0.31	0.198	0.242	0.29	0.266	0.2
0.15		0.222	0.278	0.294	0.286	0.266	0.238	0.22	0.29
1.12		0.264	0.252	0.372	0.29	0.298	0.245	0.252	0.304
12.5		0.208	0.26	0.186	0.216	0.212	0.262	0.278	0.302
25		0.24	0.308	0.236	0.208	0.206	0.286	0.228	0.232
50		0.102	0.29	0.28	0.292	0.218	0.188	0.336	0.242
100		0.332	0.288	0.252	0.204	0.334	0.33	0.276	0.312

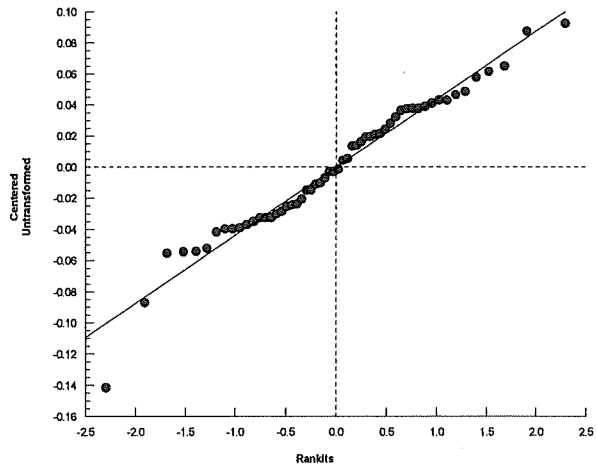
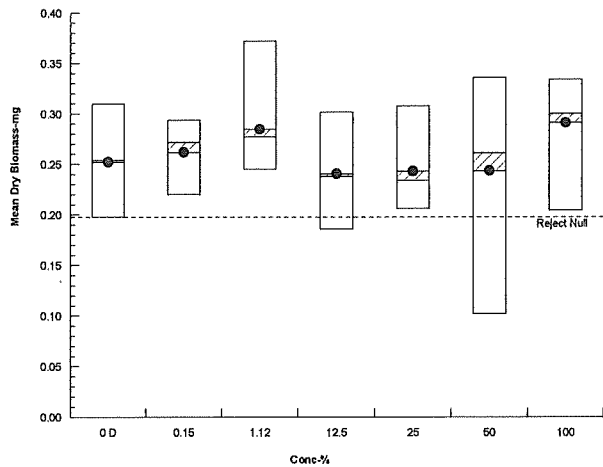
CETIS Analytical Report

Report Date: 28 Oct-19 14:32 (p 5 of 5)
Test Code/ID: 9311MYCVA / 05-1403-5673

Mysidopsis 7-d Survival, Growth and Fecundity Test King County Metro Services, WQ Lab

Analysis ID: 13-3007-1578 Endpoint: Mean Dry Biomass-mg
Analyzed: 28 Oct-19 14:32 Analysis: Parametric-Control vs Treatments CETIS Version: CETISv1.9.5
Status Level: 1

Graphics



CETIS Analytical Report

Report Date: 28 Oct-19 14:32 (p 1 of 2)

Test Code/ID: 9311MYCVA / 05-1403-5673

Mysidopsis 7-d Survival, Growth and Fecundity Test

King County Metro Services, WQ Lab

Analysis ID: 00-9484-1649	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.9.5
Analyzed: 28 Oct-19 14:31	Analysis: Linear Interpolation (ICPIN)	Status Level: 1
Batch ID: 20-8839-6101	Test Type: Growth-Survival (7d)	Analyst:
Start Date: 02 Oct-19 14:10	Protocol: EPA/821/R-02-014 (2002)	Diluent: Deionized Water
Ending Date: 09 Oct-19 13:00	Species: Mysidopsis bahia	Brine: Hawaiian Marine Mix
Test Length: 6d 23h	Taxon: Malacostraca	Source: Aquatic Biosystems, CO Age: 7d
Sample ID: 03-0724-9154	Code: L73401-3	Project: Effluent Characterization (Biannual)
Sample Date: 02 Oct-19 06:47	Material: POTW Effluent	Source: Vashon Permit WA002252-7 (WA0022
Receipt Date: 02 Oct-19 08:47	CAS (PC):	Station:
Sample Age: 7h (2 °C)	Client: Vashon Island Treatment Plant	

Vashon effluent DAY 0: L73401-1 coll. 10/1/19-10/2/19 0647h-0647h; rec'd at KCEL 10/2/19 at 0847h in 1 5-gal glass jar; at plant pH = 7.58, T= 2.2oC, TRC = 0.03mg/L; at KCEL pH=7.61, T=2.0oC, DO= 10.8 mg/L; DAY2: L73401-5 coll. 10/3/19-10/4/19 0637h-0637h, rec'd at KCEL at 0850h in 2 glass 5gal glass jars, at plant: pH=7.57, T=2.5oC, TRC= 0.04mg/L; at lab pH = 7.58/7.64, T= 1.8/1.7oC, DO = 10.3/10.4mg/L; DAY 5: L73401-6 coll. 10/6/19-10/7/19 0610h-0610h, rec'd at KCEL at 0903hh in 1 glass 5gal glass jar, at plant: pH=7.60, T=2.8oC, TRC= 0.02mg/L; at lab pH = 7.60, T=1.9oC, DO=10.5mg/L;

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1512829	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC25	>100	n/a	n/a	<1	n/a	n/a

Mean Dry Biomass-mg Summary

			Calculated Variate							Isotonic Variate	
Conc-%	Code	Count	Mean	Min	Max	Std Dev	CV%	%Effect		Mean	%Effect
0	D	8	0.2522	0.198	0.31	0.04321	17.13%	0.0%		0.2662	0.0%
0.15		8	0.2618	0.22	0.294	0.0307	11.73%	-3.77%		0.2662	0.0%
1.12		8	0.2846	0.245	0.372	0.04199	14.75%	-12.83%		0.2662	0.0%
12.5		8	0.2405	0.186	0.302	0.04048	16.83%	4.66%		0.2545	4.4%
25		8	0.243	0.206	0.308	0.036	14.81%	3.67%		0.2545	4.4%
50		8	0.2435	0.102	0.336	0.07383	30.32%	3.47%		0.2545	4.4%
100		8	0.291	0.204	0.334	0.04601	15.81%	-15.36%		0.2545	4.4%

Mean Dry Biomass-mg Detail

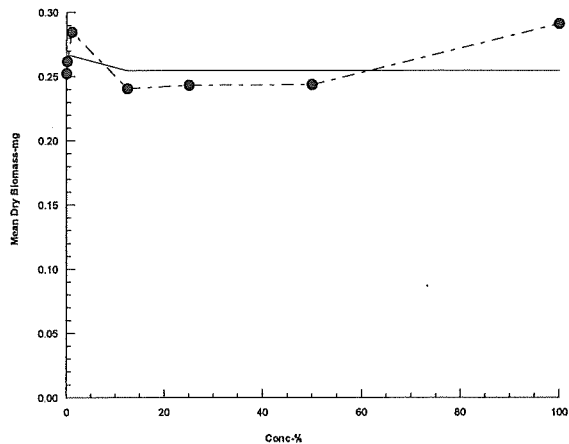
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	0.29	0.222	0.31	0.198	0.242	0.29	0.266	0.2
0.15		0.222	0.278	0.294	0.286	0.266	0.238	0.22	0.29
1.12		0.264	0.252	0.372	0.29	0.298	0.245	0.252	0.304
12.5		0.208	0.26	0.186	0.216	0.212	0.262	0.278	0.302
25		0.24	0.308	0.236	0.208	0.206	0.286	0.228	0.232
50		0.102	0.29	0.28	0.292	0.218	0.188	0.336	0.242
100		0.332	0.288	0.252	0.204	0.334	0.33	0.276	0.312

CETIS Analytical Report

Report Date: 28 Oct-19 14:32 (p 2 of 2)
Test Code/ID: 9311MYCVA / 05-1403-5673

Mysidopsis 7-d Survival, Growth and Fecundity Test				King County Metro Services, WQ Lab	
Analysis ID:	00-9484-1649	Endpoint:	Mean Dry Biomass-mg	CETIS Version:	CETISv1.9.5
Analyzed:	28 Oct-19 14:31	Analysis:	Linear Interpolation (ICPIN)	Status Level:	1

Graphics



CETIS Summary Report

Report Date: 28 Oct-19 14:32 (p 1 of 2)
 Test Code/ID: 9311MYCVA / 05-1403-5673

Mysidopsis 7-d Survival, Growth and Fecundity Test

King County Metro Services, WQ Lab

Batch ID: 20-8839-6101	Test Type: Growth-Survival (7d)	Analyst:
Start Date: 02 Oct-19 14:10	Protocol: EPA/821/R-02-014 (2002)	Diluent: Deionized Water
Ending Date: 09 Oct-19 13:00	Species: Mysidopsis bahia	Brine: Hawaiian Marine Mix
Test Length: 6d 23h	Taxon: Malacostraca	Source: Aquatic Biosystems, CO Age: 7d
Sample ID: 03-0724-9154	Code: L73401-3	Project: Effluent Characterization (Biannual)
Sample Date: 02 Oct-19 06:47	Material: POTW Effluent	Source: Vashon Permit WA002252-7 (WA0022
Receipt Date: 02 Oct-19 08:47	CAS (PC):	Station:
Sample Age: 7h (2 °C)	Client: Vashon Island Treatment Plant	

Vashon effluent DAY 0: L73401-1 coll. 10/1/19-10/2/19 0647h-0647h; rec'd at KCEL 10/2/19 at 0847h in 1 5-gal glass jar; at plant pH = 7.58, T= 2.2oC, TRC = 0.03mg/L; at KCEL pH=7.61, T=2.0oC, DO= 10.8 mg/L; DAY2: L73401-5 coll. 10/3/19-10/4/19 0637h-0637h, rec'd at KCEL at 0850h in 2 glass 5gal glass jars, at plant: pH=7.57, T=2.5oC, TRC= 0.04mg/L; at lab pH = 7.58/7.64, T= 1.8/1.7oC, DO = 10.3/10.4mg/L; DAY 5: L73401-6 coll. 10/6/19-10/7/19 0610h-0610h, rec'd at KCEL at 0903hh in 1 glass 5gal glass jar, at plant: pH=7.60, T=2.8oC, TRC= 0.02mg/L; at lab pH = 7.60, T=1.9oC, DO=10.5mg/L;

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	TU	PMSD	S
01-1020-7541	7d Survival Rate	Steel Many-One Rank Sum Test		100	>100	n/a	1	12.5%	1
13-3007-1578	Mean Dry Biomass-mg	Dunnett Multiple Comparison Test		100	>100	n/a	1	21.7%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	%	95% LCL	95% UCL	TU	S
00-9484-1649	Mean Dry Biomass-mg	Linear Interpolation (ICPIN)		IC25	>100	n/a	n/a	<1	1

7d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	8	0.9500	0.8726	1.0000	0.8000	1.0000	0.0327	0.0926	9.75%	0.00%
0.15		8	0.9500	0.8726	1.0000	0.8000	1.0000	0.0327	0.0926	9.75%	0.00%
1.12		8	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-5.26%
12.5		8	0.9000	0.7736	1.0000	0.6000	1.0000	0.0535	0.1512	16.80%	5.26%
25		8	0.9500	0.8726	1.0000	0.8000	1.0000	0.0327	0.0926	9.75%	0.00%
50		8	0.8750	0.7506	0.9994	0.6000	1.0000	0.0526	0.1488	17.01%	7.89%
100		8	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-5.26%

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	8	0.2522	0.2161	0.2884	0.198	0.31	0.01528	0.04321	17.13%	0.00%
0.15		8	0.2618	0.2361	0.2874	0.22	0.294	0.01085	0.0307	11.73%	-3.77%
1.12		8	0.2846	0.2495	0.3197	0.245	0.372	0.01485	0.04199	14.75%	-12.83%
12.5		8	0.2405	0.2067	0.2743	0.186	0.302	0.01431	0.04048	16.83%	4.66%
25		8	0.243	0.2129	0.2731	0.206	0.308	0.01273	0.036	14.81%	3.67%
50		8	0.2435	0.1818	0.3052	0.102	0.336	0.0261	0.07383	30.32%	3.47%
100		8	0.291	0.2525	0.3295	0.204	0.334	0.01627	0.04601	15.81%	-15.36%

CETIS Summary Report

Report Date: 28 Oct-19 14:32 (p 2 of 2)

Test Code/ID: 9311MYCVA / 05-1403-5673

Mysidopsis 7-d Survival, Growth and Fecundity Test

King County Metro Services, WQ Lab

7d Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	1.0000	0.8000	1.0000	1.0000	1.0000	1.0000	1.0000	0.8000
0.15		0.8000	1.0000	1.0000	1.0000	1.0000	0.8000	1.0000	1.0000
1.12		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
12.5		0.8000	1.0000	0.6000	1.0000	0.8000	1.0000	1.0000	1.0000
25		0.8000	1.0000	1.0000	1.0000	0.8000	1.0000	1.0000	1.0000
50		0.6000	1.0000	1.0000	1.0000	0.8000	0.8000	1.0000	0.8000
100		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Mean Dry Biomass-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	0.29	0.222	0.31	0.198	0.242	0.29	0.266	0.2
0.15		0.222	0.278	0.294	0.286	0.266	0.238	0.22	0.29
1.12		0.264	0.252	0.372	0.29	0.298	0.245	0.252	0.304
12.5		0.208	0.26	0.186	0.216	0.212	0.262	0.278	0.302
25		0.24	0.308	0.236	0.208	0.206	0.286	0.228	0.232
50		0.102	0.29	0.28	0.292	0.218	0.188	0.336	0.242
100		0.332	0.288	0.252	0.204	0.334	0.33	0.276	0.312

7d Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	5/5	4/5	5/5	5/5	5/5	5/5	5/5	4/5
0.15		4/5	5/5	5/5	5/5	5/5	4/5	5/5	5/5
1.12		5/5	5/5	5/5	5/5	5/5	4/4	5/5	5/5
12.5		4/5	5/5	3/5	5/5	4/5	5/5	5/5	5/5
25		4/5	5/5	5/5	5/5	4/5	5/5	5/5	5/5
50		3/5	5/5	5/5	5/5	4/5	4/5	5/5	4/5
100		5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

King County Environmental Laboratory

Lab Review Report

Reported: 28-Oct-19 15:06 ~ Data Source: ELD

Listtype / Method: AQMYSID-CHRONIC / EPA821-R-02-014
Run ID / Workgroup: R238568 / WG167051

CollectDate	Tspan	Project	Mat	Locator	Sample	Parameter	Value	Units	Qual	Mdl	Rdl	Textvalue
2019/10/01 08:47:00		421488	LC	VS_EFF	L73401-3	Survival NOEC Growth NOEC	100 100	%sample %sample				t-Test: control vs ACEC not significant; p > 0.05
						Growth IC25 Test Number		%sample	TA			Indeterminate (>100% effluent) 9311
						Date Analyzed		none				02-OCT-19 14:10
						Prep Date		none				02-OCT-19 13:00

No products missing

Reference Toxicant Tests:

**Bench Sheets
and
Precision Tables**

REFERENCE TOXICANT (Cu), PROJECT #421190/ 1034296Topsmelt (*Atherinops affinis*) 7-Day Chronic Renewal TestTest #: 9324Test Date: 10/2/19**ORGANISMS (Held 24h before test)**

see T 9312

Received from _____ via _____ as _____ days old (Hatch date: _____).
Arrived at KCEL at _____ h on _____ in _____._____ dead removed. At Arrival: pH _____, D.O. _____ mg/L, Temp _____ °C, Salinity _____ ppt. Placed in _____ 1.5L crystallizing dishes. Fed _____ mL *Artemia* nauplii/dish at _____ h. Acclimation: Placed in 20°C EC at _____ h with light aeration. Replaced _____ % with HMM.Fed _____ mL *Artemia*/dish at _____ h on _____ Analyst _____
Fed _____ mL *Artemia*/dish at _____ h on _____ Analyst _____
Fed _____ mL *Artemia*/dish at _____ h on _____ Analyst _____**DILUTION WATER/ SOLUTIONS**

- HMM:** Hawaiian Marine Mix #HW-1016 (artificial sea salts) lot# _____ rec'd 3/14/19
opened 10/4/19
a. Add 35.7 g HMM salt + 0.2g NaHCO₃ in 1L MilliQ Water;
b. Aerate > 2h and filter to 0.45 µm before use.
c. Salinity: 30 ppt;
- Cu Stock Soln** (500 mg/L nominal as CuCl₂·2H₂O): Measured 938 mg/L on 11-16-05.
Prepped on: 05-10-08 by adding 1.341 g CuCl₂·2H₂O ⊆ 500 mL DW.
(Mfr 051108 JT Baker # I-1850, rec'd _____, opened _____, lot # _____)
- LIMS Sample #:** WG 166820-1 **WKGP #:** WG 166820

DILUTIONS

Code	Cu (µg/L)	Cu Stock (mL)	HMM (mL)	Sample #	Measured µg/L Cu in DW
Blue	0	HMM only	1000		
Green	56	0.060	⊆ 1000	73433 -1	62.2
Yellow	100	0.107	⊆ 1000	-2	114
Orange	180	0.192	⊆ 1000	-3	205
Red	320	0.341	⊆ 1000	-4	362
White	572	0.610	⊆ 1000	-5	635

REFERENCE TOXICANT (Cu), PROJECT #421190/ 1034296Topsmelt (*Atherinops affinis*) 7-Day Chronic Renewal TestTest #: 9324Test Date: 10/2/19**PROCEDURE**

1. Prep solutions as above in 1000-mL graduated cylinder; decant to 1 L glass flasks. HMM stored in 20°C environmental chamber.
2. Add 200 mL each treatment to each of 5 (five) 600-mL beakers/treatment (reps A→E).
3. Place beakers randomly on bench in 20°C EC # 8556 and bring solutions to 20°C. Setup at 1300 h.
4. Measure 0h temp in 1 rep/treatment plus in 6 locations (4 corner + 2 center beakers). Measure 0h D.O. and pH (collect 10 mL/rep from 5 reps of "Blue" and "White" treatments).
5. Add 5 larvae per beaker directly into solutions using a nylon screen; rinse with DW between beakers.
6. Start test at ⁸⁸13450 h on 10/2/19. Start count verified by JR & —. Place into 20°C EC# 8556, QC (1st) shelf. Place Tidbit temp recorder (SN 10080645) in beaker w/DW into EC.
7. Take Cu sample prepared in DW ✓; Acidify Logan Analyst Gy.
8. Feed larvae 1 drop *Artemia* nauplii/beaker 2x/day.
9. Record number alive and remove dead larvae daily at renewal.
10. Measure temp daily in 1 rep/trtmt and in 6 positions (4 corner + 2 center beakers). Measure D.O., pH and salinity daily in 0h (new) and 24h (old) solns (Blue and White treatments).
11. Renew solutions daily (75% renewal):
 - a. Remove approximately 150 mL soln with waste + excess food by decanting and/or pipet + bulb.
 - b. Replace \leq 200 mL with new prepared solution (warmed to 20°C) by pouring down side of beaker.
 - c. Count larvae before and after renewal.
12. End test at 1400 h on 10-9-19.
 - a. Record survival.
 - b. Inactivate larvae with ice water; rinse larvae onto screen and place into tared weigh pans.
 - c. Process by rep (1 analyst/rep):

Rep A RR Rep B RR Rep C RR Rep D RR Rep E JA
13. Pans into 60°C oven at 1457 h on 10-9-19.
14. Pans into desiccator at 0600 h on 10-10-19.
15. Weigh at 1015 h on 10-10-19 by Gy using Mettler XP105 balance.

REFERENCE TOXICANT (Cu), PROJECT #421190/ 1034296
Topsmelt (*Atherinops affinis*) 7-Day Chronic Renewal Test

Test #: 9324
 Test Date: 10/2/19

Feeding Schedule (Time, h) (1 drop/beaker)

Day	1 st	2 nd	Analyst
0	13455	1750	JS / JA
1	0920	1800	EF / JA
2	0735	1830	Gy / JA
3	0930	1850	FS / JA
4	1030	1915	Gy / JA
5	0730	1710	Gy / JA
6	0950	1700	Gy / JA

HMM Artificial Seawater Batches

Day	Batch #	Prep Date	D.O. mg/L	Sal ppt	pH	Analyst
0	1	9-30-19	7.0	30	7.964	Gy
1	1	9-30-19	7.0	30	7.964	EF
2	4	10-1	7.1	30	7.847	Gy
3	6	10-2	6.9	30	8.227	Gy
4	8	10-3	6.7	30	7.641	Gy
5	11	10-4	6.9	30	7.897	EF
6	13	10/6	6.8	30	7.250	FS

CHEMISTRY

Blue (HMM Control)

Day	D.O. (mg/L)		pH		Sal (ppt)		Analyst
	0h	24h	0h	24h	0h	24h	
0	7.0		8.052		30		Gy
1	6.8	6.6	8.070	8.045	30	30	Gy
2	7.0	6.4	7.962	8.012	30	30	EF
3	6.9	6.7	8.208	8.013	30	30	Gy
4	7.1	6.6	8.000	7.980	30	30	JS/RR
5	7.1	6.4	7.954	7.883	30	30	EF
6	7.1	6.6	7.995	7.945	30	30	JS
7		6.7		7.985		30	JA

REFERENCE TOXICANT (Cu), PROJECT #421190/ 1034296

Topsmelt (*Atherinops affinis*) 7-Day Chronic Renewal Test

Test #: 9324

Test Date: 10/2/19

White (Highest Conc.)							
Day	D.O. (mg/L)		pH		Sal (ppt)		Analyst
	0h	24h	0h	24h	0h	24h	
0	7.1		8.074		30		GY
1	6.9	6.4	8.079	8.020	30	30	GY
2	7.0	6.5	8.015	8.072	30	30	EF
3	7.0	6.4	8.204	7.974	30	30	GY
4	7.2	6.10	7.997	7.988	30	30	JS/RR
5	7.0	6.9	7.882	7.967	30	30	EF
6	7.1	6.9	7.984	8.020	30	30	JS
7		6.7		8.016		30	JA

"Red" conc
"Red" conc

Temperature °C (SN 170786325)							
Day	Blue	Green	Yellow	Orange	Red	White	Analyst
0	20.8	20.9	20.8	20.7	20.8	20.7	FS
1	20.7	20.7	20.7	20.7	20.5	20.5	FS
2	20.7	20.7	20.7	20.7	20.7	20.7	EF
3	20.9	20.7	20.5	20.5	20.7	20.7	FS
4	21.2	20.9	21.2	21.0	20.9	20.9	JS
5	20.8	20.9	20.9	20.8	20.8	20.7	FS
6	20.6	20.4	20.4	20.4	20.4	—	FF
7	20.4	20.3	20.4	20.4	20.4	—	GY

Temperature °C in 6 Positions (4 Corner + 2 Center)									
Code	Rep	0d	1d	2d	3d	4d	5d	6d	7d
Yellow	A	20.8	20.7	20.7	20.7	21.2	20.9	20.5	20.5
Yellow	B	20.8	20.7	20.6	20.7	21.2	20.8	20.6	20.6
Blue	C	20.9	20.7	20.8	20.7	21.0	20.8	20.5	20.4
Yellow	E	20.7	20.7	20.6	20.5	21.0	20.5	20.4	20.4
Orange	C	20.7	20.5	20.7	20.5	21.0	20.8	20.4	20.3
Green	P	20.7	20.5	20.7	20.5	20.9	20.7	20.4	20.3

FS

JS

REFERENCE TOXICANT (Cu), PROJECT #421190/ 1034296
Topsmelt (*Atherinops affinis*) 7-Day Chronic Renewal Test

Test #: 8324
 Test Date: 10/2/19

Cumulative Survival

Trtmnt	Cumulative Daily Survival (#Alive/Rep)						Tot # Surv	% Surv*	Analyst
	Day	A	B	C	D	E			
Blue (0 µg/L)	1	5	5	5	5	5			FS
	2	5	5	5	5	5			EF
	3	5	5	5	5	5			FS
	4	5	5	5	5	5			FS
	5	5	5	5	5	5			GY
	6	5	5	5	5	5			EF
	7	5	5	5	5	5	25	100	RRJ
Green (56 µg/L)	1	5	5	5	5	5			FS
	2	5	5	5	5	5			EF
	3	5	5	5	5	5			FS
	4	5	5	5	5	5			GY
	5	5	5	5	5	5			GY
	6	5	5	5	5	5			EF
	7	5	5	5	5	5	25	100	RRJ
Yellow (100 µg/L)	1	5	5	5	5	5			FS
	2	5	5	5	5	5			EF
	3	4	5	5	5	5			FS
	4	4	4	5	5	4			FS
	5	4	4	5	5	4			GY
	6	4	4	5	5	4			EF
	7	4	4	5	5	4	22	88	RRJ
Orange (180 µg/L)	1	4	4	5	5	5			FS
	2	4	4	5	5	4			GY
	3	1	4	5	5	4			FS
	4	1	4	5	3	4			GY
	5	1	4	4	3	3			GY
	6	1	4	4	3	3			EF
	7	1	3	4	3	3	14	56	RRJ
Red (320 µg/L)	1	5	4	5	5	5			FS
	2	4	4	5	4	5			EF
	3	3	2	5	2	3			FS
	4	1	2	2	2	2			FS
	5	1	2	1	2	2			EF
	6	1	2	1	1	2			EF
	7	1	2	0	1	2	7	28	RRJ
White (572 µg/L)	1	1	2	0	1	2			FS
	2	0	0	0	0	0			GY
	3								
	4								
	5								
	6								
	7						0	0	

s = stressed

*Pass if control survival $\geq 80\%$

REFERENCE TOXICANT (Cu), PROJECT #421190/ 1034296

Topsmelt (*Atherinops affinis*) 7-Day Chronic Renewal Test

Test #: 9324

Test Date: 10/2/19

Growth

Dry Weight (mg) per Larvae at 7 Days							
Trtmt		A	B	C	D	E	Mean Wt**
Blue (0 µg/L)	Pan#	1	2	3	4	5	
	Tot Wt (mg)	82.42	73.44	77.52	82.93	86.92	
	Tare Wt (mg)	77.37	69.17	71.76	76.81	80.23	
	Net Wt (mg)	5.05	4.27	5.76	6.12	6.69	
	N	5	5	5	5	5	
	mg/indiv	1.010	0.854	1.152	1.224	1.338	
Green (56 µg/L)	Pan#	6	7	8	9	10	
	Tot Wt (mg)	93.02	93.08	85.84	84.63	96.38	
	Tare Wt (mg)	86.57	87.29	80.09	79.03	91.11	
	Net Wt (mg)	6.45	5.80	5.77	5.60	5.27	
	N	5	5	5	5	5	
	mg/indiv	1.290	1.160	1.154	1.120	1.054	
Yellow (100 µg/L)	Pan#	11	12	13	14	15	
	Tot Wt (mg)	87.35	84.51	86.04	86.01	90.20	
	Tare Wt (mg)	81.67	79.85	81.16	79.75	84.38	
	Net Wt (mg)	5.68	4.66	4.88	6.26	5.82	
	N	5	5	5	5	5	
	mg/indiv	1.136	0.932	0.976	1.252	1.164	
Orange (180 µg/L)	Pan#	16	17	18	19	20	
	Tot Wt (mg)	76.86	89.36	74.18	82.27	83.64	
	Tare Wt (mg)	75.56	84.89	70.15	78.89	78.91	
	Net Wt (mg)	1.30	4.47	4.03	3.38	4.73	
	N	5	5	5	5	5	
	mg/indiv	0.260	0.894	0.806	0.676	0.946	
Red (320 µg/L)	Pan#	21	22	23	24	25	
	Tot Wt (mg)	73.87	85.89	77.58	80.71	80.49	
	Tare Wt (mg)	72.76	84.10	76.54	79.70	78.62	
	Net Wt (mg)	1.11	1.79	1.04	1.01	1.87	
	N	5	5	5	5	5	
	mg/indiv	0.222	0.358	0.208	0.202	0.374	
White (572 µg/L)	Pan#	26	27	28	29	30	
	Tot Wt (mg)	77.72	88.82	80.17	70.68	71.76	
	Tare Wt (mg)	77.72	88.82	80.17	70.68	71.76	
	Net Wt (mg)	0	0	0	0	0	
	N	5	5	5	5	5	
	mg/indiv	0	0	0	0	0	

**Pass if mean control weight ≥ 0.85 mg/ind

Blank Tare Weights		
Pan #	Before	After
31	71.32	71.34
32	76.89	76.89
33	67.39	67.39

REFERENCE TOXICANT (Cu), PROJECT #421190/ 1034296
Topsmelt (*Atherinops affinis*) 7-Day Chronic Renewal Test

Test #: 9324
 Test Date: 10/2/19

Treatment/Rep (Facing Shelf)

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

Random Beaker Position

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

NOTES

Glassware rinsed with hot tap and DW before use.

King County Environmental Laboratory

Lab QC Report - 10/18/1913:28

Run ID: R238333 Workgroup: WG166820 ()

RT:WG166820-1 Matrix:SALT WTR Listtype:AQTOPSMELT-CHRONIC Method:EPA600/R-95/136 Project: Pkey:STD
(Reference Toxicant)

Parameter	Unit	RT Value	Ctrl-Limits	Mean	Qual	CV
Growth IC25	ug/L	151.18	55.58-179.58	117.58		26.37
Survival LC50	ug/L	203.68	82.37-253.03	167.7		25.44

King County Environmental Laboratory
Lab Review Report

Reported: 18-Oct-19 13:28 ~ Data Source: ELD

Listtype / Method: AQTOPSMELT-CHRONIC / EPA600/R-95/136
Run ID / Workgroup: R238333 / WG166820

CollectDate	Tspan	Project	Mat	Locator	Sample	Parameter	Value	Units	Qual	Mdl	Rdl	Textvalue
			LL	RT	WG166820-1	Growth IC25	151.18	ug/L	TA			95% Confidence Interval: 112.84 to 206.56. Reference toxicant is Copper.
						Survival LC50	203.68	ug/L	TA			95% Confidence Interval: 171.35 to 242.11. Reference toxicant is Copper.
						Test Number		none				9324
						Date Analyzed		none				02-OCT-19 13:50
						Prep Date		none				02-OCT-19 13:50

No products missing

CETIS Analytical Report

Report Date: 18 Oct-19 06:47 (p 1 of 2)

Test Code/ID: 9324AACQC / 01-2177-0066

Pacific Topsmelt 7-d Survival and Growth Test						King County Metro Services, WQ Lab					
Analysis ID: 12-2778-5837		Endpoint: 7d Survival Rate		CETIS Version: CETISv1.9.5							
Analyzed: 18 Oct-19 6:43		Analysis: Untrimmed Spearman-Kärber		Status Level: 1							
Batch ID: 16-3109-5052		Test Type: Growth-Survival (7d)		Analyst: GY							
Start Date: 02 Oct-19 13:50		Protocol: EPA/600/R-95/136 (1995)		Diluent: Deionized Water							
Ending Date: 09 Oct-19 14:00		Species: Atherinops affinis		Brine: Hawaiian Marine Mix							
Test Length: 7d 0h		Taxon: Actinopterygii		Source: Aquatic Biosystems, CO Age: 10d							
Sample ID: 11-3666-8822		Code: WG166820-1		Project: Reference Toxicant							
Sample Date: 02 Oct-19 13:00		Material: Copper chloride		Source:							
Receipt Date:		CAS (PC):		Station:							
Sample Age: 50m		Client: Internal Lab									
Spearman-Kärber Estimates											
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL				
Control Threshold	0	0.00%	2.309	0.03753	203.7	171.4	242.1				
Test Acceptability Criteria											
		TAC Limits									
Attribute	Test Stat	Lower	Upper	Overlap	Decision						
Control Resp	1	0.8	>>	Yes	Passes Criteria						
7d Survival Rate Summary											
			Calculated Variate(A/B)					Isotonic Variate			
Conc-µg/L	Code	Count	Mean	Min	Max	Std Dev	CV%	%Effect	A/B	Mean	%Effect
0	D	5	1.0000	1.0000	1.0000	0.0000	0.00%	0.0%	25/25	1	0.0%
56		5	1.0000	1.0000	1.0000	0.0000	0.00%	0.0%	25/25	1	0.0%
100		5	0.8800	0.8000	1.0000	0.1095	12.45%	12.0%	22/25	0.88	12.0%
180		5	0.5600	0.2000	0.8000	0.2191	39.12%	44.0%	14/25	0.56	44.0%
320		5	0.2800	0.2000	0.4000	0.1095	39.12%	72.0%	7/25	0.28	72.0%
572		5	0.0000	0.0000	0.0000	0.0000		100.0%	0/25	0	100.0%
7d Survival Rate Detail											
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	D	1.0000	1.0000	1.0000	1.0000	1.0000					
56		1.0000	1.0000	1.0000	1.0000	1.0000					
100		0.8000	0.8000	1.0000	1.0000	0.8000					
180		0.2000	0.6000	0.8000	0.6000	0.6000					
320		0.2000	0.4000	0.2000	0.2000	0.4000					
572		0.0000	0.0000	0.0000	0.0000	0.0000					
7d Survival Rate Binomials											
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	D	5/5	5/5	5/5	5/5	5/5					
56		5/5	5/5	5/5	5/5	5/5					
100		4/5	4/5	5/5	5/5	4/5					
180		1/5	3/5	4/5	3/5	3/5					
320		1/5	2/5	1/5	1/5	2/5					
572		0/5	0/5	0/5	0/5	0/5					

FS

CETIS Analytical Report

Report Date: 18 Oct-19 06:47 (p 2 of 2)
 Test Code/ID: 9324AACQC / 01-2177-0066

Pacific Topsmelt 7-d Survival and Growth Test

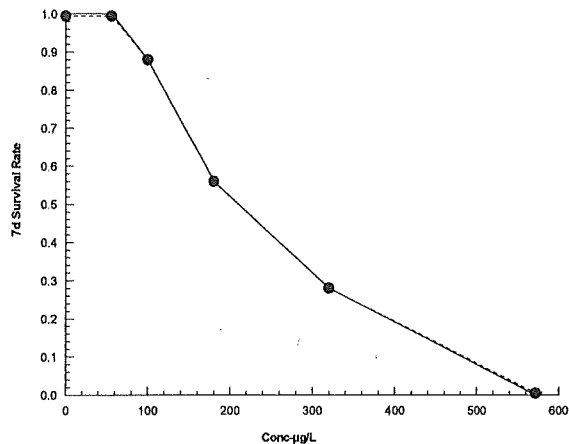
King County Metro Services, WQ Lab

Analysis ID: 12-2778-5837
 Analyzed: 18 Oct-19 6:43

Endpoint: 7d Survival Rate
 Analysis: Untrimmed Spearman-Kärber

CETIS Version: CETISv1.9.5
 Status Level: 1

Graphics



CETIS Summary Report

Report Date: 18 Oct-19 06:47 (p 1 of 1)
 Test Code/ID: 9324AACQC / 01-2177-0066

Pacific Topsmelt 7-d Survival and Growth Test

King County Metro Services, WQ Lab

Batch ID: 16-3109-5052	Test Type: Growth-Survival (7d)	Analyst: GY
Start Date: 02 Oct-19 13:50	Protocol: EPA/600/R-95/136 (1995)	Diluent: Deionized Water
Ending Date: 09 Oct-19 14:00	Species: Atherinops affinis	Brine: Hawaiian Marine Mix
Test Length: 7d 0h	Taxon: Actinopterygii	Source: Aquatic Biosystems, CO Age: 10d

Sample ID: 11-3666-8822	Code: WG166820-1	Project: Reference Toxicant
Sample Date: 02 Oct-19 13:00	Material: Copper chloride	Source:
Receipt Date:	CAS (PC):	Station:
Sample Age: 50m	Client: Internal Lab	

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	TU	S
12-2778-5837	7d Survival Rate	Spearman-Kärber	EC50	203.7	171.4	242.1		1

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
12-2778-5837	7d Survival Rate	Control Resp	1	0.8	>>	Yes	Passes Criteria

7d Survival Rate Summary

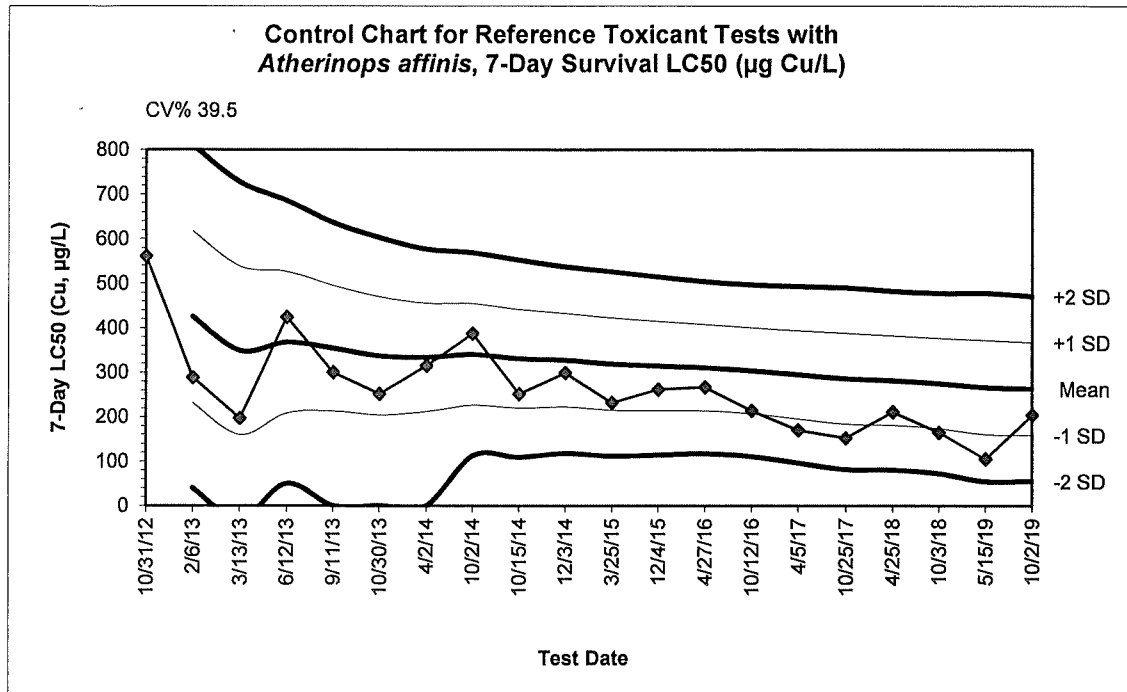
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	5	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
56		5	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
100		5	0.8800	0.7440	1.0000	0.8000	1.0000	0.0490	0.1095	12.45%	12.00%
180		5	0.5600	0.2880	0.8320	0.2000	0.8000	0.0980	0.2191	39.12%	44.00%
320		5	0.2800	0.1440	0.4160	0.2000	0.4000	0.0490	0.1095	39.12%	72.00%
572		5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		100.00%

7d Survival Rate Detail

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.0000	1.0000	1.0000	1.0000	1.0000
56		1.0000	1.0000	1.0000	1.0000	1.0000
100		0.8000	0.8000	1.0000	1.0000	0.8000
180		0.2000	0.6000	0.8000	0.6000	0.6000
320		0.2000	0.4000	0.2000	0.2000	0.4000
572		0.0000	0.0000	0.0000	0.0000	0.0000

7d Survival Rate Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	5/5	5/5	5/5	5/5	5/5
56		5/5	5/5	5/5	5/5	5/5
100		4/5	4/5	5/5	5/5	4/5
180		1/5	3/5	4/5	3/5	3/5
320		1/5	2/5	1/5	1/5	2/5
572		0/5	0/5	0/5	0/5	0/5



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
10/31/12	561.0					
2/6/13	288.8	424.90	232.4255	39.9511	617.3745	809.8489
3/13/13	197.2	349.00	159.7762	-29.4476	538.2238	727.4476
6/12/13	423.7	367.67	208.7186	49.7722	526.6114	685.5578
9/11/13	299.7	354.07	213.1046	0.0000	495.0394	636.0069
10/30/13	252.2	337.09	204.3262	0.0000	469.8604	602.6275
4/2/14	315.4	333.99	212.5180	0.0000	455.4705	576.9468
10/2/14	387.3	340.66	226.6242	112.5908	454.6908	568.7242
10/15/14	252.2	330.83	220.1602	109.4915	441.4976	552.1663
12/3/14	299.3	327.68	222.8613	118.0465	432.4907	537.3055
3/25/15	232.0	318.98	215.4422	111.9063	422.5141	526.0501
12/4/15	262.4	314.26	214.2037	114.1441	414.3230	514.3826
4/27/16	266.7	310.60	213.9008	117.1970	407.3084	504.0122
10/12/16	214.6	303.75	207.3593	110.9714	400.1350	496.5228
4/5/17	170.3	294.85	195.7840	96.7173	393.9174	492.9841
10/25/17	152.8	285.97	183.8888	81.8051	388.0562	490.1399
4/25/18	211.3	281.58	181.0924	80.6048	382.0676	482.5552
10/3/18	165.1	275.11	173.8294	72.5500	376.3884	477.6678
5/15/19	105.6	266.19	160.3576	54.5278	372.0172	477.8469
10/2/19	203.7	263.06	159.1125	55.1620	367.0135	470.9640

***Atherinops affinis*, Chronic Test Precision**
7-Day Survival LC50, µg Cu/L
Table 2 of 2

Date	Test #	Dilution Series, NaCl, g/L			Stock Soln Prep Date	Water	Control Surv, %	Pass/ Fail**	Survival LC50	Stats	Control Limits	% COV		
120411	6149	50	100	200	400	800	111130	HMM	96	P	486.3	PA	11.31 – 646.2	48.3
121003	6442	50	100	200	400	800	111130	HMM	100	P	386.7	PA	24.67 – 650.8	46.3
121031	6491	50	100	200	400	800	111130	HMM	100	P	561	PA	32.71 – 677.6	45.4
130206	6578	50	100	200	400	800	111130	HMM	80	P	288.8	SK	38.12 – 677.6	44.7
130313	6597	50	100	200	400	800	111130	HMM	100	P	197.2	SK	49.9 – 674.7	42.5
130612	6662	50	100	200	400	800	111130	HMM	100	P	423.7	PA	51.9 – 622.5	42.3
130911	6816	50	100	200	400	800	111130	HMM	96	P	299.7	SK	57.09 – 622.6	41.6
101030	6909	50	100	200	400	800	111130	HMM	100	P	252.2	PA	56.2 – 622.7	41.7
140402	7015	50	100	200	400	800	111130	HMM	100	P	315.4	SK	76.4 – 618.0	39.0
141002	7277	50	100	200	400	800	111130	HMM	100	P	387.3	SK	76.3 – 617.8	39.0
141015	7317	50	100	200	400	800	140430	HMM	100	P	252.2	PA	76.8 – 617.6	38.9
141203	7363	50	100	200	400	800	140430	HMM	100	P	299.3	PA	74.0 – 616.5	39.3
150325	7459	50	100	200	400	800	140430	HMM	100	P	232	PA	68.3 – 617.3	40.0
151204	7843	50	100	200	400	800	140430	HMM	96	P	262.4	SK	70.9 – 616.9	39.7
160427	7933	56	100	180	320	572	051108	HMM	88	P	266.7	SK	72.0 – 616.7	39.5
161012	8171	56	100	180	320	572	051108	HMM	100	P	214.6	TSK	69.0 – 617.6	40.0
170405	8276	56	100	180	320	572	051108	HMM	96	P	170.3	SK	66.3 – 618.8	40.3
171025	8527	56	100	180	320	572	051108	HMM	96	P	152.8	SK	91.1 – 536.8	35.5
180425	8627	56	100	180	320	572	051108	HMM	100	P	211.3	TSK	80.5 – 534.7	36.9
181003	8915	56	100	180	320	572	051108	HMM	100	P	165.1	SK	73.0 – 509.5	37.5
190515	9061	56	100	180	320	572	051108	HMM	96	P	105.6	TSK	59.3 – 485.2	39.1
191002	9324	56	100	180	320	572	051108	HMM	100	P	203.7	SK	55.2 – 471.0	39.5

PA = Probit Analysis
MA = Moving Average
(T)SK = (Trimmed) Spearman Karber
GI = Graphical Interpolation
**Pass if control survival ≥ 80%; control weight ≥ 0.85 mg

I = Indeterminate Value
* = Value Outside Control Limits
HMM = Hawaiian Marine Mix ASW
NSW = Natural Seawater

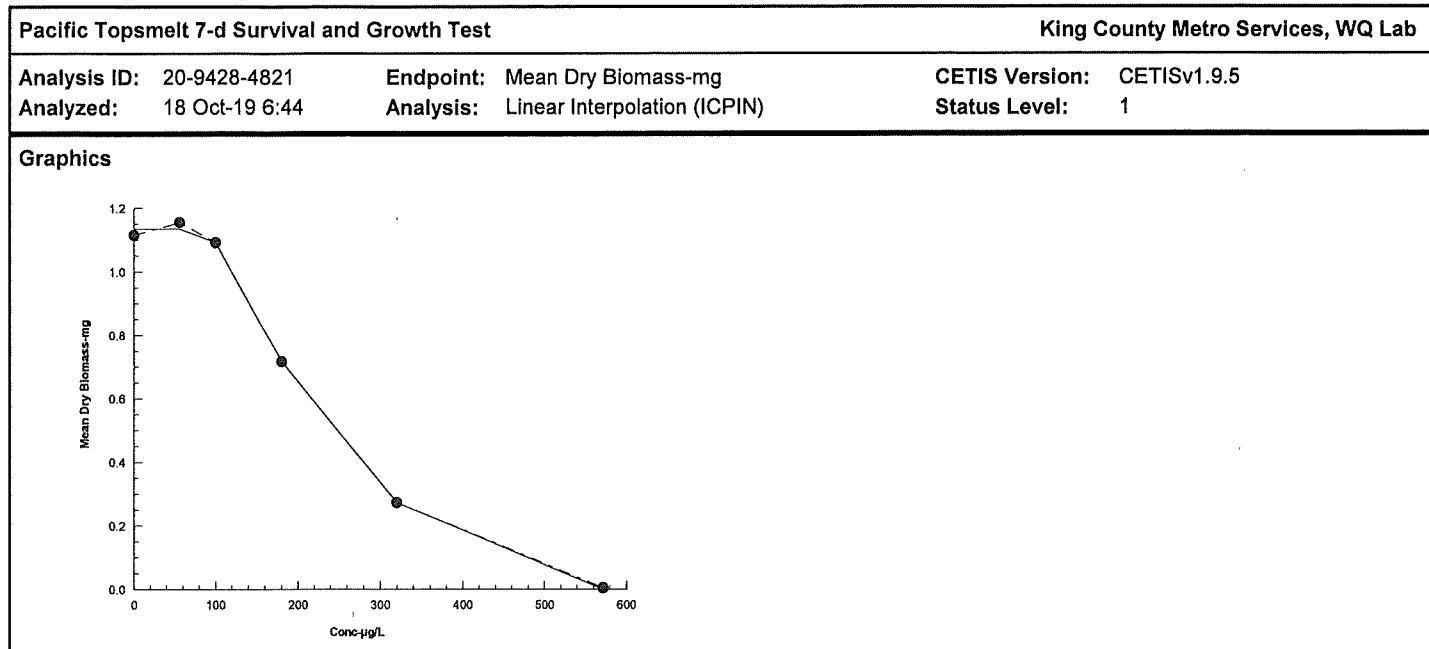
CETIS Analytical Report

Report Date: 18 Oct-19 07:21 (p 1 of 2)
Test Code/ID: 9324AACQC / 01-2177-0066

Pacific Topsmelt 7-d Survival and Growth Test						King County Metro Services, WQ Lab				
Analysis ID: 20-9428-4821		Endpoint: Mean Dry Biomass-mg		CETIS Version: CETISv1.9.5						
Analyzed: 18 Oct-19 6:44		Analysis: Linear Interpolation (ICPIN)		Status Level: 1						
Batch ID: 16-3109-5052		Test Type: Growth-Survival (7d)		Analyst: GY						
Start Date: 02 Oct-19 13:50		Protocol: EPA/600/R-95/136 (1995)		Diluent: Deionized Water						
Ending Date: 09 Oct-19 14:00		Species: Atherinops affinis		Brine: Hawaiian Marine Mix						
Test Length: 7d 0h		Taxon: Actinopterygii		Source: Aquatic Biosystems, CO		Age: 10d				
Sample ID: 11-3666-8822		Code: WG166820-1		Project: Reference Toxicant						
Sample Date: 02 Oct-19 13:00		Material: Copper chloride		Source:						
Receipt Date:		CAS (PC):		Station:						
Sample Age: 50m		Client: Internal Lab								
Linear Interpolation Options										
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method					
Linear	Linear	1146286	200	Yes	Two-Point Interpolation					
Test Acceptability Criteria										
		TAC Limits								
Attribute	Test Stat	Lower	Upper	Overlap	Decision					
Control Resp	1.116	0.85	>>	Yes	Passes Criteria					
Point Estimates										
Level	µg/L	95% LCL	95% UCL							
IC25	151.2	112.8	206.6							
Mean Dry Biomass-mg Summary										
		Calculated Variate							Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Min	Max	Std Dev	CV%	%Effect	Mean	%Effect
0	D	5	1.116	0.854	1.338	0.1885	16.90%	0.0%	1.136	0.0%
56		5	1.156	1.054	1.29	0.08613	7.45%	-3.59%	1.136	0.0%
100		5	1.092	0.932	1.252	0.134	12.27%	2.12%	1.092	3.84%
180		5	0.7164	0.26	0.946	0.2749	38.37%	35.78%	0.7164	36.91%
320		5	0.2728	0.202	0.374	0.08558	31.37%	75.55%	0.2728	75.98%
572		5	0	0	0	0		100.0%	0	100.0%
Mean Dry Biomass-mg Detail										
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
0	D	1.01	0.854	1.152	1.224	1.338				
56		1.29	1.16	1.154	1.12	1.054				
100		1.136	0.932	0.976	1.252	1.164				
180		0.26	0.894	0.806	0.676	0.946				
320		0.222	0.358	0.208	0.202	0.374				
572		0	0	0	0	0				

CETIS Analytical Report

Report Date: 18 Oct-19 07:21 (p 2 of 2)
Test Code/ID: 9324AACQC / 01-2177-0066



CETIS Summary Report

Report Date: 18 Oct-19 07:21 (p 1 of 1)
Test Code/ID: 9324AACQC / 01-2177-0066

Pacific Topsmelt 7-d Survival and Growth Test				King County Metro Services, WQ Lab			
Batch ID: 16-3109-5052	Test Type: Growth-Survival (7d)	Analyst: GY					
Start Date: 02 Oct-19 13:50	Protocol: EPA/600/R-95/136 (1995)	Diluent: Deionized Water					
Ending Date: 09 Oct-19 14:00	Species: Atherinops affinis	Brine: Hawaiian Marine Mix					
Test Length: 7d 0h	Taxon: Actinopterygii	Source: Aquatic Biosystems, CO	Age: 10d				
Sample ID: 11-3666-8822	Code: WG166820-1	Project: Reference Toxicant					
Sample Date: 02 Oct-19 13:00	Material: Copper chloride	Source:					
Receipt Date:	CAS (PC):	Station:					
Sample Age: 50m	Client: Internal Lab						

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	TU	S
20-9428-4821	Mean Dry Biomass-mg	Linear Interpolation (ICPIN)	IC25	151.2	112.8	206.6		1

Test Acceptability

				TAC Limits			
Analysis ID	Endpoint	Attribute	Test Stat	Lower	Upper	Overlap	Decision
20-9428-4821	Mean Dry Biomass-mg	Control Resp	1.116	0.85	>>	Yes	Passes Criteria

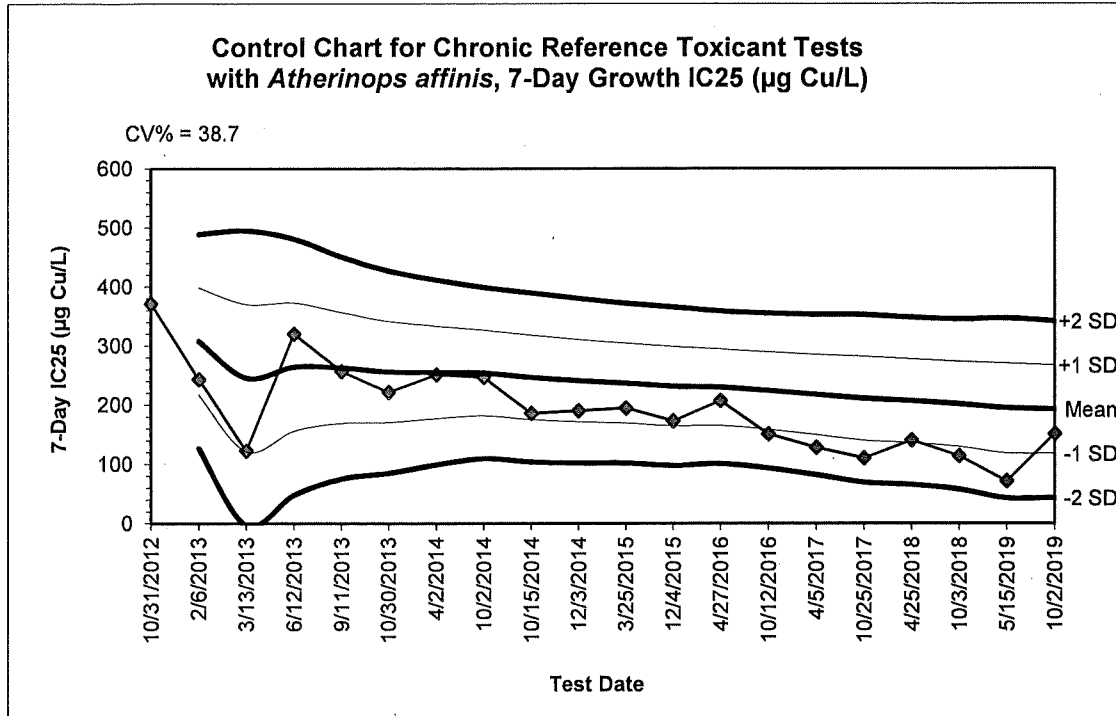
Mean Dry Biomass-mg Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	5	1.116	0.8816	1.35	0.854	1.338	0.0843	0.1885	16.90%	0.00%
56		5	1.156	1.049	1.263	1.054	1.29	0.03852	0.08613	7.45%	-3.59%
100		5	1.092	0.9257	1.258	0.932	1.252	0.05991	0.134	12.27%	2.12%
180		5	0.7164	0.3751	1.058	0.26	0.946	0.1229	0.2749	38.37%	35.78%
320		5	0.2728	0.1665	0.3791	0.202	0.374	0.03827	0.08558	31.37%	75.55%
572		5	0	0	0	0	0	0	0		100.00%

Mean Dry Biomass-mg Detail

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.01	0.854	1.152	1.224	1.338
56		1.29	1.16	1.154	1.12	1.054
100		1.136	0.932	0.976	1.252	1.164
180		0.26	0.894	0.806	0.676	0.946
320		0.222	0.358	0.208	0.202	0.374
572		0	0	0	0	0

ATH IC25



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
10/31/2012	371.6					
2/6/2013	243.5	307.5500	216.9696	126.3892	398.1304	488.7108
3/13/2013	122.6	245.9000	121.3827	-3.1347	370.4173	494.9347
6/12/2013	320.2	264.4750	156.2322	47.9894	372.7178	480.9606
9/11/2013	256.6	262.9000	169.0929	75.2857	356.7071	450.5143
10/30/2013	221.6	256.0167	170.4357	84.8547	341.5977	427.1786
4/2/2014	251.2	255.3286	177.1830	99.0374	333.4742	411.6198
10/2/2014	247.2	254.3125	181.9067	109.5008	326.7183	399.1242
10/15/2014	186.2	246.7444	175.3109	103.8773	318.1780	389.6116
12/3/2014	190.5	241.1200	171.4628	101.8056	310.7772	380.4344
3/25/2015	194.5	236.8818	169.3207	101.7597	304.4429	372.0040
12/4/2015	172.9	231.5500	164.5374	97.5249	298.5626	365.5751
4/27/2016	207.3	229.6846	165.1734	100.6622	294.1958	358.7070
10/12/2016	151.0	224.0643	158.6135	93.1628	289.5150	354.9658
4/5/2017	127.9	217.6533	149.8719	82.0905	285.4347	353.2161
10/25/2017	110.3	210.9438	140.1742	69.4047	281.7133	352.4828
4/25/2018	140.8	206.8176	136.2151	65.6125	277.4202	348.0228
10/3/2018	114.1	201.6667	129.7703	57.8738	273.5631	345.4595
5/15/2019	71.6	194.8226	118.8496	42.8766	270.7956	346.7686
10/2/2019	151.2	192.6415	118.0542	43.4670	267.2288	341.8160

TOPSMELT (*Atherinops affinis*) CHRONIC TEST PRECISION
7-Day Growth IC25, µg Cu/L
Table 1 of 1

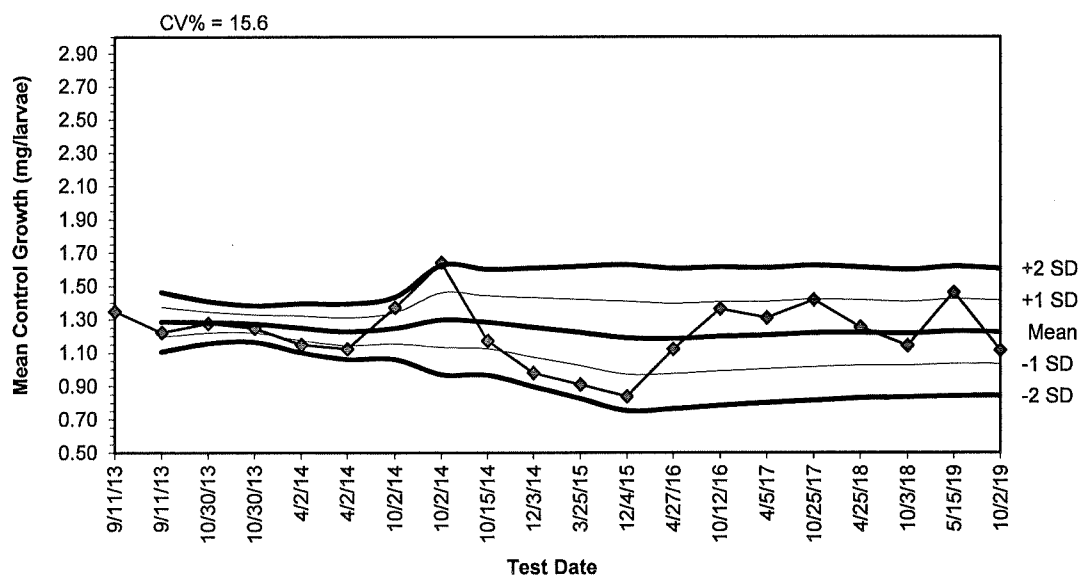
Date	Test #	Dilution Series (Cu, µg/L)			Stock Soln Prep Date	Water	Control Surv. %	Control Weight (mg)	Pass/ Fail **	NOEC	Control Limits	Stats	Growth IC25	Control Limits (± 2SD)	% COV
130612	6662	50	100	200	400	800	100	1.194	P	-	-	-	320.2	20.7 – 382.2	44.9
130911	6816	50	100	200	400	800	96	1.223	P	-	-	-	256.6	24.2 – 387.5	44.1
131030	6909	50	100	200	400	800	100	1.248	P	-	-	-	221.6	38.0 – 386.3	41.0
140402	7015	50	100	200	400	800	100	1.149	P	-	-	-	251.2	48.8 – 388.3	38.8
141002	7277	50	100	200	400	800	100	1.643	P	-	-	-	247.2	53.6 – 391.1	38.0
141015	7317	50	100	200	400	800	100	1.174	P	-	-	-	186.2	55.8 – 390.9	37.5
141203	7363	50	100	200	400	800	100	0.977	P	-	-	-	190.5	61.8 – 389.7	36.3
150325	7459	50	100	200	400	800	100	0.910	P	-	-	-	194.5	76.2 – 384.6	33.5
151204	7843	50	100	200	400	800	96	0.839	P	(used	CuCl ₂	-2H ₂ O)	172.9	74.0 – 384.9	33.9
160427	7933	56	100	180	320	572	88	1.123	P	"	"	"	207.3	76.9 – 384.9	33.4
161012	8171	56	100	180	320	572	100	1.364	P	"	"	"	151	70.3 – 374.8	34.2
170405	8276	56	100	180	320	572	96	1.311	P	"	"	"	127.9	72.7 – 373.6	33.7
171025	8527	56	100	180	320	572	96	1.418	P	"	"	"	110.3	64.5 – 358.4	34.8
180425	8627	56	100	180	320	572	100	1.254	P	"	"	"	140.8	59.1 – 359.1	35.9
181003	8915	56	100	180	320	572	100	1.144	P	"	"	"	114.1	56.6 – 338.3	35.7
190515	9061	56	100	180	320	572	96	1.464	P	"	"	"	71.6	39.4 – 342.7	39.7
191002	9324	56	100	180	320	572	100	1.116	P	"	"	"	151.2	43.5 – 341.8	38.7

D = Dunnett's Multiple Comparison Test
B = Bonferroni T-Test
W = Williams T-test
Wc = Wilcoxon's w/ B

*Value Outside Control Limits

**Pass if control survival ≥ 80%; control weight ≥ 0.85 mg
I = Indeterminate Result
HMM = Hawaiian Marine Mix ASW
NSW = Natural Seawater

**Control Chart for 7-Day Chronic Tests
with *Atherinops affinis*, Mean Control Growth (mg)**



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
9/11/13	1.349					
9/11/13	1.223	1.2860	1.1969	1.1078	1.3751	1.4642
10/30/13	1.278	1.2833	1.2202	1.1570	1.3465	1.4097
10/30/13	1.248	1.2745	1.2200	1.1655	1.3290	1.3835
4/2/14	1.149	1.2494	1.1761	1.1027	1.3227	1.3961
4/2/14	1.124	1.2285	1.1453	1.0621	1.3117	1.3949
10/2/14	1.373	1.2491	1.1556	1.0620	1.3427	1.4363
10/2/14	1.643	1.2984	1.1344	0.9704	1.4624	1.6264
10/15/14	1.174	1.2846	1.1257	0.9667	1.4435	1.6024
12/3/14	0.980	1.2541	1.0760	0.8979	1.4322	1.6103
3/25/15	0.910	1.2228	1.0245	0.8263	1.4211	1.6194
12/4/15	0.839	1.1908	0.9717	0.7526	1.4100	1.6291
4/27/16	1.123	1.1856	0.9750	0.7643	1.3963	1.6069
10/12/16	1.364	1.1984	0.9904	0.7825	1.4063	1.6142
4/5/17	1.311	1.2059	1.0034	0.8010	1.4083	1.6108
10/25/17	1.418	1.2191	1.0165	0.8138	1.4218	1.6244
4/25/18	1.254	1.2212	1.0248	0.8284	1.4176	1.6140
10/3/18	1.144	1.2169	1.0255	0.8341	1.4083	1.5997
5/15/19	1.464	1.2299	1.0354	0.8410	1.4243	1.6188
10/2/19	1.116	1.2242	1.0332	0.8423	1.4152	1.6061

Communications & COC

REFERENCE TOXICANT TEST (Cu), Project 421190/1034296

Mysidopsis bahia 7-Day Chronic Renewal Test

Test#: 9325
Test Date: 10/2/19

ORGANISMS

See T 9311
Mysids received from _____ via _____ as _____ days old (Hatch Date: _____)
) Arrived at _____ h on _____
At Arrival: Salinity _____ ppt, Temp _____ °C; pH _____; D.O. _____
_____ mg/L. Shipped in _____ dead removed.
Acclimation: Placed into _____ 1.5L cryst dishes. Into _____ °C waterbath at _____ h. Fed _____
_____ mL *Artemia* nauplii/dish at _____ h.
At _____ h incr WB temp to _____ °C
_____ h " " " _____ °C
_____ h " " " _____ °C
_____ h " " " _____ °C

DILUTION WATER/ SOLUTIONS

1. HMM: Hawaiian Marine Mix #HW-1016 (artificial sea salts) lot# _____ rec'd 3/6/19 opened 10/4/19.

35.7g HMM salt + 0.2g NaHCO₃ in 1L MilliQ Water;
Aerate > 2h and filter to 0.45 um before use.
Salinity: 30 ppt.

2. Cu Stock Soln: Prep: 4-30-14 by adding 1.26 g CuSO₄ ≤ 1000 mL DW.
(JT Baker # I 1850 rec'd _____, opened _____, lot # 020104)
a. Measured 376 mg Cu/L on 5-13-14. (Nominal Cu 500 mg/L.)
b. Daily add 2.12 mL ≤ 2000 mL with HMM = 400 µg/L Cu.
3. MYC LIMS Sample #: WG 166820 -1; Wkqp #: WG 166820

DILUTIONS

Code	Cu, µg/L	HMM, mL	Decant, mL	Sample #	Measured µg/L Cu in DW
White	400	2000	1000	73432-1	468.5
Red	200	≤ 2000	↓		
Orange	100	↓	↓		
Yellow	50	↓	↓		
Green	25	↓	↓		
Blue	0	HMM only	HMM only		

HMM Artificial Sea Water Batches

Day	Batch	Prep Date	DO (mg/L)	Salinity (ppt)	pH	Analyst
0	2	9-30-19	7.2	30	7.924	Gy
1	3	10-1-19	7.1	30	7.793	Gy
2	5	10-2-19	6.8	30	7.991	Gy
3	7	10-3-19	6.6	30	7.997	EF
4	6	10-2-19	6.9	30	8.227	Gy
5	10	10-4-19	6.8	30	8.058	Gy
6	9	10-4-19	6.6	30	7.930	Gy

REFERENCE TOXICANT TEST (Cu), Project 421190/1034296

Mysidopsis bahia 7-Day Chronic Renewal Test

Test#: 9325

Test Date: 10/2/19

PROCEDURE

1. Prep solutions as above in 2000-mL graduated cylinder; decant to 1-L glass flasks.
 2. Pour 250 mL each treatment to each of (4) 400-mL beakers (Reps A-D)/trtmt.
 3. Place beakers randomly in waterbath # A and bring solns to 26°C. Setup at _____ h.
 4. Add 5 mysids/beaker directly into solutions with polyscreen; rinse screen w/ DW between beakers.
 5. Start test at 1433 h on 10/2/19. Counts verified by RL & JS. Place HOBO/Tidbit temp recorder (SN 20067201) in beaker w/DW into waterbath.
 6. Prep sample for Cu analysis in DW: ✓ Acidify: ✓ Analyst: GY.
 7. Feed mysids 2 drops *Artemia* nauplii/beaker 2x/day.
 8. Renew solutions daily:
 - a) Remove approximately 200 mL soln with waste + excess food by decanting and/or pipet+bulb.
 - b) Replace 250 mL with new prepared solution (26°C) by pouring down side of beaker.
 - c) Count larvae before and after renewal.
 9. Record survival and remove dead larvae daily at renewal.
 10. Measure temp daily in 1 rep/trtmt and in 6 positions (4 corners + 2 center). Measure D.O., pH, and Salinity daily in 0h (new) and 24h (old) solutions (Blue and White treatments).
 11. End test at 1340 h on 10-9-19.
 - a) Record survival.
 - b) Rinse larvae with ice water onto screen and place into tared weigh pans.
 - c) Process by rep (1 analyst/rep):
- Rep A JA Rep B GY Rep C JA Rep D JA
yu
12. Into 60°C oven at 1407 h on 10-9-19.
 13. Into desiccator at 0600 h on 10-10-19.
 14. Weigh at 0955 h on 10-10-19 by GY with Mettler XP105 balance.

FEEDING SCHEDULE (Time, h) (2 drops/beaker)

Day	1 st	2 nd	Analyst
0	1438	1750	JA / JA
1	0800	1800	EF / JA
2	0705	1830	GY / JA
3	0755	1845	GY / JA
4	1040	1915	JS / JA
5	0650	1705	EF / JA
6	0950	1655	GY / JA

REFERENCE TOXICANT TEST (Cu), Project 421190/1034296
Mysidopsis bahia 7-Day Chronic Renewal Test

Test#: 9325
 Test Date: 10/2/19

CHEMISTRY

Temperature °C (SN 170786325)							
Day	Blue	Green	Yellow	Orange	Red	White	Analyst
0	25.9	25.9	25.8	25.8	25.8	25.7	Gy
1	26.1	26.1	26.1	26.1	26.1	26.1	EF
2	26.1	26.0	26.1	26.0	26.1	26.1	Gy
3	26.1	26.1	26.1	26.1	26.1	26.1	EF
4	26.1	26.0	26.1	26.1	26.2	26.0	JS
5	26.1	26.0	26.1	26.1	26.1	26.1	EF
6	26.1	26.0	26.0	26.1	26.1	26.0	EF
7	26.0	26.0	26.0	26.0	26.0	26.0	Gy

Temperature °C (4 Corner + 2 Center)									
Code	Rep	0d	1d	2d	3d	4d	5d	6d	7d
Blue	A	25.9	26.1	26.2	26.2	26.1	26.1	26.1	26.1
Green	A	25.6	26.0	26.1	26.1	26.0	26.0	26.0	26.0
Green	B	25.8	26.1	26.1	26.1	26.2	26.1	26.1	26.1
Yellow	D	25.8	26.1	26.1	26.1	26.1	26.1	26.0	26.0
Orange	B	25.8	26.1	26.1	26.1	26.1	26.1	26.1	26.0
Red	D	25.8	26.1	26.1	26.1	26.2	26.1	26.1	26.0
Analyst:		Gy	EF	Gy	EF	JS	EF	EF	Gy

Blue (HMM Control)							
Day	D.O. (mg/L)		pH		Sal (ppt)		Analyst
	0h	24h	0h	24h	0h	24h	
0	7.3		8.147		30		Gy
1	6.9	5.8	8.089	7.978	30	30	Gy
2	6.9	5.8	8.089	7.845	30	30	EF
3	6.8	5.4	8.003	7.957	30	30	Gy
4	7.0	5.5	8.130	7.820	30	30	JS/RR
5	6.9	5.4	7.973	7.883	30	30	EF
6	7.0	5.7	8.058	7.844	30	30	JS
7		6.4		8.012		32	JA

White							
Day	D.O. (mg/L)		pH		Sal (ppt)		Analyst
	0h	24h	0h	24h	0h	24h	
0	7.4		8.020		30		Gy
1	7.0	6.0	8.087	7.990	30	30	Gy
2	6.9	5.3	8.081	7.938	30	30	EF
3	6.7	5.6	8.015	8.034	30	30	Gy
4	7.0	5.9	8.131	7.956	30	30	JS/RR
5	6.7	5.3	7.949	7.808	29.5	30	EF
6	7.0	6.0	8.054	7.994	30	30	JS
7		6.4		8.012		31	JA

REFERENCE TOXICANT TEST (Cu), Project 421190/1034296
Mysidopsis bahia 7-Day Chronic Renewal Test

Test#: 9325
Test Date: 10/2/19

Code	Cumulative Survival (#Alive/Rep) at 7d							Dry Weight (mg) at 7d**					
	Day	A	B	C	D	Tot # Surv	% Surv*	Ana- lyst		A	B	C	D
Blue (WW)	1	5	5	5	5			EF	Pan #	1	2	3	4
	2	5	5	5	5			EF	Tot Wt	84.77	69.01	80.05	82.11
	3	5	5	5	5			EF	Tare Wt	83.77	67.83	79.06	81.06
	4	4	5	5	5			SS	Net Wt	1.00	1.18	0.99	1.05
	5	4	5	5	5			EF	N	5	5	5	5
	6	4	5	5	5			EF	mg/ind	0.200	0.236	0.198	0.210
	7	4	5	4	5	18	90		Mean**	0.211			
Green	1	5	5	5	5			EF	Pan #	5	6	7	8
	2	5	5	5	5			GY	Tot Wt	73.31	77.79	86.36	90.83
	3	5	5	5	5			GY	Tare Wt	72.15	76.48	85.16	89.54
	4	5	5	5	5			SS	Net Wt	1.160	1.310	1.200	1.290
	5	5	5	5	5			GY	N	5	5	5	5
	6	5	5	5	5			EF	mg/ind	0.232	0.242	0.240	0.258
	7	5	5	5	5	20	100		Mean	0.248			
Yellow	1	5	5	5	5			EF	Pan #	9	10	11	12
	2	5	5	5	5			EF	Tot Wt	78.99	85.67	70.16	88.52
	3	4	5	5	5			EF	Tare Wt	78.05	84.61	68.82	87.34
	4	4	5	5	5			SS	Net Wt	0.940	1.06	1.34	1.18
	5	4	5	5	5			EF	N	5	5	5	5
	6	4	5	5	5			EF	mg/ind	0.188	0.212	0.268	0.236
	7	4	5	5	5	19	95		Mean	0.212			
Orange	1	5	5	4	5			EF	Pan #	13	14	15	16
	2	5	5	4	5			G	Tot Wt	78.88	88.53	86.00	81.10
	3	5	5	4	5			GY	Tare Wt	77.78	87.17	84.98	79.51
	4	5	5	4	5			SS	Net Wt	1.100	1.36	1.02	1.59
	5	5	5	4	5			GY	N	5	5	5	5
	6	5	5	4	5			EF	mg/ind	0.220	0.272	0.204	0.318
	7	5	5	4	5	19	95		Mean	0.254			
Red	1	5	4	5	4			EF	Pan #	17	18	19	20
	2	5	4	5	4			EF	Tot Wt	90.49	85.94	83.09	82.02
	3	5	4	5	4			EF	Tare Wt	89.38	84.80	82.10	81.39
	4	5	4	5	3			SS	Net Wt	1.11	1.14	0.99	0.63
	5	5	4	5	3			EF	N	5	5	5	5
	6	5	4	5	3			EF	mg/ind	0.222	0.228	0.198	0.126
	7	5	4	5	3	17	85		Mean	0.194			

*Pass if mean control survival $\geq 80\%$

**Pass if mean control weight ≥ 0.2 mg/ind

REFERENCE TOXICANT TEST (Cu), Project 421190/1034296
Mysidopsis bahia 7-Day Chronic Renewal Test

Test#: 9325
 Test Date: 10/2/19

Code	Cumulative Survival (#Alive/Rep) at 7d								Dry Weight (mg) at 7d**				
	Day	A	B	C	D	Tot # Surv	% Surv*	Analyst		A	B	C	D
White	1	3	4	5	5			EF	Pan #	21	22	23	24
	2	1	3	4	5			Gy	Tot Wt	71.63	84.50	91.52	89.90
	3	1	3	4	2			Gy	Tare Wt	71.45	84.36	91.19	89.48
	4	1	1	4	1			ES	Net Wt	0.18	0.14	0.33	0.02
	5	1	1	3	1			EF	N	5	5	5	5
	6	1	1	3	1	5	25	EF	mg/ind	0.026	0.028	0.066	0.004
	7	1	1	2	1				Mean	0.034			

*Pass if mean control survival $\geq 80\%$

**Pass if mean control weight ≥ 0.2 mg/ind

Blank Tare Pan Weights (mg)		
Pan #	Before	After
25	90.46	90.46
26	101.28	101.29
27	64.52	64.53

Random # Position

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

NOTES

Glassware rinsed with hot tap and DW before use.

King County Environmental Laboratory

Lab QC Report - 10/18/19 13:27

Run ID: R238332 Workgroup: WG166819 0

RT:WG166819-1 Matrix:SALT WTR Listtype:AQMYSID-CHRONIC Method:EPA821-R-02-014 Project: Pkey:STD

(Reference Toxicant)

Parameter	Unit	RT Value	Ctrl-Limits	Mean	Qual	CV
Growth IC25	ug/L	221.91	22.72-227.85	125.28		40.93

King County Environmental Laboratory
Lab Review Report

Reported: 18-Oct-19 13:28 ~ Data Source: ELD

Listtype / Method: AQMYSID-CHRONIC / EPA821-R-02-014
Run ID / Workgroup: R238332 / WG166819

CollectDate	Tspan	Project	Mat	Locator	Sample	Parameter	Value	Units	Qual	Mdl	Rdl	Textvalue
			LL	RT	WG166819-1	Growth IC25	221.91	ug/L	TA			95% Confidence Interval: 129.97 to 288.44. Reference toxicant is Copper. 9325
					Test Number			none				02-OCT-19 14:33
					Date Analyzed			none				02-OCT-19 14:33
					Prep Date							

No products missing

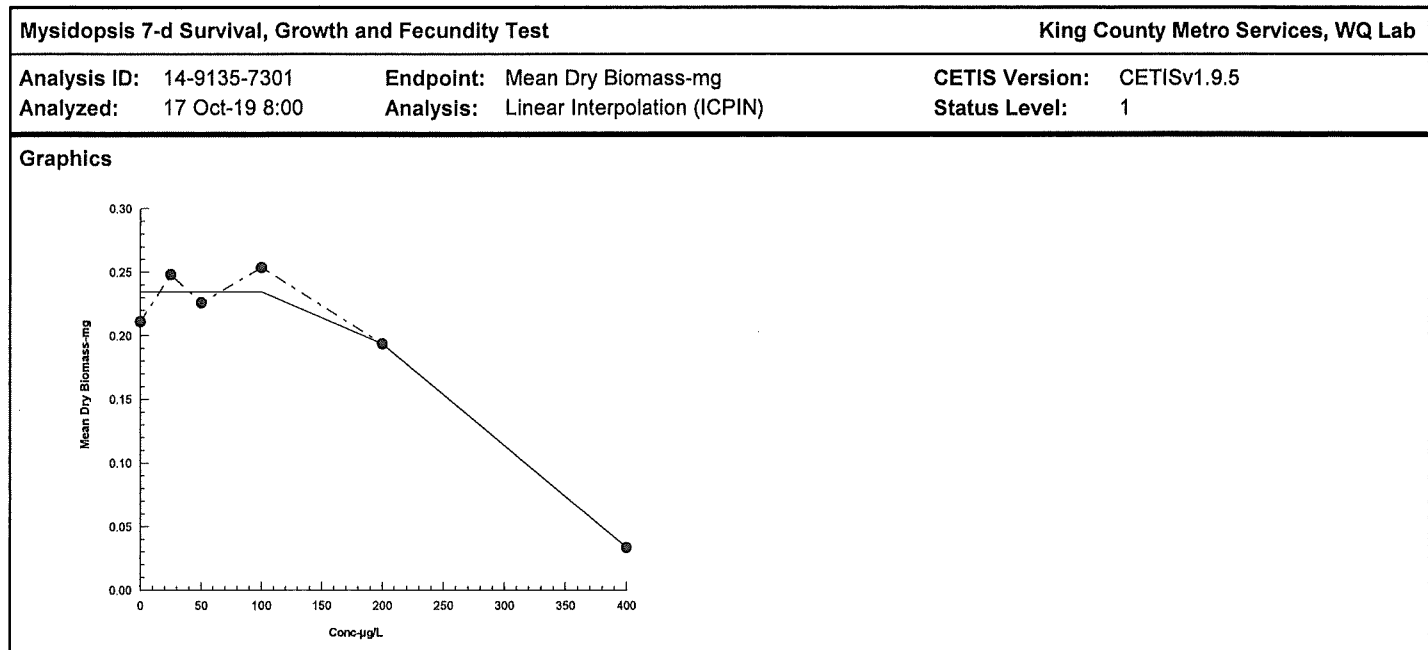
CETIS Analytical Report

 Report Date: 18 Oct-19 07:07 (p 1 of 2)
 Test Code/ID: 9325MYCQC / 00-3948-1764

Mysidopsis 7-d Survival, Growth and Fecundity Test						King County Metro Services, WQ Lab				
Analysis ID: 14-9135-7301		Endpoint: Mean Dry Biomass-mg		CETIS Version: CETISv1.9.5						
Analyzed: 17 Oct-19 8:00		Analysis: Linear Interpolation (ICPIN)		Status Level: 1						
Batch ID: 13-7755-2668		Test Type: Growth-Survival-Fec (7d)		Analyst: GY						
Start Date: 02 Oct-19 14:33		Protocol: EPA/821/R-02-014 (2002)		Diluent: Deionized Water						
Ending Date: 09 Oct-19 13:40		Species: Mysidopsis bahia		Brine: Hawaiian Marine Mix						
Test Length: 6d 23h		Taxon: Malacostraca		Source: Aquatic Biosystems, CO		Age: 7d				
Sample ID: 04-6618-9588		Code: wg166819-1		Project: Reference Toxicant						
Sample Date: 02 Oct-19 14:00		Material: Copper sulfate		Source: Reference Toxicant						
Receipt Date:		CAS (PC):		Station:						
Sample Age: 33m		Client: Internal Lab								
Linear Interpolation Options										
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method					
Linear	Linear	1232529	200	Yes	Two-Point Interpolation					
Test Acceptability Criteria										
		TAC Limits								
Attribute	Test Stat	Lower	Upper	Overlap	Decision					
Control Resp	0.211	0.2	>>	Yes	Passes Criteria					
Point Estimates										
Level	µg/L	95% LCL	95% UCL							
IC25	221.9	130	268.4							
Mean Dry Biomass-mg Summary										
			Calculated Variate						Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Min	Max	Std Dev	CV%	%Effect	Mean	%Effect
0	D	4	0.211	0.198	0.236	0.01747	8.28%	0.0%	0.2346	0.0%
25		4	0.248	0.232	0.262	0.01433	5.78%	-17.54%	0.2346	0.0%
50		4	0.226	0.188	0.268	0.03418	15.12%	-7.11%	0.2346	0.0%
100		4	0.2535	0.204	0.318	0.05188	20.47%	-20.14%	0.2346	0.0%
200		4	0.1935	0.126	0.228	0.04683	24.20%	8.29%	0.1935	17.53%
400		4	0.0335	0.004001	0.066	0.02558	76.36%	84.12%	0.0335	85.72%
Mean Dry Biomass-mg Detail										
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4					
0	D	0.2	0.236	0.198	0.21					
25		0.232	0.262	0.24	0.258					
50		0.188	0.212	0.268	0.236					
100		0.22	0.272	0.204	0.318					
200		0.222	0.228	0.198	0.126					
400		0.036	0.028	0.066	0.004001					

CETIS Analytical Report

Report Date: 18 Oct-19 07:07 (p 2 of 2)
 Test Code/ID: 9325MYCQC / 00-3948-1764



CETIS Summary Report

Report Date: 18 Oct-19 07:07 (p 1 of 1)
Test Code/ID: 9325MYCQC / 00-3948-1764

Mysidopsis 7-d Survival, Growth and Fecundity Test

King County Metro Services, WQ Lab

Batch ID: 13-7755-2668	Test Type: Growth-Survival-Fec (7d)	Analyst: GY
Start Date: 02 Oct-19 14:33	Protocol: EPA/821/R-02-014 (2002)	Diluent: Deionized Water
Ending Date: 09 Oct-19 13:40	Species: Mysidopsis bahia	Brine: Hawaiian Marine Mix
Test Length: 6d 23h	Taxon: Malacostraca	Source: Aquatic Biosystems, CO Age: 7d
Sample ID: 04-6618-9588	Code: wg166819-1	Project: Reference Toxicant
Sample Date: 02 Oct-19 14:00	Material: Copper sulfate	Source: Reference Toxicant
Receipt Date:	CAS (PC):	Station:
Sample Age: 33m	Client: Internal Lab	

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	TU	S
14-9135-7301	Mean Dry Biomass-mg	Linear Interpolation (ICPIN)	IC25	221.9	130	268.4		1

Test Acceptability

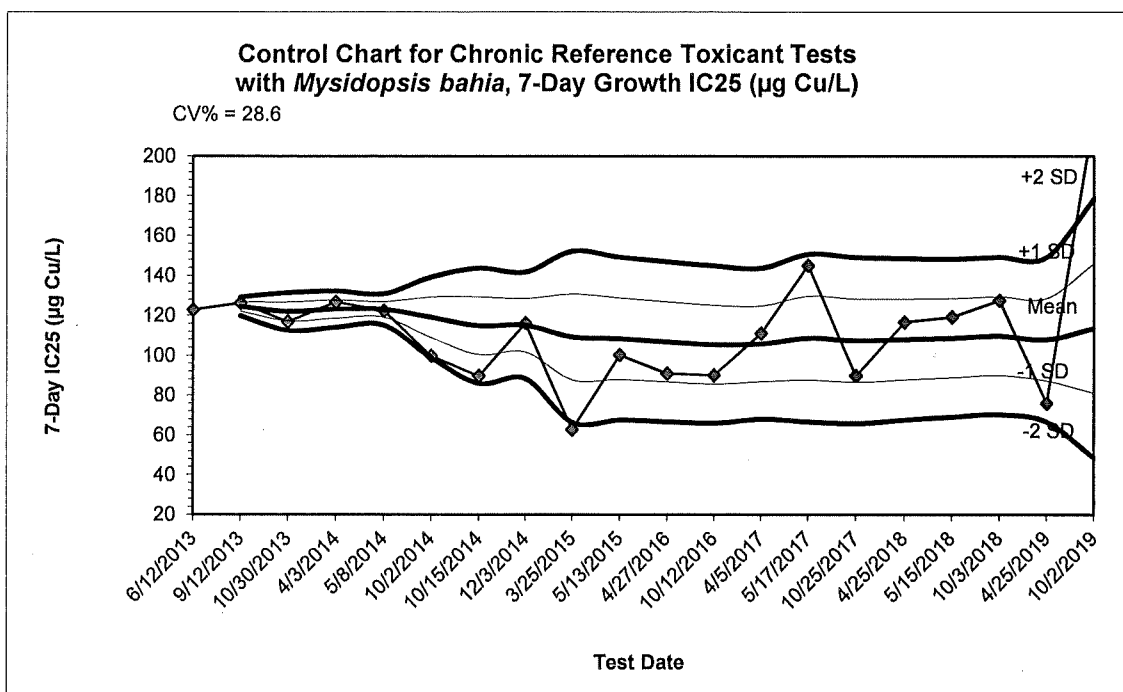
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
14-9135-7301	Mean Dry Biomass-mg	Control Resp	0.211	0.2	>>	Yes	Passes Criteria

Mean Dry Biomass-mg Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.211	0.1832	0.2388	0.198	0.236	0.008737	0.01747	8.28%	0.00%
25		4	0.248	0.2252	0.2708	0.232	0.262	0.007165	0.01433	5.78%	-17.54%
50		4	0.226	0.1716	0.2804	0.188	0.268	0.01709	0.03418	15.12%	-7.11%
100		4	0.2535	0.1709	0.3361	0.204	0.318	0.02594	0.05188	20.47%	-20.14%
200		4	0.1935	0.119	0.268	0.126	0.228	0.02341	0.04683	24.20%	8.29%
400		4	0.0335	-0.007202	0.0742	0.004001	0.066	0.01279	0.02558	76.36%	84.12%

Mean Dry Biomass-mg Detail

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.2	0.236	0.198	0.21
25		0.232	0.262	0.24	0.258
50		0.188	0.212	0.268	0.236
100		0.22	0.272	0.204	0.318
200		0.222	0.228	0.198	0.126
400		0.036	0.028	0.066	0.004001

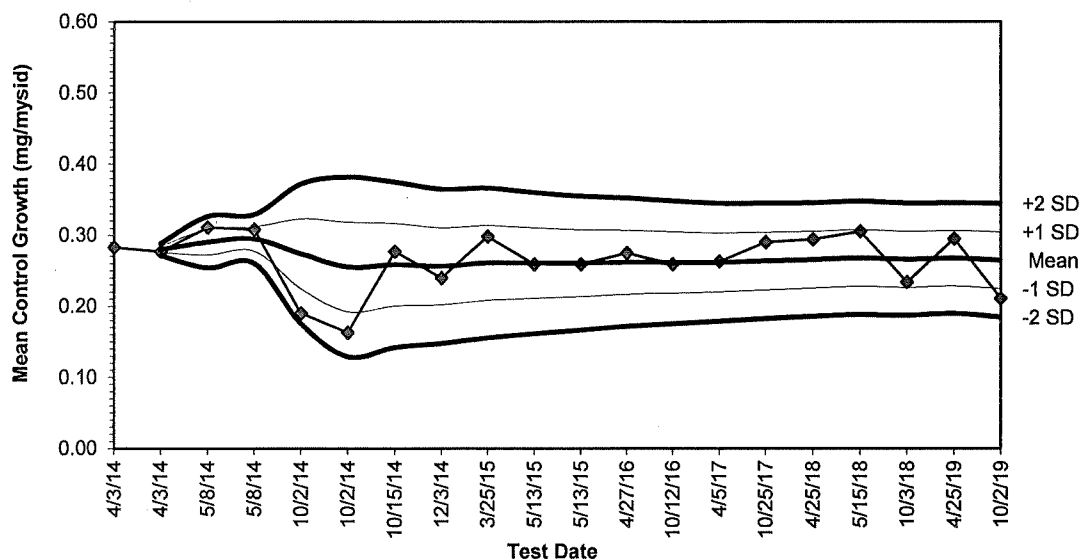


Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
6/12/2013	122.8					
9/12/2013	126.1	124.4500	122.1165	119.7831	126.7835	129.1169
10/30/2013	116.8	121.9000	117.1851	112.4703	126.6149	131.3297
4/3/2014	126.7	123.1000	118.5635	114.0270	127.6365	132.1730
5/8/2014	122.4	122.9600	119.0188	115.0776	126.9012	130.8424
10/2/2014	99.9	119.1083	109.0367	98.9650	129.1800	139.2517
10/15/2014	89.7	114.9071	100.4821	86.0570	129.3322	143.7572
12/3/2014	116.1	115.0563	101.6946	88.3329	128.4179	141.7796
3/25/2015	62.7	109.2422	87.7843	66.3263	130.7002	152.1581
5/13/2015	100.2	108.3380	87.9062	67.4744	128.7698	149.2016
4/27/2016	91.0	106.7636	86.6893	66.6150	126.8380	146.9123
10/12/2016	90.1	105.3750	85.6397	65.9043	125.1103	144.8457
4/5/2017	111.1	105.8154	86.8536	67.8919	124.7771	143.7389
5/17/2017	145.0	108.6143	87.6009	66.5874	129.6277	150.6411
10/25/2017	89.8	107.3600	86.5364	65.7128	128.1836	149.0072
4/25/2018	116.6	107.9375	87.6878	67.4381	128.1872	148.4369
5/15/2018	119.2	108.6000	88.8040	69.0079	128.3960	148.1921
10/3/2018	127.5	109.6500	89.9351	70.2202	129.3649	149.0798
4/25/2019	75.8	107.8684	87.1950	66.5216	128.5418	149.2152
10/2/2019	221.9	113.5700	81.0884	48.6068	146.0516	178.5332

CV%
28.6

**Control Chart for 7-Day Chronic Tests
with *Mysidopsis bahia*, Mean Control Growth (mg)**

CV% = 15.1



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
4/3/14	0.283					
4/3/14	0.277	0.2800	0.2758	0.2715	0.2842	0.2885
5/8/14	0.311	0.2903	0.2722	0.2540	0.3085	0.3266
5/8/14	0.308	0.2948	0.2775	0.2602	0.3120	0.3293
10/2/14	0.190	0.2738	0.2246	0.1755	0.3230	0.3721
10/2/14	0.163	0.2553	0.1922	0.1292	0.3184	0.3815
10/15/14	0.277	0.2584	0.2003	0.1421	0.3166	0.3748
12/3/14	0.240	0.2561	0.2019	0.1476	0.3104	0.3646
3/25/15	0.298	0.2608	0.2081	0.1555	0.3134	0.3660
5/13/15	0.259	0.2606	0.2110	0.1614	0.3102	0.3598
5/13/15	0.259	0.2605	0.2134	0.1663	0.3075	0.3546
4/27/16	0.275	0.2617	0.2166	0.1715	0.3068	0.3518
10/12/16	0.259	0.2615	0.2183	0.1751	0.3046	0.3478
4/5/17	0.263	0.2616	0.2201	0.1786	0.3031	0.3445
10/25/17	0.290	0.2635	0.2228	0.1822	0.3041	0.3447
4/25/18	0.294	0.2654	0.2254	0.1854	0.3054	0.3454
5/15/18	0.305	0.2677	0.2278	0.1879	0.3076	0.3475
10/3/18	0.234	0.2658	0.2263	0.1868	0.3053	0.3449
4/25/19	0.295	0.2674	0.2284	0.1894	0.3064	0.3453
10/2/19	0.211	0.2646	0.2246	0.1846	0.3045	0.3445

MYSID SHRIMP (*Mysidopsis bahia*) CHRONIC TEST PRECISION
7-Day Exposure to Reference Toxicant, Cu, µg/L
Table 3 of 3

Date	Test #	Dilution Series (Cu, µg/L)	Ref. Tox. Lot #	Water	Control Surv. %	Control Weight (mg)	Pass/Fail **	NOEC	Control Limits	Stats	Growth IC25	Control Limits	% COV
170405	8277	25, 50, 100, 200, 400	140430	HMM	90	0.263	P	-	-	-	111.1	70.2–145.8	17.2
170517	8293	25, 50, 100, 200, 400	140430	HMM	90	0.233	P	-	-	-	145.0	71.5–148.7	17.5
171025	8528	25, 50, 100, 200, 400	140430	HMM	90	0.290	P	-	-	-	89.8	69.5–147.0	17.9
180425	8628	25, 50, 100, 200, 400	140430	HMM	95	0.294	P	-	-	-	116.6	70.5–144.1	17.1
180515	8651	25, 50, 100, 200, 400	140430	HMM	90	0.305	P	-	-	-	119.2	71.8–145.2	16.9
181003	8916	25, 50, 100, 200, 400	140430	HMM	85	0.234	P	-	-	-	127.5	72.6–147.3	17.0
190425	9050	25, 50, 100, 200, 400	140430	HMM	95	0.295	P	-	-	-	75.8 ^s	67.8–148.4	18.6
191002	9325	25, 50, 100, 200, 400	140430	HMM	90	0.211	P	-	-	-	221.9*	48.6–178.5	28.6

D = Dunnett's Multiple Comparison Test
B = Bonferroni T-Test
W = Williams T-test
S = Based On Surviving #

*Value Outside Control Limits

**Pass if control surv ≥ 80%; control wt ≥ 0.2 mg/mysid
Indet. Indeterminate Result
Wc = Wilcoxon's w/ B

Communications & COC

Sweeney, Francis

From: Dudenhoeffer, Chris (ECY) <cdud461@ECY.WA.GOV>
Sent: Friday, October 25, 2019 12:16 PM
To: Sweeney, Francis
Subject: RE: Vashon WET testing - Mysid control limits

[EXTERNAL Email Notice!] External communication is important to us. Be cautious of phishing attempts. Do not click or open suspicious links or attachments.

Thanks for the email Francis. I have no concerns with this test data, or your conclusion with regards to the ref tox control limit. These tests are acceptable. Thank you for the summary document and explanation.

-Chris

Chris Dudenhoeffer

Lead for Aquatic Toxicity Assessments
Water Quality Program | Dept of Ecology
cdud461@ecy.wa.gov | 360.407.6445



"This communication is public record and may be subject to disclosure as per the Washington State Public Records Act, RCW 42.56."

From: Sweeney, Francis <Francis.Sweeney@kingcounty.gov>
Sent: Friday, October 25, 2019 11:56 AM
To: Dudenhoeffer, Chris (ECY) <cdud461@ECY.WA.GOV>
Subject: FW: Vashon WET testing - Mysid control limits

THIS EMAIL ORIGINATED FROM OUTSIDE THE WASHINGTON STATE EMAIL SYSTEM - Take caution not to open attachments or links unless you know the sender AND were expecting the attachment or the link

Not sure if I had the correct email on the first try.

From: Sweeney, Francis
Sent: Tuesday, October 22, 2019 9:22 AM
To: Dudenhoeffer, Chris (ECY) (cdud461@ECY.WA.GOV) <cdud461@ECY.WA.GOV>
Subject: Vashon WET testing - Mysid control limits

Hi Chris, hope you are well.

I wanted to give you a heads up that we will be submitting a chronic test result for the Vashon Treatment Plant that had a control chart exceedance. We tested Vashon Effluent 10/2/19 using topsmelt and mysids. Topsmelt testing had acceptable QC and no toxicity. Mysid testing had no toxicity, QC was acceptable with the exception that the mysid reference toxicant was slightly above the upper control limit. We reviewed our test procedures and did not find any errors or problems. Therefore, we believe it's just a random statistical result and the data is acceptable for permit

reporting. Data reports are due with permit renewal July 2021, although the plant may be submitting data along with their DMRs through PARIS before that date.

A data summary table is attached, please let us know if you have any questions or concerns.

Fran Sweeney | Aquatic Toxicology Supervisor | King County Environmental Laboratory | 206.477.7117 | 322 West Ewing Place, Seattle WA 98119 | LAB-NR-0100 | [@KCEnviroLab on Instagram](#) | [Nwtoxicalgae.org](#) |

Login: P73401

NPDES Vashon T. P. Chronic Bio-Monitoring_October 2019

TC: _____

Project: 421488

CHAIN OF CUSTODY

LPM: Erin McCabe

Relinquished by <i>David Robinson</i>	Date <i>10/2/2019</i>	Time <i>0847</i>
Received by <i>Sav Vashtip</i>	Date <i>10-2-19</i>	Time <i>0847</i>
Sample Numbers <i>73401-1</i>		[All]

Sample Number	P73401-1	P73401-2	P73401-3
QC Link			
Locator	VS_EFF	VS_EFF	VS_EFF
Short Loc Desc	VS_EFF	VS_EFF	VS_EFF
Locator Desc	VASHON TP/FINAL EFFLUENT	VASHON TP/FINAL EFFLUENT	VASHON TP/FINAL EFFLUENT
Site	VASHON INPLANT	VASHON INPLANT	VASHON INPLANT
Comments	field info (chronic); Day 0	final effluent	final effluent
Start Date/Time	<i>10/1/2019 0647</i>		
End Date/Time	<i>10/2/2019 0647</i>		
Time Span			
Sample Depth			
CLIENT LOC	<i>Vashon T.P. pre final discharge pipe</i>	<i>8312</i>	<i>8311</i>
DATE, INDIV	*****	<i>10-2-19</i>	<i>10-2-19</i>
DIS VOL		*****	*****
FLOW, MGD		*****	*****
PERSONNEL	<i>DR</i>	*****	*****
PH, FIELD	<i>7.58</i>	*****	*****
SAMP DESCRIP	<i>BAAS</i>	*****	*****
SAMP METH	<i>11012 / 6030.7</i>	*****	*****
SAMP TEMP	<i>2.229</i>	*****	*****
SAMPLE UNIT	<i>73</i>	*****	*****
Dept, Matrix, Prod	<i>C12 = 0.03</i> <i>1 sampler @ 225 ml per 20 minutes</i>	<i>4 LC MENIDIA-CHRONIC</i> <i>Tepsmelt</i>	<i>4 LC MYSID-CHRONIC</i>

CHAIN OF CUSTODY

RELINQUISHED BY <i>Sav Vashtip</i>	Date <i>10-2-19</i>	Time <i>0925</i>
RECEIVED BY <i>D.SAV</i>	Date <i>10/2/19</i>	Time <i>0925</i>
Sample Number(s)		

*↑ please change
to Tepsmelt-chronic
✓ DCS 10/2/19*

Login: P73401

NPDES Vashon T. P. Chronic Bio-Monitoring, October 2019

TC: _____

Project: 421488

LPM: Erin McCabe

RELINQUISHED BY <i>David Robinson</i>	Date 10/4/19	Time 0850
RECEIVED BY <i>Harry Vasquez</i>	Date 10-4-19	Time 0850
Sample Number(s) 73401-5		

Sample Number	P73401-4	P73401-5	P73401-6
QC Link			
Locator	VS_EFF	VS_EFF	VS_EFF
Short Loc Desc	VS_EFF	VS_EFF	VS_EFF
Locator Desc	VASHON TP/FINAL EFFLUENT	VASHON TP/FINAL EFFLUENT	VASHON TP/FINAL EFFLUENT
Site	VASHON INPLANT	VASHON INPLANT	VASHON INPLANT
Comments	final effluent; Day 0	field info (chronic); Day 2	field info (chronic); Day 5
Start Date/Time		10/3/2019 0637	10/6/2019 0610
End Date/Time		10/4/2019 0637	10/7 0610
Time Span		24	24
Sample Depth		93H, 9312	
CLIENT LOC	9311, 9312	2 1500 3710 sampler 225ml per 20 mins	9301, 9312, 1500
DATE, INDIV	10-2-19	10-2-19	10-7-19
DIS VOL			*****
FLOW, MOD			*****
PERS, NAME		DR	DR
PH, FIELD		7.57	7.60
SAMP, DESC		*****	*****
SAMP, METH		01012 etc...	...1.....
SAMP, TEMP		2.5	2.797
SAMPL, CONC		73	73
Dept, Matrix	3 LC ALK 3 LC NH3 6 LC HARDNESS CALC-ICP	3 LC ALK 3 LC NH3 6 LC CA-ICP — * 6 LC ICP-HARDNESS 6 LC MG-ICP — * * Not Needed	3 LC ALK 3 LC NH3 6 LC HARDNESS CALC-ICP

CHAIN OF CUSTODY

RELINQUISHED BY <i>Harry Vasquez</i>	Date 10-4-19	Time 1030
RECEIVED BY <i>D. Sku</i>	Date 10/4/19	Time 1030
Sample Number(s) 73401-5		

Cl₂ = 0.04Cl₂ = 0.02