Rainier ENVIRONMENTAL

Whole Effluent Toxicity Test Report
Anacortes WWTP

February 2020

Report date: February 18, 2020

Submitted to:

City of Anacortes WWTP
P.O. Box 547/500 T Ave
Anacortes, WA 98221

5013 Pacific Hwy East
Suite 20
Tacoma, WA 98424

1.0 INTRODUCTION

An acute toxicity test was conducted using an effluent sample collected from the City of Anacortes Wastewater Treatment Plant in February 2020. The bioassay was conducted using the test organism *Ceriodaphnia dubia* (*Ceriodaphnia*). Testing was performed at Rainier Environmental Laboratory located in Tacoma, Washington.

2.0 METHODS

2.1 Sample Collection and Transport

City of Anacortes personnel collected a 24-hr. composite effluent sample in an LDPE cubitainer. The sample was packed in a cooler containing ice and shipped overnight to Rainier Environmental. Appropriate chain-of-custody procedures were employed during collection and transport.

2.2 Sample Receipt

Upon arrival at Rainier Environmental, the cooler was opened, the sample inspected, and the contents verified against information on the chain-of-custody form. Receipt temperature was measured and recorded on the chain-of-custody form. Standard water quality parameters were measured and recorded in the sample check-in sheet (Appendix B). The sample was stored at 4°C in the dark until used for testing.

2.3 Test Methods

An acute toxicity test was conducted according to procedures presented by USEPA (2002) and is summarized in Table 1.

Table 1. Summary of methods for the 48h *Ceriodaphnia* acute survival test.

Test initiation date and time	2/5/2020; 1340h
Test termination date and time	2/7/2020; 1315h
Test organism	*Ceriodaphnia dubia*
Test organism source	In-house cultures
Test organism age	< 24 hours
Test duration	48 hours
Feeding	50:50 mixture YTC:algal suspension during organism holding time. No feeding during test.
Test chamber	30 mL plastic cup
Test solution volume	15 mL
Test temperature	20 ± 1°C
Dilution water	Moderately Hard Synthetic Water
Test concentrations (% sample)	100, 50, 25, 12.5, 2.6, laboratory control
Number of organisms/chamber	5
Number of replicates	4
Photoperiod	16 hours light/8 hours dark
Aeration	None
Test protocol	EPA-821-R-02-012
Test acceptability criterion for controls	≥ 90% survival
Reference toxicant	Copper sulfate

3.0 RESULTS

Details of standard water quality measurements conducted upon receipt of sample are provided in Table 2.

Table 2. Sample information.

Sample ID	Final Effluent
Rainier Environmental Log-In Number	20-019
Collection date; time	2/4/2020; 0740h
Receipt date; time	2/5/2020; 1030h
Receipt temperature (°C)	0.5
Dissolved oxygen (mg/L)	9.0
pH	7.35
Conductivity (µS/cm)	454
Hardness (mg/L $CaCO_3$)	124
Alkalinity (mg/L $CaCO_3$)	100
Total Chlorine (mg/L)	<0.03
Total Ammonia (mg/L)	8.2

Survival was evaluated in the *Ceriodaphnia* acute toxicity test after 48 hours of exposure. Results are summarized in Table 3. Mean survival was 100 percent in 100 percent effluent. There was no statistically significant difference between the control and the acute critical effluent concentration (ACEC) of 2.6 percent sample.

Table 3. Summary of results for the acute toxicity test.

Species	Concentration (%)	Survival (%)	NOEC[a] (% effluent)	LOEC[b] (% effluent)	LC_{50}[c] (% effluent)
Ceriodaphnia	0.0	100	100	>100	>100
	2.6	100			
	12.5	100			
	25	100			
	50	100			
	100	100			

[a] No Observed Effect Concentration, [b] Lowest Observed Effect Concentration, [c] Predicted lethal concentration for 50% of test organisms

Statistical summary for the test and a copy of the laboratory bench sheet and a copy of the chain-of-custody form are provided in Appendices A and C.

4.0 QA/QC

The sample was received in good condition and within the temperature range specified by WDOE (2016). The toxicity test met acceptability criteria for performance of control organisms. There were no deviations from the protocol and water quality parameters remained within the ranges specified in the corresponding test method throughout the test.

Results for the reference toxicant test used to monitor laboratory performance and test organism sensitivity are summarized in Table 4. The results for the reference toxicant test fell within the acceptable range of mean ± two standard deviations of historical test results, indicating that the test organisms were of an appropriate degree of sensitivity. The coefficient of variation (CV) for the test is also shown in the table.

Table 4. Reference toxicant test results.

Species	Date initiated	Endpoint	LC_{50} (g/L NaCl)	Acceptable Range (g/L NaCl)	CV (%)
Ceriodaphnia	1/23/2020	48h survival	14.4	10.0 - 24.1	24.5

REFERENCES

Tidepool Scientific Software. 2000-2010. CETIS Comprehensive Environmental Toxicity Information System Software, Version 1.8.0.8.

USEPA. 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. EPA-821-R-02-012. Pp 55-56.

WDOE. 2016. Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria. Washington State Department of Ecology. Water Quality Program. Publication number: WQ-R-95-80, Revised June 2016.

Appendix A
Ceriodaphnia dubia Acute Toxicity Test
Statistical Summary and Raw Bench Sheet

CETIS Summary Report

Ceriodaphnia 48-h Acute Survival Test — **Rainier Environmental Laboratory**

Batch ID:	14-8298-5015	**Test Type:**	Survival (48h)	**Analyst:**	Eric Tollefson
Start Date:	05 Feb-20 13:40	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	07 Feb-20 13:15	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	48h	**Source:**	In-House Culture	**Age:**	<24h
Sample ID:	16-4553-9843	**Code:**	20-019	**Client:**	Anacortes
Sample Date:	04 Feb-20 07:40	**Material:**	POTW Effluent	**Project:**	
Receive Date:	05 Feb-20 10:30	**Source:**	Anacortes (WA0020257)		
Sample Age:	30h (0.5 °C)	**Station:**			

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
05-5895-9184	48h Survival Rate	100	>100	NA	5.0%	1	Steel Many-One Rank Sum Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
05-5895-9184	48h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

48h Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	1	1	1	1	1	0	0	0.0%	0.0%
2.6		4	1	1	1	1	1	0	0	0.0%	0.0%
12.5		4	1	1	1	1	1	0	0	0.0%	0.0%
25		4	1	1	1	1	1	0	0	0.0%	0.0%
50		4	1	1	1	1	1	0	0	0.0%	0.0%
100		4	1	1	1	1	1	0	0	0.0%	0.0%

48h Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
2.6		1	1	1	1
12.5		1	1	1	1
25		1	1	1	1
50		1	1	1	1
100		1	1	1	1

48h Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	5/5	5/5	5/5	5/5
2.6		5/5	5/5	5/5	5/5
12.5		5/5	5/5	5/5	5/5
25		5/5	5/5	5/5	5/5
50		5/5	5/5	5/5	5/5
100		5/5	5/5	5/5	5/5

Freshwater Acute
48 Hour Toxicity Test Data Sheet

Client: Anacortes
Sample ID: Final Effluent
Test #: 2002-007
Log-In #: 20-019

Start Date & Time: 2/5/2020 1340
End Date & Time: 2/7/2020 1315
Test Organism: Ceriodaphnia dubia

Rep. #	Conc. or %	Cont. #	Number of Live Organisms 0	24	48	Dissolved Oxygen (mg/L) 0	24	48	pH (units) 0	24	48	Cond (uhom-cm) 0	24	48	Temperature (°C) 0	24	48	Mean Percent Survival
1	CON	15	5	5	5	7.7	7.0	7.4	7.86	7.92	7.94	314	310	313	20.3	19.8	19.7	
2		4	5	5	5													
3		18	5	5	5													
4		10	5	5	5													
1	2.6	2	5	5	5	7.9	7.1	7.6	7.84	7.95	7.96	313	310	313	20.4	19.9	19.8	
2		11	5	5	5													
3		17	5	5	5													
4		8	5	5	5													
1	12.5	16	5	5	5	7.7	7.1	7.4	7.76	7.93	7.98	328	325	327	20.4	19.8	19.8	
2		1	5	5	5													
3		20	5	5	5													
4		9	5	5	5													
1	25	7	5	5	5	7.7	7.0	7.1	7.67	7.91	7.97	347	343	345	20.1	19.8	19.8	
2		19	5	5	5													
3		3	5	5	5													
4		14	5	5	5													
1	50	21	5	5	5	7.7	6.9	7.5	7.57	7.90	7.99	385	380	381	20.1	19.7	19.8	
2		5	5	5	5													
3		12	5	5	5													
4		23	5	5	5													
1	100	6	5	5	5	8.2	6.5	6.9	7.41	7.83	7.99	462	451	450	20.1	19.8	19.8	
2		22	5	5	5													
3		24	5	5	5													
4		13	5	5	5													
Technician Initials			AH	AH	AH	AH	AH	AH										

Sample Description:

Dilution Water Batch #: MHSW 011
Test Chamber: RM2

Animal Source: In-house cultures
Date Received: —

QA Check: AH

Comments: 0 hrs:
24 hrs.
48 hrs.

Rainier Environmental
Washington Laboratory
5013 Pacific Hwy. E. Suite 20
Tacoma, WA 98424

Appendix B
Sample check-in sheet

Rainier Environmental
5013 Pacific Hwy East, Ste. 20
Tacoma, WA 98424

Sample Check-In Information

Client: City of Anacortes WWTP

Tests Performed: Cd-a

Test ID No(s).: 2002-007

Sample ID:	Final Effluent			
Log-in No. (20-xxxx):	20-019			
Sample Collection Date & Time:	2/4/20 0740			
Sample Receipt Date & Time:	2/5/20 1030			
Check-in Temperature (°C)	0.5			
Temperature OK?	(Y) N	Y N	Y N	Y N
DO (mg/L)	9.0			
pH (units)	7.35			
Conductivity (µS/cm)	454			
Salinity (ppt)	—			
Tit. Vol / Sam. Vol. / Alkalinity (mg/L)*	2.5 / 25 / 100	/ /	/ /	/ /
Tit. Vol. / Sam. Vol. / Hardness (mg/L)* [a]	3.1 / 25 / 124	/ /	/ /	/ /
Total Chlorine (mg/L)	<0.03			
Total Ammonia Nitrogen (mg/L)	8.2			
Technician Initials	AJ			

* = mg/L as $CaCO_3$, [a] = Measured for freshwater samples only, NA = Not Applicable,
NM = Not Measured

Sample Description:

COC Complete? Y or N
1 Y 2 ___ 3 ___

Filtration? Y (N)
Pore Size: ___
Organisms or Debris

Aeration? Y (N)
Length of Time: ___
Final DO: ___
Final pH: ___

Hardness Adjustment? Y (N)
If adjusted, please see worksheet for details.

Sub-samples for additional chemistry:

Freshwater Tests:

Control/Dilution Water Source: test type: Cd-a 8:2 (DMW) (MHW) 0.11 Other: ___ Alkalinity: 64 Hardness: 96

Control/Dilution Water Source: test type: ___ 8:2 (DMW) MHW Other: ___ Alkalinity: ___ Hardness: ___

Additional Control? Y N = ___ Alkalinity: ___ Hardness: ___

Marine Tests:

Control/Dilution Water Source: test type: ___ ART SW NAT SW Alkalinity: ___ Salinity: ___

Control/Dilution Water Source: test type: ___ ART SW NAT SW Alkalinity: ___ Salinity: ___

Additional Control? Y N = ___ Alkalinity: ___ Salinity: ___

Sample Salted w/ artificial salt? Y N If yes, what ppt? ___ test type: ___

Sample salted w/brine? Y N If yes, what ppt? ___ test type: ___

Comments: Temperature for grab sample must be 0-20°C if received within 1 hour of collection time, 0-12°C if effluent received within 4 hours of collection time, and 0-6°C for all other samples.

QC Check: AJ

Appendix C
Chain-of-Custody Form

Chain of Custody

Rainier ENVIRONMENTAL

Washington
5013 Pacific Highway East, Suite 20 Fife,
WA 98424
Phone253.922.8898

Date 2/4/2020 Page 1 of 1

Sample Collection By:

Report to:		Invoice To:	
Company	City of Anacortes WWTP	**Company**	Same
Address	500 T Avenue	**Address**	
City/State/Zip	Anacortes WA, 98221	**City/State/Zip**	
Contact	Corrin Hamburg	**Contact**	
Phone	(360) 855-5237	**Phone**	
Email	corrinh@cityofanacortes.org	**Email**	

	SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	NO. OF CONTAINERS	COMMENTS	ANALYSES REQUIRED: Wet-Acute-Daphnid EPA 821-R-02-012	Receipt Temperature (°C)
1	Final Effluent	2/4/2020	0740	WW	4L cubitainer	1	Composite Sampler 24hr dd/of	X	0.5
2									
3									
4									
5									
6									
7									
8									
9									
10									

PROJECT INFORMATION		SAMPLE RECEIPT	
Client:		**Total No. of Containers**	1
PO No.:		**Received Good Condition?**	Y
Shipped Via:	UPS	**Matches Test Schedule?**	Y

SPECIAL INSTRUCTIONS/COMMENTS:

RELINQUISHED BY (CLIENT)	RELINQUISHED BY (COURIER)
(Signature) (Time) 0800	(Signature) (Time)
(Printed Name) (Date) Corrin N. Hamburg 2/4/2020	(Printed Name) (Date)
(Company) City of Anacortes WWTP	(Company)

RECEIVED BY (COURIER)	RECEIVED BY (LABORATORY)
(Signature) (Time)	(Signature) Eric Tollefson (Time) 1030
(Printed Name) (Date)	(Printed Name) ERIC TOLLEFSON (Date) 2/5/20
(Company)	(Log in #) 20-019