



**Whole Effluent Toxicity Test Report:
City of Blaine**

February 2024

Report date: March 16, 2024

Submitted to:

City of Blaine
Light House Point WWTP
272 Marine Drive
Blaine, WA 98230

1.0 INTRODUCTION

Acute toxicity tests were conducted using an effluent sample collected from the City of Blaine in february 2024. Acute bioassays were conducted using the test organisms *Ceriodaphnia dubia* (*Ceriodaphnia*) and *Pimephales promelas* (fathead minnows). Testing was performed at Rainier Environmental Laboratory located in Fife, Washington.

2.0 METHODS

2.1 Sample Collection and Transport

An effluent sample was collected into a LDPE cubitainer by City of Blaine. The sample was packed in a cooler containing ice and transported 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, sample inspected, and the contents verified against information provided 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 on the sample check-in sheet (Appendix C). The sample was stored at 4°C in the dark until used for testing.

2.3 Test Methods

Acute toxicity tests were conducted using *Ceriodaphnia* and fathead minnows according to procedures presented by USEPA (2002), and are summarized in Tables 1 and 2, respectively.

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

Test initiation date and time	2/27/2024; 0915h
Test termination date and time	2/29/2024; 0930h
Test organism	<i>Ceriodaphnia dubia</i>
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, 5.2, 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

Table 2. Summary of methods for the 96h fathead minnow acute survival test.

Test initiation date and time	2/27/2024; 1250h
Test termination date and time	3/2/2024; 1245h
Test organism	<i>Pimephales promelas</i>
Test organism source	Aquatic BioSystems; Fort Collins, CO
Test organism age	5 days post hatch
Test duration	96 hours with solution renewal at 48 hours
Feeding	<i>Artemia</i> nauplii during holding time and 2 hours prior to solution renewal
Test chamber	250 mL plastic cup
Test solution volume	200 mL
Test temperature	20 ± 1°C
Dilution water	Moderately Hard Synthetic Water
Test concentrations (% sample)	100, 50, 25, 12.5, 5.2, control
Number of organisms/chamber	10
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	Sodium chloride

3.0 RESULTS

Details of standard water quality measurements conducted upon receipt of the sample are provided in Table 3.

Table 3. Sample information.

Sample ID	Effluent
Rainier Log-In No.	24-037
Collection date and time	2/26/2024; 0800h
Receipt date and time	2/26/2024; 1055h
Receipt temperature (°C)	5.8
Dissolved oxygen (mg/L)	6.0
pH	7.08
Conductivity (µS/cm)	698
Hardness (mg/L CaCO ₃)	100
Alkalinity (mg/L CaCO ₃)	100
Total Chlorine (mg/L)	<0.03
Total Ammonia (mg/L)	<1.0

Survival was evaluated in the acute toxicity tests after 48 and 96 hours of exposure for the *Ceriodaphnia* and fathead minnows, respectively. Results are summarized in Table 4. There was 100 percent survival in the *Ceriodaphnia* acute test and 97.5 percent survival in the fathead minnow acute test in the 100 percent effluent concentration. There was no difference in organism response between the acute critical effluent concentration (ACEC) of 5.2 percent sample and the control for either test.

Table 4. Summary of results for the acute toxicity tests.

Species	Concentration (%)	Percent Survival	NOEC ^a (% effluent)	LOEC ^b (% effluent)	LC ₅₀ ^c (% effluent)
<i>Ceriodaphnia</i>	0.0	100	100	>100	>100
	5.2	100			
	12.5	100			
	25	100			
	50	100			
	100	100			
Fathead minnows	0.0	100	100	>100	>100
	5.2	100			
	12.5	100			
	25	97.5			
	50	100			
	100	97.5			

^aNo Observed Effect Concentration, ^bLowest Observed Effect Concentration, ^c Predicted lethal concentration for 50% of test organisms

Individual statistical summaries for all tests, copies of the laboratory bench sheets, sample check-in sheet and chain-of-custody forms are provided in Appendices A through D.

4.0 QA/QC

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

Results for the reference toxicant tests used to monitor laboratory performance and test organism sensitivity are summarized in Table 5. The results for the reference toxicant tests fell within the acceptable range of mean \pm two standard deviations of historical test results, indicating that the tests organisms were of an appropriate degree of sensitivity. The coefficients of variation (CV) for the tests are also shown in the table.

Table 5. Reference toxicant test results.

Species	Date initiated	Endpoint	EC ₅₀	Acceptable Range	CV (%)
<i>Ceriodaphnia</i>	2/9/2024	48h Survival	8.28 µg/L Cu	4.73-36.2 µg/L Cu	66.4
Fathead minnow	2/27/2024	96h Survival	5.69 g/L NaCl	5.10-7.88 g/L NaCl	11.5

REFERENCES

- Tidepool Scientific Software. 2000-2011. CETIS Comprehensive Environmental Toxicity Information System Software, Version 1.8.4.6.
- 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. 51-52, 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 Acute Toxicity Test
Statistical Summaries and Raw Bench Sheets

CETIS Summary Report

Report Date: 15 Mar-24 14:22 (p 1 of 1)
Test Code: 2402-069 | 07-3645-8468

Ceriodaphnia 48-h Acute Survival Test

Rainier Environmental Laboratory

Batch ID:	11-1513-1177	Test Type:	Survival (48h)	Analyst:	Eric Tollefson
Start Date:	27 Feb-24 09:15	Protocol:	EPA/821/R-02-012 (2002)	Diluent:	Mod-Hard Synthetic Water
Ending Date:	29 Feb-24 09:30	Species:	Ceriodaphnia dubia	Brine:	
Duration:	48h	Source:	In-House Culture	Age:	<24h
Sample ID:	19-9210-9202	Code:	24-037	Client:	Blaine
Sample Date:	26 Feb-24 08:00	Material:	POTW Effluent	Project:	
Receive Date:	26 Feb-24 10:55	Source:	Blaine (WA0022641)		
Sample Age:	25h (5.8 °C)	Station:			

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
16-4188-8809	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
16-4188-8809	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%
5.2		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
5.2		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
5.2		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: B. pine
Sample ID: Effluent
Test #: 2402-069
Log-In #: 34-037

Start Date & Time: 2/27/2024 0915
End Date & Time: 2/29/2024 0930
Test Organism: Ceriodaphnia dubia

Rep. #	Conc. or Cont. #	Number of Live Organisms			Dissolved Oxygen (mg/L)			pH (units)			Cond (uohm-cm)			Temperature (°C)			Mean Percent Survival
		0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	
1	CON	14	5	5	8.4	8.3	7.9	7.98	8.03	8.01	299	274	276	20.1	20.2	19.9	
2		24	5	5													
3		2	5	5													
4		17	5	5													
1	5-2	23	5	5	8.3	8.3	7.9	7.90	8.04	8.02	211	223	225	20.2	20.1	19.9	
2		5	5	5													
3		18	5	5													
4		1	5	5													
1	12.5	6	5	5	8.3	8.2	7.8	7.80	8.03	8.02	221	231	234	20.1	20.1	19.9	
2		22	5	5													
3		3	5	5													
4		21	5	5													
1	25	4	5	5	8.3	8.2	7.7	7.63	8.05	8.03	250	260	266	20.1	20.1	20.0	
2		4	5	5													
3		16	5	5													
4		4	5	5													
1	50	15	5	5	8.2	8.2	7.7	7.46	8.10	8.05	311	322	327	20.0	20.1	20.1	
2		10	5	5													
3		8	5	5													
4		19	5	5													
1	100	13	5	5	8.0	8.3	7.8	7.20	8.09	8.06	430	474	419	20.0	20.1	20.1	
2		20	5	5													
3		7	5	5													
4		12	5	5													
Technician Initials		<u>CH</u>	<u>SH</u>	<u>SH</u>	<u>CH</u>	<u>SH</u>	<u>CH</u>										

Dilution Water Batch #: MHBN 020 Animal Source: In-house cultures Sample Description: _____
Test Chamber: KM2 Date Received: _____ QA Check: if

Comments: 0 hrs: _____
24 hrs: _____
48 hrs: _____
Rainier Environmental
Washington Laboratory
5013 Pacific Hwy. E. Suite 20
Tacoma, WA 98424

Appendix B
Fathead Minnow Acute Toxicity Test
Statistical Summaries and Raw Bench Sheets

CETIS Summary Report

Report Date: 15 Mar-24 14:39 (p 1 of 1)
 Test Code: 2402-070 | 07-1965-0825

Fathead Minnow 96-h Acute Survival Test

Rainier Environmental Laboratory

Batch ID:	15-4361-1109	Test Type:	Survival (96h)	Analyst:	Eric Tollefson
Start Date:	27 Feb-24 12:50	Protocol:	EPA/821/R-02-012 (2002)	Diluent:	Mod-Hard Synthetic Water
Ending Date:	02 Mar-24 12:45	Species:	Pimephales promelas	Brine:	
Duration:	96h	Source:	Aquatic Biosystems, CO	Age:	5d
Sample ID:	19-9210-9202	Code:	24-037	Client:	Blaine
Sample Date:	26 Feb-24 08:00	Material:	POTW Effluent	Project:	
Receive Date:	26 Feb-24 10:55	Source:	Blaine (WA0022641)		
Sample Age:	29h (5.8 °C)	Station:			

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
09-8122-3497	96h Survival Rate	100	>100	NA	5.6%	1	Steel Many-One Rank Sum Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
09-8122-3497	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

96h Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	1	1	1	1	1	0	0	0.0%	0.0%
5.2		4	1	1	1	1	1	0	0	0.0%	0.0%
12.5		4	1	1	1	1	1	0	0	0.0%	0.0%
25		4	0.975	0.9563	0.9937	0.9	1	0.025	0.05	5.13%	2.5%
50		4	1	1	1	1	1	0	0	0.0%	0.0%
100		4	0.975	0.9563	0.9937	0.9	1	0.025	0.05	5.13%	2.5%

96h Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
5.2		1	1	1	1
12.5		1	1	1	1
25		0.9	1	1	1
50		1	1	1	1
100		1	0.9	1	1

96h Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	10/10	10/10	10/10	10/10
5.2		10/10	10/10	10/10	10/10
12.5		10/10	10/10	10/10	10/10
25		9/10	10/10	10/10	10/10
50		10/10	10/10	10/10	10/10
100		10/10	9/10	10/10	10/10

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

96 Hour Toxicity Test Data Sheet
Freshwater 96-hr Acute with Renewal

Client: Blaine
Sample ID: effluent
Test #: 2402-070
Rainier Check-In #: 24-037

Start Date & Time: 2/27/2024 12:50
End Date & Time: 3/2/2024 12:45
Test Organism: Pimephales promelas

Sample Conc. or %	D.O. (mg/L)				pH (mg/L)					
	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.
	0	24	48	48	72	96	0	24	48	48
CON	8.4	8.3	8.1	8.5	8.3	8.1	7.98	8.03	7.95	8.01
5.2	8.3	8.3	8.4	8.5	8.3	8.0	7.90	8.04	7.93	7.99
12.5	8.3	8.2	8.2	8.6	8.4	8.2	7.80	8.03	7.97	7.92
25	8.3	8.2	8.4	8.7	8.3	8.2	7.63	8.05	7.96	7.67
50	8.2	8.2	8.3	8.6	8.4	8.4	7.46	8.10	8.01	7.52
100	8.0	8.3	8.3	8.4	8.2	8.2	7.20	8.09	8.03	7.23

Sample Conc. or %	Conductivity μ S/cm						Test Temperature (°C)					
	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.
	0	24	48	48	72	96	0	24	48	48	72	96
CON	299	274	288	297	307	298	20.1	20.2	19.8	20.3	20.1	19.7
5.2	211	223	224	301	308	303	20.2	20.1	19.8	20.3	20.1	19.8
12.5	221	231	233	306	310	312	20.1	20.1	19.9	20.1	20.0	19.8
25	250	260	257	322	325	323	20.1	20.1	19.8	20.0	20.1	20.1
50	311	322	319	319	324	327	20.0	20.1	19.8	19.5	20.2	20.0
100	430	474	449	423	441	445	20.0	20.1	19.8	19.2	20.2	19.8
Tech. Initials	off	off	off	off	off	off						
Sample Used:	24-037											

Dilution Water Batch #: MHSW 020
Test Chamber: KM2

Comments: _____
QA Check: 7

Animal Source: ABS
Date Received: 2/27/2024
Date of Hatch: 2/22/2024

Sample Conc. or %	Rep #	Cont #	Number of Live Organisms				
			0	24	48	72	96
CON	1	15	10	10	10	10	10
	2	6	10	10	10	10	10
	3	23	10	10	10	10	10
	4	18	10	10	10	10	10
5.2	1	14	10	10	10	10	10
	2	1	10	10	10	10	10
	3	17	10	10	10	10	10
	4	7	10	10	10	10	10
12.5	1	13	10	10	10	10	10
	2	5	10	10	10	10	10
	3	24	10	10	10	10	10
	4	16	10	10	10	10	10
25	1	20	10	10	10	10	9
	2	3	10	10	10	10	10
	3	8	10	10	10	10	10
	4	22	10	10	10	10	10
50	1	10	10	10	10	10	10
	2	19	10	10	10	10	10
	3	2	10	10	10	10	10
	4	11	10	10	10	10	10
100	1	9	10	10	10	10	10
	2	4	10	10	10	10	9
	3	24	10	10	10	10	10
	4	12	10	10	10	10	10
Tech. Initials			off	off	off	off	off

48-Hr. Feeding: ✓

Appendix C
Sample Check-In Sheet

Sample Check-In Information

Client: BLAINE

Tests Performed: My-c; Aq-c; Cd-a; Pb-a

Test ID No(s): 2402-067; 2402-068; 2402-069; 2402-070

Sample ID:	EFFLUENT	Effluent	EFFLUENT
Log-in No. (20-xxxx):	24-037	24-040	24-041
Sample Collection Date & Time:	2/26/24 0800	2/28/24 0730	2/28/24 0730
Sample Receipt Date & Time:	2/26/24 1055	2/28/24 1030	2/28/24 1050
Check-in Temperature (°C)	5.4	5.5	4.7
Temperature OK?	<input checked="" type="checkbox"/> N	<input checked="" type="checkbox"/> N	<input checked="" type="checkbox"/> N
DO (mg/L)	6.0	8.2	6.8
pH (units)	7.04	7.06	7.1
Conductivity (µS/cm)	698	320	323
Salinity (ppt)	0.3	—	—
Tit. Vol / Sam. Vol. / Alkalinity (mg/L)*	2.5 / 25 / 100	2.6 / 25 / 104	2.5 / 25 / 100
Tit. Vol. / Sam. Vol. / Hardness (mg/L)* ^a	2.5 / 25 / 100	2.7 / 25 / 108	2.5 / 25 / 104
Total Chlorine (mg/L)	<0.03	<0.03	<0.03
Total Ammonia Nitrogen (mg/L)	4.10	4.10	4.10
Technician Initials	df	df	df

* = mg/L as CaCO₃, ^a = Measured for freshwater samples only, NA = Not Applicable.

NM = Not Measured

Freshwater Tests:

Control/Dilution Water Source: test type: PP-9 8.2 (DMW) MHW Other: -020 Alkalinity: 64 Hardness: 84

Control/Dilution Water Source: test type: Cd-a 8.2 (DMW) MHW Other: — Alkalinity: — Hardness: —

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

Marine Tests:

Control/Dilution Water Source: test type: Art SW ART SW NAT SW Alkalinity: 116 Salinity: 29.3

Control/Dilution Water Source: test type: Art SW 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.

Sample Description:

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

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:

QC Check: df

Appendix D
Chain-of-Custody Form

Washington
5013 Pacific Highway East, Suite 200
Fife, WA 98424
Phone: 253.922.8898

Date _____ Page _____ of _____

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