



**Whole Effluent Toxicity Test Report:
Birch Bay Water & Sewer**

July 2024

Report date: July 22, 2024

Submitted to:

Birch Bay Water & Sewer
7096 Point Whitehorn Road
Blaine, WA 98230

1.0 INTRODUCTION

Acute toxicity tests were conducted using an effluent sample collected from Birch Bay Water & Sewer in July 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 Birch Bay personnel. 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	7/9/2024; 1430h
Test termination date and time	7/11/2024; 1400h
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, 2.5, 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	7/9/2024; 1410h
Test termination date and time	7/13/2024; 1415h
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, 2.5, 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.	24-091
Collection date and time	7/8/2024; 0900h
Receipt date and time	7/9/2024; 1340h
Receipt temperature (°C)	4.7
Dissolved oxygen (mg/L)	5.6
pH	7.14
Conductivity (µS/cm)	2130
Hardness (mg/L CaCO ₃)	204
Alkalinity (mg/L CaCO ₃)	104
Total Chlorine (mg/L)	<0.03
Total Ammonia (mg/L)	6.2

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 2.5 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
	2.5	100			
	12.5	100			
	25	100			
	50	100			
	100	100			
Fathead minnows	0.0	97.5	100	>100	>100
	2.5	95.0			
	12.5	97.5			
	25	100			
	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>	7/9/2024	48h Survival	24.2 µg/L Cu	4.74-37.0 µg/L Cu	67.2
Fathead minnow	7/16/2024	96h Survival	6.25 g/L NaCl	4.82-7.75 g/L NaCl	12.6

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: 18 Jul-24 12:58 (p 1 of 1)
 Test Code: 2407-018 | 06-3041-0870

Ceriodaphnia 48-h Acute Survival Test

Rainier Environmental Laboratory

Batch ID:	01-0960-4261	Test Type:	Survival (48h)	Analyst:	Eric Tollefson
Start Date:	09 Jul-24 14:30	Protocol:	EPA/821/R-02-012 (2002)	Diluent:	Mod-Hard Synthetic Water
Ending Date:	11 Jul-24 14:00	Species:	Ceriodaphnia dubia	Brine:	
Duration:	48h	Source:	In-House Culture	Age:	<24h
Sample ID:	03-4076-1958	Code:	24-091	Client:	Birch Bay
Sample Date:	08 Jul-24 09:00	Material:	POTW Effluent	Project:	
Receive Date:	09 Jul-24 13:40	Source:	Birch Bay (WA0029556)		
Sample Age:	29h (4.7 °C)	Station:			

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
20-5117-7513	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
20-5117-7513	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%
2.5		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
2.5		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
2.5		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: Birch Bay
 Sample ID: Effluent
 Test #: 2407-018
 Log-In #: 24-091

Start Date & Time: 7/9/2024 1430
 End Date & Time: 7/11/2024 1400
 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	19	5	5	1.0	7.5	7.4	8.03	7.92	7.90	289	291	294	20.4	19.9	20.1	
2		10	5	5													
3		17	5	5													
4		3	5	5													
1	2.5	20	5	5	7.4	7.5	7.3	7.96	7.85	7.83	340	345	347	20.3	19.9	20.1	
2		2	5	5													
3		16	5	5													
4		9	5	5													
1	12.5	21	5	5	7.5	7.7	7.3	7.78	7.81	7.80	418	431	438	20.3	20.0	20.1	
2		1	5	5													
3		15	5	5													
4		6	5	5													
1	25	4	5	5	7.4	7.6	7.4	7.65	7.79	7.74	659	657	662	20.6	19.9	20.1	
2		13	5	5													
3		11	5	5													
4		12	5	5													
1	50	5	5	5	7.3	7.4	7.6	7.49	7.71	7.73	1007	1009	1010	19.9	19.8	20.1	
2		14	5	5													
3		24	5	5													
4		8	5	5													
1	100	1	5	5	7.2	7.5	7.7	7.25	7.59	7.66	1692	1695	1698	19.5	20.0	20.1	
2		18	5	5													
3		12	5	5													
4		23	5	5													
Technician Initials		gt	gt	gt	gt	gt	gt										

Dilution Water Batch #: MHSW 070
 Test Chamber: PM2

Animal Source: 14-house cultures
 Date Received: QA Check: gt

Sample Description:

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: 18 Jul-24 13:04 (p 1 of 1)
Test Code: 2407-019 | 15-1197-4077

Fathead Minnow 96-h Acute Survival Test

Rainier Environmental Laboratory

Batch ID:	20-9506-5475	Test Type:	Survival (96h)	Analyst:	Eric Tollefson
Start Date:	09 Jul-24 14:10	Protocol:	EPA/821/R-02-012 (2002)	Diluent:	Mod-Hard Synthetic Water
Ending Date:	13 Jul-24 14:15	Species:	Pimephales promelas	Brine:	
Duration:	4d 0h	Source:	Aquatic Biosystems, CO	Age:	5d
Sample ID:	03-4076-1958	Code:	24-091	Client:	Birch Bay
Sample Date:	08 Jul-24 09:00	Material:	POTW Effluent	Project:	
Receive Date:	09 Jul-24 13:40	Source:	Birch Bay (WA0029556)		
Sample Age:	29h (4.7 °C)	Station:			

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
11-7516-7572	96h Survival Rate	100	>100	NA	9.09%	1	Steel Many-One Rank Sum Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
11-7516-7572	96h Survival Rate	Control Resp	0.975	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	0.975	0.9563	0.9937	0.9	1	0.025	0.05	5.13%	0.0%
2.5		4	0.95	0.9127	0.9873	0.8	1	0.05	0.1	10.53%	2.56%
12.5		4	0.975	0.9563	0.9937	0.9	1	0.025	0.05	5.13%	0.0%
25		4	1	1	1	1	1	0	0	0.0%	-2.56%
50		4	1	1	1	1	1	0	0	0.0%	-2.56%
100		4	0.975	0.9563	0.9937	0.9	1	0.025	0.05	5.13%	0.0%

96h Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	0.9	1	1
2.5		1	1	1	0.8
12.5		1	1	1	0.9
25		1	1	1	1
50		1	1	1	1
100		1	1	0.9	1

96h Survival Rate Binomials


C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	10/10	9/10	10/10	10/10
2.5		10/10	10/10	10/10	8/10
12.5		10/10	10/10	10/10	9/10
25		10/10	10/10	10/10	10/10
50		10/10	10/10	10/10	10/10
100		10/10	10/10	9/10	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: Birch Bay
Sample ID: Effluent J
Test #: 2407-019
Rainier Check-In #: 24-091

Start Date & Time: 7/9/2024 1410
End Date & Time: 7/13/2024 1415
Test Organism: Pimephales promelas


Sample Conc. or 	D.O. (mg/L)						pH (mg/L)					
	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	7.0	7.5	7.4	7.3	7.4	7.6	8.03	7.92	7.90	8.01	7.90	7.85
2.5	7.4	7.5	7.4	7.3	7.5	7.7	7.96	7.85	7.81	7.99	7.87	7.83
12.5	7.5	7.7	7.3	7.4	7.5	7.8	7.18	7.81	7.80	7.84	7.80	7.81
25	7.4	7.6	7.2	7.2	8.0	8.1	7.05	7.79	7.18	7.16	7.15	7.78
50	7.3	7.4	7.1	7.0	7.9	7.9	7.49	7.11	7.17	7.44	7.10	7.74
100	7.2	7.5	7.1	7.0	7.8	7.9	7.25	7.59	7.02	7.27	7.02	7.71

Sample Conc. or 	Conductivity μ S/cm						Test Temperature ($^{\circ}$ 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	289	241	293	301	304	310	20.4	19.9	20.1	20.1	20.1	19.9
2.5	340	345	346	344	341	355	20.5	19.9	20.1	20.1	20.0	19.9
12.5	418	481	484	475	479	483	20.3	20.0	20.0	20.1	20.0	19.8
25	659	651	659	654	656	663	20.0	19.9	20.1	20.2	20.0	19.8
50	1007	1068	1009	1001	1004	1008	19.9	19.8	20.0	20.5	20.1	20.0
100	1692	1695	1697	1650	1654	1657	19.5	20.0	20.1	20.3	20.1	19.9

Dilution Water Batch #: MHSW 070
Test Chamber: KM2

Comments:

QA Check: Y
Animal Source: ABS
Date Received: 7/9/2024
Date of Hatch: 7/4/2024

Sample Conc. or 	Rep #	Cont #	Number of Live Organisms				
			0	24	48	72	96
			0	24	48	72	96
CON	1	16	10	10	10	10	10
	2	22	10	9	9	9	9
	3	4	10	10	10	10	10
	4	10	10	10	10	10	10
2.5	1	15	10	10	10	10	10
	2	2	10	10	10	10	10
	3	21	10	10	10	10	10
	4	9	10	9	9	8	8
12.5	1	19	10	10	10	10	10
	2	1	10	10	10	10	10
	3	14	10	10	10	10	10
	4	5	10	10	9	9	9
25	1	17	10	10	10	10	10
	2	6	10	10	10	10	10
	3	3	10	10	10	10	10
	4	11	10	10	10	10	10
50	1	18	10	10	10	10	10
	2	7	10	10	10	10	10
	3	24	10	10	10	10	10
	4	12	10	10	10	10	10
100	1	20	10	10	10	10	10
	2	8	10	10	10	10	10
	3	23	10	10	10	10	10
	4	13	10	10	10	10	10

48-Hr, Feeding: ✓

Appendix C
Sample Check-In Sheet

Client: Birch Bay Water & Sewer

Tests Performed: MR-C, AA-C, Cd-A, PP-A
Test ID No(s): 2407-016, 2407-017, 2407-018, 2407-019

Sample ID:	Effluent	Final Effluent	Final Effluent	
Log-in No. (20-xxxx):	24-091	24-093	24-096	
Sample Collection Date & Time:	7/8/24 0900	7/10/24 0710	7/13/24 0705	
Sample Receipt Date & Time:	7/9/24 1340	7/11/24 1230	7/13/24 0954	
Check-in Temperature (°C)	4.7	5.6	3.8	
Temperature OK?	<input checked="" type="radio"/> N	<input checked="" type="radio"/> N	<input checked="" type="radio"/> N	Y N
DO (mg/L)	5.6	4.9	5.2	
pH (units)	7.14	7.26	7.29	
Conductivity (µS/cm)	2130	2180	2170	
Salinity (ppt)	1.2	1.2	1.2	
Tit. Vol / Sam. Vol. / Alkalinity (mg/L)*	2.6 / 25 / 104	3.5 / 25 / 140	2.9 / 25 / 116	1 1
Tit. Vol. / Sam. Vol. / Hardness (mg/L)*	5.1 / 25 / 204	5.3 / 25 / 212	5.4 / 25 / 136	1 1
Total Chlorine (mg/L)	<0.03	<0.03	<0.03	
Total Ammonia Nitrogen (mg/L)	6.2	7.0	6.8	
Technician Initials	df	df	df	

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

NM = Not Measured

Freshwater Tests:

Control/Dilution Water Source: test type: PP-A 8.2 (DMW) Other: MHW -070

Control/Dilution Water Source: test type: PP-A 8.2 (DMW) MHW Other: MHW

Additional Control? Y N =

Marine Tests:

Control/Dilution Water Source: test type: AA-C ART SW NAT SW

Control/Dilution Water Source: test type: AA-C ART SW NAT SW

Additional Control? Y N =

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

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

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

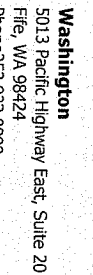
Length of Time: _____
Final DO: _____
Final pH: _____

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



Chain of Custody

Date _____ Page _____ of _____

[illegible]