



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

January 2024

Report date: February 19, 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 January 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	1/30/2024; 1125h
Test termination date and time	2/1/2024; 1125h
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	1/30/2024; 1500h
Test termination date and time	2/3/2024; 1445h
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	Final Effluent
Rainier Log-In No.	24-019
Collection date and time	1/29/2024; 0900h
Receipt date and time	1/30/2024; 0940h
Receipt temperature (°C)	4.0
Dissolved oxygen (mg/L)	9.4
pH	7.08
Conductivity (µS/cm)	1408
Hardness (mg/L CaCO ₃)	164
Alkalinity (mg/L CaCO ₃)	112
Total Chlorine (mg/L)	<0.03
Total Ammonia (mg/L)	5.3

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 100 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	100	100	>100	>100
	2.5	100			
	12.5	100			
	25	97.5			
	50	97.5			
	100	100			

^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>	1/5/2024	48h Survival	5.44 µg/L Cu	5.36-34.6 µg/L Cu	59.4
Fathead minnow	1/9/2024	96h Survival	5.55 g/L NaCl	4.86-8.10 g/L NaCl	13.6

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: 19 Feb-24 12:39 (p 1 of 1)
 Test Code: 2401-055 | 06-8361-0755

Ceriodaphnia 48-h Acute Survival Test				Rainier Environmental Laboratory			
Batch ID:	21-2140-2148	Test Type:	Survival (48h)	Analyst:	Eric Tollefson		
Start Date:	30 Jan-24 11:25	Protocol:	EPA/821/R-02-012 (2002)	Diluent:	Mod-Hard Synthetic Water		
Ending Date:	01 Feb-24 11:25	Species:	Ceriodaphnia dubia	Brine:			
Duration:	48h	Source:	In-House Culture	Age:	<24h		
Sample ID:	04-9614-8712	Code:	24-019	Client:	Birch Bay		
Sample Date:	29 Jan-24 09:00	Material:	POTW Effluent	Project:			
Receive Date:	30 Jan-24 09:40	Source:	Birch Bay (WA0029556)				
Sample Age:	26h (4 °C)	Station:					

Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
12-8430-4270	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
12-8430-4270	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: Final Effluent
 Test # 2401-055
 Log-In # 24-019

Start Date & Time: 11/30/2024 11:25
 End Date & Time: 12/1/2024 11:25
 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				
1	CON	11	5	5	8.8	8.5	8.5	8.08	797	791	327	334	328	20.4	20.1	19.8	
2		24	5	5													
3		2	5	5													
4		16	5	5													
1	2.5	23	5	5	8.4	8.7	8.6	8.08	798	795	371	359	372	19.6	20.0	19.8	
2		1	5	5													
3		10	5	5													
4		17	5	5													
1	12.5	22	5	5	8.7	8.6	8.4	799	795	792	452	455	461	19.8	20.0	19.9	
2		4	5	5													
3		13	5	5													
4		25	5	5													
1	25	3	5	5	8.7	8.5	8.4	792	792	791.0	583	582	595	20.0	20.1	19.9	
2		12	5	5													
3		6	5	5													
4		21	5	5													
1	50	19	5	5	8.5	8.4	8.5	790	789	788	986	827	822	20.2	20.1	19.8	
2		5	5	5													
3		14	5	5													
4		8	5	5													
1	100	7	5	5	8.6	8.5	8.3	779	781	784	1256	1284	1291	20.3	20.1	19.8	
2		15	5	5													
3		18	5	5													
4		9	5	5													

Technician Initials: RM2

Dilution Water Batch #: MHSW 010
 Test Chamber: RM2

Animal Source: In-house cultures
 Date Received: RM2

Sample Description: QA Check: RM2

Comments: 0 hrs:
 24 hrs:
 48 hrs:

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

CETIS Summary Report

Report Date: 19 Feb-24 12:36 (p 1 of 1)
 Test Code: 2401-054 | 11-9317-8512

Fathead Minnow 96-h Acute Survival Test

Rainier Environmental Laboratory

Batch ID: 21-1669-4799	Test Type: Survival (96h)	Analyst: Eric Tollefson
Start Date: 30 Jan-24 15:00	Protocol: EPA/821/R-02-012 (2002)	Diluent: Mod-Hard Synthetic Water
Ending Date: 03 Feb-24 14:45	Species: Pimephales promelas	Brine:
Duration: 96h	Source: Aquatic Biosystems, CO	Age: 5d

Sample ID: 04-9614-8712	Code: 24-019	Client: Birch Bay
Sample Date: 29 Jan-24 09:00	Material: POTW Effluent	Project:
Receive Date: 30 Jan-24 09:40	Source: Birch Bay (WA0029556)	
Sample Age: 30h (4 °C)	Station:	

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
04-8129-8394	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
04-8129-8394	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%
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	0.975	0.9563	0.9937	0.9	1	0.025	0.05	5.13%	2.5%
50		4	0.975	0.9563	0.9937	0.9	1	0.025	0.05	5.13%	2.5%
100		4	1	1	1	1	1	0	0	0.0%	0.0%

96h 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	0.9	1
50		1	1	0.9	1
100		1	1	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
2.5		10/10	10/10	10/10	10/10
12.5		10/10	10/10	10/10	10/10
25		10/10	10/10	9/10	10/10
50		10/10	10/10	9/10	10/10
100		10/10	10/10	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: Birch Bay
 Sample ID: Final Effluent
 Test #: 2401-054
 Rainier Check-In #: 24-019

Start Date & Time: 1/30/2024 1500
 End Date & Time: 2/3/2024 1445
 Test Organism: Pimephales promelas

Sample Conc. or ϕ	D.O. (mg/L)						pH (mg/L)					
	Init.	24	48	Init.	72	96	Init.	24	48	Init.	72	96
CON	8.8	8.5	8.1	8.5	8.4	8.3	8.08	7.97	7.93	8.11	8.07	8.04
2.5	8.8	8.7	8.4	8.5	8.5	8.4	8.08	7.99	7.91	8.10	8.05	8.01
12.5	8.7	8.6	8.5	8.4	8.5	8.5	7.99	7.95	7.91	8.05	8.01	7.95
25	8.7	8.5	8.5	8.4	8.6	8.5	7.92	7.93	7.88	7.93	7.94	7.91
50	8.6	8.4	8.4	8.6	8.6	8.3	7.90	7.89	7.87	7.91	7.91	7.91
100	8.5	8.5	8.3	8.5	9.1	8.4	7.78	7.81	7.82	7.87	7.84	7.90

Sample Conc. or ϕ	Conductivity μ S/cm						Test Temperature ($^{\circ}$ C)					
	Init.	24	48	Init.	72	96	Init.	24	48	Init.	72	96
CON	337	334	335	317	320	318	20.4	20.1	19.9	19.8	20.1	19.8
2.5	371	369	372	365	310	372	19.6	20.1	19.8	20.1	20.1	19.9
12.5	452	455	396	449	454	468	19.8	20.0	19.8	20.1	20.0	19.9
25	583	582	584	579	582	585	20.0	20.0	19.9	20.2	20.0	20.1
50	826	827	822	822	824	822	20.2	20.1	19.9	20.4	20.1	19.9
100	1286	1284	1292	1281	1290	1294	20.3	20.1	19.9	20.7	20.0	19.8

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

Comments: _____
 Dilution Water Batch #: MHSM 010
 Test Chamber: KM12
 Animal Source: AB5
 Date Received: 1/30/2024
 Date of Hatch: 1/25/2024
 QA Check: gt

48-Hr. Feeding: ✓

Appendix C
Sample Check-In Sheet

Client: Birch Bay Water & Sewer

Tests Performed: CA-a, PP-a, JMW-C, AA-C
Test ID No(s): 2401-052; 2401-053; 2401-054; 2401-055

Sample ID:	Final Effluent	Final Effluent	Final Effluent
Log-in No. (20-xxxx):	24-019	24-023	24-025
Sample Collection Date & Time:	1/29/24	0906	1/31/24 0800
Sample Receipt Date & Time:	1/30/24	0940	2/1/24 1230
Check-in Temperature (°C):	4.0	1.5	4.0
Temperature OK?	Y	N	N
DO (mg/L)	9.4	7.5	9.0
pH (units)	7.08	7.23	7.12
Conductivity (µS/cm)	1408	1409	1403
Salinity (ppt)	0.7	0.8	0.7
Tit. Vol / Sam. Vol. / Alkalinity (mg/L)*	2.8 / 25 / 112	2.6 / 25 / 104	2.5 / 25 / 100
Tit. Vol. / Sam. Vol. / Hardness (mg/L)* ^a	4.1 / 25 / 164	4.0 / 25 / 160	4.3 / 25 / 172
Total Chlorine (mg/L)	<0.03	<0.03	<0.03
Total Ammonia Nitrogen (mg/L)	5.3	4.4	5.0
Technician Initials	df	df	df

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

Freshwater Tests:

Control/Dilution Water Source: test type: FP-a 8.2 (DMW) MHW Other: 010 Alkalinity: 58 Hardness: 100
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: AA-C ART SW NAT SW Alkalinity: 112 Salinity: 29.3
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.

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

Sample Collection By:

ANALYSES REQUIRED

Report to:

Invoice To:

Company: Birch Bay Water & Sewer
Address: 7096 Pt Whitehorn Rd
City/State/zip: Blaine WA 98230
Contact: Shane Hess
Phone: 360-371-7100
Email: shane@bbwsd.com

Company: Same as Report to
Address: _____
City/State/zip: _____
Contact: _____
Phone: _____
Email: _____

SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	NO. OF CONTAINERS	COMMENTS	ANALYSES REQUIRED				Receipt Temperature (°C)	
1	Final Effluent	1-29-24	9:00	Effluent	plastic	1						40
2												
3												
4												
5												
6												
7												
8												
9												
10												

PROJECT INFORMATION		SAMPLE RECEIPT		RELINQUISHED BY (CLIENT)		RELINQUISHED BY (COURIER)	
Client:	<u>Birch Bay Water & Sewer</u>	Total No. of Containers:	<u>1</u>	(Signature)	<u>Shane Hess</u>	(Signature)	<u>[Signature]</u>
PO No.:	<u>Sewer Dept</u>	Received Good Condition?	<u>Y</u>	(Printed Name)	<u>Shane Hess</u>	(Printed Name)	<u>[Name]</u>
Shipped Via:	<u>Fed Ex</u>	Matches Test Schedule?	<u>Y</u>	(Company)	<u>Birch Bay Water & Sewer</u>	(Company)	<u>[Company]</u>
				(Time)	<u>0915</u>	(Time)	<u>0940</u>
				(Date)	<u>01/30/24</u>	(Date)	<u>1/30/24</u>

SPECIAL INSTRUCTIONS/COMMENTS:

RECEIVED BY (COURIER) _____ (Signature) _____ (Time) _____ (Date)

RECEIVED BY (LABORATORY) Eric Toljsten (Signature) 0940 (Time) 1/30/24 (Date)

Log in # 24-019