



**Whole Effluent Toxicity Test Report:
Liberty Lake**

January 2025

Report date: January 24, 2025

Submitted to:

Liberty Lake Sewer and Water District
2218 N. Harvard Road
Liberty Lake, WA 99019

1.0 INTRODUCTION

Acute toxicity tests were conducted using an effluent sample collected from Liberty Lake in January 2025. 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 Liberty Lake 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	1/14/2025; 1410h
Test termination date and time	1/16/2025; 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, 27, 12.5, 6.25, 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	1/14/2025; 1445h
Test termination date and time	1/18/2025; 1420h
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, 27, 12.5, 6.25, 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 Sample #1
Rainier Log-In No.	25-009
Collection date and time	1/13/2025; 0915h
Receipt date and time	1/14/2025; 1300h
Receipt temperature (°C)	3.7
Dissolved oxygen (mg/L)	9.4
pH	7.89
Conductivity (µS/cm)	505
Hardness (mg/L CaCO ₃)	128
Alkalinity (mg/L CaCO ₃)	124
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 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 27 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
	6.25	100			
	12.5	100			
	27	100			
	50	100			
	100	100			
Fathead minnows	0.0	100	100	>100	>100
	6.25	97.5			
	12.5	100			
	27	97.5			
	50	100			
	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>	1/7/2025	48h Survival	17.7 µg/L Cu	8.12-34.7 µg/L Cu	43.8
Fathead minnow	1/14/2025	96h Survival	5.34 g/L NaCl	4.81-7.84 g/L NaCl	13.0

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: 23 Jan-25 15:19 (p 1 of 1)
 Test Code: 2501-037 | 15-8920-4539

Ceriodaphnia 48-h Acute Survival Test

Rainier Environmental Laboratory

Batch ID:	16-5416-9033	Test Type:	Survival (48h)	Analyst:	Lab Tech
Start Date:	14 Jan-25 14:10	Protocol:	EPA/821/R-02-012 (2002)	Diluent:	Mod-Hard Synthetic Water
Ending Date:	16 Jan-25 14:00	Species:	Ceriodaphnia dubia	Brine:	
Duration:	48h	Source:	In-House Culture	Age:	<24h
Sample ID:	14-5865-8594	Code:	25-009	Client:	Liberty Lake
Sample Date:	13 Jan-25 09:15	Material:	POTW Effluent	Project:	
Receive Date:	14 Jan-25 13:00	Source:	Liberty Lake (WA0045144)		
Sample Age:	29h (3.7 °C)	Station:			

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
16-0902-0797	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
16-0902-0797	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%
6.25		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%
27		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
6.25		1	1	1	1
12.5		1	1	1	1
27		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
6.25		5/5	5/5	5/5	5/5
12.5		5/5	5/5	5/5	5/5
27		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: Liberth Lake
 Sample ID: Effluent Sample #1
 Test # 2501-637
 Log-In # 25-009

Start Date & Time: 1/14/2025 14:10
 End Date & Time: 1/16/2025 14:00
 Test Organism: Caridophyid daphnia

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	15	5	5	7.8	7.4	7.7	8.10	8.04	8.08	319	316	309	20.1	20.0	19.8	
2		24	5	5													
3		23	5	5													
4	0.25	6	5	5	7.8	7.4	7.8	8.09	8.04	8.05	310	311	312	20.0	19.9	19.9	
1		11	5	5													
2		11	5	5													
3		11	5	5													
4		11	5	5													
1	12.5	20	5	5	8.0	7.5	7.4	8.04	8.00	8.01	327	330	333	19.9	20.0	20.0	
2		5	5	5													
3		19	5	5													
4		12	5	5													
1	27	23	5	5	8.0	7.5	7.7	8.01	8.00	7.99	369	367	366	19.9	20.0	20.0	
2		4	5	5													
3		15	5	5													
4		9	5	5													
1	50	19	5	5	8.0	7.2	7.1	8.00	7.99	7.95	450	452	451	20.0	20.0	19.8	
2		24	5	5													
3		22	5	5													
4		10	5	5													
1	100	16	5	5	8.2	7.0	6.8	7.90	7.94	7.92	620	621	618	19.9	20.0	19.8	
2		12	5	5													
3		13	5	5													
4		3	5	5													

Technician Initials

ST ST ST

Sample Description:

Dilution Water Batch #: MHSW 005
 Test Chamber: VWK

Animal Source: Iron Horse Culture
 Date Received:

QA Check: ✓

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: 24 Jan-25 13:00 (p 1 of 1)
 Test Code: 2501-038 | 20-5147-6635

Fathead Minnow 96-h Acute Survival Test

Rainier Environmental Laboratory

Batch ID: 09-6828-6202	Test Type: Survival (96h)	Analyst: Eric Tollefson
Start Date: 14 Jan-25 14:45	Protocol: EPA/821/R-02-012 (2002)	Diluent: Mod-Hard Synthetic Water
Ending Date: 18 Jan-25 14:20	Species: Pimephales promelas	Brine:
Duration: 96h	Source: Aquatic Biosystems, CO	Age: 5d
Sample ID: 14-5865-8594	Code: 25-009	Client: Liberty Lake
Sample Date: 13 Jan-25 09:15	Material: POTW Effluent	Project:
Receive Date: 14 Jan-25 13:00	Source: Liberty Lake (WA0045144)	
Sample Age: 30h (3.7 °C)	Station:	

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
17-0345-4276	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
17-0345-4276	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%
6.25		4	0.975	0.9563	0.9937	0.9	1	0.025	0.05	5.13%	2.5%
12.5		4	1	1	1	1	1	0	0	0.0%	0.0%
27		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	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
6.25		0.9	1	1	1
12.5		1	1	1	1
27		0.9	1	1	1
50		1	1	1	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
6.25		9/10	10/10	10/10	10/10
12.5		10/10	10/10	10/10	10/10
27		9/10	10/10	10/10	10/10
50		10/10	10/10	10/10	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: Liberty Lake
 Sample ID: Effluent Sample #
 Test # ~~9-25-09~~ 2501-038
 Rainier Check-In #: 25-009

Start Date & Time: 1/14/2025 1445
 End Date & Time: 1/19/2025 1440
 Test Organism: Pimephales promelas

Sample Conc. or %	D.O. (mg/L)			pH (mg/L)		
	Init.	Fin.	Init.	Fin.	Init.	Fin.
CON	0	48	48	72	96	96
6.25	1.8	7.1	7.9	7.5	7.8	7.8
12.5	1.8	7.4	7.9	7.4	7.3	7.3
27	8.0	1.5	6.7	7.4	7.5	7.5
50	8.0	1.2	6.5	7.3	7.4	7.4
100	8.2	1.0	6.1	7.1	7.0	7.0

Sample Conc. or %	Conductivity μ S/cm						Test Temperature (°C)					
	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.
CON	0	24	48	48	72	96	0	24	48	48	72	96
6.25	3.14	3.16	3.14	3.02	3.05	3.03	20.1	20.0	19.9	19.8	20.0	20.3
12.5	3.16	3.11	3.11	3.06	3.05	3.05	20.0	19.9	19.9	19.9	20.1	20.0
27	3.21	3.30	3.22	3.21	3.20	3.19	19.9	20.0	20.0	19.9	20.1	20.0
50	3.69	3.67	3.65	3.58	3.59	3.55	19.9	20.0	20.0	19.9	20.0	20.1
100	4.50	4.52	4.49	4.46	4.52	4.54	20.0	20.0	20.0	20.3	20.1	20.1

Sample Conc. or %	Rep #	Cont #	Number of Live Organisms				
			0	24	48	72	96
CON	1	8	10	10	10	10	10
	2	19	10	10	10	10	10
	3	1	10	10	10	10	10
	4	23	10	10	10	10	10
6.25	1	13	10	10	10	9	9
	2	18	10	10	10	10	10
	3	7	10	10	10	10	10
	4	22	10	10	10	10	10
12.5	1	3	10	10	10	10	10
	2	17	10	10	10	10	10
	3	21	10	10	10	10	10
	4	12	10	10	10	10	10
27	1	2	10	10	10	10	9
	2	11	10	10	10	10	10
	3	6	10	10	10	10	10
	4	15	10	10	10	10	10
50	1	16	10	10	10	10	10
	2	4	10	10	10	10	10
	3	24	10	10	10	10	10
	4	9	10	10	10	10	10
100	1	20	10	10	10	10	10
	2	14	10	10	10	10	10
	3	6	10	10	10	10	10
	4	10	10	10	10	10	10

Comments: _____
 Animal Source: ARS
 Date Received: 1/14/2025
 Date of Hatch: 1/9/2025
 QA Check: ✓

Dilution Water Batch #: MHSW 005
 Test Chamber: VWV
 Tech. Initials: ST
 48-Hr, Feeding: ✓

Appendix C
Sample Check-In Sheet

Client: Liberty Lake Sewer and Water District Tests Performed: Cd-C ; Pp-C ; Cd-A ; Pp-A
 Test ID No(s): 2501-035 ; 2501-036 ; 2501-037 ; 2501-038

Log-in No. (20-xxxx):	Effluent Sample #1	Effluent Sample #2	EFFLUENT SAMPLE #3
25-009	25-010	25-013	

Sample Collection Date & Time: 1/13/25 0915 1/15/25 0920 1/17/25 0930

Sample Receipt Date & Time: 1/14/25 1300 1/16/25 0815 1/18/25 0930

Check-in Temperature (°C) 3.1 3.0 2.0

Temperature OK? Y N Y N Y N

DO (mg/L) 9.4 8.6 8.0

pH (units) 7.89 7.84 7.85

Conductivity (µS/cm) 505 531 525

Salinity (ppt) 0.3 0.3 0.3

Tit. Vol / Sam. Vol. / Alkalinity (mg/L)* 3.1 125 112 2.8 125 112 3.0 135 120

Tit. Vol. / Sam. Vol. / Hardness (mg/L)* 3.2 125 128 3.3 125 132 3.1 125 124

Total Chlorine (mg/L) <0.03 <0.03 <0.03

Total Ammonia Nitrogen (mg/L) <1.0 <1.0 <1.0

Technician Initials off off off

* = mg/L as CaCO₃, ² = Measured for freshwater samples only, NA = Not Applicable

CD-C NIM = Not Measured

Freshwater Tests:

Control/Dilution Water Source: test type: SP-C DP-C DP-C 8.2 (DMW) MHW Other: -005

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

Additional Control? Y N =

Marine Tests:

Control/Dilution Water Source: test type: ART SW NAT SW

Control/Dilution Water Source: test type: ART SW NAT SW

Additional Control? Y N =

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
 Pore Size:
 Organisms or Debris

Aeration? Y
 Length of Time:
 Final DO:
 Final pH:

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

Sub-samples for additional chemistry:

QC Check: off

Appendix D
Chain-of-Custody Form

