



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

August 2024

Report date: August 16, 2024

Submitted to:

City of Everett
3200 Cedar Street
Everett, WA 98201

Rainier Environmental
5013 Pacific Hwy East
Suite 20
Tacoma, WA 98424

1.0 INTRODUCTION

An acute toxicity test was conducted using effluent samples collected from City of Everett's Port Gardner SCE Outfall 100. Testing was conducted in August 2024 using the test organism *Pimephales promelas* (fathead minnow). Testing was performed at Rainier Environmental Laboratory.

2.0 METHODS

2.1 Sample Collection and Transport

An effluent sample was collected into LDPE cubitainers by City of Everett personnel. The sample was packed in coolers containing ice and transported to Rainier Environmental the day of collection. Appropriate chain-of-custody procedures were employed during collection and transport.

2.2 Sample Receipt

Upon arrival at the laboratory, coolers were opened, samples inspected, and the contents verified against information provided on the chain-of-custody forms. Receipt temperature was measured and recorded on the chain-of-custody form for each sample. Standard water quality parameters were measured and recorded on a sample check-in sheet (Appendix B). Samples were stored at 4°C in the dark until used for testing.

2.3 Test Methods

Acute toxicity tests were conducted using fathead minnow according to procedures presented by USEPA (2002) and summarized in Table 1.

Table 1. Summary of conditions for the fathead minnow 96-h acute survival tests.

Test initiation date and time	8/7/2024; 1425h
Test termination date and time	8/11/2024; 1445h
Test organism	<i>Pimephales promelas</i>
Test organism source	Aquatic Bio-Systems; Fort Collins, CO
Test organism age	6 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 and test solution volume	250 mL plastic cup, 200mL
Test temperature	20 ± 1°C
Dilution water	Moderately Hard Synthetic Water
Test concentrations (% sample)	100, 30, 10, 3.0, 0.64, laboratory control
Number of organisms/chamber	10
Number of replicates	4
Photoperiod	16 hours light/8 hours dark
Aeration	Tests run under CO ₂ atmosphere
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 samples are provided in Table 2.

Table 2. Sample information.

Sample ID	SCE
Log-In No.	24-115
Collection date and time	8/7/2024; 0700h
Receipt date and time	8/7/2024; 1045h
Receipt temperature (°C)	2.0
Dissolved oxygen (mg/L)	7.2
pH	7.63
Conductivity (µS/cm)	859
Hardness (mg/L CaCO ₃)	124
Alkalinity (mg/L CaCO ₃)	212
Total Chlorine (mg/L)	<0.03
Total Ammonia (mg/L)	20.2

Survival was evaluated in the acute toxicity tests after 96 hours of exposure. Results are summarized in Table 3. Mean survival in the 100 percent effluent concentration was 95 percent. There was no significant difference between the controls and the acute critical effluent concentration (ACEC) of 0.64 percent effluent.

Table 3. Summary of results

Species	Concentration (%)	Survival (%)	NOEC ^a (% effluent)	LOEC ^b (% effluent)
Sample ID: SCE	0.0	100	30	100
<i>Pimephales promelas</i>	0.64	100		
	3.0	100		
	10	100		
	30	100		
	100	95.0		

^a No Observed Effect Concentration, ^b Lowest Observed Effect Concentration

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

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. The tests were run in a chamber filled with 5 percent CO₂ to control pH drift and related ammonia toxicity. There were no other deviations from the protocols and water quality parameters remained within the ranges specified in the test method throughout the tests.

Results for the reference toxicant test used to monitor laboratory performance and test organism sensitivity are summarized in Table 4. Results for the reference toxicant test fell within the acceptable range of mean \pm 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 (%)
Fathead minnow	7/16/2024	96h survival	6.25	4.82 - 7.75	12.6

REFERENCES

- Tidepool Scientific Software. 2001-2011. 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, pg 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
Fathead Minnow Acute Toxicity Test
Statistical Summaries and Raw Bench Sheets

CETIS Summary Report

Report Date: 16 Aug-24 13:11 (p 1 of 1)
Test Code: 2408-013 | 19-9866-4861

Fathead Minnow 96-h Acute Survival Test

Rainier Environmental Laboratory

Batch ID:	20-3461-5562	Test Type:	Survival (96h)	Analyst:	Eric Tollefson
Start Date:	07 Aug-24 14:25	Protocol:	EPA/821/R-02-012 (2002)	Diluent:	Mod-Hard Synthetic Water
Ending Date:	11 Aug-24 14:45	Species:	Pimephales promelas	Brine:	
Duration:	4d 0h	Source:	Aquatic Biosystems, CO	Age:	6d
Sample ID:	14-8627-8599	Code:	24-115	Client:	Everett
Sample Date:	07 Aug-24 07:00	Material:	POTW Effluent	Project:	
Receive Date:	07 Aug-24 10:45	Source:	Everett (WA0024490)		
Sample Age:	7h (2 °C)	Station:	SCE Outfall 100		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
06-9479-0357	96h Survival Rate	30	100	54.77	4.94%	3.333	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
18-5233-5237	96h Survival Rate	LC5	100	27.93	N/A	1	Linear Interpolation (ICPIN)
		LC10	>100	N/A	N/A	<1	
		LC15	>100	N/A	N/A	<1	
		LC20	>100	N/A	N/A	<1	
		LC25	>100	N/A	N/A	<1	
		LC40	>100	N/A	N/A	<1	
		LC50	>100	N/A	N/A	<1	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
06-9479-0357	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria
18-5233-5237	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%
0.64		4	1	1	1	1	1	0	0	0.0%	0.0%
3		4	1	1	1	1	1	0	0	0.0%	0.0%
10		4	1	1	1	1	1	0	0	0.0%	0.0%
30		4	1	1	1	1	1	0	0	0.0%	0.0%
100		4	0.95	0.9284	0.9716	0.9	1	0.02887	0.05774	6.08%	5.0%

96h Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
0.64		1	1	1	1
3		1	1	1	1
10		1	1	1	1
30		1	1	1	1
100		1	0.9	0.9	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
0.64		10/10	10/10	10/10	10/10
3		10/10	10/10	10/10	10/10
10		10/10	10/10	10/10	10/10
30		10/10	10/10	10/10	10/10
100		10/10	9/10	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: Everett - SCE
Sample ID: SCE
Test #: 2408-013
Rainier Check-In #: 24-115

Start Date & Time: 8/7/2024 1425
End Date & Time: 8/11/2024 1445
Test Organism: Pimephales promelas

Sample Conc. or 	D.O. (mg/L)				pH (mg/L)			
	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.
0	24	48	48	72	96	0	24	48
CON	7.3	7.4	7.4	7.3	7.5	8.16	7.11	7.10
0.64	1.5	1.2	1.1	1.4	7.5	8.14	1.43	1.09
3	7.8	7.3	7.1	7.1	7.4	8.08	1.14	7.11
10	7.6	7.2	7.0	7.1	7.4	8.01	7.16	7.12
30	7.4	7.3	7.0	7.5	7.3	7.88	7.29	7.20
100	7.1	6.8	7.0	7.3	7.1	7.69	7.49	7.44


Sample Conc. or 	Conductivity μ S/cm				Test Temperature ($^{\circ}$ C)			
	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.
0	24	48	48	72	96	0	24	48
CON	301	303	302	303	301	20.1	20.1	19.9
0.64	271	284	285	279	301	20.0	19.9	19.9
3	275	300	301	284	289	20.0	19.9	19.7
10	291	306	304	299	301	19.9	20.0	19.8
30	388	395	397	389	391	19.8	20.0	19.9
100	681	682	684	690	695	19.6	20.1	19.9

Dilution Water Batch #: MHSW 080
Test Chamber: KM2

Comments: Yuv under 1/2 headspace
QA Check: U

Animal Source: ABS
Date Received: 8/6/2024
Date of Hatch: 8/11/2024

48-Hr. Feeding: V

Sample Conc. or 	Rep #	Cont #	Number of Live Organisms				
			0	24	48	72	96
CON	1	15	10	10	10	10	10
	2	23	10	10	10	10	10
	3	2	10	10	10	10	10
	4	21	10	10	10	10	10
0.64	1	10	10	10	10	10	10
	2	14	10	10	10	10	10
	3	1	10	10	10	10	10
	4	19	10	10	10	10	10
3	1	9	10	10	10	10	10
	2	20	10	10	10	10	10
	3	14	10	10	10	10	10
	4	5	10	10	10	10	10
10	1	13	10	10	10	10	10
	2	18	10	10	10	10	10
	3	3	10	10	10	10	10
	4	4	10	10	10	10	10
30	1	11	10	10	10	10	10
	2	8	10	10	10	10	10
	3	4	10	10	10	10	10
	4	17	10	10	10	10	10
100	1	22	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

Tech. Initials

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Appendix B
Sample Check-In Sheet

Sample Check-In Information

Client: City of Everett

Tests Performed: FP-AL
Test ID No(s): 3408-013

Sample Description:

Sample ID:	<u>SCE</u>		
Log-in No. (20-xxxx):	<u>24-115</u>		
Sample Collection Date & Time:	<u>8/7/24</u>	<u>0700</u>	
Sample Receipt Date & Time:	<u>8/7/24</u>	<u>1045</u>	
Check-in Temperature (°C)	<u>2.0</u>		
Temperature OK?	<u>(Y) N</u>	Y N	Y N
DO (mg/L)	<u>1.2</u>		
pH (units)	<u>7.63</u>		
Conductivity (µS/cm)	<u>859</u>		
Salinity (ppt)	<u>0.4</u>		
Tit. Vol / Sam. Vol. / Alkalinity (mg/L)*	<u>5.3 / 25 / 212</u>	<u>/ /</u>	<u>/ /</u>
Tit. Vol. / Sam. Vol. / Hardness (mg/L)*	<u>3.1 / 25 / 124</u>	<u>/ /</u>	<u>/ /</u>
Total Chlorine (mg/L)	<u><0.03</u>		
Total Ammonia Nitrogen (mg/L)	<u>20.3</u>		
Technician Initials	<u>AF</u>		

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

NM = Not Measured

Freshwater Tests:

Control/Dilution Water Source: test type: FP-AL 8.2 (DMW) MH-W Other: -0.80
Control/Dilution Water Source: test type: 8.2 (DMW) MH-W 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:

Alkalinity: 64 Hardness: 84
Alkalinity: Hardness:
Alkalinity: Hardness:
Alkalinity: Hardness:

Alkalinity: Salinity:
Alkalinity: Salinity:
Alkalinity: Salinity:

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: 4

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:

Appendix C
Chain-of-Custody Forms

Washington
5013 Pacific Highway East, Suite 20
Fife, WA 98424
Phone: 253.922.8898

Chain of Custody

Date _____ Page ____ of ____

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