



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

**February 2025**

Report date: February 20, 2025

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**

Acute toxicity tests were conducted using effluent samples collected from City of Everett's Port Gardner TF/SC System (SCE) and Lagoon System (FEN). Testing was conducted in February 2025 using the test organism *Pimephales promelas* (fathead minnow). Testing was performed at Rainier Environmental Laboratory.

## **2.0 METHODS**

### **2.1 Sample Collection and Transport**

Effluent samples were collected into LDPE cubitainers by City of Everett personnel. The samples were 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	FEN: 2/5/2025; 1220h SCE: 2/5/2025; 1245h
Test termination date and time	FEN: 2/9/2025; 1225h SCE: 2/9/2025; 1245h
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)	FEN: 100, 50, 25, 15.6, 6.25, laboratory control SCE: 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 <sub>2</sub> 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	FEN	SCE
Log-In No.	25-029	25-030
Collection date and time	2/5/2025; 0700h	2/5/2025; 0700h
Receipt date and time	2/5/2025; 0910h	2/5/2025; 0910h
Receipt temperature (°C)	3.5	4.2
Dissolved oxygen (mg/L)	9.8	7.8
pH	7.54	7.18
Conductivity (µS/cm)	652	748
Hardness (mg/L CaCO <sub>3</sub> )	100	76
Alkalinity (mg/L CaCO <sub>3</sub> )	172	148
Total Chlorine (mg/L)	<0.03	<0.03
Total Ammonia (mg/L)	28.4	22.3

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 for samples FEN and SCE was 100 percent. There was no significant difference between the controls and the acute critical effluent concentration (ACEC) of 15.6 percent effluent and 0.64 percent effluent for FEN and SCE, respectively.

**Table 3. Summary of results**

Species	Concentration (%)	Survival (%)	NOEC <sup>a</sup> (% effluent)	LOEC <sup>b</sup> (% effluent)
<b><u>Sample ID: FEN</u></b>	0.0	100	100	>100
<i>Pimephales promelas</i>	6.25	100		
	15.6	100		
	25	100		
	50	100		
	100	100		
<b><u>Sample ID: SCE</u></b>	0.0	100	30	100
<i>Pimephales promelas</i>	0.64	100		
	3.0	100		
	10	100		
	30	100		
	100	95.0		

<sup>a</sup> No Observed Effect Concentration, <sup>b</sup> 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

All samples were 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 CO<sub>2</sub> 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 <sub>50</sub> (g/L NaCl)	Acceptable Range (g/L NaCl)	CV (%)
Fathead minnow	2/11/2025	96h survival	4.97	4.74 - 7.82	13.3

#### 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**

## **FEN - Lagoon System**

# CETIS Summary Report

Report Date: 21 Feb-25 10:50 (p 1 of 1)  
 Test Code: 2502-026 | 00-8597-6079

## Fathead Minnow 96-h Acute Survival Test

Rainier Environmental Laboratory

Batch ID:	06-8296-9767	Test Type:	Survival (96h)	Analyst:	Eric Tollefson
Start Date:	05 Feb-25 12:20	Protocol:	EPA/821/R-02-012 (2002)	Diluent:	Mod-Hard Synthetic Water
Ending Date:	09 Feb-25 12:25	Species:	Pimephales promelas	Brine:	
Duration:	4d 0h	Source:	Aquatic Biosystems, CO	Age:	6d
Sample ID:	05-3615-3493	Code:	25-029	Client:	Everett
Sample Date:	05 Feb-25 07:00	Material:	POTW Effluent	Project:	
Receive Date:	05 Feb-25 09:10	Source:	Everett (WA0024490)		
Sample Age:	5h (3.5 °C)	Station:	FEN Outfall 015		

## Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
06-9768-9132	96h Survival Rate	100	>100	NA	2.5%	1	Steel Many-One Rank Sum Test

## Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
06-9768-9132	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	1	1	1	1	1	0	0	0.0%	0.0%
15.6		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%

## 96h 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
15.6		1	1	1	1
25		1	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		10/10	10/10	10/10	10/10
15.6		10/10	10/10	10/10	10/10
25		10/10	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: Everett  
Sample ID: ECN  
Test #: 2502-026  
Rainier Check-In #: 25-029

Start Date & Time: 2/5/2025 1220  
End Date & Time: 2/9/2025 1225  
Test Organism: Pimephales promelas

Sample Conc. or (%)	D.O. (mg/L)						pH (mg/L)					
	Init.	24	48	Init.	72	96	Init.	24	48	Init.	72	96
	Fin.	Fin.	Fin.	Fin.	Fin.	Fin.	Fin.	Fin.	Fin.	Fin.	Fin.	Fin.
CON	0	24	48	48	72	96	0	24	48	48	72	96
6.25	7.7	6.7	6.5	7.9	7.5	7.7	8.06	6.89	6.84	8.09	8.91	8.97
15.6	7.6	6.9	6.6	7.8	7.3	7.1	7.96	6.89	6.87	8.01	8.92	8.95
25	7.4	7.1	6.6	7.5	7.1	7.0	7.87	6.94	6.91	7.94	8.95	8.99
50	7.6	6.9	6.4	7.5	7.4	7.2	7.81	6.99	6.95	7.82	7.01	7.04
100	7.5	6.7	6.4	7.5	7.0	7.2	7.74	7.08	7.01	7.74	7.16	7.15
	7.7	5.4	5.9	7.4	7.2	7.3	7.61	7.22	7.20	7.64	7.30	7.33

Sample Conc. or (%)	Conductivity (µS/cm)						Test Temperature (°C)					
	Init.	24	48	Init.	72	96	Init.	24	48	Init.	72	96
	Fin.	Fin.	Fin.	Fin.	Fin.	Fin.	Fin.	Fin.	Fin.	Fin.	Fin.	Fin.
CON	0	24	48	48	72	96	0	24	48	48	72	96
6.25	31.8	34.0	38.5	32.0	31.9	32.3	20.0	20.1	20.0	20.0	20.1	20.2
15.6	31.9	30.9	31.5	32.1	33.5	33.7	20.1	20.0	20.0	19.9	20.0	20.1
25	35.0	33.7	33.6	35.4	35.7	35.1	20.1	20.1	19.9	20.1	20.0	20.1
50	38.1	36.7	36.6	38.7	39.9	39.4	20.2	20.1	19.8	20.1	20.0	20.0
100	45.7	43.7	43.2	45.9	46.3	46.6	20.1	20.2	19.9	20.1	20.1	20.0
	47.2	58.6	58.4	61.3	61.5	61.8	20.1	20.1	19.8	20.1	20.1	20.0
Tech. Initials	SA	SA	SA	SA	SA	SA						
Sample Used:	25-029			25-029								

Sample Conc. or (%)	Rep #	Cont #	Number of Live Organisms				
			0	24	48	72	96
			Fin.	Fin.	Fin.	Fin.	Fin.
CON	1	11	10	10	10	10	10
	2	24	10	10	10	10	10
	3	2	10	10	10	10	10
	4	23	10	10	10	10	10
6.25	1	12	10	10	10	10	10
	2	1	10	10	10	10	10
	3	18	10	10	10	10	10
	4	10	10	10	10	10	10
15.6	1	17	10	10	10	10	10
	2	3	10	10	10	10	10
	3	14	10	10	10	10	10
	4	6	10	10	10	10	10
25	1	22	10	10	10	10	10
	2	4	10	10	10	10	10
	3	19	10	10	10	10	10
	4	9	10	10	10	10	10
50	1	13	10	10	10	10	10
	2	5	10	10	10	10	10
	3	21	10	10	10	10	10
	4	8	10	10	10	10	10
100	1	7	10	10	10	10	10
	2	15	10	10	10	10	10
	3	16	10	10	10	10	10
	4	20	10	10	10	10	10
Tech. Initials	SA	SA	SA	SA	SA	SA	SA

Comments: CO2  
Animal Source: ABS  
Date Received: 2/4/2025  
Date of Hatch: 1/30/2025  
QA Check: u

48-Hr. Feeding: ✓

Dilution Water Batch #: MHSW 013  
Test Chamber: R001 2

**SCE - TF/SC System**

# CETIS Summary Report

Report Date: 20 Feb-25 12:25 (p 1 of 1)  
Test Code: 2502-027 | 10-7019-8540

## Fathead Minnow 96-h Acute Survival Test

Rainier Environmental Laboratory

Batch ID: 09-6203-1544	Test Type: Survival (96h)	Analyst: Eric Tollefson
Start Date: 05 Feb-25 12:45	Protocol: EPA/821/R-02-012 (2002)	Diluent: Mod-Hard Synthetic Water
Ending Date: 09 Feb-25 12:45	Species: Pimephales promelas	Brine:
Duration: 96h	Source: Aquatic Biosystems, CO	Age: 6d
Sample ID: 01-8067-0940	Code: 25-030	Client: Everett
Sample Date: 05 Feb-25 07:00	Material: POTW Effluent	Project:
Receive Date: 05 Feb-25 09:10	Source: Everett (WA0024490)	
Sample Age: 6h (4.2 °C)	Station: SCE Outfall 100	

## Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
03-8775-9611	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
08-7881-9172	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
03-8775-9611	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria
08-7881-9172	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	1	0.9

## 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	10/10	9/10

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  
Sample ID: SCE  
Test #: 2502-021  
Rainier Check-In #: 25-030

Start Date & Time: 2/5/2025 1245  
End Date & Time: 2/9/2025 1345  
Test Organism: Pimephales promelas

Sample Conc. or (%)	D.O. (mg/L)						pH (mg/L)					
	Init.	24	48	72	96	0	24	48	72	96	0	24
	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.
CON	0	24	48	48	72	96	0	24	48	48	72	96
0.64	8.0	6.8	6.7	7.8	7.1	6.9	8.0	6.8	6.7	7.1	6.9	6.8
3.0	8.0	7.0	6.6	7.7	7.0	7.2	8.0	6.8	6.7	7.1	6.9	6.8
10	7.9	7.2	6.6	7.7	7.0	7.1	7.9	6.8	6.7	7.1	6.9	6.8
30	7.7	6.4	6.4	7.5	6.5	6.7	7.8	6.8	6.8	7.1	6.9	6.8
100	7.5	6.3	6.2	7.4	6.4	6.3	7.5	6.9	6.9	7.1	6.9	6.8
	6.7	5.8	6.1	6.9	6.1	5.5	7.3	7.1	7.1	7.1	7.1	7.1

Sample Conc. or (%)	Conductivity (µS/cm)						Test Temperature (°C)					
	Init.	24	48	48	72	96	Init.	24	48	72	96	Init.
	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.	Fin.
CON	0	24	48	48	72	96	20.1	19.9	20.0	20.1	19.9	20.2
0.64	303	293	290	307	307	312	20.0	19.9	20.0	20.1	20.0	20.2
3.0	312	298	294	314	312	314	20.1	20.0	20.0	20.1	20.1	20.1
10	335	326	327	336	337	340	20.1	20.0	19.9	20.1	20.1	20.1
30	429	410	402	437	441	440	20.1	19.9	20.0	20.0	20.1	20.0
100	762	663	661	706	710	713	20.2	19.8	20.1	20.1	20.1	20.0
Tech. Initials	SA	SA	SA	SA	SA	SA						
Sample Used:	25-030											

Sample Conc. or (%)	Rep #	Cont #	Number of Live Organisms				
			0	24	48	72	96
			0	24	48	72	96
CON	1	1	10	10	10	10	10
	2	16	10	10	10	10	10
	3	24	10	10	10	10	10
	4	9	10	10	10	10	10
0.64	1	8	10	10	10	10	10
	2	15	10	10	10	10	10
	3	22	10	10	10	10	10
	4	5	10	10	10	10	10
3.0	1	14	10	10	10	10	10
	2	7	10	10	10	10	10
	3	20	10	10	10	10	10
	4	2	10	10	10	10	10
10	1	12	10	10	10	10	10
	2	19	10	10	10	10	10
	3	6	10	10	10	10	10
	4	3	10	10	10	10	10
30	1	13	10	10	10	10	10
	2	10	10	10	10	10	10
	3	21	10	10	10	10	10
	4	13	10	10	10	10	10
100	1	4	10	10	10	10	10
	2	17	10	10	10	10	10
	3	23	10	10	10	10	10
	4	11	10	10	10	10	10
Tech. Initials	SA	SA	SA	SA	SA	SA	SA

Comments: CO2  
Animal Source: ABC  
Date Received: 2/4/2025  
Date of Hatch: 1/30/2025  
QA Check: SA

48-Hr, Feeding: V

Dilution Water Batch #: MHSW 013  
Test Chamber: KCM2

**Appendix B**  
**Sample Check-In Sheet**

Client: City of Everett

Tests Performed: PP4, PP4  
Test ID No(s): 2502-026; 2502-027

Sample ID:	FEN	SCE		
Log-in No. (20-xxxx):	25-029	25-030		
Sample Collection Date & Time:	2/5/25 0700	2/5/25 0700		
Sample Receipt Date & Time:	2/5/25 0910	2/5/25 0910		
Check-in Temperature (°C)	3.5	4.2		
Temperature OK?	<input checked="" type="radio"/> Y <input type="radio"/> N	<input checked="" type="radio"/> Y <input type="radio"/> N	<input type="radio"/> Y <input type="radio"/> N	<input type="radio"/> Y <input type="radio"/> N
DO (mg/L)	9.8	7.8		
pH (units)	7.54	7.18		
Conductivity (µS/cm)	652	718		
Salinity (ppt)	0.3	0.4		
Tit. Vol / Sam. Vol. / Alkalinity (mg/L)*	4.3 / 25 / 172	3.7 / 25 / 148	1	1
Tit. Vol. / Sam. Vol. / Hardness (mg/L)*	2.5 / 25 / 100	1.9 / 25 / 76	1	1
Total Chlorine (mg/L)	<0.03	<0.03		
Total Ammonia Nitrogen (mg/L)	284	223		
Technician Initials	CH	CH		

\* = mg/L as CaCO<sub>3</sub>; <sup>a</sup> = Measured for freshwater samples only; NA = Not Applicable,  
NM = Not Measured

Freshwater Tests:

Control/Dilution Water Source: test type: PP4 8:2 (DMW) MHW Other: -013 Alkalinity: 68 Hardness: 96  
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:  ART SW NAT SW Alkalinity:  Salinity:   
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 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:

QC Check: Y

**Appendix C**  
**Chain-of-Custody Forms**

[illegible]