



**Whole Effluent Toxicity Test Report  
Pierce County**

January 2025

Report date: January 31, 2025

Submitted to:

**Pierce County Chambers Creek WWTP**  
10311 Chambers Creek Road West  
University Place, WA 98467

5013 Pacific Hwy East  
Suite 20  
Tacoma, WA 98424

## **1.0 INTRODUCTION**

An acute toxicity test was conducted using an effluent sample collected from the Pierce County Wastewater Treatment Plant. Testing was conducted in January 2025 using the test organism *Pimephales promelas* (fathead minnow). Testing was performed at Rainier Environmental Laboratory located in Tacoma, Washington.

## **2.0 METHODS**

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

Pierce County personnel collected an effluent sample into a LDPE cubitainer. The sample was packed in a cooler 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, 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 a sample check-in sheet (Appendix B). The sample was stored at 4°C in the dark until use.

### **2.3 Test Methods**

The toxicity test was conducted according to procedures presented by USEPA (2002), which are summarized in Table 1.

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

Test initiation date and time	1/22/2025; 1300h
Test termination date and time	1/26/2025; 1255h
Test organism	<i>Pimephales promelas</i>
Test organism source	Aquatic BioSystems; 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	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, 6.4, laboratory 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 sample are provided in Table 2.

**Table 2. Sample information.**

Sample ID	Final Effluent Composite
Rainier Log-In No.	25-017
Collection date and time	1/22/2025; 0815h
Receipt date and time	1/22/2025; 0945h
Receipt temperature (°C)	7.5
Dissolved oxygen (mg/L)	8.2
pH	7.15
Conductivity (µS/cm)	535
Hardness (mg/L CaCO <sub>3</sub> )	84
Alkalinity (mg/L CaCO <sub>3</sub> )	68
Total Chlorine (mg/L)	<0.03
Total Ammonia (mg/L)	<1.0

Survival was evaluated in the acute toxicity test after 96 hours of exposure. Results are summarized in Table 3. Mean survival in 100 percent effluent was 97.5 percent. There was no statistically significant difference between the control and the acute critical effluent concentration (ACEC) of 6.4 percent effluent, which had 100 percent survival.

**Table 3. Summary of results for the fathead minnow acute toxicity test.**

Species	Concentration (%)	Survival (%)	NOEC <sup>a</sup> (% effluent)	LOEC <sup>b</sup> (% effluent)	LC50 <sup>c</sup> (95% CI) (% effluent)
Fathead Minnow	0.0	100	100	>100	>100
	6.4	100			
	12.5	97.5			
	25	100			
	50	100			
	100	97.5			

<sup>a</sup> No Observed Effect Concentration; <sup>b</sup> Lowest Observed Effect Concentration; <sup>c</sup> Lethal concentration of 50% of test organisms

A summary of the statistical analysis, a copy of the laboratory bench sheets, a copy of the sample check-in sheet and chain-of-custody form 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 test met all acceptability criteria for performance of control organisms.

Results for the reference toxicant test used to monitor laboratory performance and test organism sensitivity are summarized in Table 4. Reference toxicant test results 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 Table 4

**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	1/14/2025	96h survival	5.34	4.81 – 7.84	13.0

## REFERENCES

- Tidepool Scientific Software. 2000-2010. 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. 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  
*Pimephales promelas* Acute Toxicity Test  
Statistical Summaries and Raw Bench Sheets

# CETIS Summary Report

Report Date: 31 Jan-25 12:16 (p 1 of 1)  
 Test Code: 2501-052 | 04-3549-1306

## Fathead Minnow 96-h Acute Survival Test

Rainier Environmental Laboratory

Batch ID:	11-1628-2057	Test Type:	Survival (96h)	Analyst:	Eric Tollefson
Start Date:	22 Jan-25 13:00	Protocol:	EPA/821/R-02-012 (2002)	Diluent:	Mod-Hard Synthetic Water
Ending Date:	26 Jan-25 12:55	Species:	Pimephales promelas	Brine:	
Duration:	96h	Source:	Aquatic Biosystems, CO	Age:	6d
Sample ID:	17-5228-9430	Code:	25-017	Client:	Pierce County
Sample Date:	22 Jan-25 08:15	Material:	POTW Effluent	Project:	
Receive Date:	22 Jan-25 09:45	Source:	Pierce County (WA0039624)		
Sample Age:	5h (7.5 °C)	Station:			

## Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
01-0740-8320	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
01-0740-8320	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%
6.4		4	1	1	1	1	1	0	0	0.0%	0.0%
12.5		4	0.975	0.9563	0.9937	0.9	1	0.025	0.05	5.13%	2.5%
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	0.975	0.9563	0.9937	0.9	1	0.025	0.05	5.13%	2.5%

## 96h Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
6.4		1	1	1	1
12.5		1	0.9	1	1
25		1	1	1	1
50		1	1	1	1
100		1	0.9	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
6.4		10/10	10/10	10/10	10/10
12.5		10/10	9/10	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	9/10	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: Pierce County  
Sample ID: Final Effluent Composite  
Test #: 2501-052  
Rainier Check-In #: 25-017

Start Date & Time: 1/22/2025 1300  
End Date & Time: 1/26/2025 1255  
Test Organism: Pimephales promelas

Sample Conc. or Ⓢ	D.O.						pH					
	(mg/L)						(mg/L)					
	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
6.4	7.4	7.4	7.2	1.8	8.0	7.8	8.02	8.00	7.99	8.10	7.96	7.88
12.5	7.5	7.4	7.2	7.9	7.8	8.0	7.96	7.97	7.98	8.04	7.96	7.91
25	7.7	7.3	7.1	7.6	7.8	7.7	7.86	7.92	7.94	7.92	7.89	7.90
50	7.2	7.0	7.0	7.5	7.6	7.8	7.72	7.84	7.86	7.75	7.84	7.84
100	7.0	6.8	6.9	7.3	7.5	7.4	7.56	7.67	7.69	7.57	7.61	7.63
	6.9	6.8	6.7	7.0	7.1	6.9	7.17	7.52	7.60	7.20	7.57	7.63

Sample Conc. or Ⓢ	Conductivity						Test Temperature					
	µS/cm						(°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
6.4	201	204	205	310	308	311	20.2	20.1	20.0	20.0	19.9	20.4
12.5	293	300	206	311	313	313	20.3	20.0	20.0	20.1	20.0	20.1
25	303	305	307	312	315	316	20.3	20.1	19.9	20.1	20.0	19.9
50	328	325	325	329	325	323	20.2	20.1	19.9	20.1	20.1	19.9
100	316	371	379	379	381	379	20.0	20.1	19.9	20.2	20.1	19.8
	474	476	476	470	472	474	20.0	20.2	19.8	20.4	20.1	19.9
Tech. Initials	SA	SA	SA	SA	SA	SA						
Sample Used:	25-017						25-017					

Dilution Water Batch #: MHW/07  
Test Chamber: VWR

Comments:

Animal Source: ABS  
Date Received: 1/24/2025  
Date of Hatch: 1/16/2025

Sample Conc. or Ⓢ	Rep #	Cont #	Number of Live Organisms				
			0	24	48	72	96
CON	1	11	10	10	10	10	10
	2	24	10	10	10	10	10
	3	1	10	10	10	10	10
	4	13	10	10	10	10	10
6.4	1	23	10	10	10	10	10
	2	5	10	10	10	10	10
	3	2	10	10	10	10	10
	4	12	10	10	10	10	10
12.5	1	16	10	10	10	10	10
	2	4	10	10	10	9	9
	3	15	10	10	10	10	10
	4	22	10	10	10	10	10
25	1	14	10	10	10	10	10
	2	3	10	10	10	10	10
	3	19	10	10	10	10	10
	4	8	10	10	10	10	10
50	1	17	10	10	10	10	10
	2	6	10	10	10	10	10
	3	20	10	10	10	10	10
	4	9	10	10	10	10	10
100	1	18	10	10	10	10	10
	2	7	10	9	9	9	9
	3	21	10	10	10	10	10
	4	10	10	10	10	10	10
Tech. Initials	SA	SA	SA	SA	SA	SA	SA

48-Hr, Feeding: ✓



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

Sample Check-In Information

Client: Pierce County Chambers Creek WWT

Tests Performed: PP 4a  
Test ID No(s): 3501-052

Sample Description:

Sample ID:	Final Effluent Comp	
Log-in No. (20-xxxx):	25-017	
Sample Collection Date & Time:	1/22/25 0815	
Sample Receipt Date & Time:	1/22/25 0945	
Check-in Temperature (°C)	7.5	
Temperature OK?	<input checked="" type="checkbox"/> Y <input type="checkbox"/> N	
DO (mg/L)	8.2	
pH (units)	7.15	
Conductivity (µS/cm)	535	
Salinity (ppt)	—	
Tit. Vol / Sam. Vol. / Alkalinity (mg/L)*	1.7 / 25 / 68	
Tit. Vol. / Sam. Vol. / Hardness (mg/L)* <sup>a</sup>	2.1 / 25 / 84	
Total Chlorine (mg/L)	<0.03	
Total Ammonia Nitrogen (mg/L)	<1.0	
Technician Initials	df	

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

NIM = Not Measured

Freshwater Tests:

Control/Dilution Water Source: test type: PP 2a 8.2 (DMW) MHW Other: -007 Alkalinity: 60 Hardness: 80

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.

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: df

**Appendix C**  
**Chain-of-Custody Form**

Sample Collection By:

Report to:

Company Pierce County Chambers Creek WWTP  
Address 10311 Chambers Creek Rd W  
City/State/Zip University Place, WA 98467  
Contact Melissa Didier  
Phone 253-798-3004  
Email melissa.didier@piercecountywa.gov

Invoice To:

Company Same  
Address  
City/State/Zip  
Contact  
Phone  
Email

Fathead Minnow 96-hour static-renewal  
test (Pimephales Promelas)  
EPA 821-R-02-012

ANALYSES REQUIRED

Receipt Temperature (°C)

SAMPLE ID

DATE

TIME

MATRIX

CONTAINER  
TYPE

NO. OF  
CONTAINERS

COMMENTS

Final Effluent Composite

01/21/25 to  
01/22/25

0845  
0815

WW

LDPE

1

Include ACEC 6.4% in dilutions  
along with 100% effluent

X

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