



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

**April 2024**

Report date: April 18, 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

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 April 2024 using the test organism *Daphnia pulex*. 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 *D. pulex* according to procedures presented by USEPA (2002) and summarized in Table 1.

**Table 1. Summary of conditions for the 48h *D. pulex* acute survival tests.**

Test initiation date and time	FEN: 4/10/2024; 1330h SCE: 4/10/2024; 1450h
Test termination date and time	FEN: 4/12/2024; 1315h SCE: 4/12/2024; 1425h
Test organism	<i>Daphnia pulex</i>
Test organism source	In-house cultures
Test organism age	< 24 hours
Test duration	48 hours
Feeding	YTC:algal suspension during org. holding time. No feeding during test.
Test chamber and test solution volume	30 mL plastic cup, 25 mL
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	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

### 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
Rainier Log-In No.	24-060	24-061
Collection date and time	4/10/2024; 0700h	4/10/2024; 0700h
Receipt date and time	4/10/2024; 1242h	4/10/2024; 1242h
Receipt temperature (°C)	1.7	1.7
Dissolved oxygen (mg/L)	9.3	8.9
pH	7.59	7.33
Conductivity (µS/cm)	618	615
Hardness (mg/L CaCO <sub>3</sub> )	84	92
Alkalinity (mg/L CaCO <sub>3</sub> )	156	100
Total Chlorine (mg/L)	<0.03	<0.03
Total Ammonia (mg/L)	27.3	20.1

Survival was evaluated in the acute toxicity tests after 48 hours of exposure. Results are summarized in Table 3. Mean survival in the 100 percent effluent concentration for sample FEN was 60 percent. Mean survival in the 100 percent effluent concentration for sample 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>Sample ID: FEN</b>				
<i>Daphnia pulex</i>	0.0	100	100	>100
	6.25	100		
	15.6	100		
	25	100		
	50	100		
	100	60		
	<b>Sample ID: SCE</b>			
<i>Daphnia pulex</i>	0.0	100	100	>100
	0.64	100		
	3.0	100		
	10	100		
	30	100		
	100	100		

<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. All water quality parameters remained within the ranges specified in the corresponding test methods 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> ( $\mu\text{g/L}$ copper)	Acceptable Range ( $\mu\text{g/L}$ copper)	CV (%)
<i>Daphnia pulex</i>	4/12/2024	96h survival	13.9	8.19 – 28.0	36.0

#### 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, pg. 53-54.
- 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**  
***Daphnia pulex* Acute Toxicity Test**  
**Statistical Summaries and Raw Bench Sheets**

**FEN - Lagoon System**

# CETIS Summary Report

Report Date: 18 Apr-24 09:46 (p 1 of 1)  
 Test Code: 2404-022 | 17-5957-5770

Daphnia pulex 48-h Acute Survival Test Rainier Environmental Laboratory

Batch ID: 20-3658-6934	Test Type: Survival (48h)	Analyst: Eric Tollefson
Start Date: 10 Apr-24 13:30	Protocol: EPA/821/R-02-012 (2002)	Diluent: Mod-Hard Synthetic Water
Ending Date: 12 Apr-24 13:15	Species: Daphnia pulex	Brine:
Duration: 48h	Source: In-House Culture	Age: <24h

Sample ID: 19-0425-3716	Code: 24-060	Client: Everett
Sample Date: 10 Apr-24 07:00	Material: POTW Effluent	Project:
Receive Date: 10 Apr-24 12:42	Source: Everett (WA0024490)	
Sample Age: 7h (1.7 °C)	Station: FEN Outfall 015	

### Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
09-1134-5548	48h Survival Rate	100	>100	NA	32.4%	1	Steel Many-One Rank Sum Test

### Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
09-1134-5548	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%
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	0.6	0.4171	0.7829	0	1	0.2449	0.4899	81.65%	40.0%

### 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
15.6		1	1	1	1
25		1	1	1	1
50		1	1	1	1
100		0	1	1	0.4

### 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
15.6		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		0/5	5/5	5/5	2/5

Freshwater Acute  
48 Hour Toxicity Test Data Sheet

Client: Everett  
 Sample ID: FEN  
 Test # 2404-022  
 Log-In # 24-060

Start Date & Time: 4/10/2024 1330  
 End Date & Time: 4/12/2024 1315  
 Test Organism: Daphnia pulex

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	5	5	5	5	8.8	8.2	8.5	8.04	8.01	7.92	309	311	311	20.5	19.9	19.9		
2		18	5	5	5														
3		1	5	5	5														
4		23	5	5	5														
1	0.25	16	5	5	5	8.4	8.2	8.6	7.95	7.94	7.93	385	327	322	20.5	20.1	19.9		
2		9	5	5	5														
3		22	5	5	5														
4		4	5	5	5														
1	15.6	10	5	5	5	8.5	8.0	8.6	7.98	7.90	7.87	350	354	261	20.4	20.2	19.9		
2		24	5	5	5														
3		7	5	5	5														
4		21	5	5	5														
1	25	12	5	5	5	8.3	1.8	8.4	7.93	1.86	7.90	373	386	375	20.4	20.2	19.9		
2		6	5	5	5														
3		20	5	5	5														
4		15	5	5	5														
1	50	2	5	5	5	8.4	1.8	8.5	7.75	1.80	7.85	466	469	467	20.2	20.2	19.9		
2		17	5	5	5														
3		14	5	5	5														
4		11	5	5	5														
1	100	19	5	5	5	8.5	1.8	8.4	7.54	1.74	7.81	633	637	633	19.9	20.0	19.9		
2		3	5	5	5														
3		8	5	5	5														
4		13	5	5	5														

Technician Initials gt gt gt gt

Dilution Water Batch #: M15W 040  
 Test Chamber: ROOM 2  
 Animal Source: In-house cultures  
 Date Received: \_\_\_\_\_  
 Sample Description: \_\_\_\_\_  
 QA Check: gt

Comments: 0 hrs:  
 24 hrs:  
 48 hrs:

Rainier Environmental  
 Washington Laboratory  
 5013 Pacific Hwy. E. Suite 20  
 Tacoma, WA 98424

**SCE - TE/SC System**

# CETIS Summary Report

Report Date: 18 Apr-24 09:52 (p 1 of 1)  
 Test Code: 2404-023 | 14-2731-6763

## Daphnia pulex 48-h Acute Survival Test

Rainier Environmental Laboratory

Batch ID: 20-1269-3548	Test Type: Survival (48h)	Analyst: Eric Tollefson
Start Date: 10 Apr-24 14:50	Protocol: EPA/821/R-02-012 (2002)	Diluent: Mod-Hard Synthetic Water
Ending Date: 12 Apr-24 14:25	Species: Daphnia pulex	Brine:
Duration: 48h	Source: In-House Culture	Age: <24h
Sample ID: 05-0837-0875	Code: 24-061	Client: Everett
Sample Date: 10 Apr-24 07:00	Material: POTW Effluent	Project:
Receive Date: 10 Apr-24 12:42	Source: Everett (WA0024490)	
Sample Age: 8h (1.7 °C)	Station: SCE Outfall 100	

## Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
10-8311-5889	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
10-8311-5889	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%
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	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
0.64		1	1	1	1
3		1	1	1	1
10		1	1	1	1
30		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
0.64		5/5	5/5	5/5	5/5
3		5/5	5/5	5/5	5/5
10		5/5	5/5	5/5	5/5
30		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: Everett  
 Sample ID: SCE  
 Test # 2464-023  
 Log-In # 24-061

Start Date & Time: 4/10/2024 1450  
 End Date & Time: 4/12/2024 1425  
 Test Organism: Daphnia pulex

Rep. #	Conc. or Cont.	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	19	5	5	5	8.6	8.0	8.2	8.04	8.00	7.95	311	319	320	20.4	19.9	19.9		
2		7	5	5	5														
3		17	5	5	5														
4		12	5	5	5														
1	0.64	20	5	5	5	8.4	8.1	8.4	7.94	7.98	7.95	305	304	310	20.4	20.1	19.9		
2		3	5	5	5														
3		14	5	5	5														
4		6	5	5	5														
1	3.0	9	5	5	5	8.3	8.1	8.4	7.8	7.97	7.92	307	306	308	20.6	20.1	19.9		
2		2	5	5	5														
3		21	5	5	5														
4		15	5	5	5														
1	10	10	5	5	5	8.2	8.1	8.2	7.77	7.95	7.91	330	334	332	20.6	20.0	19.9		
2		22	5	5	5														
3		4	5	5	5														
4		13	5	5	5														
1	30	9	5	5	5	8.3	7.8	8.0	7.69	7.82	7.87	317	314	318	20.2	20.0	19.9		
2		23	5	5	5														
3		1	5	5	5														
4		16	5	5	5														
1	100	18	5	5	5	8.4	7.7	7.9	7.38	7.54	7.71	314	322	325	19.9	19.9	19.9		
2		5	5	5	5														
3		24	5	5	5														
4		11	5	5	5														

Technician Initials: SL SL SL

Dilution Water Batch #: M5W 048  
 Test Chamber: DOOM 2  
 Animal Source: h-house cultures  
 Date Received:       
 Sample Description:       
 QA Check:     

Comments: 0 hrs:  
 24 hrs:  
 48 hrs:

**Appendix B**  
**Sample Check-In Sheets**

Client: CITY OF EVERETT

Tests Performed: DP-a X2  
Test ID No(s): 2404-023; 2404-023

Sample Description:

Sample ID:

Log-in No. (20-xxxx):

Sample Collection Date & Time:

Sample Receipt Date & Time:

Check-in Temperature (°C)

Temperature OK?

DO (mg/L)

pH (units)

Conductivity (µS/cm)

Salinity (ppt)

Tit. Vol / Sam. Vol. / Alkalinity (mg/L)\*

Tit. Vol. / Sam. Vol. / Hardness (mg/L)\*

Total Chlorine (mg/L)

Total Ammonia Nitrogen (mg/L)

Technician Initials

Sample ID:	<u>FEJ</u>	<u>SCE</u>			
Log-in No. (20-xxxx):	<u>24-060</u>	<u>24-061</u>			
Sample Collection Date & Time:	<u>4/10/24 0700</u>	<u>4/10/24 0700</u>			
Sample Receipt Date & Time:	<u>4/10/24 1242</u>	<u>4/10/24 1242</u>			
Check-in Temperature (°C)	<u>17</u>	<u>17</u>			
Temperature OK?	<u>(X) N</u>	<u>(X) N</u>	<u>Y</u>	<u>N</u>	<u>Y</u>
DO (mg/L)	<u>9.3</u>	<u>8.9</u>			
pH (units)	<u>7.89</u>	<u>7.33</u>			
Conductivity (µS/cm)	<u>618</u>	<u>615</u>			
Salinity (ppt)	<u>—</u>	<u>—</u>			
Tit. Vol / Sam. Vol. / Alkalinity (mg/L)*	<u>3.9 / 1.25 / 156</u>	<u>2.5 / 1.25 / 100</u>	<u>1</u>	<u>1</u>	<u>1</u>
Tit. Vol. / Sam. Vol. / Hardness (mg/L)*	<u>2.1 / 1.25 / 84</u>	<u>3.3 / 1.25 / 92</u>	<u>1</u>	<u>1</u>	<u>1</u>
Total Chlorine (mg/L)	<u>49.03</u>	<u>49.03</u>			
Total Ammonia Nitrogen (mg/L)	<u>27.3</u>	<u>20.1</u>			
Technician Initials	<u>df</u>	<u>df</u>			

\* = 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: DP-A 8:2 (DMW) Other: MHW -040  
 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.

COC Complete? Y or N  
1 2 3

Filtration? Y (X) N

Pore Size: —  
Organisms or Debris

Aeration? Y (X) N

Length of Time: —  
Final DO: —  
Final pH: —

Hardness Adjustment? Y (X) 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: City of Everett  
Address: 3200 Cedar St  
City/State/Zip: Everett, WA 98201  
Contact: Devek Kerlee  
Phone: 425-257-9220  
Email: D.Kerlee@EverettWA.gov

Invoice To:  
Company: \_\_\_\_\_  
Address: \_\_\_\_\_  
City/State/Zip: \_\_\_\_\_  
Contact: \_\_\_\_\_  
Phone: \_\_\_\_\_  
Email: \_\_\_\_\_

ANALYSES REQUIRED

Acute Daphnia Pulex									
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Receipt Temperature (°C)

SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	NO. OF CONTAINERS	COMMENTS
1 FEN	4/10/24	0700	WW	10L	1	
2 SCE	4/10/24	0700	WW	10L	1	
3						
4						
5						
6						
7						
8						
9						
10						

PROJECT INFORMATION		SAMPLE RECEIPT		RELINQUISHED BY (CLIENT)		RELINQUISHED BY (COURIER)	
Client:		Total No. of Containers:	2	Signature:	<i>[Signature]</i>	Signature:	<i>[Signature]</i>
PO No.:		Received Good Condition?	Y	Printed Name:	<i>[Name]</i>	Printed Name:	<i>[Name]</i>
Shipped Via:	<i>Unit</i>	Matches Test Schedule?	Y	Date:	4/10/24	Date:	4/10/24
SPECIAL INSTRUCTIONS/COMMENTS:		RECEIVED BY (COURIER)		RECEIVED BY (LABORATORY)		RECEIVED BY (LABORATORY)	
		Signature:		Signature:		Signature:	
		Printed Name:		Printed Name:		Printed Name:	
		Date:		Date:		Date:	
		Company:		Company:		Company:	
		RECEIVED BY (COURIER)		RECEIVED BY (LABORATORY)		RECEIVED BY (LABORATORY)	
		Signature:		Signature:		Signature:	
		Printed Name:		Printed Name:		Printed Name:	
		Date:		Date:		Date:	
		Company:		Company:		Company:	
		RECEIVED BY (COURIER)		RECEIVED BY (LABORATORY)		RECEIVED BY (LABORATORY)	
		Signature:		Signature:		Signature:	
		Printed Name:		Printed Name:		Printed Name:	
		Date:		Date:		Date:	
		Company:		Company:		Company:	