



Acute Toxicity Testing Results for City of Bremerton WWTP

Monitoring Period: January 2025

Prepared For: Luke McKinney
City of Bremerton
1600 Oyster Bay Ave. S
Bremerton, Washington 98312

Prepared By: Nautilus Environmental, Inc.

Date Submitted: February 27, 2025

Data Quality Assurance:

- Nautilus Environmental is a member of the IEH Laboratories & Consulting Group. IEH acquired the San Diego aquatic toxicology group from Enthalpy Analytical on December 7, 2024.
- Nautilus Environmental is accredited in accordance with NELAP by the State of Oregon Environmental Laboratory Accreditation Program (Lab ID 4053). It is also certified by the State of California Water Resources Control Board Environmental Laboratory Accreditation Program (Certificate No. 1802) and the State of Washington Department of Ecology (Lab ID C552).
- All data have been reviewed and verified.
- All test results have met minimum test acceptability criteria under their respective EPA protocols, unless otherwise noted in this report.
- All test results have met internal Quality Assurance Program requirements.

Results Verified by: _____

Barbara Orelo, Project Manager

Introduction

An acute toxicity test using the fathead minnow, *Pimephales promelas*, was conducted on an effluent sample collected from the City of Bremerton's Wastewater Treatment Plant in January 2025. Testing was performed at Nautilus Environmental, Inc. (Nautilus; formerly Enthalpy Analytical) located in San Diego, California, between January 28 and February 1, 2025.

Materials and Methods

Sample Collection, Transport, and Receipt

The effluent sample was collected in a low-density polyethylene cubitainer by City of Bremerton personnel. The sample was packed in a cooler containing ice and shipped to the laboratory by overnight delivery service. Appropriate chain-of-custody (COC) procedures were employed during collection and transport. Upon arrival at Nautilus, the cooler was opened, the sample inspected, and contents verified against information on the COC form. Receipt temperature was measured and recorded on the COC form. Standard water quality parameters were measured and recorded on a sample check-in sheet and are summarized in Table 1. The sample was stored in the dark at 4°C until used for testing.

Table 1. Sample Information

Sample ID	Plant Effluent
Lab Log-In No.	25-0098
Collection Date and Time	1/27/25; 08:00
Receipt Date and Time	1/28/25; 09:35
Receipt Temperature (°C)	2.1
Dissolved Oxygen (mg/L)	9.6
pH (units)	7.56
Conductivity (µS/cm)	6240
Salinity (ppt)	3.3
Alkalinity (mg/L CaCO ₃)	196
Hardness (mg/L CaCO ₃)	NM
Total Chlorine (mg/L)	0.03
Total Ammonia (mg/L as N)	30.9

NM = Not Measured

Test Methods

The acute toxicity test was conducted using fathead minnow according to procedures presented by USEPA (2002) and WDOE (2016) as summarized in Table 2.

Table 2. Summary of Methods for the 96h Fathead Minnow Acute Toxicity Test

Test Initiation Date and Time	1/28/25; 17:30
Test Termination Date and Time	2/1/25; 16:35
Test Organism	<i>Pimephales promelas</i> (fathead minnow)
Test Organism Source; Age	Aquatic BioSystems, Fort Collins, CO; 6 days
Test Duration	96 ± 2 hours
Feeding	<i>Artemia</i> nauplii during holding time and 2 hours prior to test solution renewal
Test Chamber	500 mL plastic cup
Test Solution Volume	250 mL
Test Temperature	20 ± 1°C
Dilution/Control Water	Moderately Hard Synthetic Water
Test Concentrations (% sample)	100, 50, 25, 12.5, 5.0 and 0 (laboratory control)
Number of Organisms/Chamber	10
Number of Replicates/Concentration	4
Photoperiod	16 hours light/8 hours dark
Test Protocol	EPA-821-R-02-012 and WQ-R-95-80
Additional Sample Treatments	Test chambers placed under 5% CO ₂ atmosphere to control pH drift for unionized ammonia suppression
Test Acceptability Criterion for Controls	≥ 90% mean survival
Statistical Analysis Software	CETIS™ v2.1.4.11
Reference Toxicant	Copper chloride

Statistical Analysis

Statistical analysis was conducted using USEPA flow chart specifications as outlined in the test guidance manual (USEPA 2002). Organism performance in the sample concentrations was compared to that observed in the lab control exposure. Results were used to calculate the No Observed Effect Concentration (NOEC) and mean lethal concentration (LC₅₀) values. Statistical analysis was performed using the Comprehensive Environmental Toxicity Information System™ (CETIS) by Tidepool Scientific Software, version 2.1.4.11.

Results

No statistically significant effects were observed in the acute critical effluent concentration (ACEC) of 5.0 percent sample, which exhibited 97.5 percent survival. Additionally, no statistically significant effects were observed in any other concentration, resulting in a NOEC of 100. Mean survival in the undiluted effluent was 92.5 percent, and the LC₅₀ was calculated as greater than 100 percent effluent. Results are summarized in Table 3. A statistical summary and copy of the laboratory bench sheet for the acute test can be found in Appendix A.

Table 3. Summary of Results for the Fathead Minnow Acute Toxicity Test

Concentration (% effluent)	Mean Survival (%)	NOEC (% effluent)	LOEC (% effluent)	LC ₅₀ (% effluent)
0.0 (Lab Control)	100	100	>100	>100
5	97.5			
12.5	95.0			
25	97.5			
50	97.5			
100	92.5			

NOEC = No Observed Effect Concentration

LOEC = Lowest Observed Effect Concentration

LC₅₀ = Effluent concentration estimated to produce mortality in 50 percent of the test organisms

Values in **bold** exhibited a statistically significant effect when compared to the lab control.

Quality Assurance

The sample was received within the appropriate temperature range specified by WDOE (2016), and the test was initiated within the required 36-hour sample holding time. The toxicity test met the acceptability criterion for survival of control organisms.

Statistical analysis followed standard USEPA flowchart selections, and the dose-response relationship was reviewed to ensure the validity of the data. Based on the dose response observed during testing, the calculated effect concentration is deemed reliable. The sample check-in sheet and COC form are provided in Appendices B and C, respectively. A list of qualifier codes is provided in Appendix D.

Reference Toxicant

Results for monthly reference toxicant testing used to monitor laboratory performance and test organism sensitivity are summarized in Table 4. The median effect value for the reference toxicant test fell within the acceptable range of the mean of historical test results plus or minus two standard deviations, indicating that the test organisms were as sensitive to copper as those historically tested. The coefficient of variation (CV) for the test is also provided in the table.

Table 4. Reference Toxicant Test Results

Species	Endpoint	Date Initiated	LC ₅₀ (µg/L copper)	Historical Mean ± 2 SD (µg/L copper)	CV (%)
Fathead Minnow	96h survival	1/28/25	68.5	73.3 ± 77.9	53.1

LC₅₀ = The effluent concentration estimated to produce mortality in 50 percent of the test organisms

Historical LC₅₀ ± 2 SD = The mean LC₅₀ from the previous 20 tests performed by the lab, plus or minus two standard deviations (SD)

CV = Coefficient of Variation

References

- Tidepool Scientific Software. 2000-2022. CETIS Comprehensive Environmental Toxicity Information System Software, Version 2.1.4.11.
- 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.
- WDOE. 2016. Whole Effluent Toxicity Testing Guidance and Test Review Criteria. Washington State Department of Ecology. Water Quality Program. Publication number: WQ-R-95-80, Revised June 2016.

Appendix A
Statistical Summary and Raw Bench Sheet

CETIS Summary Report

Report Date: 18 Feb-25 15:29 (p 1 of 1)
 Test Code/ID: 2501-S150 / 09-2052-4297

Fathead Minnow 96-h Acute Survival Test

Nautilus Environmental (CA)

Batch ID: 06-4145-0035	Test Type: Survival (96h)	Analyst:
Start Date: 28 Jan-25 17:30	Protocol: EPA/821/R-02-012 (2002)	Diluent: Mod-Hard Synthetic Water
Ending Date: 01 Feb-25 16:35	Species: Pimephales promelas	Brine: Not Applicable
Test Length: 95h	Taxon:	Source: Aquatic Biosystems, CO Age: 6d

Sample ID: 14-5022-6287	Code: 25-0098	Project:
Sample Date: 27 Jan-25 08:00	Material: POTW Effluent	Source: Bremerton WWTP (WA0029289)
Receipt Date: 28 Jan-25 09:35	CAS (PC):	Station: Plant Effluent
Sample Age: 33h (2.1 °C)	Client: Bremerton, City of	

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	TU	S
17-4017-6490	96h Survival Rate	Steel Many-One Rank Sum Test		100	>100	---	9.58%	1	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	%	95% LCL	95% UCL	TU	S
04-6258-3304	96h Survival Rate	Linear Interpolation (ICPIN)		EC50	>100	---	---	<1	1

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
04-6258-3304	96h Survival Rate	Control Resp	1	0.9	<<	Yes	Passes Criteria
17-4017-6490	96h Survival Rate	Control Resp	1	0.9	<<	Yes	Passes Criteria

96h Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LC	4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
5		4	0.975	0.895	1.050	0.900	1.000	0.025	0.050	5.13%	2.50%
12.5		4	0.950	0.858	1.040	0.900	1.000	0.029	0.058	6.08%	5.00%
25		4	0.975	0.895	1.050	0.900	1.000	0.025	0.050	5.13%	2.50%
50		4	0.975	0.895	1.050	0.900	1.000	0.025	0.050	5.13%	2.50%
100		4	0.925	0.773	1.080	0.800	1.000	0.048	0.096	10.35%	7.50%

96h Survival Rate Detail

MD5: 6BBC2807934AA7CF0D6160F1A5E7A37F

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LC	1.000	1.000	1.000	1.000
5		0.900	1.000	1.000	1.000
12.5		1.000	1.000	0.900	0.900
25		1.000	0.900	1.000	1.000
50		1.000	1.000	0.900	1.000
100		1.000	0.900	0.800	1.000

CETIS Analytical Report

Report Date: 17 Feb-25 09:59 (p 3 of 4)
Test Code/ID: 2501-S150 / 09-2052-4297

Fathead Minnow 96-h Acute Survival Test								Nautilus Environmental (CA)															
Analysis ID: 17-4017-6490		Endpoint: 96h Survival Rate			CETIS Version: CETISv2.1.4																		
Analyzed: 17 Feb-25 9:58		Analysis: Nonparametric-Control vs Treatments			Status Level: 1																		
Edit Date: 17 Feb-25 9:55		MD5 Hash: 6BBC2807934AA7CF0D6160F1A5E7A37F			Editor ID: 000-502-715-6																		
Data Transform		Alt Hyp		NOEL		LOEL		TOEL		Tox Units		MSDu		PMSD									
Angular (Corrected)		C > T		100		>100		---		1		0.0958		9.58%									
Steel Many-One Rank Sum Test																							
Control		vs		Conc-%		df		Test Stat		Critical		Ties		P-Type		P-Value		Decision(α:5%)					
Lab Control		5		6		16		10		1		CDF		0.6105		Non-Significant Effect							
		12.5		6		14		10		1		CDF		0.3451		Non-Significant Effect							
		25		6		16		10		1		CDF		0.6105		Non-Significant Effect							
		50		6		16		10		1		CDF		0.6105		Non-Significant Effect							
		100		6		14		10		1		CDF		0.3451		Non-Significant Effect							
ANOVA Table																							
Source		Sum Squares		Mean Square		DF		F Stat		P-Value		Decision(α:5%)											
Between		0.032643		0.0065286		5		0.778		0.5785		Non-Significant Effect											
Error		0.151103		0.0083946		18																	
Total		0.183746				23																	
ANOVA Assumptions Tests																							
Attribute		Test				Test Stat		Critical		P-Value		Decision(α:1%)											
Variance		Bartlett Equality of Variance Test										Indeterminate											
Distribution		Shapiro-Wilk W Normality Test				0.901		0.884		0.0224		Normal Distribution											
96h Survival Rate Summary																							
Conc-%		Code		Count		Mean		95% LCL		95% UCL		Median		Min		Max		Std Err		CV%		%Effect	
0		LC		4		1.000		1.000		1.000		1.000		1.000		1.000		0.000		0.00%		0.00%	
5				4		0.975		0.895		1.000		1.000		0.900		1.000		0.025		5.13%		2.50%	
12.5				4		0.950		0.858		1.000		0.950		0.900		1.000		0.029		6.08%		5.00%	
25				4		0.975		0.895		1.000		1.000		0.900		1.000		0.025		5.13%		2.50%	
50				4		0.975		0.895		1.000		1.000		0.900		1.000		0.025		5.13%		2.50%	
100				4		0.925		0.773		1.000		0.967		0.800		1.000		0.048		10.35%		7.50%	
Angular (Corrected) Transformed Summary																							
Conc-%		Code		Count		Mean		95% LCL		95% UCL		Median		Min		Max		Std Err		CV%		%Effect	
0		LC		4		1.410		1.410		1.410		1.410		1.410		1.410		0.000		0.00%		0.00%	
5				4		1.370		1.240		1.500		1.410		1.250		1.410		0.041		5.94%		2.89%	
12.5				4		1.330		1.180		1.480		1.330		1.250		1.410		0.047		7.07%		5.77%	
25				4		1.370		1.240		1.500		1.410		1.250		1.410		0.041		5.94%		2.89%	
50				4		1.370		1.240		1.500		1.410		1.250		1.410		0.041		5.94%		2.89%	
100				4		1.300		1.060		1.530		1.360		1.110		1.410		0.074		11.35%		8.28%	

CETIS Analytical Report

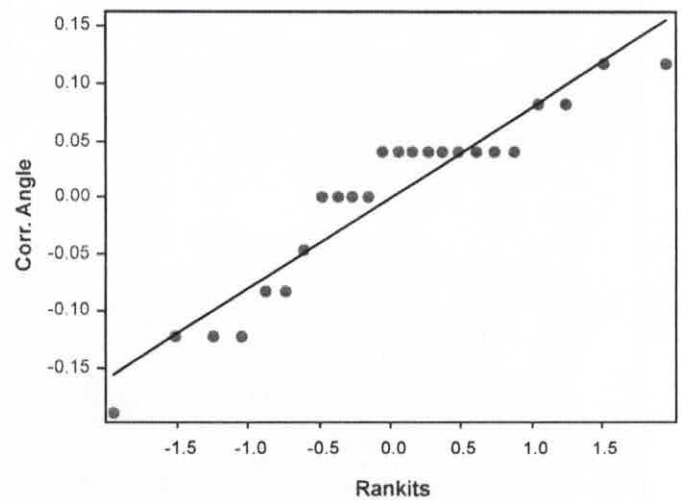
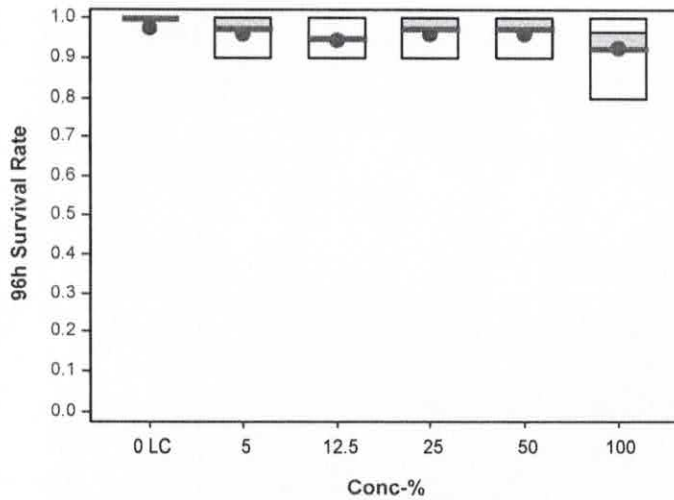
Report Date: 17 Feb-25 09:59 (p 4 of 4)
Test Code/ID: 2501-S150 / 09-2052-4297

Fathead Minnow 96-h Acute Survival Test

Nautilus Environmental (CA)

Analysis ID: 17-4017-6490	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.4
Analyzed: 17 Feb-25 9:58	Analysis: Nonparametric-Control vs Treatments	Status Level: 1
Edit Date: 17 Feb-25 9:55	MD5 Hash: 6BBC2807934AA7CF0D6160F1A5E7A37F	Editor ID: 000-502-715-6

Graphics



CETIS Analytical Report

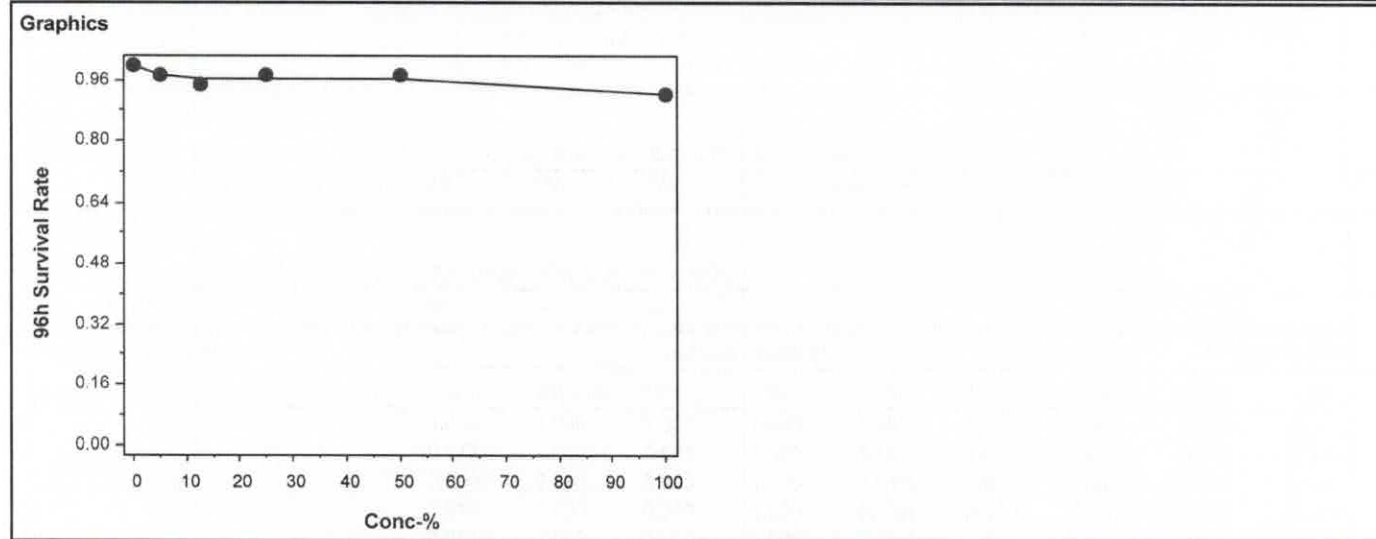
Report Date: 18 Feb-25 15:29 (p 1 of 1)
Test Code/ID: 2501-S150 / 09-2052-4297

Fathead Minnow 96-h Acute Survival Test						Nautilus Environmental (CA)					
Analysis ID:	04-6258-3304	Endpoint:	96h Survival Rate	CETIS Version:	CETISv2.1.4						
Analyzed:	18 Feb-25 15:28	Analysis:	Linear Interpolation (ICPIN)	Status Level:	1						
Edit Date:	17 Feb-25 9:55	MD5 Hash:	6BBC2807934AA7CF0D6160F1A5E7A37F	Editor ID:	000-502-715-6						

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1960969	1000	Yes	Two-Point Interpolation

Point Estimates						
Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
EC50	>100	---	---	<1	---	---

96h Survival Rate Summary			Calculated Variate(A/B)							Isotonic Variate	
Conc-%	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	LC	4	1.000	1.000	1.000	1.000	0.00%	0.00%	40/40	1.000	0.00%
5		4	0.975	1.000	0.900	1.000	5.13%	2.50%	39/40	0.975	2.50%
12.5		4	0.950	0.950	0.900	1.000	6.08%	5.00%	38/40	0.967	3.30%
25		4	0.975	1.000	0.900	1.000	5.13%	2.50%	39/40	0.967	3.30%
50		4	0.975	1.000	0.900	1.000	5.13%	2.50%	39/40	0.967	3.30%
100		4	0.925	0.967	0.800	1.000	10.35%	7.50%	37/40	0.925	7.50%



96-hour Freshwater Acute Bioassay
Static-Renewal Conditions
DF-006

Water Quality Measurements
& Test Organism Survival

Client: City of Bremerton

Test Species: P. promelas

Sample ID: Plant Effluent

Start Date/Time: 1/28/25 1730

Sample Log-In: 25-0098

End Date/Time: 2/1/25 1035

Test No.: 2501-S150

Tech Initials				
0	24	48	72	96
HH	AD	WF	WF	FM
HH	AD	WF	WF	FM
HH	AD	WF	WF	FM

Counts:

Readings:

Dilutions made by:

Concentration (%)	RAND #	Number of Live Organisms					pH (units)					Dissolved Oxygen (mg/L)					Conductivity (µmhos/cm)					Temperature (°C)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	1	10	10	10	10	10	7.40	7.31	7.53	7.52	7.45	8.9	7.4	8.6	7.7	8.3	294	306	245	307	312	20.8	20.8	21.0	20.8	20.9
(MHSW)	13	10	10	10	10	10			7.28					7.6					312						21.0	
	20	10	10	10	10	10																				
	2	10	10	10	10	10																				
5.0	4	10	9	9	9	9	7.40	7.33	7.54	7.79	7.45	8.9	7.3	8.6	7.8	8.1	537	535	591	619	629	20.9	20.7	21.0	20.7	20.7
	7	10	10	10	10	10			7.31					7.7					542						21.0	
	17	10	10	10	10	10																				
	3	10	10	10	10	10																				
12.5	16	10	10	10	10	10	7.41	7.39	7.59	7.40	7.51	8.9	7.2	8.6	7.6	8.0	101	991	1025	1100	1119	20.9	20.8	20.9	20.8	20.8
	19	10	10	10	10	10			7.30					7.4					949						20.9	
	21	10	9	9	9	9																				
	15	10	10	10	9	9																				
25	8	10	10	10	10	10	7.42	7.42	7.58	7.37	7.59	8.9	7.0	8.6	7.5	7.9	1766	1740	1740	1942	1975	21.0	20.7	20.8	20.6	20.7
	24	10	10	9	9	9			7.36					7.2					1753						20.9	
	22	10	10	10	10	10																				
	10	10	10	10	10	10																				
50	5	10	10	10	10	10	7.40	7.55	7.57	7.60	7.71	9.0	6.8	8.7	7.3	7.7	3160	3060	3150	3448	3510	21.0	20.8	20.8	20.9	20.9
	11	10	10	10	10	10			7.45					6.9					3070						20.9	
	18	10	10	10	10	9																				
	6	10	10	10	10	10																				
100	12	10	10	10	10	10	7.42	7.62	7.54	7.63	7.79	9.0	6.7	9.0	7.3	7.4	5180	5180	5939	6510	6600	21.0	20.6	20.7	20.7	20.7
	23	10	10	10	9	9			7.63					6.7					5810						20.9	
	14	10	10	10	9	8																				
	9	10	10	10	10	10																				

Rand # QC: HH

Initial Counts QC'd by: AD

Initiated by: HH

Environmental Chamber: C

Animal Source/Date Received: ABS 01/28/25

Age at Initiation: 6d

Animal Acclimation Qualifiers (circle all that apply):

Q22 / Q23 / Q24 / none

Comments:

i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal

Organisms fed prior to initiation, circle one (y / n) 018 WF 1/31/25

QC Check:

im 2/12/25

Final Review:

AS 2/18/25

Appendix B
Sample Receipt Information

Nautilus Environmental, Inc.
4340 Vandever Avenue
San Diego, CA 92120

Client: City of Bremerton
Sample ID: Plant Effluent
Test ID No(s): 2501-S150

NORTHWEST CLIENTS
Sample Check-In Information
DC-005

Sample (A, B, C):	A			
Log-in No. (25-xxxx):	0098			
Sample Collection Date & Time:	1/21/25 0800			
Sample Receipt Date & Time:	1/28/25 0935			
Number of Containers & Container Type:	1x 4L bag			
Approx. Total Volume Received (L):	4L			
Check-in Temperature (°C)	2.1			
Temperature OK? ¹	<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	<input type="radio"/> Y <input type="radio"/> N
DO (mg/L)	9.6			
pH (units)	7.56			
Conductivity (µS/cm)	6240			
Salinity (ppt)	3.3			
Alkalinity (mg/L) ²	196			
Hardness (mg/L) ^{2,3}	—			
Total Chlorine (mg/L)	0.03			
Technician Initials	DT			

Test Performed: Acute Fathead Minnow Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: MHSW
Alkalinity: 52 Hardness or Salinity: 85
Additional Control? ☐ Y ☒ N = _____ Alkalinity: _____ Hardness or Salinity: _____

Test Performed: _____ Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: _____
Alkalinity: _____ Hardness or Salinity: _____
Additional Control? ☐ Y ☐ N = _____ Alkalinity: _____ Hardness or Salinity: _____

Test Performed: _____ Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: _____
Alkalinity: _____ Hardness or Salinity: _____
Additional Control? ☐ Y ☐ N = _____ Alkalinity: _____ Hardness or Salinity: _____

Notes: ¹ Temperature of sample should be 0-6°C at receipt.

² mg/L as CaCO₃, ³ Measured for freshwater samples only, NA = Not Applicable

Additional Comments: Tested under CO₂ atmosphere to suppress ammonia

Sample Description:

A) light yellow, slightly opaque, no odor, light debris

Subsamples for Additional Chemistry Required:

NH3 (always required)

Other _____

Tech Initials A HW B C

COC Complete (Y/N)?

A Y B C

Filtration? ☐ Y ☒ N Initials: _____

Pore Size: _____

Organisms _____ or _____ Debris

Salinity Adjustment? ☐ Y ☒ N

Test: _____ Source: _____ Target ppt: _____

Test: _____ Source: _____ Target ppt: _____

Test: _____ Source: _____ Target ppt: _____

pH Adjustment? ☐ Y ☒ N

A B C

Initial pH:

Amount of HCl added:

Final pH:

Cl₂ Adjustment? ☐ Y ☒ N

A B C

Initial Free Cl₂:

STS added:

Final Free Cl₂:

Sample Aeration? ☐ Y ☒ N

A B C

Initial D.O.

Duration & Rate

Final D.O.

QC Check: GM 2/17/25

Final Review: ATS 2/18/25

DC-001

Client: City of Bremerton
Project: NPDES Toxicity Testing
Test Type: Acute fathead minnow

DI Blank: 0.0
Test Start Date: 1/28/2025

Analyst: SM
Analysis Date: 2/17/25

$$N \times 1.22$$

Nautilus Environmental, Inc. 4340 Vandever Avenue. San Diego, CA 92120.

Appendix C
Chain-of-Custody Form

Nautilus Environmental - Environmental Toxicology

4340 Vandever Avenue
San Diego, CA 92120
Phone 858.587.7333



NAUTILUS

ENVIRONMENTAL

Chain of Custody

Date 1/27/25 Page 1 of 1

Sample Collection By: <u>LM</u>							ANALYSES REQUIRED Enthalpy Matrix Codes: G = Grab C = Composite FW = Freshwater SW = Seawater Sed = Sediment STRM = Stormwater GW = Groundwater WW = Wastewater O = Other (specify)										Receipt Temperature (°C) 21			
Report to: Company <u>City of Bremerton</u> Address <u>1600 Oyster Bay Ave. S.</u> City/State/Zip <u>Bremerton WA 98312</u> Contact <u>Loke McKinney</u> Phone <u>360-473-5446</u> Email <u>Loke.McKinney@ci.bremerton.wa.us</u>				Invoice To: Same as Report to <input checked="" type="checkbox"/> Company _____ Address _____ City/State/Zip _____ Contact _____ Phone _____ Email _____			Pathhead bioassay													
SAMPLE ID	SAMPLE		MATRIX CODE (FW, SW, Sed, STRM, GW, WW, O)	Container		COMMENTS														
	Date	Time		Type (G or C)	Type		Qty													
1 <u>EFFluent</u>	<u>1/27/25</u>	<u>0800</u>	<u>C</u>	<u>WW</u>	<u>Cube</u>	<u>1</u>	<u>X</u>													
2																				
3																				
4																				
5																				
6																				
7																				
8																				
9																				
10																				
PROJECT INFORMATION			SAMPLE RECEIPT				1) RELINQUISHED BY (CLIENT)				2) RECEIVED BY (COURIER)									
Project Name:			Total No. of Containers		<u>1</u>		(Signature) <u>[Signature]</u>		(Time) <u>0850</u>		(Signature) _____		(Time) _____							
PO No.:			Received Good Condition?		<u>Y</u>		(Printed Name) <u>Loke McKinney</u>		(Date) <u>1/27/25</u>		(Printed Name) _____		(Date) _____							
Shipped Via: <u>Fedex</u>			Matches Test Schedule?		<u>Y</u>		(Company) _____				(Company) _____									
SPECIAL INSTRUCTIONS/COMMENTS:							3) RELINQUISHED BY (COURIER)				4) RECEIVED BY (LABORATORY)									
							(Signature) _____		(Time) _____		(Signature) <u>[Signature]</u>		(Time) <u>0935</u>							
							(Printed Name) _____		(Date) _____		(Printed Name) <u>Delany Torres</u>		(Date) <u>1/28/25</u>							
							(Company) _____				(Company) <u>Nautilus</u>		(Log-in #s) <u>25-0098</u>							

Additional costs may be required for sample disposal or storage. Payment net 30 unless otherwise contracted.
 Shaded areas are for lab use only
 Report turn-around-time varies depending on length of test; please inquire with your project manager.

Appendix D
Qualifier Codes

Glossary of Qualifier Codes:

- Q1 - Temperature out of recommended range; corrective action taken and recorded in Test Temperature Correction Log
- Q2 - Temperature out of recommended range; no action taken, test terminated same day
- Q3 - Sample pH adjusted to within range of 6-9 with reagent grade NaOH or HCl, as needed
- Q4 - Test aerated; D.O. levels dropped below 4.0 mg/L
- Q5 - Test initiated with continuous aeration due to an anticipated drop in D.O.
- Q6 - Airline obstructed or fell out of replicate and replaced; drop in D.O. occurred
- Q7 - Salinity out of recommended range
- Q8 - Spilled test chamber/ Unable to recover test organism(s)
- Q9 - Inadequate sample volume remaining, partial renewal performed
- Q10 - Inadequate sample volume remaining, no renewal performed
- Q11 - Sample out of holding time; refer to QA section of report
- Q12 - Replicate(s) not initiated; excluded from data analysis
- Q13 - Survival counts not recorded due to poor visibility or heavy debris
- Q14 - D.O. percent saturation was checked and was $\leq 110\%$
- Q15 - Did not meet minimum test acceptability criteria. Refer to QA section of report.
- Q16 - Percent minimum significant difference (PMSD) was below the lower bound limit for acceptability. This indicates that statistics may be over-sensitive in detecting a difference from the control due to low variability in the data set. Test results were reviewed and reported in accordance with guidance found in EPA-833-R-00-003, 2000 unless otherwise specified.
- Q17 - Percent minimum significant difference (PMSD) was above the upper bound limit for acceptability. This indicates that statistics may be under-sensitive in detecting a difference from the control due to high variability in the data set. Test results were reviewed and reported in accordance with EPA-833-R-00-003, 2000 guidance unless otherwise specified.
- Q18 - Incorrect or illegible Entry
- Q19 - Miscalculation
- Q20 - PMSD criteria do not apply to test of significant toxicity (TST) analysis
- Q21 - Other (provide reason in comments section)
- Q22 - Greater than 10% batch mortality observed upon receipt and/or in holding prior to test initiation. Organisms acclimated to test conditions at Enthelphy and ultimately deemed fit to use for testing.
- Q23 - Test organisms experienced a temperature shift greater than 3°C in holding or were received at a temperature greater than 3°C outside the recommended test temperature range and had minimal time to acclimate prior to test initiation. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate test(s). Organisms were ultimately deemed fit to use for testing.
- Q24 - Test organisms experienced a salinity shift greater than 3 ppt in holding or were received at a salinity greater than 3 ppt outside the recommended test salinity range and had minimal time to acclimate prior to test initiation. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate test(s). Organisms were ultimately deemed fit to use for testing.