

Treated Wastewater Discharge

Whole Effluent Toxicity Testing, Second Quarter 2026

Citizens for Safe Water and Air - Port Townsend

PORT TOWNSEND, WA

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1. Overview and Purpose

This document summarizes a Whole Effluent Toxicity (WET) test performed on treated wastewater discharged by Port Townsend Paper Company (PTPC). The testing laboratory, Spheros Environmental of Port Gamble, Washington, conducted the work at the request of PTPC as part of the effluent characterization required under the facility's National Pollutant Discharge Elimination System (NPDES) permit, permit number WA000092. Spheros Environmental's report is identified as Report ID PG2398.01 and was submitted June 1, 2026, covering the second quarter of 2026. The report's author and Project Manager is Michelle Bennett; its quality assurance review was performed by Dani Mulligan [1].

Whole Effluent Toxicity testing exposes live aquatic test organisms directly to samples of the facility's treated wastewater, at several dilution levels, to measure whether the effluent causes acute toxicity, meaning short-term lethal or sublethal harm, under laboratory conditions. It is a direct biological test rather than a chemical concentration measurement: the organisms themselves are the instrument. The objective stated in the report is to assess the potential toxicity of the effluent to aquatic organisms, following procedures defined under the facility's National Pollutant Discharge Elimination System (NPDES) permit [1].

Two species were tested: the water flea (*Ceriodaphnia dubia*) and the fathead minnow (*Pimephales promelas*). Each was exposed to the effluent at five concentrations, 1.9 percent, 2.2 percent, 4.4 percent, 25 percent, and 100 percent effluent, alongside an unexposed laboratory control [1]. The 2.2 percent concentration is significant because it is defined in the facility's National Pollutant Discharge Elimination System (NPDES) permit as the acute critical effluent concentration (ACEC), the specific dilution level the permit uses as the regulatory threshold for compliance [1]. This 2.2 percent dilution reflects the permit's regulatory mixing zone calculation for how the effluent is diluted in Port Townsend Bay after discharge, not the undiluted concentration of the effluent itself.

Summary of Results

For both species tested, the report states that a statistically significant difference between the laboratory control and the acute critical effluent concentration (ACEC), 2.2 percent effluent, was not detected for the acute endpoints, and that the sample does not exceed the defined permit requirements for the acute endpoints [1]. This is a narrow, specific finding: it means the effluent sample, at the permit's regulatory dilution level, did not produce statistically distinguishable short-term mortality in the two test species compared to the laboratory control, under the specific conditions of this quarter's test.

It is not a broader statement about the chemical composition of the effluent or about its potential for chronic, sublethal, or long-term effects, which this test was not designed to measure.

Test Organism	Endpoint	NOEC (%)	LOEC (%)	LC (%)
Water flea (Ceriodaphnia dubia)	48-hour survival	100	>100	>100
Fathead minnow (Pimephales promelas)	96-hour survival	100	>100	>100

Table 1. Toxicity test results summary, as reported [1]. NOEC is the No Observed Effect Concentration, the highest tested concentration producing no statistically significant effect. LOEC is the Lowest Observed Effect Concentration, the lowest tested concentration producing a statistically significant effect. LC is the Lethal Concentration, the concentration causing a defined percentage of test-organism mortality. A result of ">100" means the effect was not reached at any concentration tested, up to and including full-strength, undiluted effluent.

- Permit requirement: conduct acute toxicity testing under the conditions of the National Pollutant Discharge Elimination System (NPDES) permit. The report notes this particular testing event was ordered by the Washington State Department of Ecology outside the permit's normal testing timeline [1].
- Result: a statistically significant difference between the laboratory control and the ACEC (2.2 percent effluent) was not detected for the acute endpoints. The sample meets permit compliance for the acute endpoints [1].

2. Methods

The samples were tested using criteria set out in the Washington State Department of Ecology's Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria, publication WQ-R-95-80 (2016), further defined through the United States Environmental Protection Agency (EPA)'s most recently promulgated effluent guidance documents [1][3][4].

Bioassay Testing

The project consisted of two acute bioassays, summarized below.

Test Type	Species	Descriptor	Method
Acute, 48-hour survival	Ceriodaphnia dubia (water flea)	WDOE WQ-R-95-80 (2016)	EPA-821-R-02-012, Method 2002.0; SOP TOX004.13
Acute, 96-hour survival	Pimephales promelas (fathead minnow)	WDOE WQ-R-95-80 (2016)	EPA-821-R-02-012, Method 2000.0; SOP TOX017.10

Standard operating procedure (SOP) references above are Spheros Environmental's own internal laboratory procedures for each test method.

Sample Collection and Receipt

Collecting a valid sample for this quarter's test required four separate attempts. PTPC personnel collected the first sample on May 6, 2026, but it was delivered to the laboratory out of the required temperature range of 0 to 6 degrees Celsius, and could not be used [1]. A second sample was collected May 7, 2026, and picked up by Spheros Environmental laboratory staff the same day [1]. A laboratory error then compromised the entire *Ceriodaphnia dubia* test run on that sample, so a third sample was collected May 13, 2026, also picked up the day of collection [1].

That sample failed a test acceptability criterion for *Ceriodaphnia dubia*, requiring a fourth sample collection on May 20, 2026, again picked up the same day [1]. All effluent samples were held in a walk-in cold room at 4 degrees Celsius, plus or minus 2 degrees, in the dark until used for testing [1].

The May 7, 2026 sample (laboratory ID P260507.01) was ultimately used for the fathead minnow test, and the May 20, 2026 sample (laboratory ID P260520.01) was used for the water flea test [1].

Sample (Collected)	May 6, 2026	May 7, 2026	May 13, 2026	May 20, 2026
Laboratory ID	P260506.02	P260507.01	P260513.01	P260520.01
Date/Time Received	5/6/26, 13:30	5/7/26, 07:55	5/13/26, 08:33	5/20/26, 08:00
Dissolved Oxygen (mg/L)	7.4	5.9	6.9	8.7
Temperature Upon Receipt (deg C)	6.8	6.6	3.8	4.4
pH (units)	7.5	7.8	7.6	7.5
Conductivity (uS/cm)	1387	1186	1337	1569
Hardness (mg/L CaCO3)	446	368	380	390
Alkalinity (mg/L CaCO3)	177	202	260	151
Total Chlorine (mg/L)	<0.02	<0.02	0.02	<0.02
Total Ammonia (mg/L)	6.66	6.37	8.28	4.43

Table 2. Sample conditions upon receipt for all four collection attempts [1]. Recommended ranges per the report: dissolved oxygen greater than 4.0 mg/L; temperature upon receipt 0 to 6 degrees Celsius (if 4 or more hours elapsed since collection) or 0 to 12 degrees Celsius (if less than 4 hours elapsed); pH 6 to 9 units. The May 6, 2026 sample's 6.8 degree Celsius receipt temperature is the reading the report cites as the reason that sample was rejected as out of range.

Water Used for Bioassay Testing

The freshwater diluent used for the tests was prepared using an United States Environmental Protection Agency (EPA) method for standard, synthetic, moderately hard, reconstituted water, using reagent-grade chemicals (cited in the report as USEPA 2002a) [1][5]. The report states that extensive testing on a variety of test species has shown no significant potential for toxicity or bioaccumulation of contaminants from this water supply, and that Spheros Environmental conducts and reviews a chemical analysis of this water source annually [1].

Sample Adjustment

The sample used in the tests did not require adjustment prior to analysis [1].

Data Management and Analysis

Endpoint data were calculated for each replicate, with mean value and standard deviation determined for each test treatment. All hand-entered data were reviewed for entry errors, which were corrected before summary calculations, and a minimum of 10 percent of all calculations and data sorting were separately reviewed for errors, with review counts conducted on any apparent outliers. Statistical comparisons were made according to United States Environmental Protection Agency (EPA) guidance and performed using CETIS software, cited in the report's own body text as "CETIS 2022" [1][2].

Quality Assurance and Quality Control

The laboratory's quality assurance objectives for accuracy and precision are detailed in method-specific guidance documents and the laboratory's own quality manual (QM), and cover the source and condition of test organisms, condition of equipment, test conditions, instrument calibration, use of reference toxicants, record keeping, and data evaluation [1].

Each batch of test organisms was evaluated in a reference toxicant test, run within a month of the testing period, to establish the sensitivity of the test organisms; the reference toxicant Lethal Concentration or Effect Concentration is expected to fall within two standard deviations of the historical laboratory mean [1]. Water quality measurements were monitored to ensure they fell within prescribed limits, and the methods used in every phase of the program are detailed in Spheros Environmental's own Standard Operating Procedures, in which all staff receive regular, documented training [1].

All data collected were recorded on approved data sheets, and any deviation from protocol was evaluated to determine whether the affected test remained valid according to the regulatory agencies responsible for approving the permitting action in question [1].

3. Detailed Test Results

Water Flea (*Ceriodaphnia dubia*) 48-Hour Acute Test

The acute toxicity test with *Ceriodaphnia dubia* was initiated May 20, 2026, using sample P260520.01. The test met its survival acceptability criterion, and water quality parameters were within acceptable limits throughout the 48-hour static test. The reference toxicant test results were within two standard deviations of the laboratory's historical mean for survival, indicating the test organisms were of similar sensitivity to those previously tested at the Spheros Environmental laboratory [1].

Concentration (%)	Mean Survival (%)	Standard Deviation
Control (0)	100	0
1.9	100	0
2.2 (ACEC)	100	0
4.4	100	0
25	100	0
100	95	10

Table 3. Endpoint summary for the *Ceriodaphnia dubia* acute test, sample P260520.01 [1]. Overall test endpoints: NOEC 100 percent, LOEC greater than 100 percent, LC greater than 100 percent.

Permit compliance: under the National Pollutant Discharge Elimination System (NPDES) permit and Washington State Department of Ecology requirements, the permittee must conduct acute toxicity testing using a series of dilutions that includes the ACEC, equal to 2.2 percent effluent. No statistically significant difference between the laboratory control and the ACEC (2.2 percent) was shown [1].

Test duration / type	48-hour / static
Supplier	In-house culture
Test dates	5/20/26 to 5/22/26
Age at test initiation	Less than 24 hours (recommended: less than 24 hours)
Sample holding time at initiation	5 hours (recommended: less than 36 hours; not to exceed 72 hours)
Test location	Spheros Environmental, Port Gamble, WA
Control water / diluent	Cerio Reconstituted Freshwater (CRFW)
Test lighting	16-hour light / 8-hour dark
Test chamber	30-mL plastic chamber; 15 mL exposure volume
Replicates per treatment	4, with 5 organisms per replicate
Feeding / renewal	None
Test dissolved oxygen	5.6 to 8.8 mg/L (recommended: greater than 4.0 mg/L)
Test temperature	20.2 to 20.7 deg C (recommended: 20 plus or minus 1 deg C)
Test conductivity	368 to 1548 uS/cm
Test pH	7.6 to 8.3 units (targeted range: 6 to 9 units)
Control survival	100%; meets acceptability criterion (recommended: 90% or greater)
Power standard	0%; meets criterion (standard: 29% or less)
Reference toxicant date	5/13/26
Reference toxicant LC	9.33 ug Cu/L
Laboratory mean LC range (plus or minus 2 SD)	10.8 ug Cu/L (5.39 to 21.4 ug Cu/L)
Deviations from test protocol	None

Table 4. Test condition summary for the Ceriodaphnia dubia acute test [1].

Fathead Minnow (*Pimephales promelas*) 96-Hour Acute Test

The acute toxicity test with *Pimephales promelas* was initiated May 7, 2026, using sample P260507.01. The test met its control acceptability criterion. On day 1, measured dissolved oxygen fell below the test's recommended range; the report gives this recommended range as 0 to 4 degrees Celsius, though dissolved oxygen is conventionally measured in mg/L rather than degrees Celsius, and this figure is reproduced here exactly as it appears in the underlying report rather than corrected. The test chambers

were aerated and remained within the recommended range for the rest of the test. Other than this noted deviation, water quality parameters were within acceptable limits throughout the 96-hour static-renewal test.

The reference toxicant test results were within two standard deviations of the laboratory mean for survival at the time of testing, indicating the organisms used were of normal sensitivity to those previously tested at the Spheros Environmental laboratory [1].

Concentration (%)	Mean Survival (%)	Standard Deviation
Control (0)	100	0.0
1.9	100	0.0
2.2 (ACEC)	97.5	5.0
4.4	100	0.0
25	100	0.0
100	92.5	9.6

Table 5. Endpoint summary for the *Pimephales promelas* acute test, sample P260507.01 [1]. Overall test endpoints: NOEC 100 percent, LOEC greater than 100 percent, LC greater than 100 percent.

Permit compliance: under the National Pollutant Discharge Elimination System (NPDES) permit and Washington State Department of Ecology requirements, the permittee must conduct acute toxicity testing using a series of dilutions that includes the ACEC, equal to 2.2 percent effluent. No statistically significant difference between the laboratory control and the ACEC (2.2 percent) was shown [1].

Test duration / type	96-hour / static renewal
Supplier	Spheros Environmental, Florida; acquired 5/6/26
Test dates	5/7/26 to 5/11/26
Age at test initiation	10 days (recommended: 1 to 14 days)
Sample holding time at initiation	7 hours (recommended: less than 36 hours; not to exceed 72 hours)
Test location	Spheros Environmental laboratory
Control water / diluent	Reconstituted Fresh Water (RFW)
Test lighting	16-hour light / 8-hour dark
Test chamber	12 oz. plastic chamber; 250 mL exposure volume
Replicates per treatment	4, with 10 organisms per replicate
Feeding	0.2 mL concentrated <i>Artemia</i> nauplii, two hours prior to renewal
Test solution renewal	Day 2
Test dissolved oxygen	0.9 to 9.3 mg/L (recommended: greater than 2.0 mg/L)
Test temperature	19.8 to 20.9 deg C (recommended: 20 plus or minus 1 deg C)

Test conductivity	285 to 1368 uS/cm
Test pH	7.5 to 8.4 units (targeted range: 6 to 9 units)
Control survival	100%; meets acceptability criterion (recommended: 90% or greater)
Power standard	3%; meets criterion (standard: 29% or less)
Reference toxicant date	5/7/26
Reference toxicant LC	253 ug/L copper
Laboratory mean LC range (plus or minus 2 SD)	162 ug/L copper (39.3 to 671 ug/L copper)
Deviations from test protocol	Dissolved oxygen (see day 1 note above)

Table 6. Test condition summary for the *Pimephales promelas* acute test [1].

4. Supporting Laboratory Documentation

The full underlying report includes two appendices of bench-level laboratory records, which this summary condenses to their reported results rather than reproducing in full. Appendix A contains statistical comparisons and laboratory documents; Appendix B contains chain-of-custody and sample receipt forms [1].

Appendix A's power standard calculations, the statistical backup for the survival results above, show a control mean of 5 organisms surviving versus an ACEC mean of 5 for *Ceriodaphnia dubia*, a difference of 0, expressed as 0 percent, which is well within the laboratory's 29 percent or less power standard threshold. For *Pimephales promelas*, the control mean was 10 organisms surviving versus an ACEC mean of 9.75, a difference of 0.25, expressed as 3 percent, also within the 29 percent threshold [1]. Both calculations are marked as a pass in the underlying report.

Appendix A also includes reference toxicant data sheets, recording organism survival at each copper concentration in a serial dilution series, for both species [1].

The report defines a standard set of error codes used on its laboratory bench data sheets, reproduced here for completeness:

- CA: called away, task completed by another technician
- DC: test solution too turbid or too dark to count organisms
- FB: found body, an animal found that was previously noted as missing
- IE: incorrect entry
- IW: illegible writing
- MC: miscount
- MR: meter reading changed, or meter not ready
- NB: no body, meaning no organism found

- SM: stray mark
- WC: wrong cell, an incorrect data box used
- WD: wrong date, an incorrect date entered
- WN: wrong number, an incorrect number entered
- WP: wrong page, an incorrect data sheet
- WT: wrong time, an incorrect time entered

Appendix B's chain-of-custody records include a handwritten note reading, "Sample not used due to being out of temperature range," dated May 29, with a temperature reading of 6.6 degrees Celsius, associated with sample ID P260507.01 [1]. This appears inconsistent with Section 2's account, which identifies the May 6, 2026 sample, not the May 7, 2026 sample bearing ID P260507.01, as the one rejected for being out of temperature range; that discrepancy exists in the underlying report and is not resolved here.

Sources and Citations

All factual claims in this document are drawn from the following sources, verified during research for this document.

1. Spheros Environmental: Whole Effluent Toxicity Testing: Q2 2026, prepared for Port Townsend Paper Company, 100 Mill Road, Port Townsend, WA 98363. Spheros Environmental, 4770 NE View Drive, PO Box 216, Port Gamble, WA 98364. National Pollutant Discharge Elimination System Permit # WA000092. Spheros Environmental Report ID PG2398.01. Submittal date June 1, 2026. Report author and Project Manager: Michelle Bennett. Quality assurance review: Dani Mulligan.
2. CETIS. 2025 CETIS Comprehensive Environmental Toxicity Information System User's Guide. Tidepool Scientific Software, McKinleyville, California. (The Q2 2026 report's own body text describes its statistical software specifically as "CETIS 2022" while its formal reference list cites the 2025 user's guide edition; both citations are preserved here exactly as they appear in the underlying report.)
3. United States Environmental Protection Agency. 2002b. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. EPA-821-R-02-012.
4. Washington State Department of Ecology. 2016. Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria. Water Quality Program. Publication number WQ-R-95-80, revised June 2016.
5. The Q2 2026 report also references a separate United States Environmental Protection Agency method, cited in its own text only as "USEPA 2002a," governing preparation of the standard reconstituted dilution water used in testing. The report's own formal reference list does not include a full citation for this second 2002 method; it is flagged here rather than invented.