

**Standard Operating Procedure (SOP)<sup>1</sup> for Clean Water Program  
Whole Effluent Toxicity (WET)  
SOP No. BCW-PMT-031  
Final, November 9, 2012  
Revised, November 14, 2025  
Version 1.5**

This SOP describes the methods by which the Clean Water Program will conduct reviews of WET test reports and determine NPDES permit requirements for WET. This SOP applies only to any facility that currently conducts WET testing or should begin conducting testing. This SOP is referred to within the SOPs for New and Reissuance Industrial Waste and Industrial Stormwater Individual NPDES Permit Applications (BPNPSM-PMT-001) and New and Reissuance Sewage Individual NPDES Permit Applications (BPNPSM-PMT-002). **Attachment A** provides definitions of terms used in this SOP. These definitions are provided only to facilitate understanding of the approaches taken in this SOP, and are used solely to describe DEP's WET program.

**I. Permit Application Reviews (Application Managers)**

- A. Application managers will determine reasonable potential (RP) for effluents to cause toxicity as follows when a permit renewal application includes the results of at least four WET tests:
1. If the existing permit does not contain the language referenced in paragraph B of this section, application managers will evaluate the four most recent WET test reports by comparing the statistically determined "LC<sub>50</sub>" or "IC<sub>25</sub>" concentrations (for acute tests) or the "NOEC" concentration (for chronic tests) to the target in-stream waste concentration (TIWC) for the discharge. A minimum of 8 endpoints should be evaluated for acute tests (two species, survival endpoints) and a minimum of 16 endpoints should be evaluated for chronic tests (two species, growth, reproduction and survival endpoints). If the LC<sub>50</sub>, IC<sub>25</sub> or NOEC concentrations for any endpoint is less than the TIWC, reasonable potential will have been demonstrated, and WET limitations will generally be established in the reissued permit unless the application manager can document, in the fact sheet, that either of the following conditions apply:
    - a. There is one endpoint failure in four consecutive tests, however, (1) the application manager can document there is no history of endpoint failures in the five years prior to the WET tests under review, (2) no significant changes have occurred at the facility (e.g., no increased influent pollutant loads) in that time, and (3) use of DEP's *WET Analysis Spreadsheet* determines that the test would be considered a passing result using EPA's Test of Significant Toxicity (TST) statistical approach. Under these circumstances, the application manager may assume that the sole failure is a false positive.

**NOTE** – For permit renewal applications in which the permit language referenced in paragraph B is not in the existing permit, application managers should generally not review WET results using the *WET Analysis Spreadsheet* where the results are considered "not toxic" under the conventional data analysis method (i.e., LC<sub>50</sub>, IC<sub>25</sub> or NOEC).

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<sup>1</sup> **DISCLAIMER:** The process and procedures outlined in this SOP are intended to supplement existing requirements. Nothing in the SOP shall affect regulatory requirements. The process, procedures and interpretations herein are not an adjudication or a regulation. There is no intent on the part of DEP to give the rules in this SOP that weight or deference. This document establishes the framework within which DEP will exercise its administrative discretion in the future. DEP reserves the discretion to deviate from this policy statement if circumstances warrant.

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- b. Review of the application determines that reasonable potential exists for one or more toxic pollutants, which have not been limited in the existing NPDES permit. If the application manager believes that establishing limitations for such pollutant(s) will control the toxicity, the application manager may postpone the inclusion of WET limitations until further WET data are reviewed.
  2. For permit renewals where the existing permit language conforms to paragraph B of this section, application managers will evaluate reasonable potential based on DEP's *WET Analysis Spreadsheet* using the last 4 consecutive WET test results, and will generally establish WET limitations in reissued permits when reasonable potential is indicated by DEP's *WET Analysis Spreadsheet* unless the criteria in paragraphs A.1.a (conditions (1) and (2)) and/or A.1.b are met.
  3. If WET limitations are required, the species with endpoints that failed within the four consecutive tests will receive limitations in the form of Toxicity Units (TU<sub>a</sub> or TU<sub>c</sub>). WET tests must be completed for both species, but if one had failure(s) and the other did not, only the species with failure(s) require a permit limit.
  4. Where it is determined that acute testing is required, the permit limit should be expressed as 1/TIWC<sub>a</sub>, and where chronic testing is required, the permit limit should be expressed as 1/TIWC<sub>c</sub>, as Maximum Daily Limits (MDLs). Note that MDLs will be used rather than average monthly limits (AMLs) because of the expectation that WET monitoring will not occur at a frequency of 1/month or more frequent.
  5. Permit limits will be specified in effluent limits tables in Part A of the permit, where the endpoints are identified in the name of the parameter. For example, if limits will be imposed on *Pimephales promelas* for chronic WET, the parameters that will be specified in Part A of the permit with the calculated limit will be:  
  
Chronic Toxicity – *Pimephales* Survival; and  
Chronic Toxicity – *Pimephales* Growth.  
  
The limits should also be specified on the Discharge Monitoring Report (DMR) accompanying the permit, for ongoing compliance assessment.  
  
The sample type should be “24-hour composite” and the sample frequency should be “See Permit” (since the frequency of WET testing may vary throughout the permit term).
  6. Where there is no WET limit, WET parameters should not be identified in Part A of the permit nor on the DMR.
- B. The application manager will determine the appropriate Part C permit language to use in the reissued permit pertaining to WET.
1. If WET limits will not be established in the permit, the application manager should use the Part C condition in WMS named “Part C 114” (see **Attachment B**).
  2. If WET limits will be established in the permit, the application manager should use the Part C condition in WMS named “Part C 115” (see **Attachment C**).
- C. In general, the application manager will determine the type of WET tests to be completed for new or renewed permits as follows:
1. If RP has been determined for WET and WET limits will be established in the permit, application managers will generally specify the type of test used as part of the RP evaluation in the renewed permit.
  2. For new permits and renewed permits where WET limits will not be established, acute tests will be specified when the In-Stream Waste Concentration – acute (IWC<sub>a</sub>) is less than 1% (i.e., where dilution

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is greater than 100:1 at the edge of the “mixing zone” following 15 minutes) and chronic tests will be specified when the IWC<sub>a</sub> is greater than or equal to 1%.

**NOTE** – For permit renewals, the *WET Analysis Spreadsheet* will automatically evaluate Steps 1 and 2 and make a recommendation on the type of test for the permit based on these criteria.

3. For discharges to lakes and impoundments, it is recommended that a Target In-Stream Waste Concentration (TIWC) value of 10% be assumed unless there is a technical basis for a different value that is documented in the fact sheet. At a TIWC of 10%, chronic tests would be performed.

**NOTE** – For discharges to the tidal portion of the Delaware River, the application manager may need to consider DRBC’s dilution factor in calculating the IWC<sub>a</sub> and determining the type of test to specify in the permit.

4. Application managers will not generally require performance of both acute and chronic tests unless there are unique circumstances that are documented in the fact sheet.
- D. Once the type of test is determined for the permit, the application manager will determine the dilution series to be used for the permit by 1) calculating the TIWC<sub>c</sub> (for chronic tests) or TIWC<sub>a</sub> (for acute tests) and 2) determining the dilution series to be used for the reissued permit by using the TIWC value to look up the appropriate series in **Attachment D** of this SOP.

**NOTE** – The *WET Analysis Spreadsheet* will determine dilution series automatically upon entry of discharge and stream flow data.

- E. For facilities that do not conduct WET testing in their existing permit, the application manager will consider the criteria below to determine whether to establish new WET monitoring requirements in reissued permits.
1. Major sewage facilities with an average annual design flow greater than or equal to 1.0 MGD (25 Pa. Code § 92a.27(a)(1)(i)).
  2. Sewage facilities with EPA-approved pretreatment programs or will be required in the permit to develop a program (25 Pa. Code § 92a.27(a)(1)(i)).
  3. Other facilities that are considered candidates for WET testing by one or more of the factors contained in 25 Pa. Code § 92a.27(a)(2).

## **II. WET Test Report Reviews During the Permit Term (Application Managers or Biologists)**

Application managers or biologists may review WETT test reports submitted during the permit review, at their discretion. If a review is completed, it will focus on verifying that the WET test met the quality assurance and quality control requirements of 25 Pa. Code § 252.403. If the application manager or biologist believes that such requirements have not been met, the Bureau of Laboratories’ Laboratory Accreditation Program will be notified.

## ATTACHMENT A

### DEFINITIONS OF TERMS USED IN SOP

**Acute WET Test** is a test to determine the concentration of effluent or ambient waters that causes an adverse effect (usually death) on a group of test organisms during a short-term exposure (e.g., 24, 48, or 96 hours). Acute toxicity is measured using statistical procedures (e.g., point estimate or hypothesis testing approaches).

**Chronic WET Test** is a test typically 96 hours or longer in duration in which sublethal effects (e.g., significantly reduced growth or reproduction) are measured in addition to lethality using statistical procedures.

**Discharge Flow ( $Q_d$ )** is the design flow, in cubic feet per second (cfs), of a wastewater discharge from an NPDES-permitted facility that is used to calculate water quality-based effluent limits.

**Endpoint** is a quantitative measurement of the biological responses of test organisms (e.g., survival, growth, reproduction, etc.) to exposure to an aqueous sample.

**In-Stream Waste Concentration (IWC)** is the concentration of effluent in the receiving waters after allowable dilution, expressed as a percentage, given by the following formula:

$$IWC (\%) = (Q_d / ((Q_s \times PMF) + Q_d)) \times 100$$

The Acute IWC value ( $IWC_a$ ) is determined when the Acute PMF is used in the calculation, and the Chronic IWC value ( $IWC_c$ ) is determined when the Chronic PMF is used in the calculation.

**Lethal Concentration, 50 Percent ( $LC_{50}$ )** is the concentration of effluent in a serial dilution of effluent that causes death in 50 percent of the test organisms over a specified period of time.

**No Observed Effect Concentration (NOEC)** is the highest tested concentration of an effluent in which there is no observable adverse effect on the test organisms, or the highest concentration of toxicity at which the values for the observed responses do not statistically differ from the control condition.

**Partial Mix Factor (PMF)** means the portion of the receiving waters, expressed as a fraction, that the Discharge Flow mixes with after periods of time known as "criteria compliance time" (CCTs). The Acute PMF ( $PMF_a$ ) uses 15 minutes for the CCT, and the Chronic PMF ( $PMF_c$ ) uses 72 hours for the CCT. DEP's Pentoxsd model is generally used to calculate PMF values.

**Reasonable Potential (RP)** is the likelihood that an effluent will cause or contribute to an excursion above a water quality standard. An RP process for WET is provided in this document.

**Stream Flow ( $Q_s$ )** is the flow of the water receiving the discharge at the design condition, expressed in cfs. The design stream flow condition for WET is the  $Q_{7-10}$  flow, which is the actual or estimated lowest 7 consecutive-day average flow that occurs once in 10 years for a stream with unregulated flow, or the estimated minimum flow for a stream with regulated flow.

**Target In-Stream Waste Concentration – Acute ( $TIWC_a$ )** is the value used to determine whether Acute WET results demonstrate reasonable potential to exceed the Acute criterion in 25 Pa. Code § 16.52, expressed as a percentage. The  $TIWC_a$  is the  $IWC_a$  divided by the Acute criterion:  $TIWC_a = ((Q_d / ((Q_s \times PMF_a) + Q_d)) \times 100) / 0.3$

**Target In-Stream Waste Concentration – Chronic ( $TIWC_c$ )** is the value used to determine whether Chronic WET results demonstrate reasonable potential to exceed the Chronic criterion in 25 Pa. Code § 16.52, expressed as a percentage. The  $TIWC_c$  is the  $IWC_c$ , divided by the Chronic criterion:  $TIWC_c = ((Q_d / ((Q_s \times PMF_c) + Q_d)) \times 100) / 1.0$

ATTACHMENT B

PART C CONDITION – NO PERMIT LIMITS FOR WET

I. WHOLE EFFLUENT TOXICITY (WET)

A. General Requirements

1. The permittee shall conduct (chronic / acute) WET tests as specified in this section. The permittee shall collect discharge samples and perform WET tests to generate (chronic survival and reproduction / acute survival) data for the cladoceran, *Ceriodaphnia dubia* and (chronic survival and growth / acute survival) data for the fathead minnow, *Pimephales promelas*.
2. Samples shall be collected at Outfall [REDACTED] in accordance with paragraph E.
3. The permittee shall perform testing using the following dilution series: [REDACTED]%, [REDACTED]%, [REDACTED]%, [REDACTED]%, and 100% effluent, with a control, where [REDACTED]% is the facility-specific Target In-Stream Waste Concentration (TIWC).
4. The determination of whether a test endpoint passes or fails shall be made using DEP's WET Analysis Spreadsheet (available at [www.depweb.state.pa.us/wett](http://www.depweb.state.pa.us/wett)) by comparing replicate data for the control with replicate data for the TIWC dilution.
5. The permittee shall submit only valid WET test results to DEP.

B. Test Frequency and Reporting

1. WET testing shall be conducted annually, at a minimum, during the period January 1 – December 31. Annual WET tests must be completed at least 6 months apart, and shall start in the year the permit becomes effective if the permit effective date is prior to October 1.
2. A complete WET test report shall be submitted to the DEP regional office that issued the permit within 45 days of test completion. A complete WET test report submission shall include the information contained in paragraph H, below. The permittee shall continue annual WET monitoring, at a minimum, during the permit renewal review period and during any period of administrative extension of this permit.
3. If a test failure is determined for any endpoint during annual monitoring, the permittee shall initiate a re-test for the species with the failure within 45 days of test completion. All endpoints for the species shall be evaluated in the re-test. The results of the re-test shall be submitted to the DEP regional office that issued the permit.
4. If a passing result is determined for all endpoints in a re-test, the permittee may resume annual monitoring.
5. If there is a failure for one or more endpoints in a re-test, the permittee shall initiate or continue quarterly WET testing for both species until there are four consecutive passing results for all endpoints. The results of all tests shall be submitted to the DEP regional office that issued the permit. In addition, the permittee shall initiate a Phase I Toxicity Reduction Evaluation (TRE) as specified in paragraph C, below.
6. The permittee shall attach the WET Analysis Spreadsheet for the latest four consecutive WET tests to the NPDES permit renewal application that is submitted to DEP at least 180 days prior to the permit expiration date.

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C. Phase I Toxicity Reduction Evaluation (TRE)

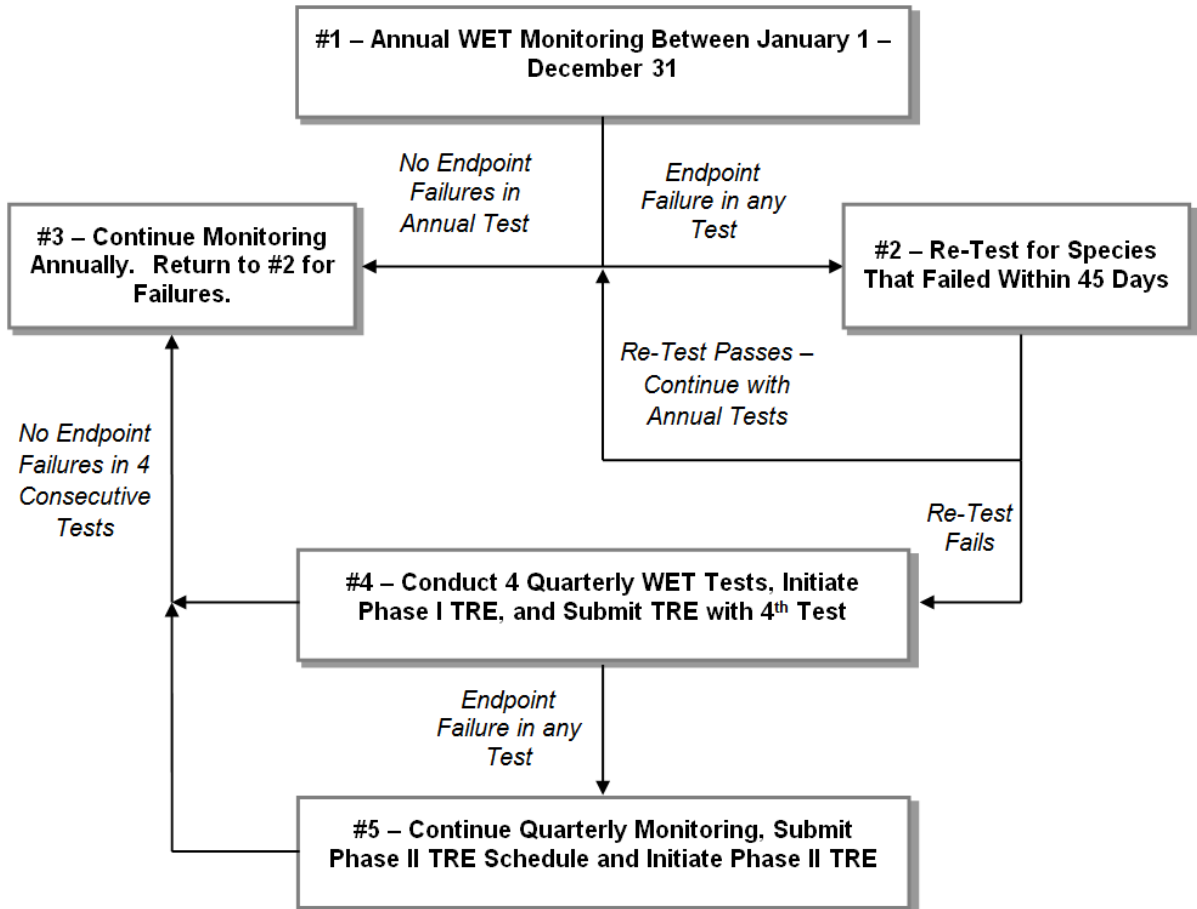
1. The Phase I TRE trigger is one WET endpoint failure followed by a re-test that confirms the failure for the same species. When the TRE process is triggered, quarterly WET testing shall be initiated for both species until there are four consecutive passing results for all endpoints. The Phase I TRE may include a Toxicity Identification Evaluation (TIE) if the permittee cannot immediately identify the possible causes of the effluent toxicity and the possible sources of the causative agents.
2. The permittee shall, within one year following the Phase I TRE trigger, submit a Phase I TRE report to the DEP regional office that issued the permit. The Phase I TRE shall be conducted in accordance with EPA's guidance, "Toxicity Reduction Evaluation for Municipal Wastewater Treatment Plants" (EPA/833B-99/002), "Generalized Methodology for Conducting Industrial Toxicity Reduction Evaluations" (EPA/600/2-88/070), and other relevant EPA guidance, as applicable. If a TIE is conducted as part of the Phase I TRE, it shall conform to EPA's guidance, "Methods for Aquatic Toxicity Identification Evaluations Phase I" (EPA/600/6-91/003), "Phase II" (EPA/600/R-92/080), "Phase III" (EPA/600/R-92/081) and other relevant EPA guidance. The Phase I TRE report shall be submitted with the fourth quarterly WET test report that is completed following the Phase I TRE trigger. The TRE shall include all activities undertaken to identify the cause(s) and source(s) of toxicity and any control efforts.
3. If all four quarterly WET tests produce passing results for all endpoints during the Phase I TRE process, performance of a Phase II TRE is not required, and annual WET testing in accordance with paragraph B.1 may resume.
4. If the four WET tests produce at least one failing result during the Phase I TRE process, the permittee shall continue quarterly WETT monitoring for both species and initiate a Phase II TRE in accordance with paragraph D. In this case, the Phase I TRE must include a schedule for completion of the Phase II TRE. The schedule must include interim milestones and a final completion date not to exceed two years from the initiation of the Phase II TRE. The permittee shall implement the Phase II TRE in accordance with the schedule unless DEP issues written approval to modify the schedule or cease performance of the Phase II TRE.
5. Re-tests during the TRE process are required for invalid tests but are optional and at the discretion of the permittee for valid tests. The results of all re-tests must be submitted to the DEP regional office that issued the permit along with the required elements in paragraph H.
6. ( OPTIONAL - If a Phase I TRE is in progress at the time of renewal, specify the date by which the Phase I TRE report is due ).

D. Phase II Toxicity Reduction Evaluation (TRE)

1. The Phase II TRE trigger is one WET endpoint failure during performance of the Phase I TRE. A Phase II TRE, if required, shall conform to EPA's guidance, "Toxicity Reduction Evaluation for Municipal Wastewater Treatment Plants" (EPA/833B-99/002), "Generalized Methodology for Conducting Industrial Toxicity Reduction Evaluations" (EPA/600/2-88/070), and other relevant EPA guidance, as applicable. A Phase II TRE evaluates the possible control options to reduce or eliminate the effluent toxicity and the implementation of controls.
2. Once initiated, the Phase II TRE must continue until the source(s) of toxicity are controlled as evidenced by four consecutive WET test passing results for all endpoints, and a final TRE report must be submitted on or before the date specified in the schedule, unless otherwise approved by DEP in writing.
3. If four consecutive quarterly WET tests produce passing results for all endpoints during the Phase II TRE process, annual WET testing in accordance with paragraph B.1 may be initiated or resume.

4. ( OPTIONAL - If a Phase II TRE is in progress at the time of renewal, specify the date(s) remaining in the Phase II TRE schedule submitted by the permittee ).

An overview of the process described in paragraphs B, C and D is presented below:



**E. Sample Collection**

For each acute testing event, a 24-hour flow-proportioned composite sample shall be collected. For each chronic testing event, three 24-hour flow-proportioned, composite samples shall be collected over a seven day exposure period. The samples must be collected at a frequency of not greater than every two hours and must be flow-proportioned. The samples must be collected at the permit compliance sampling location. Samples must be analyzed within 36 hours from the end of the compositing period and must be placed on ice and held at  $\leq 6^{\circ}\text{C}$ . Refer to the sample handling and preservation regulations set forth in 40 CFR 136, 25 Pa. Code Chapter 252, The NELAC Institute (TNI) Standard, and the appropriate EPA methods.

**F. Test Conditions and Methods**

Laboratories must be accredited by the DEP Laboratory Accreditation Program in order to perform and report WET tests for NPDES permit compliance. Laboratories must be either State or NELAP accredited.

1. Acute tests shall be completed in accordance with EPA’s “Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms” (EPA-821-R-02-012, latest edition). Forty eight (48) hour static non-renewal tests shall be used.

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2. Chronic tests shall be completed in accordance with EPA's "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" (EPA-821-R-02-013, latest edition). Seven (7) day tests shall be used with renewal every 24 hours.
3. The quality assurance and control (QA/QC) requirements and test acceptability standards specified in EPA's test methods and the requirements set forth in 25 Pa Code Chapter 252 or the TNI Standard must be followed.
4. If the permittee or its accredited laboratory determines that QA/QC requirements and/or test acceptability standards have not been met, a re-test shall be initiated within 45 days. Original test data must be maintained by the laboratory and be submitted to DEP upon request. The justification for a re-test must be clearly documented and kept on file with the sample results.

**G. Chemical Analyses**

Chemical analyses must follow the requirements of the EPA methods and applicable State and/or Federal regulations.

1. Chemical analysis on effluent samples shall include pH, Conductivity, Total Alkalinity, Total Hardness, Total Residual Chlorine, Total Ammonia (Unionized Ammonia), Dissolved Oxygen and temperature. Chemical analyses as described in the EPA Methods (above) shall be performed for each sampling event, including each new batch of dilution water and each testing event.
2. In addition to the chemical analyses required above, those parameters listed in Part A of the NPDES permit for the outfall(s) tested shall be analyzed concurrently with the WET test by using the method(s) specified in the permit.

**H. WET Report Elements**

WET test reports that are submitted to DEP must include the requirements identified in 25 Pa. Code § 252.401(j)(1) – (15) or in the TNI Standard, or equivalent, as well as the following information:

1. A general test description, including the origin and age of test organisms, dates and results of reference toxicant tests, light and temperature regimes, and other documentation that QA and test acceptability criteria as specified in EPA's methods and DEP's QA Summaries have been met.
2. A description of sample collection procedures and sampling location.
3. Name(s) of individual(s) collecting and transporting samples, including sample renewals, and the date(s) and time(s) of sample collection.
4. All chemical and physical data including laboratory quantitation limits and observations made on the species. The hardness shall be reported for each test condition.
5. Copies of raw data sheets and/or bench sheets with data entries and signatures.
6. When effluents are dechlorinated, dechlorination procedures must be described and if applicable a thiosulfate control used in addition to the normal dilution water control. If the thiosulfate control results are significantly different from the normal control, as determined using DEP's WET Analysis Spreadsheet, the thiosulfate control shall be used in the spreadsheet for comparison with the TIWC condition. The WET report must specify which control was used to determine whether the test result is pass or fail.
7. A description of all observations or test conditions that may have affected the test outcome.

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8. Control charts for the species tested regarding age, temperature test range, mortality data and all reference toxicant tests.
9. A completed WET test summary report (3800-FM-BPNPSM0485).
10. A DEP WET Analysis Spreadsheet printout that provides control and TIWC replicate data and displays the outcome of the test (pass or fail) for each endpoint tested.

WETT reports shall be submitted to the DEP regional office that issued the permit and, for discharges to the Delaware River basin, the Delaware River Basin Commission (DRBC).

## ATTACHMENT C

### PART C CONDITION – PERMIT LIMITS FOR WET

#### II. WHOLE EFFLUENT TOXICITY (WET)

##### A. General Requirements

1. The permittee shall conduct ( chronic / acute ) WET tests as specified in this section. The permittee shall collect discharge samples and perform WET tests to generate ( chronic survival and reproduction / acute survival ) data for the cladoceran, *Ceriodaphnia dubia* and ( chronic survival and growth / acute survival ) data for the fathead minnow, *Pimephales promelas*.
2. Samples shall be collected at Outfall [REDACTED] in accordance with paragraph E.
3. The permittee shall perform testing using the following dilution series: [REDACTED]%, [REDACTED]%, [REDACTED]%, [REDACTED]%, and 100% effluent, with a control, where [REDACTED]% is the facility-specific Target In-Stream Waste Concentration (TIWC).
4. The determination of whether a test endpoint passes or fails shall be made using DEP's WET Analysis Spreadsheet (available at [www.depweb.state.pa.us/wett](http://www.depweb.state.pa.us/wett)) by comparing replicate data for the control with replicate data for the TIWC dilution.
5. The permittee shall submit only valid WET test results to DEP.

##### B. Test Frequency and Reporting

1. WET testing shall be conducted quarterly, beginning within 30 days of the permit effective date and continuing until four tests have been completed. Tests shall be completed within calendar quarters, i.e., one test each during the periods of January 1 – March 31, April 1 – June 30, July 1 – September 30, and October 1 – December 31. A complete WET test report shall be submitted to the DEP regional office that issued the permit within 45 days of test completion. A complete WET test report submission shall include the information contained in paragraph H, below.
2. If no endpoint failures occur in the initial four quarterly tests, the permittee may reduce WET monitoring to annually during the period January 1 – December 31. This minimum WET monitoring frequency will remain in place until the permit is reissued, unless more frequent monitoring is triggered in accordance with paragraph B.5. The permittee must continue annual WET monitoring, at a minimum, during the permit renewal review period and during any period of administrative extension of this permit.
3. If a test failure is determined for any endpoint during quarterly or annual monitoring, the permittee shall perform a re-test for the species with the failure, at a minimum, within 45 days of test completion. All endpoints for the species shall be evaluated in the re-test. The results of the re-test shall be submitted to the DEP regional office that issued the permit.
4. If a passing result is determined for all endpoints in a re-test, the permittee may resume quarterly or annual monitoring, as applicable.
5. If there is a failure for one or more endpoints in a re-test, the permittee shall initiate or continue quarterly WET testing for both species until there are four consecutive passing results for all endpoints. The results of all tests shall be submitted to the DEP regional office that issued the permit. In addition, the permittee shall initiate a Phase I Toxicity Reduction Evaluation (TRE) as specified in paragraph C, below.

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6. The permittee must report the results of each test endpoint that has a WET limit in Part A of this permit on the Discharge Monitoring Report (DMR). Test results shall be reported on the DMR in terms of acute or chronic Toxicity Units (TUa or TUc), where TUa is used for acute tests and TUc is used for chronic tests. If DEP's WET Analysis Spreadsheet indicates a passing result for an endpoint, report the value obtained from the expression "1/TIWC", which is equivalent to the permit limit. If the Spreadsheet indicates a failure, report the value obtained from the expression "> 1/TIWC". For example, an acute test endpoint failure at a TIWC dilution of 50% would be reported as "> 2.0 TUa" (1/0.5).
7. The permittee shall attach a completed WET Analysis Spreadsheet for the latest four consecutive WET tests to the NPDES permit renewal application that is submitted to DEP at least 180 days prior to the permit expiration date.

C. Phase I Toxicity Reduction Evaluation (TRE)

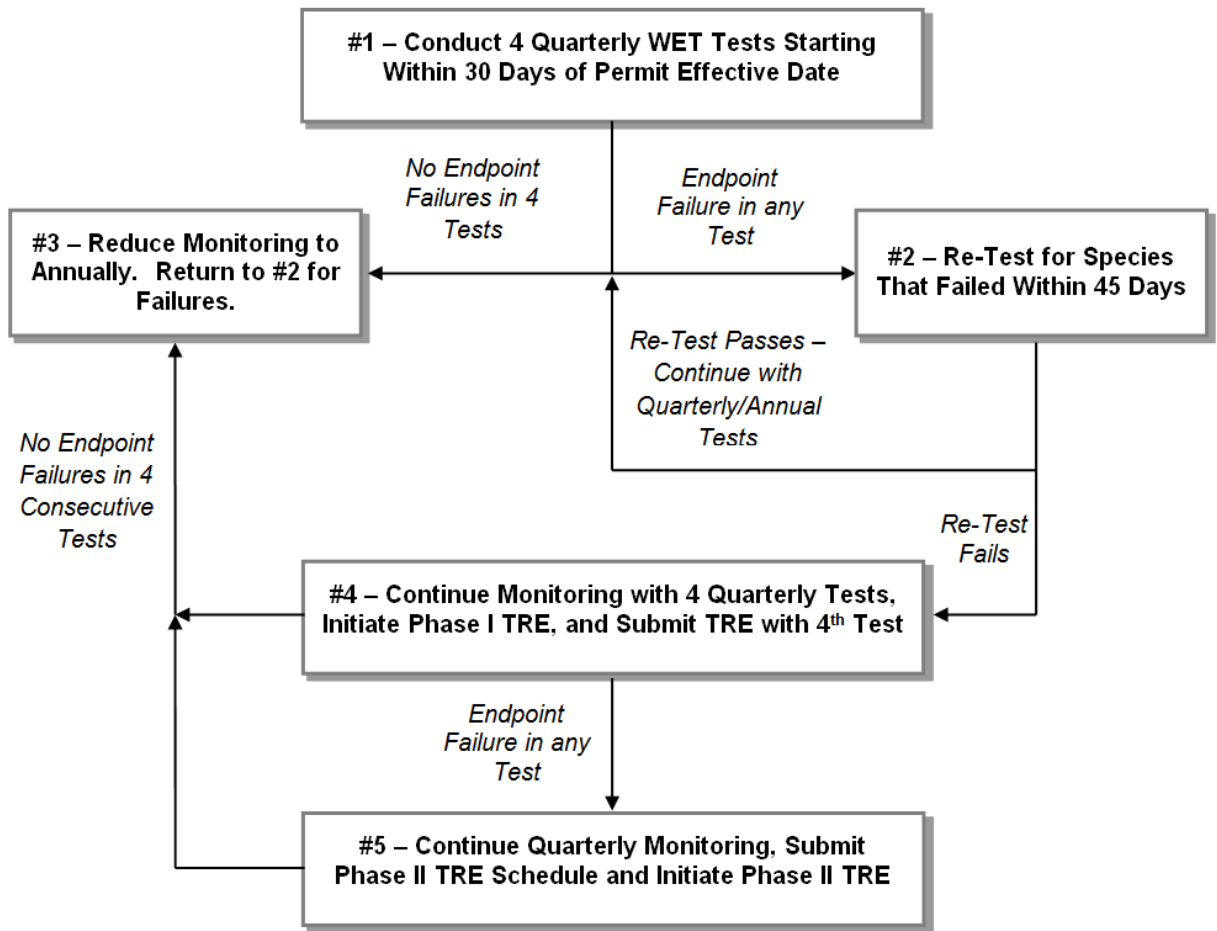
1. The Phase I TRE trigger is one WET endpoint failure followed by a re-test that confirms the failure for the same species. When the Phase I TRE process is triggered, quarterly WET testing shall be initiated for both species and continue until there are four consecutive passing results for all endpoints. The Phase I TRE may include a Toxicity Identification Evaluation (TIE) if the permittee cannot immediately identify the possible causes of the effluent toxicity and the possible sources of the causative agents.
2. The permittee shall, within one year following the Phase I TRE trigger, submit a Phase I TRE report to the DEP regional office that issued the permit. The Phase I TRE shall be conducted in accordance with EPA's guidance, "Toxicity Reduction Evaluation for Municipal Wastewater Treatment Plants" (EPA/833B-99/002), "Generalized Methodology for Conducting Industrial Toxicity Reduction Evaluations" (EPA/600/2-88/070), and other relevant EPA guidance, as applicable. If a TIE is conducted as part of the Phase I TRE, it shall conform to EPA's guidance, "Methods for Aquatic Toxicity Identification Evaluations Phase I" (EPA/600/6-91/003), "Phase II" (EPA/600/R-92/080), "Phase III" (EPA/600/R-92/081) and other relevant EPA guidance. The Phase I TRE report shall be submitted with the fourth quarterly WET test report that is completed following the Phase I TRE trigger. The TRE report shall include all activities undertaken to identify the cause(s) and source(s) of toxicity and any control efforts.
3. If all four quarterly WET tests produce passing results for all endpoints during the Phase I TRE process, performance of a Phase II TRE is not required, and annual WET testing in accordance with paragraph B.2 may be initiated or resume.
4. If the four WET tests produce at least one failing result during the Phase I TRE process, the permittee shall continue quarterly WET monitoring for both species and initiate a Phase II TRE in accordance with paragraph D. In this case, the Phase I TRE must include a schedule for completion of the Phase II TRE. The schedule must include interim milestones and a final completion date not to exceed two years from the initiation of the Phase II TRE. The permittee shall implement the Phase II TRE in accordance with the schedule unless DEP issues written approval to modify the schedule or cease performance of the Phase II TRE.
5. Re-tests during the TRE process are required for invalid tests but are optional and at the discretion of the permittee for valid tests. The results of all re-tests must be submitted to the DEP regional office that issued the permit along with the required elements in paragraph H.
6. ( OPTIONAL - If a Phase I TRE is in progress at the time of renewal, specify the date by which the Phase I TRE report is due ).

D. Phase II Toxicity Reduction Evaluation (TRE)

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1. The Phase II TRE trigger is one WET endpoint failure during performance of the Phase I TRE. A Phase II TRE, if required, shall conform to EPA’s guidance, “Toxicity Reduction Evaluation for Municipal Wastewater Treatment Plants” (EPA/833B-99/002), “Generalized Methodology for Conducting Industrial Toxicity Reduction Evaluations” (EPA/600/2-88/070), and other relevant EPA guidance, as applicable. A Phase II TRE evaluates the possible control options to reduce or eliminate the effluent toxicity and the implementation of controls.
2. Once initiated, the Phase II TRE must continue until the source(s) of toxicity are controlled as evidenced by four consecutive WET test passing results for all endpoints, and a final TRE report must be submitted on or before the date specified in the schedule, unless otherwise approved by DEP in writing.
3. If four consecutive quarterly WET tests produce passing results for all endpoints during the Phase II TRE process, annual WET testing in accordance with paragraph B.2 may be initiated or resume.
4. ( OPTIONAL - If a Phase II TRE is in progress at the time of renewal, specify the date(s) remaining in the Phase II TRE schedule submitted by the permittee ).

An overview of the process described in paragraphs B, C and D is presented below:



**E. Sample Collection**

For each acute testing event, a 24-hour flow-proportioned composite sample shall be collected. For each chronic testing event, three 24-hour flow-proportioned, composite samples shall be collected over a

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seven day exposure period. The samples must be collected at a frequency of not greater than every two hours and must be flow-proportioned. The samples must be collected at the permit compliance sampling location. Samples must be analyzed within 36 hours from the end of the compositing period and must be placed on ice and held at  $\leq 6^{\circ}\text{C}$ . Refer to the sample handling and preservation regulations set forth in 40 CFR 136, 25 Pa. Code Chapter 252, The NELAC Institute (TNI) Standard, and the appropriate EPA methods.

**F. Test Conditions and Methods**

Laboratories must be accredited by the DEP Laboratory Accreditation Program in order to perform and report WET tests for NPDES permit compliance. Laboratories must be either State or NELAP accredited.

1. Acute tests shall be completed in accordance with EPA's "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms" (EPA-821-R-02-012, latest edition). Forty eight (48) hour static non-renewal tests shall be used.
2. Chronic tests shall be completed in accordance with EPA's "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" (EPA-821-R-02-013, latest edition). Seven (7) day tests shall be used with renewal every 24 hours.
3. The quality assurance and control (QA/QC) requirements and test acceptability standards specified in EPA's test methods and the requirements set forth in 25 Pa Code Chapter 252 or the TNI Standard must be followed
4. If the permittee or its accredited laboratory determines that QA/QC requirements and/or test acceptability standards have not been met, a re-test shall be initiated within 45 days. Original test data must be maintained by the laboratory and be submitted to DEP upon request. The justification for a re-test must be clearly documented and kept on file with the sample results.

**G. Chemical Analyses**

Chemical analyses must follow the requirements of the EPA methods and applicable State and/or Federal regulations.

1. Chemical analysis on effluent samples shall include pH, Conductivity, Total Alkalinity, Total Hardness, Total Residual Chlorine, Total Ammonia (Unionized Ammonia), Dissolved Oxygen and temperature. Chemical analyses as described in the EPA Methods (above) shall be performed for each sampling event, including each new batch of dilution water and each testing event.
2. In addition to the chemical analyses required above, those parameters listed in Part A of the NPDES permit for the outfall(s) tested shall be analyzed concurrently with the WET test by using the method(s) specified in the permit.

**H. WET Report Elements**

WET test reports that are submitted to DEP must include the requirements identified in 25 Pa. Code § 252.401(j)(1) – (15) or in the TNI Standard, or equivalent, as well as the following information:

1. A general test description, including the origin and age of test organisms, dates and results of reference toxicant tests, light and temperature regimes, and other documentation that QA and test acceptability criteria as specified in EPA's methods and DEP's QA Summaries have been met.
2. A description of sample collection procedures and sampling location.
3. Name(s) of individual(s) collecting and transporting samples, including sample renewals, and the date(s) and time(s) of sample collection.

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4. All chemical and physical data including laboratory quantitation limits and observations made on the species. The hardness shall be reported for each test condition.
5. Copies of raw data sheets and/or bench sheets with data entries and signatures.
6. When effluents are dechlorinated, dechlorination procedures must be described and if applicable a thiosulfate control used in addition to the normal dilution water control. If the thiosulfate control results are significantly different from the normal control, as determined using DEP's WET Analysis Spreadsheet, the thiosulfate control shall be used in the spreadsheet for comparison with the TIWC condition. The WET report must specify which control was used to determine whether the test result is pass or fail.
7. A description of all observations or test conditions that may have affected the test outcome.
8. Control charts for the species tested regarding age, temperature test range, mortality data and all reference toxicant tests.
9. A completed WET test summary report (3800-FM-BPNPSM0485).
10. A DEP WET Analysis Spreadsheet printout that provides control and TIWC replicate data and displays the outcome of the test (pass or fail) for each endpoint tested.

WETT reports shall be submitted to the DEP regional office that issued the permit and, for discharges to the Delaware River basin, the Delaware River Basin Commission (DRBC).

ATTACHMENT D  
 WETT DILUTION SERIES

TIWC	Dilutions (% Effluent)				
	1st	2nd	3rd	4th	5th
1	1	2	30	60	100
2	1	2	30	60	100
3	1	3	30	60	100
4	2	4	30	60	100
5	2	5	30	60	100
6	3	6	30	60	100
7	3	7	30	60	100
8	4	8	30	60	100
9	4	9	30	60	100
10	5	10	30	60	100
11	3	6	11	56	100
12	3	6	12	56	100
13	3	7	13	57	100
14	4	7	14	57	100
15	4	8	15	58	100
16	4	8	16	58	100
17	4	9	17	59	100
18	5	9	18	59	100
19	5	10	19	60	100
20	5	10	20	60	100
21	5	11	21	61	100
22	6	11	22	61	100
23	6	12	23	62	100
24	6	12	24	62	100
25	6	13	25	63	100
26	7	13	26	63	100
27	7	14	27	64	100
28	7	14	28	64	100
29	7	15	29	65	100
30	8	15	30	65	100
31	8	16	31	66	100
32	8	16	32	66	100
33	8	17	33	67	100
34	9	17	34	67	100
35	9	18	35	68	100
36	9	18	36	68	100
37	9	19	37	69	100

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TIWC	Dilutions (% Effluent)				
	1st	2nd	3rd	4th	5th
38	10	19	38	69	100
39	10	20	39	70	100
40	10	20	40	70	100
41	10	21	41	71	100
42	11	21	42	71	100
43	11	22	43	72	100
44	11	22	44	72	100
45	11	23	45	73	100
46	12	23	46	73	100
47	12	24	47	74	100
48	12	24	48	74	100
49	12	25	49	75	100
50	13	25	50	75	100
51	13	26	51	76	100
52	13	26	52	76	100
53	13	27	53	77	100
54	14	27	54	77	100
55	14	28	55	78	100
56	14	28	56	78	100
57	14	29	57	79	100
58	15	29	58	79	100
59	15	30	59	80	100
60	15	30	60	80	100
61	15	31	61	81	100
62	16	31	62	81	100
63	16	32	63	82	100
64	16	32	64	82	100
65	16	33	65	83	100
66	17	33	66	83	100
67	17	34	67	84	100
68	17	34	68	84	100
69	17	35	69	85	100
70	18	35	70	85	100
71	18	36	71	86	100
72	18	36	72	86	100
73	18	37	73	87	100
74	19	37	74	87	100
75	19	38	75	88	100
76	19	38	76	88	100
77	19	39	77	89	100

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TIWC	Dilutions (% Effluent)				
	1st	2nd	3rd	4th	5th
78	20	39	78	89	100
79	20	40	79	90	100
80	20	40	80	90	100
81	20	41	81	91	100
82	21	41	82	91	100
83	21	42	83	92	100
84	21	42	84	92	100
85	21	43	85	93	100
86	22	43	86	93	100
87	22	44	87	94	100
88	22	44	88	94	100
89	22	45	89	95	100
90	23	45	90	95	100
91	23	46	91	96	100
92	23	46	92	96	100
93	23	47	93	97	100
94	24	47	94	97	100
95	24	48	95	98	100
96	24	48	72	96	100
97	24	49	73	97	100
98	25	49	73	98	100
99	25	50	74	99	100
100	25	50	70	90	100

**Version History**

<b>Date</b>	<b>Version</b>	<b>Revision Reason</b>
11/14/25	1.5	Updated Attachment C to remove a sentence related to using a dilution higher than the TIWC.
5/13/14	1.4	Changed requirement for the submission of WET reports and for re-testing from 30 days to 45 days in Attachments B and C (permit language).
8/7/13	1.3	Added Attachment A, definitions. Added a note to paragraph I.A.1.a to clarify that application managers should not generally use the WET Analysis Spreadsheet to evaluate results that pass using the conventional data analysis method. Paragraph I.C.1 was modified to clarify that 1) if RP is determined and limits will be imposed based on a certain type of test, that type of test should be specified in the renewed permit, and 2) in general application managers should not include both acute and chronic tests in permits.
6/27/13	1.2	Added clarification to Section I.A.1.a that DEP's WET Analysis Spreadsheet will be used to evaluate results before concluding that one endpoint failure based on LC50, IC25 or NOEC results do not warrant WET limits. Added clarification to Section I.A.2 that DEP's WET Analysis Spreadsheet may be used to evaluate WET results even if the permit does not require its use by the permittee in the existing permit. Added Sections I.A.3 – A.6 to provide guidance on implementing WET limits and monitoring requirements in Part A of the permit and on DMRs.
12/31/2012	1.1	Changed requirement for re-testing within 14 days to within 30 days in Attachments A and B (permit language).
11/9/2012	1.0	Original