NPDES Compliance Inspection Manual

Chapter 8

EPA Publication Number: 305-K-17-001
Interim Revised Version, January 2017

CHAPTER 8 – TOXICITY

Contents

List of Tables

Related Websites

Office of Science and Technology/Engineering and Analysis Division Methods home page (including Whole Effluent Toxicity): https://www.epa.gov/cwa-methods/whole-effluent-toxicity-methods

Office of Wastewater Management/Water Permits Division National Pollutant Discharge Elimination System Permits Program—Whole Effluent Toxicity home page: https://www.epa.gov/npdes/npdes-permit-limits#wet

Office of Wastewater Management/Water Permits Division – Recorded Webinars and Training – Whole Effluent Toxicity (WET) Training: https://www.epa.gov/npdes/npdes-training#wettraining (Note: Module 8, *NPDES WET Compliance and Enforcement*)

A. OBJECTIVES

Toxicity is a characteristic of a substance (or group of substances) that causes adverse effects in organisms. Adverse effects include an increased rate of morbidity (the rate of occurrence of disease) and mortality (the rate of occurrence of death), as well as those effects that limit an organism's ability to survive in nature, such as impaired reproductive ability, mobility or growth. Toxicity of a substance is measured by observing the responses of organisms to increasing concentrations of that substance. One substance is more toxic than another when it causes the same adverse effects at a lower concentration.

Whole Effluent Toxicity (WET) is a National Pollutant Discharge Elimination System (NPDES) permits program parameter designed to evaluate the toxicity of the entire wastestream as opposed to its individual components. WET testing may be performed or evaluated as part of one of five NPDES inspections:

- Compliance Evaluation Inspection (CEI)
- Compliance Sampling Inspection (CSI)
- Performance Audit Inspection (PAI)
- Toxics Sampling Inspection (XSI)
- Compliance Biomonitoring Inspection

In addition, an inspector should consider the toxicity of a municipal treatment plant's effluent as part of Pretreatment Compliance Inspections (PCIs), since the effluent toxicity may originate from industrial or commercial discharges to the municipal treatment plant.

EPA test methods manuals for Whole Effluent Toxicity testing can be accessed at: https://www.epa.gov/cwa-methods/whole-effluent-toxicity-methods.

The inspector should understand the permittee's WET testing requirements so that the appropriate objectives can be met. These objectives may include:

- Assess compliance with NPDES permit conditions.
- Assess NPDES permit conditions for clear and inclusive language.
- Consider overall laboratory WET test performance (reference toxicants and other WET quality assurance/quality control (QA/QC) requirements) especially EPA's minimum WET test methods' Test Acceptability Criteria (TAC).
- Evaluate quality of self-monitoring data.
- Assess adequacy of self-monitoring procedures.
- Document presence or absence of toxic conditions.
- Identify need to perform Toxicity Reduction Evaluation (TRE) and/or a Toxicity Identification Evaluation (TIE).
- Identify permit terms and conditions that may not be strong enough to ensure state WET water quality standards are met.

B. REQUIREMENTS OF WET TESTING

WET tests are techniques to determine the toxicity of a permittee's discharge or effluent by measuring the responses of organisms to varying concentrations of the facility's effluent and test dilution water. The EPA WET test methods, as revised November 2002, are specified in 40 CFR Part 136 and described in the EPA WET test methods manuals (accessible at https://www.epa.gov/cwa-methods/whole-effluent-toxicity-methods). This section provides general background on WET tests and guidance for inspectors to consider when performing various types of inspections concerning WET tests (laboratory performance, effluent sampling, shipping, records, etc.).

TYPES OF WET TESTING

Depending on the EPA WET test required under a NPDES permit, the WET test designs may vary according to nationally standardized testing and where applicable, regional specific protocols. They vary in the number of test organisms used, duration of the test (acute or chronic), or in the way in which the effluent contacts the organism (flow-through, static, static renewal). The permitting authority will select the appropriate WET test design depending on the suspected toxicants present and the intended use of the WET test results. For example, a preliminary Range screening or T-test WET uses comparatively fewer organisms than the full scale WET test (five test concentrations plus a control treatment) because the results are derived from the comparison of a single effluent test concentration to the control treatment. This initial screening WET test is usually conducted to assess if toxicity is present and should be followed up with a multiple concentration WET test to generate a dose-response curve unless the statistical analysis used was designed for a two concentration WET test and is sufficiently robust for interpreting WET data generated from a T-test. WET data interpretation and analysis is discussed in more detail in Section C of this chapter. The more common EPA WET tests have requirements that include a multi-concentration dilution series consisting of a control treatment (no effluent) and five effluent test concentrations (serial dilutions of effluent sample plus dilution water, except for the 100-percent effluent test concentration). EPA WET test methods have minimum mandatory test acceptability criteria (TAC) that must be met for the WET test and its results to be considered a valid WET test.

EPA WET tests have method specific requirements that include: the number of test organisms per test chamber, the number of test replicates per test dilution, a test design of a control treatment plus five effluent dilution test concentrations, and specified test durations for acute and chronic testing. See the EPA WET test methods for more details. The response of each organism in each test concentration is observed and recorded. The toxicity of the effluent sample is determined by analyzing the response of the test organisms in relation to the effluent test concentration to which the organisms were exposed.

WET testing may be performed as either acute or chronic tests in accordance with standardized EPA WET test methods. The terms acute and chronic refer to the length of time that the organisms are exposed to the toxicant, and the respective WET test endpoints (i.e., acute-lethal, chronic-lethal and sub-lethal). The duration of the tests is prescribed in the WET test

method specified in the NPDES permit. Generally, acute tests measure short-term extreme negative effect responses, such as death or a debilitating physiological disorder. A test organism response to toxicity observed within 96 hours or less is typically considered an acute measurement. Chronic tests involve a causative agent that lingers or continues for a relatively longer period, often one-tenth of an organism's lifespan or more. "Chronic" should be considered a relative term depending on the lifespan of an organism. WET chronic tests typically run for seven days. A chronic effect may result in negative responses such as death (lethal endpoint), as well as stunted growth and reduced mobility or reproductive rates (sub-lethal endpoints).

Common test responses indicating the presence of toxic conditions include:

- Death—increase in number of organisms killed by a test solution when compared to the control treatment.
- Inhibited growth—measurement of reduction in growth (including mean weight of an organism) compared to the control treatment.
- Reduced reproduction or mobility—measurement of reduction in reproductive rates or mobility compared to the control treatment.
- Terata—increase in number of gross abnormalities shown in early life stages compared to the control treatment.

Other WET test design terms describe the way that test organisms are physically exposed to WET test concentrations such as: flow-through, static renewal, and static. In a flow-through test, effluent and dilution water are mechanically renewed continuously. This test setup requires specialized equipment (a serial or proportional dilutor or syringe pumps) and has higher operating costs than a static test. In a static renewal test, the test solutions are replaced periodically (usually daily) with fresh effluent and dilution water. In a static test, the solutions used at the start of the test are not replaced for the test's duration. Both static renewal and static tests require less sophisticated equipment. The decision of which WET test design type is required should be specified in the NPDES permit for both acute and/or chronic tests according to the respective EPA's WET test methods (40 CFR Part 136 and EPA Pacific West Coast methods (EPA, 1995)), which can be incorporated by reference.

WET TEST COMPONENTS

The following discussions pertain primarily to issues in a laboratory audit.

WET tests, as defined in EPA WET test methods (40 CFR Part 136 or EPA's Pacific West Coast WET methods), consist of the following components:

- Sampling, including a chain-of-custody form.
- Effluent.
- Receiving water.
- Dilution water (preferably the receiving water but in some instances a synthetic water approved by the regulatory agency).

- Testing system.
- Test organisms (in house mass cultures or externally purchased).
- QA/QC requirements, including EPA WET test method TACs.
- Reference toxicants.
- WET test data evaluation and analysis.

As described in the EPA approved WET test methods, organisms in the testing system are exposed to a combination of effluent and dilution water to produce WET test results. Each component of the test, including food items, must be of a specific quality for successful toxicity testing. The inspector should determine if the test components adhere to the requirements specified in the NPDES permit and the NPDES EPA WET test method referenced or incorporated into the NPDES permit's general conditions section (e.g., EPA's WET test methods at 40 CFR Part 136). The inspector should review the permittee's sampling logbook, chain-of-custody forms, source of WET test organisms used and the testing laboratory reports for the information necessary to assess the quality of the test components.

Each component has specific requirements (e.g., sample location for the effluent, maximum sample holding time, dilution water constituents, health of the test organisms, appropriate choice of test apparatus materials). Accurate and reproducible test results can only be expected when the critical test components are handled properly. It is, therefore, very important to understand the relationships between these test components and the critical factors that determine the acceptability (e.g., to be considered a valid WET test) of each based on quality assurance requirements and to ensure the validity of the generated WET test results. During a NPDES inspection, the inspector is likely to encounter the critical factors described in the following sections.

EFFLUENT

The effluent sampling strategy should be specified in the NPDES permit. Effluent samples must be representative of the entire final effluent discharge and free of contamination from other sources. The monitoring frequency selected by the permitting authority should be specified in the NPDES permit and should be representative of the permitted effluent discharge including accounting for the variability of the effluent due to several possible factors including but not limited to seasonal changes, facility process variations, available receiving water dilution (if allowed by state water quality standards or permitting regulations for mixing zones), etc. Samples collected to be shipped to an off-site laboratory must be maintained at a temperature ranging from 0° to 6°C by chilling the sample(s) to 6°C during or immediately after collection, shipped in ice to the designated testing laboratory accompanied by a chain-of-custody form, and refrigerated (0° to 6°C) upon receipt by the testing laboratory.

The type and frequency of samples taken (e.g., grab, composite) must be consistent with those required in the NPDES permit. For flow-through tests that are not done by pumping effluent directly into dilutors, daily sample sizes must be sufficient to supply the dilutor for periods ranging from 24 to 36 hours. This volume will depend on the type of WET test being conducted and the number of dilutions being run. For static renewal tests, daily sample volumes should be

sufficient to replenish all dilutions in the test series and provide separate containers of the dilutions to allow for dissolved oxygen (DO), pH, salinity, temperature and other chemical analyses without contaminating the test dilutions. This volume will depend on the type of WET test being conducted and dilutions being run. For static-renewal toxicity tests, composite and grab samples for 7-day chronic testing requires the use of an original sample and two renewal samples over the duration of the test. Preferably, and after using the original sample, renewal samples should be put into use on days 3 and 5 of testing. Table 8-1 provides guidance as to representative sampling strategies for various situations. For some volatile toxicants that are acutely toxic (e.g., chlorine), standard composite sampling does not yield an effluent sample that is representative of the actual permitted effluent discharge due to volatilization of chlorine during sampling, shipping and holding. On-site flow-through testing would yield more appropriate WET test results where, considering available dilution, the effluent contains measurable amounts of chlorine.

Samples for on-site laboratory testing should be used immediately when practical, but must be used within 36 hours of collection. It is usually not possible to refrigerate the large-volume samples (200 liters or more) that are required for flow-through fish tests, but all other samples should be either iced or refrigerated if they are not to be used immediately. Note: hand-delivered samples used on the same day of collection do not need to be cooled at 0° to 6°C prior to WET test initiation.

As a minimum requirement in all cases, tests should be initiated within 36 hours of collection. In the case of short-term chronic tests, samples taken on days one, three, and five may be held for a longer period of time to complete the test. In no case should preservatives be added to or chemical disinfection performed on the effluent sample(s) prior to being tested for toxicity, nor should the effluent samples be dechlorinated unless the permit specifically allows for sample dechlorination.

DILUTION WATER

The choice of dilution water to use in WET tests should be specified in the NPDES permit and depends on the purpose of the toxicity test. Synthetic dilution water is used to evaluate the inherent toxicity of the effluent. Dilution water from the receiving stream or a nontoxic equivalent is used to test for interactions after an effluent discharge thoroughly mixes with the receiving water (where state laws allow for a mixing zone). Receiving waters, synthetic waters, or synthetic waters adjusted to approximate receiving water characteristics may be used for dilution water, if the water meets the qualifications for an acceptable dilution water. EPA WET test methods manuals describe various techniques for the preparation of synthetic dilution water that may be necessary to use if the natural receiving water exhibits unacceptable levels of toxicity. Under no circumstances should the dilution water cause toxic responses in the WET test organisms. A lack of toxic responses or observed impacts to the control treatment organisms is one indicator of the possible suitability of the dilution water. EPA WET test methods specify mandatory TACs for test organisms in control treatments for each test species for both acute and chronic tests for both lethal and sub-lethal endpoints. TAC is further discussed in Section C of this chapter.

Dilution water obtained from receiving waters should be collected following all sampling procedures including the use of a chain-of-custody form, and should be used immediately for testing. If the dilution water will not be used within 24 hours, it should be refrigerated (0° to 6°C) as soon as it is collected. In any case, to ensure that no appreciable change in toxic characteristics occurs before testing, the holding time from the time the receiving water sample is collected to the first use of the receiving water sample in the WET test initiation must not exceed 36 hours unless a variance has been granted. If a delay in the WET test initiation of up to 36 hours is necessary, the receiving water samples must be stored under strict conditions (i.e., temperatures of 0° to 6°C). The location of the receiving water sample should be noted in the permittee's sampling log and the chain-of-custody form. It should be upstream and out of the influence of the permitted outfall. The location should be free of other sources of contamination (e.g., other facility outfalls).

Table 8-1. Recommended Effluent Sampling Strategies for Continuous and Intermittent Discharges for Flow-Through, Static Renewal, and Static Toxicity Tests[a]

Continuous Discharge			
TEST TYPE	**CHRONIC**	**ACUTE Retention Time < 14 Days**	**ACUTE Retention Time >14 Days**
Flow-through**	-	Two Grab samples daily; early a.m. and late p.m.	One grab sample daily.
Static Renewal	3x 24-hour composite samples, every other day.	Four separate grab samples each day for four concurrent tests.	One grab sample on first day.
Static	Single 24-hour composite sample on first day.	Four separate grab samples on first day for four concurrent tests.	One grab sample on first day.

Intermittent Discharge				
TEST TYPE	**CHRONIC**	**ACUTE Continuous Discharge During 1 or 2 Adjacent 8-Hour Shifts**	**ACUTE Discharge from Batch Treatment**	**ACUTE Discharge to Estuary on Outgoing Tide**
Flow-Through[b]	-	One grab sample midway through shifts daily.	One grab sample of discharge daily.	One grab sample of discharge daily.
Static Renewal	3x 24-hour composite samples collected for duration of discharge unless discharge ceases.	One grab sample midway through shifts on first day.	One grab sample of discharge daily.	One grab sample of discharge daily.
Static	Composite sample collected for duration of discharge, first day.	One grab sample midway through shifts on first day.	One grab sample of discharge on first day.	One grab sample of discharge on first day.

[a] Sampling requirements should be clearly specified in the permit.

[b] For flow-through tests, it is always preferable to pump directly to the dilutor.

TEST SYSTEM

WET tests may be performed in a fixed or mobile laboratory. Depending on the scope of the program, facilities may include equipment for rearing, holding, and acclimating test organisms. Temperature control is achieved using circulating water baths, heat exchangers, or environmental chambers. Holding, acclimation, and dilution water should be temperature controlled and aerated whenever possible. Air used for aeration must be free of oil and fumes; filters to remove oil in the air are desirable. Test facilities must be well-ventilated and free of fumes. During holding, acclimating, and testing, conditions should remain as constant as possible and test organisms should be shielded from external disturbances (held under the same conditions as those used for testing). Reference toxicants should be properly stored in a closed area separate from the WET testing areas.

Any materials that contact either the effluent or dilution water must not release, absorb, or adsorb toxicants. Many choices for test equipment are available. Properly prepared (see discussion at end of this section) glassware and stainless steel are generally acceptable for effluent freshwater holding, mixing, and transfer to WET test chambers. Stainless steel, however, is not acceptable for saltwater systems. Square-sided glass aquaria should be held together with small beads of silicone adhesive, with any unnecessary adhesive removed from inside the aquaria. If stainless steel containers are used, they must be welded, not soldered. Other specialized containers of Nitex or Teflon™ are also acceptable. Tanks for storing effluents and dilution water may also be made of fiberglass. All containers or tubes made from these materials are reusable with appropriate cleaning (see below).

Polyethylene, polypropylene, polyvinyl chloride, polystyrene, and Tygon® may also be used for containers or tubing, but should be checked for toxicity before being used in a WET test. Because these materials may absorb toxicants during a test, their reuse is discouraged to prevent absorbed toxicants from leaching into new effluent or dilution water.

Copper, galvanized metal, brass, lead, and rubber must not contact the testing solutions at any time.

New plastic ware (from a known nontoxic source) can be used after rinsing with dilution water. New glassware should be soaked overnight in dilute (20 percent) nitric or hydrochloric acid, rinsed in tap water, and then rinsed with dilution water before use.

Glassware and stainless steel components that must be reused should be soaked in an appropriate detergent used for toxicity testing and scrubbed (or washed in a laboratory dishwasher), rinsed twice with tap water, rinsed with dilute acid, rinsed twice with tap water, rinsed with full strength acetone, rinsed twice with tap water, and then rinsed with dilution water before use. Glassware for algae tests should be neutralized in sodium bicarbonate before use.

TEST ORGANISMS

Organisms used for toxicity testing are limited to certain species for which there are established EPA WET testing protocols (40 CFR Part 136 and EPA Pacific West Coast WET Test methods

(EPA, 1995)). Some examples of freshwater and saltwater test species commonly used in WET tests include: a) freshwater—daphnids (water flea, invertebrate) and fathead minnows (fish vertebrate); b) saltwater—algae (plant), mysids (shrimp, invertebrate) and silversides (fish vertebrate). The life stage, source, acclimation and feeding procedures, presence of disease, and the number of organisms placed in test chambers all affect the degree to which test organisms respond to toxicants. Therefore, it is important that these factors comply with EPA's required WET test method procedures. Test conditions for various types of tests and organisms are summarized in the test acceptability criteria tables that can be accessed at https://www.epa.gov/cwa-methods/whole-effluent-toxicity-methods.

The inspector should ascertain, as closely as possible, that the following procedures are being observed:

- The correct test organisms (including the choice of test organisms to account for *species sensitivity* for the tested effluent, the most sensitive species must be used under the NPDES permit regulations for reasonable potential determinations (40 CFR 122.44(d)(1)(ii)) must be utilized in the test (most often as specified in the NPDES permit). "Wild" (e.g., collected from the receiving stream) organisms are rarely appropriate in WET testing.
- The laboratory should record the source of test organisms (hatchery, in-house, or elsewhere). Also, test organisms used in toxicity testing must be of known history, free of disease, and acclimated to test conditions. Culture information should be recorded. Test organisms must be of the appropriate age and the appropriate number of organisms must be used in each WET test chamber before initiating a WET test.
- A daily log (that is a daily bench sheet for each WET test being performed) should be kept by the laboratory concerning the WET test organisms including: feeding, mortality, reproduction, growth, mobility, and any abnormal behavioral observations. Measurements for each test chamber should be recorded such as pH, temperature, dissolved oxygen, conductivity, etc. to ensure optimal testing conditions are maintained.
- The testing laboratory must adhere to the following procedures for holding test organisms:
  - Test organisms purchased may be used to start mass cultures. However, if the organisms are to be used for WET chronic testing, then at the start of the test they must be no more than 48 hours old (if fish, purchased and shipped) or no more than 24 hours old (if fish, not shipped, or if freshwater invertebrates such as *Ceriodaphnia dubia*). Freshwater invertebrates used in a test must have been released within an 8-hour period, to avoid impacts on reproductive performance.
  - Maintain DO levels above 4 mg/L for warm water species and above 6 mg/L for cold water species.
- Test organisms should not be subjected to changes of more than 2 units of pH in any 24-hour period or 3 degrees of temperature in any 12-hour period.

- Test organisms should be fed according to the EPA WET test method requirements for the WET test. When feeding is necessary for mysid or fish tests, excess food should be removed daily during renewal by aspirating with a pipette, to avoid problems such as food buildup leading to excessive oxygen demand.
- Test organisms should be handled as little as possible to minimize stress:
  - Dip nets should be used for large organisms (e.g., salmonids).
  - Pipettes should be used for transferring small organisms such as juvenile fathead minnow, fry minnows, silverside fry, and, daphnid or midge larvae.

REFERENCE TOXICANTS

Reference toxicants are used to evaluate the health and sensitivity of WET test organisms over time and for documenting initial and ongoing laboratory performance. A laboratory performs a definitive toxicity test with a reference toxicant at least once per month for each toxicity test method conducted in that month. The monthly WET test results are plotted on a control chart to track trends in organism health or sensitivity.

Although EPA does not require the use of specific reference toxicants or set required acceptance ranges for reference toxicants for reference toxicant testing, EPA does recommend that laboratories conduct frequent reference toxicant tests. EPA recommends that the results of these reference toxicant tests be used to evaluate the health and sensitivity of the test organisms over time and for documenting initial and ongoing laboratory performance. Testing laboratories must perform at least one acceptable reference toxicant test per month for each type of toxicity test method conducted in that month regardless of the source of test organisms. If a test method is conducted only monthly, or less frequently, a reference toxicant test must be performed concurrently with each effluent toxicity test to document ongoing laboratory performance and to assess organism sensitivity and consistency when organisms are cultured in-house. When organisms are obtained from external suppliers, concurrent reference toxicant tests must be performed with each effluent sample tested, unless the test organism supplier provides control chart data from at least the past five months of reference toxicant testing, which will assess organism sensitivity and health. The EPA WET test method manuals require a laboratory to obtain consistent, precise results with reference toxicant toxicity tests with effluents under the NPDES permits. It is important that the reference toxicants should be securely stored in an area separate and away from the laboratory's mass cultures or purchased test organisms to prevent unintended exposure or contamination of test organisms by the reference toxicants. This should be one of the inspector's checklist items when inspecting a WET laboratory.

An attempt should be made to match the type of reference toxicant used (e.g., metal or chlorinated organic) to the major pollutant in the wastewater tested. Reference toxicant data must be included with the testing laboratory report.

Reference toxicant test results should not be used as *de facto* criteria for rejection of individual effluent or receiving water tests. The EPA WET test methods manuals provide guidance for what to do when more than 1 reference test in 20 reference toxicant tests falls outside of

control chart limits, or when a reference toxicant test result falls "well" outside of the control treatment limits. However, when reference toxicity tests indicate possible anomalies, the laboratory should investigate sources of variability, take corrective actions to reduce identified sources of variability, and perform an additional reference toxicant test during the same month.

CONDUCT OF THE TEST(S)

EPA WET test methods should be carried out by analysts who are experienced in the use or conduct of aquatic tests and the interpretation of data from aquatic toxicity testing. Test conditions should match those specified in the summary of test condition tables provided for each EPA WET test method. Physical and chemical measurements taken during the test (e.g., temperature, pH, and DO) must be conducted at the minimum frequency specified in the EPA WET test method manuals. The appropriate procedures are described in each EPA WET test method section of the manuals, by following the table of specified test conditions and required TACs.

RECORDKEEPING AND DATA REPORTING

Proper recordkeeping is essential to an effective NPDES WET test monitoring program. Entities collecting samples for WET testing should consistently use chain-of-custody (COC) procedures to document effluent or receiving water sample transfer. Hand-written entries on bench sheets and COC tags must generally be clear and legible. The analyst should maintain a sample log containing information as to the date, time, and type of sample taken as well as the sampler's name. Unusual conditions should be noted. When evaluating the contract lab's WET test data reporting, the inspector should verify that the following are included:

- Summary of test results, description of test conditions, material tested, test dilution water and other data for quality assurance.
- Methods used for all analyses. The method title, method number, and method source should be provided in the laboratory standard operating procedure (SOP) and test report. Tests must be conducted as stated in the SOP, and the laboratory should verify the test was conducted according to the SOP.
- Date and time test started, date and time test terminated, type and volume of test chambers, volume of solution used per chamber, number of organisms per test chamber, number of replicate test chambers per treatment.
- The test temperature (mean and range), details of whether test was aerated or not, feeding frequency, amount and type of food, and any pH control measures taken.
- The test endpoint(s), and any deviation(s) from EPA's WET test methods (40 CFR Part 136 or EPA Pacific West Coast WET test methods (EPA, 1995)) must be clearly noted.
- The reference toxicity results for WET tests conducted for the test period with specific test details to verify species, temperature, and dilution water used in reference toxicant test.
- Any acclimation of test organisms (temperature mean and range) and the reason(s) for acclimation.

- Any other relevant information.

Any deviations from specifications, as contained in EPA's WET test methods, should be documented and described in the data report by the testing laboratory. Data results for each WET test should include the raw toxicity data in tabular form, including daily records of affected test organisms in each concentration (including control treatments and effluent test concentration replicates); data in graphical form (plots of toxicity data); and a table of LC_{50}s, NOECs, IC_{25}, IC_{50}, etc. (as required in the respective NPDES permit). Records should indicate the statistical approach used to calculate endpoints, include a summary table of physical and chemical data, and include laboratory documentation of variability as part of the quality assurance/quality control (QA/QC). For more information on possible contributing factors to WET variability and recommendations for reducing it, see section 7.3 of EPA's *Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System Program* (EPA, 2000a).

REVIEW CHECKLIST

While WET test reviews are performed as part of a routine NPDES facility inspection and usually are not comprehensive, the inspector and the permittee should carefully prepare in advance for the inspection. Laboratory inspection reviews can quickly ascertain if the facility is following their NPDES permit requirements and, secondarily, identify any obvious problems with reporting or laboratory performance. Inspectors should refer to the following checklist of possible issues that can be identified during a NPDES facility inspection.

Yes	No	N/A	Does the facility have a copy of its NPDES permit readily available? (Recommended: The inspector should bring a copy of the NPDES permit in the event the permittee does not have a complete copy at the time of inspection)
Yes	No	N/A	Were the WET tests required by the NPDES permit performed? Check the permit for the WET testing frequency and any special conditions related to WET testing, including whether a testing frequency decrease is authorized and the basis or rationale for decreasing the WET testing frequency (which should be documented in the NPDES permit fact sheet). This can be done prior to arriving on-site including contacting the NPDES state permitting authority or EPA if the state is not NPDES authorized.
Yes	No	N/A	Are all test reports for WET tests performed over the last three years available for review?
Yes	No	N/A	Are the test reports complete (e.g., bench data sheets for chemicals and test organisms, reference toxicant test results, chain of custody forms or tags, statistical analyses)?
Yes	No	N/A	Was the correct type of WET test performed including the choice of an appropriate (*most sensitive species*) WET test species used?

Yes	No	N/A	Did the effluent samples contain any measurable chlorine, or > 10 mg/l ammonia?
Yes	No	N/A	Was the WET test initiated within 36 hours of the first effluent sample being taken? This can be verified by checking the dates and times on the chain-of-custody forms or tags and bench sheets.
Yes	No	N/A	Did the laboratory or permittee make any judgment decisions beyond their authority? If Yes, describe:
Yes	No	N/A	Were there any deviations from the appropriate EPA WET test method? See NPDES permit and EPA WET test methods' test acceptability criteria.
Yes	No	N/A	Were the valid WET test results recorded and did they indicate non-compliance with the NPDES permits? If Yes, what follow-up actions were taken by the permittee and/or the permitting authority?
Yes	No	N/A	Were the WET test results reported correctly by the permittee and on the DMR?
Yes	No	N/A	Was the WET test determined to be invalid due to poor test organism performance in the control treatment?
Yes	No	N/A	If the WET test was declared invalid, was a new effluent sample collected, a new WET test performed and reported?

In the case of a PAI, both the laboratory performing the WET tests and the NPDES permittee are evaluated. This type of inspection requires more extensive information than is presented in this section. The inspector is therefore referred to the EPA's *Manual for the Evaluation of Laboratories Performing Aquatic Toxicity Tests* (EPA, 1991a) for the protocol to perform a PAI.

C. ANALYSIS OF WET DATA

WET test review should be conducted by both the testing laboratory, the permittee, and the NPDES regulatory authority. A review of WET tests includes: checking the WET test conditions; checking WET data or WET test results; and checking EPA WET test methods' TAC for test organisms in the control treatment(s) (and WET test variability for non-lethal endpoints such as the EPA WET test method's required percent minimum significant different (PMSD) determinations). Considerations for each of these WET test reviews are discussed below.

WET test results or WET data need to be interpreted so that compliance with the NPDES permittee's WET permit limits can be determined. For the NPDES permits program, each of EPA WET test methods contain several recommended statistical approaches. In addition, in 2010 EPA HQ (Water Permits Division/Office of Wastewater Management) developed a statistical approach referred to as the Test of Significant Toxicity (TST) as another option for statistically analyzing and interpreting valid WET test data—see EPA's *National Pollutant Discharge Elimination System Test of Significant Toxicity Technical Document* (EPA, 2010a).

The following definitions may help the inspector to interpret the WET test results:

- The LC_{50} (for lethal concentration) is the calculated percentage of effluent (point estimate) at which 50 percent of the organisms die during the test period. Usually, the LC_{50} is calculated statistically by computer programs that fit the dose-response curve to a mathematical function. Computer-based calculation procedures usually print an estimate of the error associated with the LC_{50} estimate.
- The EC_{50} (for effect concentration) is the calculated concentration (point estimate) at which 50 percent of the organisms indicate a particular impaired response or WET test measured effect (not necessarily death) due to exposure to a toxicant. For some species (e.g., *Ceriodaphnia dubia*—freshwater water flea, invertebrate) where the point of death is not certain, immobility is often used as a surrogate for death. Results for responses like the immobility responses in *Daphnia* (water flea, invertebrate) may be reported as an EC_{50} (calculated in the same manner as the LC_{50}). Often, however, no distinction is made between the EC_{50} and the LC_{50} when the response is a surrogate for death.
- The No Observed Effect Concentration (NOEC) is the highest tested concentration at which the organisms' responses are not statistically different from the control treatment organisms' responses. The NOEC (like the Lowest Observed Effect Concentration (LOEC) and Chronic Value (ChV) defined in the following paragraph) is normally determined only for chronic tests.
- The LOEC is the lowest tested effluent test concentration at which the organisms' responses are statistically different from those in the control treatments.
- The ChV is the calculated geometric mean of the NOEC and LOEC (the square root of the product of the NOEC and LOEC).
- The Inhibition Concentration (IC25) is the calculated percentage of effluent (point estimate) at which the organisms exhibit a 25-percent reduction in a non-quantal biological measurement such as fecundity or growth.
- The percent effect response measured at the critical dilution is reported. For example, state water quality standard (WQS) or NPDES permit WET limit may prohibit toxicity at 100 percent effluent or less. In this case, the observed percent effect response at 100 percent effluent would be reported.
- The response may be reported in Toxic Units (TU), either for Acute (TU_a) or Chronic (TU_c) test endpoints.
- A *no significant toxicity* assessment is a recommended statistical analysis alternative type of NPDES permit limit to a NOEC permit limit, as determined by the EPA's recommended TST statistical approach. No significant toxicity applies when the value calculated using a Welch's t-test is *significantly different* (i.e., greater) than a critical value. Thus, for NPDES permits, the assessment for no significant toxicity is based on statistically analyzing the measured effects at the control treatment to an effluent test concentration, which for NPDES permitting is usually the in-stream waste concentration or IWC. The IWC should be one of the effluent test concentrations in the WET test

usually bracketed by the other effluent test concentrations in a multiple test concentration test design.

Overall, there is an inverse relationship between the degree of toxicity and the effluent concentration percentage causing a toxic response. Therefore, the same toxicity test response (e.g., LC_{50}), at lower percentages of an effluent concentration indicates higher toxicity than WET test results at higher percentages of an effluent concentration. So, the magnitude of a TU indicates the degree of toxicity. TUs are defined as $100/LC_{50}$ for acute and 100/NOEC for chronic, with the LC_{50} or NOEC expressed as a percent effluent concentration. An effluent with an LC_{50} of 50 percent has an acute toxicity of 2 acute toxic units ($100/50 = 2\ TU_a$). Similarly, an effluent with a NOEC of 25-percent effluent has a chronic toxicity of 4 chronic toxic units ($100/25= 4\ TU_c$). The major advantage of using toxic units to express toxicity test results is that toxic units increase linearly as the toxicity of the effluent increases and so the higher the numeric TU, the greater the magnitude of measured toxicity. Therefore, an effluent with a TU_a of 4 is twice as toxic as an effluent with a TU_a of 2. Additionally, the NOEC, LC_{50}, and other statistical analyses are entered into the national enforcement database, ICIS, as pass/fail, whereas TUs are entered as a discrete number and can therefore reveal more about toxicity over time. EPA's *Technical Support Document for Water Quality-based Toxics Control* (EPA, 1991b) provides a more extensive discussion of the application of toxic units and the relevance to NPDES permits.

Review of Test Conditions. For WET test data submitted under NPDES permits, all required EPA WET test conditions must be met or the WET test is considered invalid and a new WET test is required using a newly collected effluent sample. Deviations from recommended EPA WET test *mandatory requirements* be evaluated on a case-by-case basis to determine the validity of the WET test results. Deviations from *recommended* test conditions may or may not invalidate a WET test result depending on: the degree of the departure from WET test conditions, the objective of the WET test, and the potential or observed impact of the deviation on the WET test result. Consideration of these factors should be carefully considered before rejecting or accepting a WET test result as valid. For example, if dissolved oxygen is measured below 4.0 mg/L in one WET test chamber, the reviewer should consider whether the observed mortality in that WET test chamber corresponds with the drop in dissolved oxygen. Whereas slight deviations in WET test conditions may not invalidate an individual WET test result, test condition deviations that continue to occur frequently in a laboratory may indicate the need for improved quality control in that laboratory.

Each WET test method has specified acceptable ranges of test conditions that are to be met, such as temperature, dissolved oxygen concentration, salinity, pH, light intensity and duration of photoperiod, organism loading (numbers or weight per volume), feeding, and cleaning procedures. WET tests not meeting the test conditions, Test Acceptability Criteria (TAC), and the non-lethal endpoint percent minimum significant difference (PMSD) for a specific WET test method should be carefully reviewed by the inspector. Also, the WET test and the WET test results should be referred to the EPA or state regional biologist and the NPDES regulatory authority (or permit writer). For each parameter discussed in these tables, the parameter is either *recommended (should do)* or *required (must do)*. For example, the chronic *Ceriodaphnia*

dubia test type is required (must) to be conducted. The inspector should review the EPA WET test methods for a more extensive discussion of each of the *recommended (should)* and *required (must)* WET test specifications. The EPA WET test methods manuals for Whole Effluent Toxicity testing can be accessed at https://www.epa.gov/cwa-methods/whole-effluent-toxicity-methods.

Review of Calculated WET Test Results. Inspectors should review WET test results (from multi-concentration tests) reported under the NPDES permits program according to EPA guidance on the evaluation of concentration-response relationships (EPA, 2000a). This guidance provides review steps for 10 different concentration-response patterns that may be encountered in WET test data. Based on the review, the guidance provides one of three determinations:

1. The calculated effect concentrations are reliable and should be reported.
2. The calculated effect concentrations are anomalous and should be explained.
3. The test was inconclusive and a new WET test should be conducted using a newly collected effluent sample.

It should be noted that the determination of a valid concentration-response relationship is not always clear cut. Data from some WET tests may suggest consultation with professional toxicologists and/or NPDES regulatory officials. Tests that exhibit unexpected concentration-response relationships may indicate a need for further investigation and possibly require a new WET test to be conducted using a newly collected effluent sample.

Questionable results in an acute test include:

- Higher mortalities in lower effluent test concentrations than in higher effluent test concentrations.
- 100-percent mortality in all effluent test concentrations.
- Greater percent mortality in the control treatment than in the lower effluent test concentrations.

Questionable results in a chronic test include:

- Greater growth or reproduction or fewer terata at higher effluent test concentrations than at lower effluent test concentrations.
- No growth or reproduction or 100-percent terata at all effluent test concentrations.
- Less growth or reproduction or more terata in control treatments than in lower effluent test concentrations.

When any of these abnormalities occur (outside of experimental error), the results and test conditions should be reviewed by the EPA and/or state regional biologist or NPDES toxicologist and reported to the NPDES regulatory authority (permit writer). Part of the inspector's review may also include a review of the laboratory's WET test data results and an explanation or interpretation of the WET test results. DMRs are expected to include this information.

In addition to reviewing the concentration-dose response relationship, the inspector should review within-test variability of individual WET tests. For example, when NPDES permits require chronic sub-lethal hypothesis testing endpoints (e.g., reproduction for the *Ceriodaphnia dubia* test), within-test variability should be reviewed and variability criteria applied as described in the chapter "Report Preparation and Test Review" of each WET test method.

Within-test variability is measured as the percent minimum significant difference (PMSD), and is calculated by the test reporting entity, then compared to established upper and lower bounds for test PMSDs. WET tests conducted under NPDES permits that fail to meet these variability criteria and that show "no toxicity" at the permitted receiving water concentration (i.e., not significantly different from the control treatment) are considered invalid WET tests and a new WET test must be conducted using a newly collected effluent sample. Circumstances that indicate that the results of the WET test may be questionable include: pH of the water was less than 6 or greater than 9, feeding schedule used during the test differed from the feeding schedule recommended in the methods manuals, organism culture was contaminated with rotifers, or if the test was repeated due to laboratory error. For additional circumstances that may yield WET test results with questionable variability, the inspector should refer to EPA's *Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods* (EPA, 2001a).

To avoid penalizing laboratories that achieve unusually high precision, lower PMSD bounds are applied when a hypothesis WET test result (e.g., no observed effect concentration NOEC) or lowest observed effect concentration (LOEC) is reported. Lower PMSD bounds are based on the 10th percentiles of national PMSD data. The 10th percentile PMSD represents a practical limit to the sensitivity of the WET test method because few laboratories can achieve such precision on a regular basis and most do not achieve it even occasionally. In determining hypothesis WET test results, an effluent test concentration is not considered toxic if the relative difference from the control treatment is less than the lower PMSD bounds. See EPA's *Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System Program* (EPA, 2000a), for specific examples of implementing lower PMSD bounds.

Review of Test Acceptability Criteria (TAC) for Controls. Each EPA WET test method also has specific required WET test acceptability criteria or TAC (e.g., minimum control survival) that must be achieved to be considered a valid WET test result. See the summary of test conditions and TAC for each specific EPA WET test method. In general, the valid interpretation of WET test results requires that control treatment organisms must meet minimum TAC for survival, growth, and/or reproduction as required by the respective EPA WET test methods. A summary of TACs per EPA WET test method can be found in Table 8-2.

Mortality in control treatments must not exceed 10 percent for acute toxicity tests and 20 percent for chronic tests (or other values as required by states through their regulations). If organism survival in the control treatments does not meet 90 or 80 percent for an acute or chronic test, respectively, then the WET test results should not be used for calculating summary statistics, and a determination of compliance using the WET test results cannot be made. For

chronic tests, test organism in the control treatments must also meet minimum requirements for growth and reproduction contained in the EPA WET test methods manuals. When using dual controls, the dilution water control treatment should, through statistical analysis, be used to determine the acceptability of the WET test control treatment, and for comparisons against the effluent test concentrations.

Table 8-2. Summary of TAC per EPA Method

EPA Method	Organism with Scientific Name	Endpoint Type	Test Type	Minimum # per Test Chamber	Minimum # of Rep per Conc.	Minimum # Effluent Conc.	Test Duration	Test Acceptance Criteria (TAC)
2000.0	Fathead minnow *(Pimephales promelas)*	Survival	Acute	10	2	5	48–96 hours	> 90% survival in controls
1000.0	Fathead minnow *(Pimephales promelas)*	Survival and growth (larval)	Chronic	10	4	5	7 days	> 80% survival in controls; average dry weight per surviving organism in control chambers equals or exceeds 0.25 mg
1002.0	Water flea *(Ceriodaphnia dubia)*	Survival and reproduction	Chronic	1	10	5	Until 60% of surviving control organisms have 3 broods (6–8 days)	> 80% survival and an average of 15 or more young per surviving female in the control solutions. 60% of surviving control organisms must produce three broods
1007.0	Mysid shrimp *(Americamysis bahia)*	Survival and growth	Chronic	5	8	5	7 days	> 80% survival; average dry weight > 0.20 mg in controls

Table 8-2. Summary of TAC per EPA Method

EPA Method	Organism with Scientific Name	Endpoint Type	Test Type	Minimum # per Test Chamber	Minimum # of Rep per Conc.	Minimum # Effluent Conc.	Test Duration	Test Acceptance Criteria (TAC)
1016.0	Purple urchin *(Strongylocentrotus purpuratus)* or Sand dollar *(Dendraster excentricus)*	Fertilization	Chronic	100	4	4	40 min (20 min plus 20 min)	> 70% egg fertilization in controls; %MSD < 25%; and appropriate sperm counts
1017.0	Giant kelp *(Macrocystis pyrifera)*	Germination and germ-tube length	Chronic	100 for germination 10 for germ-tube length	5	4	48 hours	≥ 70% germination in controls; ≥ 10 µm germ-tube lengths in controls; %MSD of < 20% for both germination and germ-tube length NOEC must be below 35 µg/L in reference toxicant test
1014.0	Red abalone *(Haliotis rufescens)*	Larval development	Chronic	100	5	4	48 hours	≥ 80% normal larval development in controls Statistical significance @ 56 µg/L zinc % MSD < 20%

Table 8-2. Summary of TAC per EPA Method

EPA Method	Organism with Scientific Name	Endpoint Type	Test Type	Minimum # per Test Chamber	Minimum # of Rep per Conc.	Minimum # Effluent Conc.	Test Duration	Test Acceptance Criteria (TAC)
2002.0	Water flea *(Ceriodaphnia dubia)*	Survival	Acute	5	4	5	24, 48, or 96 hours	> 90% survival in controls
1003.0	Green algae *(Selenastrum capricornutum)*	Growth (cell counts, chlorophyll fluorescence, absorbance, or biomass)	Chronic	10,000cells/ mL	4	5	96 hours	Mean cell density of at least 1 X 106 cells/mL in the controls; variability (CV%) among control replicates less than or equal to 20%

D. TOXICITY REDUCTION EVALUTIONS AND TOXICITY IDENTIFICATION EVALUATIONS (TRES/TIES)

Toxicity Reduction Evaluations (TREs) and Toxicity Identification Evaluation (TIEs) are procedures used with the EPA's NPDES permits program to enable permittees to identify and reduce toxicity that is observed using WET tests. EPA's TRE and TIE procedures manuals can be found at the following website: https://www.epa.gov/npdes/npdes-permit-limits#wet.

A TRE is a site-specific study of the effluent or wastewater at a treatment facility. The TRE process is generally a stepwise process that attempts to identify the class of potential toxicants and, if possible, isolate the chemical causing toxicity. A TRE generally consists of six steps, but all six steps may not be required depending on the facility site-specific situation. Once the identification/isolation process has confirmed the potential cause of toxicity, the evaluation step uses techniques to determine what action(s) is needed to reduce or treat the chemical or chemicals causing toxicity in the effluent. If the evaluation step is completed successfully, the TRE should confirm that the actions chosen to reduce toxicity are successful. There are many possible ways to reduce toxicity depending on the cause of toxicity.

The need for a permittee to conduct a TRE may arise when the NPDES WET permit limit is exceeded during WET monitoring in accordance with the NPDES permit. NPDES WET permit limits are established to prevent excursions from state WET water quality standards, so an exceedance of a WET permit limit can sometimes trigger additional permit requirements. These permit triggers are actions the permittee must take to identify and resolve the toxicity to come back into compliance with the permit. Accelerated WET monitoring is a common permit trigger that can vary from state to state, but there's usually a requirement for more frequent WET testing over a short time period, generally a few weeks, to determine if the toxicity is persistent. If the effluent toxicity is not measured at a level that exceeds the permit limit, based on the data generated by the accelerated WET testing, the permit usually allows for a return to the previous WET monitoring frequency schedule. If toxicity continues to measure in exceedance of the WET permit limit, based on the accelerated WET testing data, then the TRE process is initiated. It is extremely important for the permittee and the permitting authority to agree upon an adequate work plan (developed by the permittee) that includes a schedule and reporting requirements throughout the TRE/TIE process, and especially when the TRE is first initiated.

In practice, most of the TRE work completed by the permittee is conducted through the permittee's labs or consultants. Therefore, it is important for the EPA or state NPDES permitting authority to ensure that the TRE process is on track and that the permittee resolves the toxicity problem in an appropriate and timely manner. The NPDES permitting authority can provide key recommendations to the permittee to ensure that all available information and possible strategies are considered in the evaluation. An important recommendation is that the permittee has a TRE work plan that is sufficiently detailed and includes frequent communication with the NPDES permitting authority. TRE work plan requirements vary from

state to state, but commonly include schedule and reporting requirements to ensure effluent toxicity is reduced or eliminated and compliance with the permit is achieved.

A TRE is most likely to be successful if there is a good partnership between the people who know the facility and the experts in engineering, toxicology, and perhaps hydrology, who know how to determine the causes of the effluent toxicity. For example, the toxicologist on the team can help link water quality characteristics to toxicity for different USEPA WET test species.

Regardless of the facility, a TRE almost always starts with a review of available data, such as influent and effluent chemical and physiochemical data, facility treatment data, and WET test data. Often, a thorough review of these data can be very useful in helping to determine what might be causing toxicity in the effluent. Facility treatment information that is often useful in conjunction with the effluent toxicity data include parameters such as effluent carbonaceous oxygen demand (COD), biological oxygen demand (BOD), mixed liquor solids, volatile solids, and removal rates of COD and BOD based on influent and effluent concentrations. The work plan should include the data and other information available for the evaluation, any interim reports or other deliverables to be sent to the NPDES permitting authority, and the roles and responsibilities of the TRE plan's team members.

One optional step in the six-step TRE approach is to identify the *exact cause of effluent toxicity*. This is commonly referred to as a Toxicity Identification Evaluation or TIE. Although not necessary, a TIE can often be very helpful in a TRE because toxicity can be more certainly controlled if the identity of the toxicant(s) is known. In general, the TIE is a three-phase process that characterizes, identifies and confirms the cause or causes of toxicity. Guidance documents for each of the three phases of toxicity identification evaluations and the Phase I TIE for chronically toxic effluents can be found at the EPA website provided at the beginning of this section. A TIE couples effluent chemical analysis and WET test results. Although sometimes it may take additional effort to identify the exact cause of effluent toxicity, particularly in very complex effluent situations, experienced WET testing laboratories and consultants can help ensure that the TIE is not an expensive, time-consuming venture. TIEs are applicable to evaluating toxicity of permitted effluents, ambient waters and sediments including bulk sediment or pore waters.

The role of the NPDES permitting authority in TIEs is to support innovative approaches that are technically feasible and scientifically sound, and to discourage approaches that are costly and/or not results-oriented. In some instances, the discharger may need to use novel approaches to identify the cause of toxicity. The NPDES permitting authority can assist the permittee by providing technical information where appropriate. However, conducting the TIE/TRE is the responsibility the permittee. The role of the NPDES permitting authority is to allow the TIE/TRE process to proceed and to confirm that the permittee is making good progress towards completing the TRE.

In addition to NPDES permit conditions, there are several other mechanisms that the NPDES permitting authority can use to require a permittee to conduct a TRE. The NPDES permitting authority can require a TRE through a CWA section 308 letter, a CWA section 309

administrative order, or as part of the Consent Decree requirements in the settlement of a civil judicial enforcement action. The role of the inspector is to evaluate whether the permittee has met the TRE/TIE milestones and to verify whether the permittee has implemented the selected controls and eliminated toxicity.

E. REFERENCES

The following is a list of resources providing additional information on toxicity and testing.

U.S. Environmental Protection Agency. (1991a). *Manual for the Evaluation of Laboratories Performing Aquatic Toxicity Tests*. EPA 600-4-90-031.

U.S. Environmental Protection Agency. (1991b). *Technical Support Document for Water Quality-based Toxics Control*. EPA 505-2-90-01.

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U.S. Environmental Protection Agency. (2002a). *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*. EPA 821-R-02-012.

U.S. Environmental Protection Agency. (2002b). *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*. EPA 821-R-02-013.

U.S. Environmental Protection Agency. (2002c). *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms*. EPA 821-R-02-014.

U.S. Environmental Protection Agency. (2002d). *Guidelines Establishing Test Procedures for the Analysis of Pollutants; Whole Effluent Toxicity Test Methods; Final Rule*. 40 CFR Part 136.

U.S. Environmental Protection Agency. (2010a). *National Pollutant Discharge Elimination System Test of Significant Toxicity Technical Document*. EPA 833-R-10-004.

U.S. Environmental Protection Agency. (2010b). *National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document*. EPA 833-R-10-003.

U.S. Environmental Protection Agency. (2010c). *NPDES Permit Writer's Manual: Chapter 6. Water Quality-Based Effluent Limitations*. EPA 833-K-10-001.

U.S. Environmental Protection Agency. (2016). *Clean Water Act Methods Update Rule for the Analysis of Effluent - Final Rule*. Available at: https://www.epa.gov/cwa-methods/methods-update-rule-support-documents