SECTION VIII

WHOLE EFFLUENT TOXICITY (WET) PROGRAM

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A. Definitions, Acronyms, and Abbreviations

Acute to Chronic Ratio (ACR) – The ratio of the acute toxicity of an effluent or a toxicant to its chronic toxicity. It is used as a factor for estimating chronic toxicity based on acute toxicity data, or for estimating acute toxicity based on chronic toxicity data.

Acute Toxicity – An effect that usually occurs shortly after the administration of either a single dose or multiple doses of a pollutant. Lethality to an organism is the usual measure of acute toxicity. Where death is not easily detected, immobilization is considered equivalent to death.

Criteria Continuous Concentration (CCC) – The EPA national water quality criteria recommendation for the highest instream concentration of a toxicant or an effluent to which organisms can be exposed indefinitely without causing unacceptable chronic effects. Numerically, this equates to 1.0 TU_c.

Chronic Toxicity – An effect that is irreversible or progressive or occurs because the rate of injury is greater than the rate of repair during prolonged exposure to a pollutant. This includes low level, long-term effects such as reduction in growth, reproduction, or fecundity.

Criteria Maximum Concentration (CMC) – The EPA national water quality criteria recommendation for the highest instream concentration of a toxicant or an effluent to which organisms can be exposed for a brief period without causing an acute effect. Numerically, this equates to 0.3 TU_a.

Contaminated Non-Process Wastewater – Any water which, during manufacturing or processing, comes into incidental contact with any raw material, intermediate product, finished product, by-product, or waste product by means of rainfall runoff, accidental spills, leaks caused by failure of process equipment or discharges from safety showers and related personal safety equipment.

Continuous Discharge - A discharge which occurs without interruption throughout the operating hours of the facility, except for infrequent shutdowns for maintenance, process changes, or other similar activities.

Coefficient of Variation (CV) – A standard statistical measure of the relative variation of a distribution or set of data, defined as the standard deviation divided by the mean. It is also called the relative standard deviation (RSD). The CV can be used as a measure of precision within and among laboratories, or among replicates for each treatment concentration.

Discharge Monitoring Report (DMR) – The form supplied by the Department, or an equivalent form developed by the permittee and approved by the Department, for the reporting of self-monitoring results by permittees.

Flows: 7Q10 – The critical receiving stream flow used to calculate chronic aquatic life water quality standards. It is the low flow which, on a statistical basis, would occur for a 7 consecutive day period once every 10 years.

1Q10 – The critical receiving stream flow used to calculate acute aquatic life water quality standards. It is the lowest stream flow which, on a statistical basis, would occur over a 1-day period once every 10 years.

30Q5 – The critical receiving stream flow which is used to calculate the non-carcinogenic human health water quality standards. It is the lowest stream flow which, on a statistical basis, would occur for a 30-day consecutive period once every 5 years.

Inhibition Concentration (IC) – Usually seen as IC_{25}, the estimated concentration that would cause a 25% reduction in effect from the control organisms.

Instream Waste Concentration (IWC) – The concentration of an effluent, expressed as a percentage, which occurs in the receiving waterbody after mixing. To calculate the IWC, divide the effluent flow by the 7Q10 (chronic IWC, or IWC_c) or 1Q10 (acute IWC, or IWC_a) added to the effluent flow. Also known as receiving water concentration (RWC).

Intermittent Stream – A stream that contains flowing water for extended periods during a year but does not always carry flow.

Lethal Concentration (LC) – Usually seen as LC_{50}, the concentration of a toxic pollutant or effluent expressed as percent volume that is lethal to 50% of the test organisms within the prescribed period.

Lowest Observed Effect Concentration (LOEC) – The lowest concentration of an effluent or toxicant that results in statistically adverse effects on the test organisms (i.e., where the values for the observed endpoint are statistically different from the control. It is seen as a secondary end point for chronic tests.

Minimum Significant Difference (MSD) – The magnitude of difference from the control where the null hypothesis is rejected in a statistical test comparing a treatment with a control. MSD is based on the number of replicates, control performance, and power of the test.

No Observed Adverse Effect Concentration (NOAEC) – An acute test endpoint, the highest concentration at which survival is not significantly different from the controls., and below which there is no statistically significant adverse effect.

No Observed Effect Concentration (NOEC) – A chronic test endpoint, the highest concentration of toxicant to which organisms are exposed in which the values for the observed responses are not statistically different from the controls, and below which there is no statistically significant adverse effect.

Non-Contact Cooling Water – Water which is used to reduce temperature which does not come into direct contact with any raw material, intermediate product, waste product (other than heat), by-product or finished product.

Publicly Owned Treatment Works (POTW) – Any device or system used in the treatment of municipal sewage or industrial wastes of a liquid nature which is owned by a state or municipality. Sewers, pipes, or other conveyances are included in this definition only if they convey wastewater to a POTW providing treatment.

Reasonable Potential – Where an effluent is projected or calculated to cause an excursion above a water quality standard based on several factors including, as a minimum, the four factors listed in 40 CFR 122.44(d)(1)(ii).

Reference Toxicant Test – A toxicity test performed with a quantified chemical in accordance with the procedures required for effluent tests. It checks the sensitivity of the organisms being used and the suitability of the test methodology. Reference toxicant data are part of a routine QA/QC program to evaluate the performance of laboratory personnel, and the robustness and sensitivity of the test organisms.

Significant Industrial User (SIU) – This includes, except as provided in paragraph 3. of this definition:

1. All industrial users subject to Categorical Pretreatment Standards under 9VAC25-31-780 and incorporated by reference in 9VAC25-31-30; and
2. Any other industrial user that:
   - discharges an average of 25,000 gallons per day or more of process wastewater to the POTW (excluding sanitary, noncontact cooling and boiler blowdown wastewater);
   - contributes a process wastestream which makes up 5 percent or more of the average dry weather hydraulic or organic capacity of the POTW treatment plant; or
   - Is designated as such by the Control Authority (DEQ), as defined in 9 VAC 25-31-840A, on the basis that the industrial user has a reasonable potential for adversely affecting the POTW's operation or for violating any pretreatment standard or requirement.
3. Upon a finding that an industrial user meeting the criteria in paragraph 2. of this definition has no reasonable potential for adversely affecting the POTW's operation or for violating any pretreatment standard or requirement, the control authority may at any time, on its own initiative or in response to a petition received from an industrial user or POTW, and in accordance with Part VII (9VAC25-31-730 et seq.) of this regulation, determine that such industrial user is not a significant industrial user.

Test Acceptability Criteria (TAC) – In order that toxicity test results be considered acceptable, the effluent and the reference toxicant must meet specific criteria as defined in the test method (e.g., for the chronic *Ceriodaphnia dubia* survival and reproduction test, the criteria are as follows: the test must achieve at least 80 percent survival and an average of 15 young per surviving female in the controls).

Toxicity – The inherent potential or capacity of a material to cause adverse effects in a living organism, including acute or chronic effects to aquatic life, bioaccumulation of pollutants in the tissues of aquatic organisms at levels which result in potential harm to the organism or pose a risk to organisms in the food chain, or detrimental effects on human health or other adverse environmental effects.

Technical Support Document (TSD) – EPA's Technical Support Document for Water Quality-based Toxics Control (March 1991, EPA505/2-90-001).

Toxic Unit (TU) – Units utilized to measure Whole Effluent Toxicity, TU_a refers to an acute toxicity unit and TU_c refers to a chronic toxicity unit.

Waste Load Allocation (WLA) – Wasteload Allocation is the portion of a receiving water's total maximum daily load that is allocated to one of its existing or future point sources of pollution.

Water Quality Standards (WQS) - regulations that describe water quality requirements in general terms or numerical limits for specific physical, chemical, and biological characteristics of

water. Water quality standards consist of numeric or narrative water quality criteria, use designations for state waters and an antidegradation policy. These statements and limits serve as the enforceable means, particularly through their use in VPDES permit limits and certification of 401 applications, to protect the beneficial use of State waters such as swimming, fishing, propagation and growth of aquatic life, and domestic water supply. (See 9VAC25-260-00 et seq.)

B. WET Applicability

1. Introduction

The U.S. Environmental Protection Agency (EPA) established an integrated toxics control program in 1984 following the analysis of effluent data that suggested the previously established technology-based effluent limits (TBELs) were not fully protective of aquatic life. This finding supported that complex effluents may contain numerous toxicants that lead to possible additive, synergistic, or antagonistic effects. The Clean Water Act (CWA) goals of "protection and propagation" and the CWA's national policy that the "discharge of pollutants in toxic amounts be prohibited" provide a basis for the implementation of a program to control such effects. This approach, and consequent regulations, established the requirement for NPDES permits to include water quality-based effluent limits (WQBELs), when necessary, to achieve water quality standards (WQS). One such requirement is 40 CFR 122.44(d)(1)(iv), which states "When the permitting authority determines, using the procedures in paragraph (d)(1)(ii) of this section, that a discharge causes, has the reasonable potential to cause, or contributes to an in-stream excursion above the numeric criterion for whole effluent toxicity, the permit must contain effluent limits for whole effluent toxicity (WET)."

Virginia utilizes the EPA recommended numeric criteria for WET of 0.3 toxic unit-acute (TU_a) and 1.0 toxic unit-chronic (TU_c), and the narrative criteria of "no toxics in toxic amounts." The WET program employs acute and chronic toxicity tests to measure such aggregate toxicity of pollutants present in wastewater. An acute toxicity test is designed to measure mortality or lethality and has an exposure time that is generally 96 hours or less. A chronic toxicity test is usually conducted with species of an age that they represent a critical life phase and can have exposure times that range from minutes to days. Chronic tests measure mortality and immobility as well as sublethal effects such as growth or reproduction. In addition to the type of test required, test species, monitoring period, and frequency also need to be established to generate data that is representative of the effects of the effluent on the receiving water's biology. The test requirements are determined by several factors, including the evaluation of the instream waste concentration (IWC), which is a representation of the possible dilution or mixing of an effluent in the receiving water, the variability of the effluent, and chemical composition of the effluent.

The determination that a facility is subject to WET requirements depends on the classification of the facility (municipal or industrial), the design flow, and the characterization of the effluent. Generally, effluents that are known or believed to contain pollutants that cause or may contribute to toxicity in the receiving water are subject to WET requirements. Once an effluent has been thoroughly characterized, a determination can be made for monitoring requirements, including test species, monitoring frequency, and reporting requirements.

All permit applications should be carefully reviewed to determine if there is "reasonable potential for toxicity" from the discharger. Reasonable potential (RP) is demonstrated if a discharge causes, has the potential to cause, or contributes to toxicity in the receiving water. An RP analysis can be performed with or without WET testing data and can determine if a WET limit is necessary. RP

analyses can be performed as frequently as new information is made available but should be performed at a minimum of once every permit cycle before a permit is issued or reissued. See Section F, Reasonable Potential Analysis, for more detailed information on when and how to perform these analyses.

2. Municipal Facilities

The following criteria have been developed by Virginia DEQ for municipal facilities, including privately or Publicly Owned Treatment Works (PVOTWs and POTWs, respectively), to determine if the discharger is subject to WET requirements:

a. All facilities permitted as a major municipal facility with design flow rates greater than or equal to 1.0 MGD.
b. All facilities with an approved pretreatment program or required to develop a pretreatment program.
c. Other facilities based on consideration of the following factors:
    i. The variability of pollutants or pollutant parameters in the effluent; or
    ii. The Instream Waste Concentration (IWC); or
    iii. Existing controls on point or non-point sources, including total maximum daily load (TMDL) calculations for the receiving stream segment and the relative contribution of the facility; or
    iv. Receiving stream characteristics; or
    v. Other considerations that could cause or contribute to adverse water quality impacts.

The main determining factor for municipal facilities that are subject to WET requirements concerns the variability of the influent. As municipal influent consists of wastewater from several sources, it is challenging to reliably qualify the pollutants that may be a part of the waste stream.

3. Industrial Facilities

The following criteria have been developed for industrial facilities to determine if the discharger is subject to WET requirements:

a. Any industry whose Standard Industrial Classification (SIC) code(s) are included in Appendix A of the WET Guidance (Guidance Memo No. 00-2012).
b. Any industry with an IWC greater than or equal to 33%.
c. Any other discharge that is determined to have the potential for toxicity or instream impact based on evaluation of manufacturing processes, indirect dischargers, treatment processes, effluent or receiving stream data, or other relevant information. Possible candidates for this criterion are:
    i. Bulk Oil Storage Facilities
    ii. Water Treatment Plants
    iii. Tunnels
    iv. Coal Mining Operations, including coal pile runoff.
    v. Water Conditioning Facilities
    vi. Facilities that do not discharge process water but may discharge contaminated stormwater.
    vii. Heating/Cooling Compressor wastewater
    viii. Boiler blowdown/Steam condensate
    ix. Wastewater treated through an oil/water separator.
    x. Effluents with significant concentrations of degreasers
    xi. Noncontact cooling water discharges with an IWC less than 1% but which are treated with chemical additives.

Industries that may be excluded from the WET program are:

a. Discharges of noncontact cooling waters with an IWC of less than 1% that are not treated with chemical additives.
b. Pump-outs of non-contaminated groundwater and pump-outs of petroleum contaminated groundwater which receive appropriate treatment and where BTEX limits are applied.
c. Hydrostatic tests at petroleum pipeline pump stations (excluding bulk oil storage facilities) if the permit is drafted in accordance with EPA Guidance.
d. Corrective Action Plan (CAP) permits which involve discharges to surface waters.

4. Stormwater Discharges

Discharges that contain or are believed to contain contaminated industrial stormwater are subject to WET requirements. Previously, WET requirements for industrial stormwater discharges were limited to monitoring only. Following a call with EPA on February 6, 2024, the decision was made that WET limits on stormwater discharges would be supported when determined to be appropriate. Currently, it is recommended that all stormwater discharges are evaluated for WET. This includes calculating the IWC, evaluating previous compliance data, and performing an accelerated RP analysis (See Section F for additional information).

C. Test Determinations

1. Sample Type

As is for any sample collection, the accuracy of WET test results relies on proper sampling and sample handling to maintain the condition and representativeness of the sample. Detailed instructions for sample collection, storage, and transport can be found in the EPA WET test methods, and do not need to be included in the permit directly. However, it is vital to require a sample collection type that provides the most representative sample of the discharge. Without proper sample collection, the samples, and therefore the tests, are invalid and cannot be used to evaluate an effluent. The two most used WET sampling methods in VPDES permits are grab samples and composite samples. Each sample type serves a different purpose when it comes to capturing the toxicity of an effluent.

It is important to note that regardless of the sample type, sampling should occur after all steps of treatment have concluded to get a sample that represents the final effluent that would enter the receiving stream. For facilities that disinfect with chlorine, the total residual chlorine (TRC) concentration of the sample should be measured within 15 minutes. TRC should again be measured as the sample is received at the laboratory prior to toxicity testing.

Grab Samples

Grab samples are discrete samples that are collected for a short time frame (less than 15 minutes) to represent the conditions at that time. Grab samples are useful for intermittent discharges, where compositing is difficult or impossible. For facilities with a highly variable effluent, grab samples are more likely to represent the peak toxicity of the effluent without allowing for dilution. In addition to being more representative of the discharge, a grab sample would be more appropriate for discharges to receiving waters where there is little or no mixing or dilution, tidal waters, or high velocity waters, as organisms in the receiving water would be subject to longer exposure to higher concentrations of effluent. The time that a grab sample is collected should be noted in the permit to require a sample that represents the highest potential for toxicity in the effluent. For example, an industrial discharge should sample when the concentration of process wastewater is highest, or a municipal discharge should sample when there are large contributions from industrial users.

Composite Samples
Composite samples consist of grab samples taken at a minimum frequency of one per hour and combined in proportion to flow. The number of grab samples should be determined by the variability of the composition and flow of the discharge. Composite samples average the characteristics of the effluent over the sampling period, which increases the possibility that a spike in toxicity would be captured but would also dilute the toxicity with the remaining composition of the sample. This makes composite samples more useful when the goal is to evaluate short-term chronic effects where the prolonged exposure of peak toxicity concentrations is less of a concern, or for discharges that are not variable. Flow-weighted composites, instead of time-weighted composites, may be useful in situations where the flow is variable such as municipal discharges, or in some cases, stormwater discharges.

2. Discharge Frequency

The permit writer should consider if the discharge is continuous or intermittent to require applicable sampling requirements. The determination of sampling requirements is facility specific, and should be considered as such. The flow monitoring requirements can be applied to WET sampling requirements if there is not a strong need for one type of sampling over another. It is important to note that what is representative of the discharge is not necessarily representative of the potential toxicity of the discharge.

a. Continuous Discharges
Continuous discharges occur constantly or near constantly. EPA recommends the sampling requirements be based on retention time, with estimated retention times of less than 14 days having a recommended sampling schedule of a minimum of four grab or four composite samples collected over a 24-hour period and used for separate toxicity tests. For example, four grab samples could be taken at a frequency of once every six hours or four successive 6-hour composite samples. For continuous discharges with detention times (the time it takes for the influent to make its way to the discharge) of longer than 14 days or with less than 10% WET variability over a 24-hour period regardless of retention time, EPA recommends a single grab sample collected for a single WET test as sufficiently representative of the effluent.

b. Intermittent Discharges
Intermittent Discharges are more periodic, occurring at frequencies such as several hours per day, month, or year. For the purposes of Virginia DEQ's WET Guidance, intermittent is defined as having a continuous discharge for less than four consecutive days. EPA suggests that intermittent discharges are sampled with grab samples collected midway through the discharge period. Refer to Part III of the permit for stormwater discharge sampling requirements. Virginia DEQ's WET Guidance asserts that chronic toxicity testing may be discontinued for facilities with intermittent discharges, due to the short exposure duration resulting from intermittent discharges.

3. Test Type and Species

a. Test Type
Following the characterization of the effluent through comprehensive WET testing, a determination may be made to select one test type to best represent the potential toxic impact of the discharge in the receiving water. To do so, the scope of each test type, as well as the utility of the data generated, needs to be fully understood.

i. Acute Toxicity Tests

All facilities that are subject to WET requirements need to be assessed for acute toxicity. The statistical endpoint measured by an acute toxicity test is expressed as the effluent concentration that is lethal to 50% of the test organisms (LC_{50}) and the No Observed Adverse Effect Concentration (NOAEC).

(a) LC_{50} Test

The LC_{50} test statistically estimates the concentration of the sample that is lethal to 50% of the test organisms. It can be run as a 48-hour static test, or a 96-hour static renewal test. A minimum of 5 concentrations of the sample is set up in a geometrically derived dilution series along with controls. Dilutions may need to be added at the lower end of the series to achieve a calculable LC_{50}.

(b) NOAEC Test

The NOAEC test is recommended when the acute IWC (IWC_a) is greater than 33%. The test determines the highest effluent concentration that is not significantly different from the control. This is interpreted as the highest percent concentration where there is no significant difference when compared to the controls and below which there is no statistically significant adverse effect. This test can be run as a single dilution with replicates, usually 100% effluent and controls, or as a multi-dilution test, with a 48-hour duration. The single dilution test may only be used when there is a WET limitation, and only when approved by DEQ Central Office and EPA. The LC_{50} can also be calculated from this test.

The rationale for using the NOAEC test when the IWC_a is greater than 33% is due to the requirement to meet EPA's Criteria Maximum Concentration (CMC) of 0.3 TU_a which is to be met at the end-of-pipe. The CMC is used to adjust the LC_{50} point estimate of 50% mortality to an LC_1, or a test with virtually no mortality. The equivalent LC_{50} concentration is 333.333% effluent, which is impossible to test. Testing with the highest concentration that is possible to test (100%) would still allow for a test that was compliant with the test endpoint to have 50% mortality, which is not protective of the acute criterion of "no discharge of toxic chemicals in toxic amounts." The TSD (page 35) states that the CMC of 0.3 includes 91% of observed LC_1 to LC_{50} ratios in acute tests. As a result, a dilution ratio of less than approximately three parts receiving water to one part effluent (3:1), the resulting wasteload allocation (WLA) will be lower than the minimum level of acute toxicity than the LC_{50} test can measure. Hence, the NOAEC test is more accurate, in that it statistically determines whether the 100% effluent is significantly different than the controls.

ii. Chronic Toxicity Tests

A facility should monitor for chronic toxicity if the chronic IWC (IWC_c) is greater than or equal to 1%, and the discharge is continuous. An IWC_c of less than 1% present little to no effects of chronic toxicity. A chronic test is performed with a minimum of 5 effluent dilutions and the controls for a duration of 6-8 days. The statistics compare each dilution to the controls to see if there is a significant difference. The statistical endpoints for chronic toxicity tests are typically

expressed as the no observed effect concentration (NOEC) or the inhibition concentration (IC). The inhibition concentration is typically expressed as the IC_{25}, or the concentration of effluent that is lethal or sublethal to 25% of test organisms.

(a) (NOEC)
The NOEC is the highest concentration of toxicant that organisms can be exposed to in which the values for the observed responses are not statistically different from the controls, and below which there is no statistically significant adverse effect.

(b) (IC_{25})
The IC_{25} is the Inhibition Concentration (IC) of toxicant that causes a given percent reduction (25%) in effect as compared to the controls. The IC_{25} is calculated by the linear interpolation method and is a point estimate.

b. Test Species
EPA's TSD recommends that three species are tested, one from each tropic level, to fully assess the impact the effluent has on the biology of the receiving water. EPA generally recommends that freshwater test species be used in toxicity testing when the receiving water salinity is less than 1.0 ppt and that an estuarine or marine test species be used when the receiving water salinity equals or exceeds 1.0 ppt. There should be additional consideration about which species to use dependent on the salinity of the discharge itself if the species selected may not survive in testing due to salinity. Below are the tests and the species used to determine acute and chronic toxicity in Virginia.

Acute Tests			
Freshwater			
Test Method Number	**Organism Type**	**Organism Name**	**Duration and Endpoint**
2002.0	Invertebrate	*Ceriodaphnia dubia* (Water Flea, daphnid)	48-Hour Static Acute – LC_{50}, NOAEC
2000.0	Vertebrate	*Pimephales promelas* (Fathead Minnow)	48-Hour Static Acute, LC_{50}, NOAEC 96-Hour Static Renewal Acute – LC_{50}
2019.0	Vertebrate	*Oncorhynchus mykiss* (Rainbow trout)	48-Hour Static Acute, LC_{50}, NOAEC 96-Hour Static Renewal Acute – LC_{50}
Marine/Estuarine			
Test Method Number	**Organism Type**	**Organism Name**	**Duration and Endpoint**
2007.0	Invertebrate	*Americamysis bahia* (Opossum Shrimp)	48-Hour Static Acute, LC_{50}, NOAEC 96-Hour Static Renewal Acute – LC_{50}
2004.0	Vertebrate	*Cyprinodon variegatus* (Sheepshead Minnow)	48-Hour Static Acute, LC_{50}, NOAEC 96-Hour Static Renewal Acute – LC_{50}

Chronic Tests

Freshwater			
Test Method Number	**Organism Type**	**Organism Name**	**Duration and Endpoint**
1002.0	Invertebrate	*Ceriodaphnia dubia* (Water Flea, daphnid)	Chronic Static Renewal 3-Brood Survival and Reproduction Test
1000.0	Vertebrate	*Pimephales promelas* (Fathead Minnow)	Chronic Static Renewal 7-Day Survival and Growth Test
1003.0	Plant	*Selenastrum capricornutum* (Green alga)	Chronic Static Renewal 96-Hour Cell Density, Biomass, Chlorophyll Content, Absorbance
Marine/Estuarine			
Test Method Number	**Organism Type**	**Organism Name**	**Duration and Endpoint**
2007.0	Invertebrate	*Americamysis bahia* (Opossum Shrimp)	Chronic Static Renewal 7-Day Survival, Growth, and Fecundity Test
2004.0	Vertebrate	*Cyprinodon variegatus* (Sheepshead Minnow)	Chronic Static Renewal 7-Day Survival and Growth Test
1009.0	Plant	*Champia parvula* (Red macroalga)	Chronic Static Renewal 7-Day Cystocarp Production Test

Previously, the determination had been made that facilities could demonstrate one of their test species as the “most sensitive species” and could continue testing with one species. The EPA does not recommend this approach, and a minimum of two species should be required for each type of toxicity testing for WET monitoring. The inclusion of multiple species for toxicity testing is purposeful to address the variability of the effluent and the various effects it has on different organisms in the receiving water, and limiting the number of species limits our understanding of the potential impacts. It is strongly recommended that each facility tests with multiple species, as each species is expected to have different sensitivities to different types of toxicity.

4. Test Frequency

Test frequency is determined on a case-by-case basis, with the major considerations being the variability of the effluent and the potential for impacts to the receiving water. Each of these factors can be assessed by characterizing the effluent.

When characterizing the effluent, multiple factors need to be considered to adequately determine the test frequency that has the highest probability of capturing toxicity in the effluent. Examples of the types of information relating to these factors are listed below.

- Existing controls on point and nonpoint sources of pollution
  - Industry type: Primary, secondary, raw materials used, products produced, best management practices, control equipment, treatment efficiency, etc.
  - Publicly owned treatment work type: Pretreatment, industrial loadings, unit processes, treatment efficiencies, chlorination/ammonia, metals, problems, etc.
- Variability of the pollutant or pollutant parameter in the effluent
  - Compliance history
  - Existing chemical data from discharge monitoring reports and applications.

- Sensitivity of the species to toxicity testing
    - Adopted State water quality criteria, or EPA criteria.
    - Any available in-stream survey data applied under independent application of water quality standards.
    - Receiving water type and designated/existing uses
- Dilution of the effluent in the receiving water
    - Dilution calculations.

In addition to reasonable potential, another consideration is the variability of the effluent. There is a possibility that the amount of toxicity in an effluent can vary based on time of year, weather events, treatment process, and other conditions. It is important to select a monitoring frequency that captures the "worst case" conditions to accurately assess the potential for instream impact. The most frequent testing required is typically monthly and is required for effluents that have high variability and a high potential for toxicity and should only be required if there are ongoing toxicity issues or other conditions that require assessment. Quarterly monitoring is believed to capture most variations in both effluent and environmental conditions. It is for this reason that quarterly monitoring is required for new discharges and should be used to characterize the effluent. Once an effluent is characterized, the decision can be made to reduce the monitoring to semi-annual or annual, if there is confidence in the characterization of the effluent and the reasonable potential is low.

5. Endpoint Determination

The permit writer should specify in the permit the statistical test endpoint for each WET test. It is the current procedure of Virginia DEQ to not require EPA's Test of Significant Toxicity (TST) statistical approach, which evaluates whether the biological response measured by the test is significantly different than the control. The procedure for calculating the applicable test endpoint can be found in Section F, Reasonable Potential Analysis.

6. Test Dilution Water and Dilution Series

a. Test Dilution Water

Typically, Virginia DEQ requires that reconstituted (standard) laboratory water is used in WET tests. However, EPA toxicity test methods authorize the use of receiving water as the dilution water for testing, depending on the purpose of the test.

If the purpose of the testing is to estimate the absolute toxicity of the effluent, standard laboratory water is typically used. Absolute toxicity refers to the toxicity of the effluent alone, without any influence of the receiving water. In some cases, the receiving water can decrease, increase, or otherwise affect the toxicity of the effluent. These effects may or may not be measurable or testable in the receiving water, so knowing the unaffected toxicity of the effluent may be helpful in determining direct impact on surrogate organisms. The toxicity test methods outline how laboratories may make the reconstituted fresh or saltwater, with respect to the approximated salinity and hardness.

If the purpose of the testing is to directly observe the effects of the effluent in the receiving water, a grab sample of receiving water that is outside the influence of the outfall (upstream) should be used for the dilution water. If receiving water is used for the test dilution water, an additional control test using laboratory water should be included. In this

case, the receiving water should be tested to ensure that it is not independently toxic to the test organisms, and it is representative of the water conditions at the point of discharge.

b. Dilution Series

The dilutions used in a toxicity test are calculated to ensure that the test best captures the toxicity of the effluent. The dilutions generally consist of two concentrations that are greater than the IWC, the IWC itself, and two concentrations that are less than the IWC. These concentrations are calculated using the WETLIM Program, and are facility, and therefore IWC, specific. Instructions for how to generate this can be found in Section F, Reasonable Potential Analysis.

7. Other Toxicity Testing

WET tests can also measure the aggregate toxic effect of a reference toxicant or an ambient sample from receiving water. In these tests, organisms of surrogate species for the biology of the receiving stream are held in test chambers and exposed to different concentrations of a sample. Observations are then made and recorded on data sheets for predetermined exposure periods. At the conclusion of the test period, the responses of test organisms are recorded, and data is generated to represent the effects of the effluent.

D. WET Permitting

This section describes how to determine which requirements to include in VPDES permits. Considerations that should be included in the VPDES permit or fact sheet include, but are not limited to the following:

- Type of Effluent Sample(s), as discussed in Section C.1
- IWC Evaluation
- Test Acceptability Criteria (TAC) and Other Criteria for Valid WET Testing (found in the WET Test Methods)
- Monitoring Frequency, as discussed in Section C.4
- Accelerated Toxicity Requirements, as discussed in D.6.a
- Toxicity Identification Evaluation (TIE) or Toxicity Reduction Evaluation (TRE) Requirements, as discussed in Section D.6.d
- Compliance Schedule(s), as discussed in Section D.6.c

Determinations on these and all other considerations should be clearly explained and documented in the permit fact sheet.

1. New Discharges, Issuances, or Facilities with Changing Operating Conditions

Facilities with WET testing data that is not representative of the discharge, or nonexistent altogether, should have requirements to characterize the new discharge to ensure the WET testing data is representative of the potential toxicity. Data collected prior to modifications to the treatment process, pretreatment, or pollution prevention program should be evaluated to determine if it is still representative of the discharge.

a. New Discharges or Issuances

For facilities that do not have prior WET data, the permit application should contain all necessary information to determine if the facility is subject to WET requirements. When there are no data to evaluate, a more detailed review of the pollutants contained or believed to be contained in the effluent should be performed to determine if toxicity is suspected. If the facility

is subject to WET requirements, an initial toxicity determination will need to occur to characterize the effluent and determine the approach that best captures the RP of the effluent. To do so, quarterly acute and chronic testing with the respective selected species should be required until four consecutive tests are received. A sufficient number and diversity of test species should be selected for each test to fully evaluate the potential impact on the biology of the receiving water. Following the receipt of the four consecutive quarters of monitoring data, the data should be evaluated following the instructions in Section F of this manual. Following this evaluation, the WET requirements may be revised as necessary.

b. Facilities with Changing Operating Conditions

For facilities that have previously submitted WET data, but have since changed operating conditions, the data should be evaluated to determine if it is still representative of the discharge. Sufficient changes to the facility include process changes, facility upgrades, or other changes that will affect the composition of the effluent. Normal variances due to seasonal use of chemical additives, equipment uses, routine testing, or other situations attributable to facility operation are not sufficient to invalidate WET data. The frequency of variances should be considered under such situations to adequately assess the reasonable potential for reoccurrence. For example, pilot testing of a new treatment condition may cause toxicity, and the argument could be made that the result would not be valid due to the pilot testing being discontinued. This can only be true if pilot testing, even of a different nature, is not a routine condition of facility operation. If pilot testing is a common occurrence throughout the plant, the tests are considered part of typical operation and will not support data invalidation.

Plant expansions or the addition of new industrial users may or may not constitute sufficient change to reclassify the effluent. In these situations, any existing limits should remain in the permit until such time that operations stabilize, and then toxicity should be re-evaluated to determine if the limit should be adapted or removed. **Any decision to reclassify an effluent, invalidate WET data, or remove or adapt a WET limit should be coordinated with Central Office and documented in the fact sheet.**

If sufficient changes have occurred to invalidate previous WET test data, an additional initial toxicity determination will be required to characterize the “new” effluent. To do so, quarterly acute and chronic testing with the respective selected species should be required until four consecutive tests are received. A sufficient number and diversity of test species should be selected for each test to fully evaluate the potential impact on the biology of the receiving water. Following the receipt of the four consecutive quarters of monitoring data, the data should be evaluated following the instructions in Section F of this manual. After this evaluation, the WET requirements may be revised as necessary.

2. Instream Waste Concentration Evaluation

The IWC is the concentration of effluent in the receiving water after mixing or dilution. It is the inverse of the receiving water concentration (RWC), or dilution factor, which refers to the amount of dilution available in the receiving water. The IWC is calculated by dividing the design flow in MGD by the sum of the design flow and the critical low flow of the receiving water in MGD. To get the IWC in proper units, multiply by 100 to get the percentage of effluent. The 1Q10 and the 7Q10 are used to calculate the IWC_a and the IWC_c, respectively. The IWC is calculated using the WETLIM program and is used to calculate the applicable wasteload allocations (WLAs). The IWC is used to determine other test conditions such as endpoint and should be considered when determining reasonable potential.

3. Test Report Submittal

It is recommended that permittees send the full test report by email, even if they have a WET limit on their DMR. It is important that test reports are reviewed as soon as possible, so that any toxicity measured can be addressed as soon as possible. It is requested that permittees submit WET test reports as soon as they receive them from the WET lab, preferably within 48 hours. WET Monitoring requirements should not be required on the DMR, as permittees should not wait until the DMR date to submit the WET data. WET Monitoring compliance should be evaluated through a compliance schedule event (CSE) in CEDS, see Section E.3.a. for more information. This allows permit writers, compliance staff, and permittees the necessary flexibility to submit, review, and record WET test reports as they are received.

4. Fact Sheet Language

The fact sheet should contain the rationale for each of the determinations made for the WET requirements. The OneDEQ fact sheet template includes headings and sample language for most conditions, but a review should be completed to ensure that the fact sheet thoroughly explains each requirement. The fact sheet attachments should also contain the WETLIM Program output, STATs output, recommended dilutions, test summary table, and any other supporting information.

5. Permit Language

The permit should outline the specific requirements for each outfall, including the test determinations explained in the fact sheet. The OneDEQ template includes sample language for most conditions, but sections or formatting may need to be adjusted due to additional outfalls, flow tiers, or special conditions.

6. Special Conditions in VPDES Permits

Some WET Conditions have special conditions included to better assess toxicity. Some of these conditions are listed below.

a. Accelerated Toxicity Testing

Per EPA Guidance, if a submitted valid WET test result indicates noncompliance with a VPDES WET limit, exceedance of a WLA, or an excursion of applicable WQS, permit conditions should specify follow-up or accelerated testing requirements. Accelerated WET monitoring requirements are common NPDES permitting requirements that vary depending on the permitting authority. For example, a requirement could be conducting more frequent WET testing over a short period, like every two weeks, to determine if toxicity is considered persistent. If the results do not show toxicity, the original monitoring schedule can recommence. If the results do show toxicity, a toxicity reduction evaluation (TRE) is recommended as a follow-up requirement, or the permit can be reopened to include a limit.

The number of toxicity tests and the duration of the accelerated monitoring should be designed to determine the persistence of the toxicity. The EPA recommendation for this number and duration is at least six additional toxicity tests to be conducted at 14-day intervals. This recommendation of a minimum of six additional toxicity tests is based on the probability of encountering at least one exceedance of permit requirements assuming that the effluent is toxic, but at an unknown level of toxic impact on aquatic life.

b. Sample Adjustments

Virginia's WET Guidance outlines five common sample adjustments that may be approved for WET testing. Supporting information must be provided by the permittee prior to approval for sample adjustments. For each sample adjustment, it is strongly recommended that the permittee runs parallel tests to demonstrate the sample adjustment does impact toxicity. In addition to the adjustments affecting toxicity, the adjustments should still result in a test that is still considered representative of toxicity in the receiving water. **Central Office concurrence is required before final sample adjustment approval can be transmitted to the permittee.**

i. Dissolved Oxygen (DO)

The dissolved oxygen (DO) concentration in the sample (and dilution water) should be at or below saturation prior to use. The DO saturation point should be determined from the table in the applicable WET test methods. If the sample (or dilution water) is supersaturated, the DO level must be reduced by aeration, shaking, or stirring until the DO stabilizes at an acceptable level. Samples (or dilution water) that have a DO less than 4.0 mg/L for warm and saltwater species, or less than 6.0 mg/L for cold water species must be aerated to increase the DO to acceptable levels prior to use in a test. Tests that are set up with either the sample or dilution water greater than 100% saturation or less than 4.0 mg/L (or less than 6.0 mg/L for cold water species) may be considered not acceptable.

ii. pH

Tests for compliance should be performed on the sample without pH adjustment, to better assess the effects of the effluent on the organisms. If the effluent is out of the pH 6-9 range, it is recommended that the lab check with the permittee to see if they want a parallel test set up with pH adjusted effluent and controls. This would enable the permittee to see if there are toxicants present without the effects of the "out of range" sample pH. Compliance will be determined from the unadjusted samples test result.

iii. Chlorine

Tests for compliance should be performed on the sample "as is", unless noted in the VPDES permit to dechlorinate, or if the VPDES permit has a schedule for the facility to complete dechlorination. The chlorine residual should be reported for all effluent samples. Again, it may be to the permittee's benefit to run a parallel test to see if chlorine is the toxicant.

iv. Solids

Samples that contain debris or organisms may be filtered through a sieve having 60 µm mesh openings prior to use.

v. Ultraviolet Irradiation

Samples containing filamentous bacteria or fungi may be exposed to UV light prior to use.

Test variations other than sample adjustments described above require Central Office and EPA coordination.

c. Schedules of Compliance

Schedules of Compliance should only be included if there are sufficient facility-specific conditions that provide the rationale for extenuating circumstances. Historically, 40 CFR 122.47, which requires compliance with state WQS as soon as possible, has been cited to recommend against schedules of compliance.

The fact sheet outlines the limit triggers narratively and numerically, so both DEQ staff and permittees can be aware of when a limit has been or may be triggered. Any actions to influence the limit calculations (additional testing, toxicity reductions, etc.) should occur immediately after the test with the toxic value is received. These actions are the responsibility of the permittee. If the toxicity isn't reduced or eliminated in a statistically significant way that allows a limit to not be triggered, the limit should be included at reissuance. If there is not enough time remaining in the permit term, <1 year, a schedule of compliance may be included with the limit to allow for the toxicity reduction effort to conclude. Unless there are sufficient additional results provided to alter the results of the STATs evaluation, a limit should be included, even if toxicity reduction efforts have not concluded.

d. Toxicity Identification and Toxicity Reduction Evaluations

Toxicity Identification Evaluations (TIEs) and Toxicity Reduction Evaluations (TREs) enable permittees to identify and reduce toxicity found in their effluent. These evaluations can be performed voluntarily or as a requirement of the permit to provide more insight into the type and severity of toxicity, as well as possible methods to reduce or eliminate toxicity. It is recommended that these are required when data suggests frequent or persistent toxicity, or where the cause of toxicity is unclear. TIEs and TREs can also be included in permits for facilities that has reoccurring noncompliance. EPA's TIE and TRE testing manual can be found at https://www.epa.gov/npdes/permit-limits-whole-effluent-toxicity-wet. The methods for these evaluations can be found here:

- TIE Phase I – Characterization of Physical/Chemical Nature of Toxic Constituents
- TIE Phase II – Identification of Non-Polar Organic, Ammonia, or Metal Toxicants
- TIE Phase III – Confirmation of Suspected Toxicants
- Marine TIE
- Chronic TIE
- Industrial TRE
- Municipal TRE

The permit should identify when or if a TIE and/or TRE is required, but permittees may elect to perform a TIE or TRE at any point. The TRE results can then be evaluated by DEQ and may lead to a WET limit, a chemical-specific limit, or compliance requirements.

Some TREs can be resolved by an internal evaluation on procedures, policy, and management practices, and in those cases a TIE may not be required. EPA recommends the following requirements when a TRE is triggered:

- Notice of TRE study implementation to be submitted to the NPDES permitting authority within 10 days of activation of this TRE trigger.
- A TRE schedule and TRE action plan to be submitted to the NPDES permitting authority within 60 days of the initiation of the TRE.

- The initial term of the TRE should be no longer than 24 months as follows: The "TRE initiation date" should be the date the toxicity test that confirms toxicity is initiated and the "TRE termination date" is the date corrective actions to resolve toxicity are to be identified and be no more than 24 months from the TRE initiation date. There are circumstances that could extend this recommended schedule, including intermittent toxicity or seasonal toxicity.
- A quarterly TRE progress report should be submitted with the discharge monitoring report (DMR) to the NPDES permitting authority at the end of each quarter, based on the TRE initiation date. The progress report should list all activities and findings related to resolving toxicity, including all WET and chemical test data. The data summaries of the TRE also should be provided in a tabulated format with explanations of the procedures used and the recorded findings from the study.
- Any exceedance of an NPDES WET monitoring trigger or permit limit during the implementation of a TRE should be reported within 5 working days to the NPDES permitting authority. A final TRE report should be submitted to the NPDES permitting authority within 45 days of the TRE termination date and should summarize the TRE activities and findings, propose the corrective action(s) to be taken, and propose a schedule to complete any identified corrective action(s).
- The minimum monitoring frequency for the affected test species should be noted in the TRE work plan. The NPDES permitting authority, however, might recommend additional toxicity testing, which might include streamlined toxicity tests using a single test concentration of the sample compared against the control to find toxic samples for further investigation as part of the iterative process used in a TRE. This iterative process could include using toxicity tests and chemical analysis of portions of effluent treated in the TRE and identified to be toxic.
- All samples used for toxicity testing during the TRE should be analyzed for any toxicant identified as being a potential source of toxicity. If later toxicity testing determines the toxicant to be a probable source of toxicity, the analysis may be discontinued when all the findings and analytical results are clearly documented in the quarterly TRE progress report. The objective of this testing is to ascertain whether the same level of toxicity occurs when the suspected toxicant level varies, indicating the potential for more than one source of toxicity. This information might lead to finding additional toxicants or confirming or eliminating the suspected toxicant and possibly its source.
- Where toxicity is intermittent, the NPDES permitting authority may include additional requirements based on PJ.
- TRE triggers and the actions that follow are the initial recommended responses to the confirmation of a demonstrated toxicity above the NPDES WET limit or WET numeric monitoring trigger.

E. WET Compliance

1. Introduction

Chapter 8 in the EPA Office of Compliance's NPDES Compliance Inspection Manual describes the objectives for compliance monitoring activities, such as inspections, audits, and records review, for WET data. These objectives may include:

- Documenting the presence or absence of effluent toxicity based on valid WET data;
- Assessing compliance with the conditions and limits in the NPDES permit;

- Assessing a permittee's laboratory WET test performance, including reference toxicant testing and other WET QA/QC requirements;
- Evaluating the quality of self-monitoring data; and
- Assessing the adequacy of self-monitoring procedures.

Based on these evaluations, regional staff may recommend to enforcement personnel and/or the permit writer that the permittee be required to perform a TRE or TIE. Inspectors are encouraged to coordinate with the permit writer if they identify language in a permit that could be clarified and/or strengthened.

The NPDES Compliance Inspection Manual provides examples of procedures and records that might be reviewed during an inspection, including:

- The VPDES permit;
- WET test results from the last 3 years;
- Effluent sample collection and chain-of-custody procedures for WET testing; and
- Permittee sampling logs that should include the date, time, type of sample taken, and the sampler's name.

Regional staff also should review the following:

- WET test data interpretations
- Calculations
- WET test concentration response relationship (CRR) based on multiple concentration WET tests
- Whether the WET tests meet all of EPA's mandatory TAC specific to each EPA toxicity test method
- The percent minimum significant difference (PMSD) evaluation of WET test variability

Many of the considerations for evaluating WET data when conducting an RP analysis for evaluating whether WET permit limits are needed are applicable to evaluating WET data for compliance purposes. This section of the manual provides an in-depth discussion of reviewing and evaluating WET data and factors, such as mandatory TAC that impact the quality of WET data.

2. WET Test Review

WET Tests should be reviewed as soon as possible following their receipt. The test should be reviewed for validity before being reviewed for toxicity.

a. Validity Review

The test validity is reviewed by the lab performing the test and the permittee, so a full review should only be performed if requested, or if invalidity is suspected. EPA has provided a guide for reviewing the test acceptability criteria (TAC), which has been summarized into DEQ's Acute and Chronic Review List, effective September 1, 2017. This list can be found in the WET folder on SharePoint. For tests where no invalidity is suspected, an accelerated test review should be performed to ensure that the correct test was performed and to evaluate the results. An accelerated test review list can also be found on SharePoint at Whole Effluent Toxicity Accelerated Test Review.docx.

b. Toxicity Review

Following the validity review, the test should be reviewed for toxicity. A test that reports value(s) of >1.0 TU does demonstrate some toxicity, but it does not equate a toxic result. A

test result is considered toxic if the result is greater than the facility specific WLA associated with the test. For test results of 1.0 TU, it can be assumed that the test is not toxic, and no comparison to the WLA is required. For results greater than 1.0 TU, the result should be compared to the WLA from WETLIM associated with the permit and specific outfall. If the result is greater than the WLA, the result is considered toxic, and it may trigger a limit at reissuance. It is recommended that the permittee provides any information they have on the suspected or known cause of the toxicity to be used in the RP analysis.

Permittees may request an accelerated RP analysis at any time, including after they submit a toxic test result. It is recommended that if a test exceeds the WLA, even without a request by the permittee, an accelerated RP analysis is performed to determine if a limit will be triggered at reissuance. The procedures for this can be found in Section F, Reasonable Potential Analysis.

It is important to note that a rationale for the cause of the toxicity does not provide grounds to exclude it from the RP analysis. **Results may only be excluded if the test is deemed invalid, or there is sufficient evidence to show that the test is not representative of the effluent. Any decision to exclude a test from the analysis should be coordinated with Central Office and documented in the fact sheet.**

It is recommended that the summary table below is maintained in the permit folder as tests are reviewed to reduce the amount of time spent searching and compiling WET data at reissuance. The table should include the test start date, test type, test species, monitoring period, and data summary. The table should be representative of the data submitted to DEQ, the examples below may or may not be representative of the data. It is recommended that, in addition to the results required for the permit, the percent survival in 100 percent effluent be recorded. While some labs may not report this number on the data summary sheet, it can be determined from the test bench sheets.

Summary of Chronic Toxicity Testing

Monitoring Period	**Test Date**	**Chronic 3-Brood Static Renewal Survival and Reproduction *Ceriodaphnia dubia***		**48-Hour LC_{50} (%)**	**Survival in 100% Effluent (%)**
		Survival (TU_c)	Reproduction (TU_c)		
1st Annual (January 1-December 31, 20XX)	2/13/20XX	1.0	1.0	>100	100
2nd Annual (January 1-December 31, 20XX)	5/13/20XX	1.0	1.0	>100	100

Summary of Acute Toxicity Testing

Monitoring Period	**Test Date**	**48-Hour Static *Ceriodaphnia dubia***			**Survival in 100% Effluent (%)**
		NOAEC %	48-Hour LC_{50}	TU_a	
1st Annual (January 1-December 31, 20XX)	2/13/20XX	>100%	>100	1.0	100

2nd Annual (January 1-December 31, 20XX)	5/13/20XX	>100%	>100	1.0	100

The table should be kept up to date and should be formatted to be inserted into the fact sheet for the reissued permit. It is important to note that the test date above references the first date of the test, which is found on the test report.

3. Data Management

WET Data is tracked in CEDS and in ECM in addition to the data summary table compiled and maintained for reissuance.

a. CEDS

WET Monitoring is tracked in CEDS through compliance schedule events (CSE). As permittees fulfill the requirements of the test report submittal, the respective CSE should be updated with the received date. The test report should then be reviewed as outlined in Section E, WET Compliance. Following the review of the CSE should be updated with the reviewed and completed date. It is recommended that the contact tab on the VPDES permitting page in CEDS is reviewed to ensure that there is a designated facility WET contact, and it is up to date. Some WET Monitoring CSEs are outfall specific, and those CSEs will be found on the outfall compliance schedule events on the CSEs tab.

b. ECM

Following the recording of the data, the test report should be uploaded into the Enterprise Content Management (ECM) system. If there are different monitoring periods for tests that were submitted together, they can either be uploaded separately or as a joint document. Any correspondence should be compiled into the same file as the test report. The relevant regional office should be selected, followed by Water for the Division/Media, and VPDES Individual for the Program. The document date should be the date the test was received by the regional office. The file name should follow the following convention: 20XX WET Monitoring-Xth Annual/Semi-Annual/Quarterly/Monthly Acute and Chronic/Acute/Chronic Cd/Pp/Cd and Pp. The retention schedule is 440-005 Water Quality, the file series is 006002 VPDES Individual and General Permits – Records and Reports, and the document type is TMP Data/Reports/Reviews. The keywords are 20XX WET, and the Case ID is the permit number.

F. Reasonable Potential Analysis

1. Introduction

The WET Program seeks to identify and limit the possible negative effects of a facility's effluent on the receiving water. This is done by evaluating a facility's effluent, treatment process, waste stream contributors, and other factors to determine if there is the reasonable potential for toxicity. Reasonable Potential (RP) is the determination that an effluent is projected or calculated to cause, have the potential to cause, or contribute to instream toxicity. Facilities do not need testing results to demonstrate reasonable potential, although testing results that show toxicity do demonstrate reasonable potential. Meeting the applicability requirements listed in Section B demonstrates that the facility needs to be evaluated for RP.

2. Reasonable Potential Without WET Data

After a facility is determined to be subject to WET requirements, the effluent needs to be characterized to evaluate RP. To do so, any facility that has not previously performed toxicity testing, or any facility that has had changes in their process or facility that prompt the effluent to be reassessed, shall be required to perform a minimum of four consecutive quarterly acute and chronic toxicity tests with a minimum of two species. Such testing shall assess the overall toxicity of the effluent, the species-specific impacts of the effluent, and the scale of toxicity present. Following the receipt of four consecutive quarterly test reports, the data can be evaluated for reasonable potential using the RP Analysis Procedures outlined below. Following this analysis, the facility may have their test type, frequency, and required species revised. To fully understand the test data and the main program concerns, the following information concerning facility type should be considered.

a. For municipal discharges, the applicability criteria are concerned with the variability of the effluent, as these effluents originate from several sources. The large number of contributors increases the amount of possible aggregate effects, as there is an increased potential for more diverse pollutants to be present. Hence, the basis of these criteria concern design flow rates, with pretreatment programs, types of contributors, and compliance issues of the facility. To best address these facilities, both chronic and acute toxicity testing should be required with at least two species and on a testing frequency that adequately assesses seasonal variations.

b. For industrial discharges, the applicability criteria are less concerned with variation of pollutant types, and more concerned with the concentration of known pollutants. Hence, the basis of these criteria concerns the Standard Industrial Classification (SIC) code(s), the IWC, and the manufacturing and treatment processes. To best assess these facilities, acute and chronic testing should be required with at least two species and on a testing frequency that adequately assesses peak processing for the facility. If the effluent is thoroughly understood, the facility may move to either acute or chronic testing.

c. For stormwater discharges, the applicability criteria are based on the potential for the stormwater to be contaminated. This can include the criteria for the industrial or municipal discharges, if applicable. To best assess these facilities, acute toxicity testing should be required with at least two species and on a testing frequency that adequately allows for sampling in concurrence with storm events that are believed to create a discharge that is representative of the potential toxicity of the effluent. Evaluating stormwater discharges for reasonable potential may not require as thorough of an assessment as municipal and industrial discharges. An accelerated RP analysis can be performed where only the factors that are related to the specific discharge should be considered, including but not limited to discharge frequency, volume, pollutant composition, and variability.

3. Reasonable Potential Evaluations with WET Data

Following the initial assessment of RP and the receipt of test results, a more informed RP analysis can be performed utilizing the test data. The steps for an RP analysis for a discharge to freshwater are:

Step 1: WETLIM Analysis

Current facility and receiving water data should be inputted into DEQ's WETLIM program found at https://rconnect.deq.virginia.gov/WETLIM/. This program will calculate the acute and chronic

WLAs, as well as proposed test endpoints and dilution series. The program will also calculate a site-specific acute to chronic ratio (ACR) or coefficient of variation (CV) if necessary.

Acute to Chronic Ratio

The acute to chronic ratio is the ratio of the acute toxicity of an effluent or a toxicant to its chronic toxicity. It is used as a factor to estimate chronic toxicity based on acute toxicity data, or for estimating chronic toxicity based on acute toxicity data. We develop this ratio to ensure that a WET limit, if required, is protective of both acute and chronic toxicity. This requires a consideration of both the acute and chronic WLA, since only considering one would require making assumptions about the unknown relationship of the chronic and acute toxicity of the effluent. To avoid this, a site-specific ACR should be calculated where possible.

The ACR relates acute and chronic toxicity as follows: ACR = LC_{50}/NOEC or ACR = LC_{50}/IC_{25}. The ACR can be calculated using test data from the same species run on the same dates. Separate acute and chronic tests should be conducted, and acute data extrapolated from chronic tests should not be used to develop an ACR. The ACR can be calculated using the WETLIM Program, linked above. If more than one ACR is calculated (for more than one species), the lowest ACR value should be used in the calculations.

Where the discharge is continuous and the IWC > 1%, a limit calculated when only acute data was provided should be based purely on the WLA_a and be in TU_a units. Chronic monitoring should be required so a full evaluation can be performed, and the limit should be recalculated following the receipt of the chronic data. The chronic monitoring requirements can be required before the acute limit is established in the permit, within a schedule of compliance, or as a separate WET requirement. The limit will still be included, even if data from the chronic monitoring period does not show sufficient toxicity to retrigger a limit.

Coefficient of Variation

The Coefficient of Variation is a standard statistical measure of the relative variation of a distribution of a set of data, defined as the standard deviation divided by the mean. It is also called the relative standard deviation (RSV). The CV can be calculated using the WETLIM Program, linked above. There must be at least 10 data points to calculate a site-specific CV, or the default value of 0.6 will be used.

The WETLIM output should be included in the Appendix to the fact sheet that corresponds to the WET section.

Step 2: STATs Analysis

Using the WLAs calculated by WETLIM, use STATs to determine if a limit is necessary. This should be run at every reissuance with the data from the previous permit term. Historical data, or data from before the previous permit term, should only be included if there is a facility-specific rationale for calculating with an expanded data set. STATs should be run with at least four data points but can be run with as few as one. It is important to note that running STATs with greater than 10 data points should prompt the possibility of a calculation of a site-specific CV, as described above. A sufficiently low WLA will trigger a limit in STATs inappropriately so the STATs evaluation may be excluded, given that a justification is provided in the fact sheet.

The facility name should be entered, and the chemical name should be either acute or chronic and the species (i.e., Chronic C. dubia). For an acute limit evaluation, only the WLA_a should be entered into STATs, the WLA_c should be left blank. For a chronic limit evaluation, the $WLA_{a,c}$ and the WLA_c should be entered into the respective boxes. The units are TU, Q.L. is 1.0, and the number of samples per month is always 1. Test results for each outfall, species, and test type should be evaluated separately.

The data input should be the TU value from each test that has the most toxicity. For example, if a chronic *Ceriodaphnia dubia* test report had a 1.0 TU for survival, but a 2.3 TU for reproduction, the 2.3 should be the value inputted for that test. The 1.0 should not also be inputted, only one value should be inputted per test. Every valid test that was received during the permit term should be entered. When evaluating chronic data, the STATs output may say that a limit was triggered based on acute toxicity. In this case, it is important to note that the value that STATs is evaluating for the WLA_a is actually the $WLA_{a,c}$, which is derived from the WLA_c and the ACR, and is not equal to the WLA_a.

The STATs output should be included in the Appendix to the fact sheet that corresponds to the WET section.

Step 3: Determine Reissued Test Requirements

When considering which WET requirements would be most appropriate following a reasonable potential analysis, there are three important considerations for variability.

(1) Effluent Variability: Caused by changes in the composition of the effluent. Virtually all effluents vary in composition over time. Sampling measurements should be tailored to the toxic effect of concern (acute or chronic) and the need to design testing that accounts for effluent variability.

(2) Exposure Variability: Caused by changes in flow rates of both effluent and receiving water. There also are variable receiving water parameters that may be independent of flow, such as background toxicant levels, pH, salinity, tides, suspended solids, hardness, dissolved oxygen, and temperature, that can be important in assessing impact. This can be assessed by assuming a steady state exposure condition (worst case) using a critical receiving water flow or condition and a typical effluent flow.

(3) Species sensitivity: Differences are caused by the differences in response to toxicants between species. Often, differences of several orders of magnitude exist for a given individual toxicant between the least sensitive and most sensitive species. Since the measured toxicity of an effluent will be caused by unknown toxic constituents, the relative sensitivities of various test species will also be unknown. Therefore, proper effluent toxicity analysis requires an assessment of a range of sensitivities of different species to the effluent. The only way to assess the range of sensitivities is to test several different species from different taxonomic groups, as in the development of the national ambient water quality criteria. To provide sufficient information for permitting decisions, EPA recommends a minimum number of three species, representing three different phyla (e.g. a fish, invertebrate, and plant species) be used to test an effluent for toxicity.

4. Marine/Estuarine Procedures

Due to of the difference in mixing characteristics, the waste load allocations for discharges to estuarine waters are different from those for discharges to flowing streams. Once the WLA has been determined, however, the procedures and calculations are the same as in Steps 2 and 3 above.

a. Acute Evaluation
For marine and estuarine facilities with no available dilution data, the WLA_a should be set at twice the acute water quality criteria, or 0.6 TU_a. Otherwise, the acute dilution value should be converted to the respective IWC_a by the following equation:

$$WLA_a = 0.3(Acute\ Water\ Quality\ Criterion) * Acute\ dilution$$

This TU_a value will serve as the WLA_a, as needed for test review and RP analysis.

b. Chronic Evaluation
For marine and estuarine facilities with no available dilution data, the WLA_c should be set at 50 TU_c, assuming a 50:1 dilution ratio. Otherwise, the WLA_c can be determined as follows:

$$WLA_c = 1.0\ (Chronic\ Water\ Quality\ Criterion) * Chronic\ dilution$$

This TU_c value will serve as the WLA_c, as needed for test review and RP analysis.

5. Following Draft Permit Concurrence

Once owner concurrence has been received on the draft, the CSE(s) for monitoring or limit(s) will need to be added to the facility page in CEDS. This may also be done before the public notice comment period ends. The special condition for the CSE is "WET Acute Test" or "WET Chronic Test," and the due date should correspond to the dates provided in the fact sheet. For a limit, refer to the following table for the correct parameter code:

Test Duration and Organism	Reporting Endpoint	CEDS Code	EPA Code	Units Code	Units
Acute 48-Hour Static *C. dubia*	NOAEC	704	TDA3B	23	Percent
Acute 48-Hour Static *P. promelas*	NOAEC	705	TDA6C	23	Percent
Acute 48-Hour Static *A. bahia*	NOAEC	707	TDA3Z	23	Percent
Acute 48-Hour Static *C. variegatus*	NOAEC	708	TDA6A	23	Percent
Acute 48-Hour Static *C. dubia*	LC_{50} as TUa	711	TSA3B	2F	TU_a
Acute 48-Hour Static *P. promelas*	LC_{50} as TUa	712	TSA6C	2F	TU_a
Acute 48-Hour Static *O. mykiss*	LC_{50} as TUa	713	TSA6I	2F	TU_a
Acute 48-Hour Static Renewal *P. promelas*	LC_{50} as TUa	714	TSN6C	2F	TU_a
Acute 48-Hour Static Renewal *O. mykiss*	LC_{50} as TUa	715	TSN6I	2F	TU_a
Acute 48-Hour Static *A. bahia*	LC_{50} as TUa	717	TSA3Z	2F	TU_a
Acute 48-Hour Static *C. variegatus*	LC_{50} as TUa	718	TSA6A	2F	TU_a
Chronic 3-Brood Static Renewal *C. dubia*	NOEC as TU_c	720	TTP3B	2G	TU_c
Chronic 7-day Static Renewal *P. promelas*	NOEC as TU_c	721	TTP6C	2G	TU_c
Chronic 7-day Static Renewal *A. bahia*	NOEC as TU_c	723	TTP3Z	2G	TU_c
Chronic 7-day Static Renewal *C. variegatus*	NOEC as TU_c	724	TTP6A	2G	TU_c