

WATER POLLUTION CONTROL PROGRAM

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Permits - 1
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**IMPLEMENTATION OF THE NARRATIVE STANDARD
FOR TOXICITY IN DISCHARGE PERMITS USING
WHOLE EFFLUENT TOXICITY (WET) TESTING**

Water Pollution Control Program

This document is to serve as Water Pollution Control Program Policy for Whole Effluent Toxicity (WET) testing, to implement section 61.8(2)(b)(i) of The Colorado Discharge Permit System Regulations, in accordance with the narrative standard for toxicity in Section 31.11 of the Basic Standards and Methodologies for Surface Water. This policy replaces the previous Biomonitoring Guidance Document, originally issued July 1, 1993, and last revised in September 2007.

I. BACKGROUND

Subsection 61.2 (121) of *the Colorado Discharge Permit System Regulations* defines whole effluent toxicity (WET) as “a biological activity effect by which effluents exhibit antagonism to the aquatic organisms used in biomonitoring tests in the form of acute or chronic toxicity.” Such effects are measured using WET tests. Aquatic toxicity tests are laboratory experiments designed to measure the effects of an effluent on aquatic life species in regards to the survival, growth, and reproduction of aquatic organisms. In these tests, specified organisms are exposed to different concentrations of an effluent for specified exposure periods. The responses of test organisms are used to estimate the toxicity of the effluent based on survival of the organisms, and/or effects on the growth and reproductive ability of the organisms.

The State of Colorado does not have numeric water quality standards for toxicity, and therefore is implementing the narrative standard in Regulation 31.11(1), which provides that: “state water shall be free from substances attributable to human-caused point source or nonpoint source discharge in amounts, concentrations or combinations which are harmful to the beneficial uses or toxic to humans, animals, plants or aquatic life.” Note that WET testing is not the standard for toxicity, but is an approved 40 CFR Part 136 method that the Division intends to use as a tool for implementation of the narrative standard.

The WET testing methodology, like other promulgated methods in 40 CFR Part 136, must be followed when performing WET testing in accordance with the requirements of a discharge permit. Therefore this WET policy will not alter or describe the method. However, the Division has flexibility in determining: where chronic WET testing is appropriate; how an effluent limitation is derived; how reasonable potential will be determined; what qualifies as a violation of the limitation and; the appropriate enforcement response.

This updated policy is in response to the Division's agreement with EPA to begin adding sublethal WET limitations to permit, where reasonable potential for an exceedance of the narrative standard (described below) is determined. The Division determined that the first step should be to update its WET policy through a stakeholder process. The workgroup met on four occasions from January to April 2010, and considered numerous alternatives on how to implement sublethal WET limits as well as whether revisions to the existing policy were necessary or appropriate. This policy document is the outcome of this stakeholder process as well as feedback received in conjunction with an informational hearing before the Water Quality Control Commission in August of 2010.

The major topics discussed during the workgroup process included: 1) when would chronic or acute WET testing be appropriate; 2) how should a chronic limitation be implemented in a permit; 3) how will reasonable potential be determined and; 4) how will compliance be determined and how will violations of the WET-related requirements (e.g., a limit) be addressed. There was discussion regarding changing the current 10:1 dilution criteria that defines whether chronic or acute testing will be required, however, no strong reason to change the Division's historic approach was presented and therefore this provision did not change. Consideration was given to 1) using a single statistical endpoint (either the IC₂₅ or the NOEC) with the limit being implemented as a median value of all tests during the monitoring period, 2) using both statistical endpoints as a dual limitation, and 3) implementing WET as a narrative condition in a permit. The difficulty of implementing a limitation for WET as a median, due in part to lab scheduling of testing, missing a toxic event due to reporting the median value, and costs associated with doing numerous WET tests led to the Division not choosing this option. The Division did not choose to implement WET as a narrative condition due to the difficulty and resources necessary to develop language specific to every permit with WET, and to do an independent evaluation of WET testing results for every permit. The dual endpoint was chosen as it provides greater certainty that a toxic event occurred, that the event was both statistically and biologically significant, and it is consistent with the EPA test methods. The compliance determination and violation discussion was determined to best be documented in an ERG separate from this policy. Changes to the acute WET limitations and permit conditions were not specifically considered during the workgroup process.

II. DEFINITIONS

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

Chronic Toxicity Test is a short-term test, usually 96 hours or longer in duration, in which sublethal effects (growth or reproduction) are usually measured in addition to lethality.

Endpoint is a biological measurement used to quantify the results obtained from analytical methods such as whole effluent toxicity testing [e.g., lethal concentration (LC₅₀); inhibition concentration (IC₂₅); and no observed effect concentration (NOEC)]. Such endpoints are quantitative measurements of the responses of test organisms (e.g., survival, growth, mobility, reproduction, and weight gain or loss) in response to exposure to a serial dilution of effluent.

Inhibition Concentration (IC) is a point estimate of the toxicant concentration that would cause a given percent reduction in a non-lethal biological measurement (e.g., reproduction or growth), calculated from a continuous model (i.e., Interpolation Method). IC₂₅ is a point estimate of the toxic concentration that would cause a 25-percent reduction in a non-lethal biological measurement.

Instream Waste Concentration (IWC) is the percentage of effluent in the receiving water after allowable mixing.

Lethal Concentration, 50 Percent (LC₅₀) is the toxic or effluent concentration that would cause death in 50 percent of the test organisms over a specified period of time.

No-Observed-Effect-Concentration (NOEC) - The highest concentration of toxicant to which organisms are exposed in a full life-cycle or partial life-cycle (short-term) test, that causes no observable adverse effects on the test organisms (i.e., the highest concentration of toxicant in which the values for the observed responses are not statistically significantly different from the controls). This value is used, along with other factors, to determine toxicity limits in permits.

Reasonable Potential (RP) is the likelihood that an effluent will cause or contribute to an excursion above a water quality standard based on a number of factors, including the use of data (e.g., whole effluent toxicity test data). In the context of this document, references to RP and WET limits include both lethal and sublethal effects.

Toxicity Test is a procedure using living organisms to determine whether a chemical or an effluent is toxic. A toxicity test measures the degree of the effect of a specific chemical or effluent on exposed test organisms.

Toxicity Identification Evaluation (TIE) is a set of site-specific procedures used to identify the specific chemical(s) causing effluent toxicity.

Toxicity Reduction Evaluation (TRE) is a site-specific study conducted in a step-wise process to identify the causative agents of effluent toxicity, isolate the source of toxicity, evaluate the effectiveness of toxicity control options, and then confirm the reduction in effluent toxicity after the control measures are put in place.

Whole Effluent Toxicity (WET) is the total toxic effect of an effluent measured directly on selected organisms, with a toxicity test.

III. ACUTE VS CHRONIC REQUIREMENTS

1. Instream Waste Concentration (IWC)

The determination of whether acute or a chronic WET requirements would be applicable in a permit will typically be based on the ratio of the “chronic low flow” to the effluent design flow, where the chronic low flow is the 30E3 as defined in Regulation 31. If this ratio is less than 10:1, the need for chronic WET testing will normally be evaluated. Conversely, if the ratio is greater than or equal to 10:1, the need for acute WET testing will normally be evaluated. Expressed as the Instream Waste Concentration (IWC), this 10:1 ratio is equal to 9.1 % effluent (where greater than 9.1% effluent would result in chronic WET requirements and less than or equal to 9.1% effluent would result in acute WET requirements) using the following equation:

$$\text{IWC} = [\text{facility design flow} / (\text{facility design flow} + \text{chronic low flow})] * 100$$

Note that different IWCs can be used upon request for the different monitoring periods, using the lowest of the monthly low flows in the monitoring period to determine the IWC for that monitoring period. However, the determination of whether acute or chronic WET testing is

applicable will be based on the annual low flow. The Division maintains the ability to assign acute or chronic WET testing requirements outside of the IWC determination outlined above, or to assign both acute and chronic WET requirements.

If a mixing zone is denied due to circumstances such as but not limited to, discharge to a stream segment designated as threatened and endangered species habitat, the low flow is considered to be zero and end of pipe limitations apply. In this circumstance, chronic WET requirements will normally be applied. Note that in this example, denial of a mixing zone depends on the various options in the MOU with the US Fish and Wildlife Service.

2. Exemptions From the Normal Acute vs. Chronic Determination

- a. WET testing will not be required where there is not an aquatic life designated use on the stream segment, unless such testing is determined to be necessary to protect downstream aquatic life designated uses.
- b. Regardless of the dilution ratio, acute WET testing will be required where there is an aquatic life designated use, but most of the aquatic life standards (e.g. chlorine, and the TVS equations such as ammonia and metals standards) are not in the site-specific segment standards, unless it is determined that chronic WET testing is necessary to protect downstream aquatic life designated uses, or other evidence exists that would make chronic WET requirements appropriate.
- c. Where the discharge is intermittent, as defined below, acute WET testing may be substituted for chronic WET testing. The basis for this is that there would not be chronic exposure of aquatic life to the effluent.

Definition of Intermittent discharges – For the purposes of this policy, to be considered an intermittent discharge one of the following must apply:

- A) the maximum discharge frequency is less than 3 consecutive days (72 hours), and less than 3 days per 7 day period, and less than 10 days total per month
- B) the maximum discharge frequency is less than 5 consecutive days (120 hours) and less than 5 total days per month
- C) It can be shown that discharge frequency and duration is tied solely to precipitation events, where the discharge starts and stops shortly after the precipitation event starts/stops.

IV. WET LIMITATIONS, MONITORING REQUIREMENTS, TEST SPECIES, TEST METHODOLOGIES, DILUTION SERIES AND REASONABLE POTENTIAL DETERMINATION

1. Limitations

WET limits will be written into permits as a daily minimum as compliance with the limitation is intended to be determined based on the results of any single WET test conducted during the monitoring period.

- a. Acute WET Limits - The limit shall be expressed as the LC50 which represents an estimate of the effluent concentration which is lethal to 50% of the test organisms in the time period prescribed by the test. This test will be conducted on *Ceriodaphnia dubia* (or substituted species, see Section IV.3) using a 48 hour static replacement test, and on fathead minnow (or substituted species, see Section IV.3) using a 96-hour static replacement test. If no instantaneous mixing is provided, the acute WET limit shall be no LC50 at effluent concentration less than or equal to 100% effluent. If instantaneous mixing (e.g., discharge via a diffuser) is provided, an IWC will normally be calculated and the limitation may be something less than 100% effluent, based on how much of the low flow is demonstrated to be instantaneously mixed with the effluent. This limit will be written into a permit as a daily maximum. This limitation will appear in a permit in the following typical format:

<u>Effluent Parameter</u>	<u>Effluent Limitations Maximum Concentrations</u>				<u>Monitoring Requirements</u>	
	<u>30-Day Average</u>	<u>7-Day Average</u>	<u>Daily Maximum</u>	<u>2-Year Average</u>	<u>Frequency</u>	<u>Sample Type</u>
WET, acute LC50 Statre 96Hr Acute <i>Pimephales</i>			LC50 ≥ 100% (daily min)		Quarterly	Grab
LC50 Statre 48Hr Acute <i>Ceriodaphnia</i>			LC50 ≥ 100% (daily min)		Quarterly	Grab

- b. Chronic WET Limits – Chronic WET refers to toxicity related to lethality, growth or reproduction.

The Chronic Methodology states in Section 9.2.4, “In summary, the assessment of a "safe" or "no-effect" concentration cannot be made from the results of statistical analysis alone, unless (1) the assumptions of a strict threshold model are accepted, and (2) it is assumed that the amount of adverse effect present at the threshold is statistically detectable by hypothesis testing. In this case, estimates obtained from a statistical analysis are indeed estimates of a "no-effect" concentration. If the assumptions are not deemed tenable, then estimates from a statistical analysis can only be used in conjunction with an assessment from a biological standpoint of what magnitude of adverse effect constitutes a "safe" concentration. In this instance, a "safe" concentration is not necessarily a truly "no-effect" concentration, but rather a concentration at which the effects are judged to be of no biological significance.”

The Division will define WET limits based on use of a dual statistical endpoint approach. The purpose of the dual limit is to show that there is a biological effect (IC25) and that the biological effect is also statistically significant in relation to the control (NOEC). Upon a test failure, the use of both statistical endpoints allows for the determination that there is a significant statistical difference (NOEC) between the allowable IWC-based effluent dilution and the control, and that this difference is biologically significant.

The chronic WET limitation shall be expressed as a combination of the IC25 and the NOEC and be stated as: “the effluent discharge shall not result in both; 1) an NOEC less than the IWC, and 2) an IC25 less than the IWC.” This test shall be conducted on both *Ceriodaphnia dubia* and *Pimephales promelas* (fathead minnows), using a static renewal 7-day test.

- b. Note that although the limitation is defined as having to fail both statistical endpoints to be considered a violation of the permit, if the permittee has two consecutive instances, (i.e. 1st and 2nd quarter if quarterly monitoring frequency, or if monthly frequency, June then July) of failing one of the two statistical endpoints, the Division will require the automatic compliance response actions be taken (See Section V of this policy). The Division may also require the automatic compliance response actions if there are multiple instances of one test failing during the course of the permit, but they have not been consecutive. Notification will be sent to the permittee if/when this is required, or such circumstances will be written into a renewal permit.

The chronic WET testing limitation will appear in a permit in the following typical format:

<u>Effluent Parameter</u>	<u>Effluent Limitations Maximum Concentrations</u>				<u>Monitoring Requirements</u>	
	<u>30-Day Average</u>	<u>7-Day Average</u>	<u>Daily Maximum</u>	<u>2-Year Average</u>	<u>Frequency</u>	<u>Sample Type</u>
WET, chronic						
Static Renewal 7 Day Chronic <i>Pimephales promelas</i>			NOEC or IC25 > X% (daily min)		Quarterly	3 Composites / Test
Static Renewal 7 Day Chronic <i>Ceriodaphnia dubia</i>			NOEC or IC25 > X% (daily min)		Quarterly	3 Composites / Test

Grab samples may be substituted for composite sampling, when the facility is a lagoon type system (allowing for consistent, fully mixed conditions), or when circumstances dictate that a grab sample is more appropriate or representative of the discharge quality (such as when a volatile toxic substance is expected to be present in the discharge or when sampling logistics dictate).

2. Monitoring Frequencies

WET testing shall normally be required on a quarterly basis, although the Division retains authority to vary the frequency as warranted by site-specific circumstances. For instance, frequency may be increased to monthly where there have been instances of WET failures, for a new facility where there are indications that toxicity could be a concern.

Monitoring frequencies may also be reduced based on such information as facility performance in regards to WET testing results, limited variation in effluent quality, or other factors deemed appropriate. See Part VI of this policy for additional information regarding reduced monitoring or relief from WET testing.

3. Test Species

The following test species are approved by EPA for use with the acute and chronic WET tests, as outlined in the specific acute or chronic method documents. If a different species is desired for WET testing, an alternate test procedure (ATP) must be submitted for approval. The process for submitting an ATP is outlined in 40 CFR Part 136.4.

a. Acute WET Testing Species

For acute testing, the Division may allow the use of those 6 organisms identified in EPA document, Methods for Measuring the Acute Toxicity of Effluents and Receiving Water to Freshwater and Marine Organisms, Fifth Edition, October 2002 (EPA-821-R-02-012 or the most recent version), in Section 6.1.2. The six organisms are:

Invertebrates: *Ceriodaphnia dubia*, *Daphnia pulex*, *Daphnia magna*;

Vertebrates: *Pimephales promelas* (fathead minnow), rainbow trout, brook trout.

The Division will normally specify *Ceriodaphnia dubia* and *Pimephales promelas*, when a permittee has not requested use of an alternate species. Random alternating of species by the permittee is not allowed. Trading of species is restricted to those within the common family, vertebrate and invertebrate. Any request for a change in species is subject to Division approval and must be reflected in the permit.

b. Chronic WET Testing Species

Outside of an approved alternate test procedure (ATP), the only allowable test species for chronic WET testing are *Ceriodaphnia dubia*, *Pimephales promelas*, and *Pseudokirchneriella subcapitata*, as outlined in EPA document, Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms, Fourth Edition, October 2002 (EPA-821-R-02-013 or the most recent version), in Section 6.1.3. If additional species are approved, this policy will be amended to include those species at that time.

4. Test Methodologies

a. Default Methodologies

All WET testing methods, analyses, and validity determinations shall be conducted in accordance with EPA's approved 40 CFR Part 136 methods: Methods for Measuring the Acute Toxicity of Effluents and Receiving Water to Freshwater and Marine Organisms, Fifth Edition, October 2002 (EPA-821-R-02-012) and; Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms, Fourth Edition, October 2002 (EPA-821-R-02-013) or most current editions.

At the request of the permittee the following revisions to portions of the EPA methodologies referred to above will be added to the permit. These revisions have been included based on there being flexibility due to the applicable EPA method citation being a recommended approach. These revisions will be referenced in the permit and will be required to be followed for all WET testing procedures. *Note that these additions may increase the likelihood that a test is considered invalid and that a new test must be scheduled and performed within the same monitoring period (Ceriodaphnia reproduction, alpha/confidence interval) and will increase the number of replicates that must be done within a specific WET test (alpha/confidence interval). Invalid tests cannot be used to meet the required monitoring frequency. If a facility does not get a valid test result during the monitoring period, it will be considered a failure to monitor, and the permittee will be in violation of the permit.* If not requested, the permit conditions will default to the EPA recommendations included in the appropriate method. See Section VII of this policy for more information regarding valid, invalid and inconclusive tests.

Ceriodaphnia Reproduction – The EPA chronic method recommends that the test should be terminated when 60% or more of the surviving control females have produced their 3rd brood. On request, this portion of the method will be changed to 80% or more of the surviving control females have produced their 3rd brood. This will be written into the permit and must be done on every test done under the requirements of the permit.

Confidence Intervals – The EPA methods recommend that a 95% confidence interval ($\alpha = 0.05$) be used in the statistical analyses performed under the method. On request, this will be changed to a 99% confidence interval ($\alpha = 0.01$). This will be written into the permit and must be used on every test done under the requirements of the permit. The use of this alpha level decreases the potential for false positives but increases the potential for false negatives. Therefore, if this option is chosen, the Division will require an increase in the number of replicates within each individual test as needed, to assure that the test meets the minimum sensitivity requirements.

EPA has a guidance document that shows how to calculate the minimum significant difference (MSD) using an alpha of 0.01. This is contained in Chapter 2 of the Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing, EPA 821-B-00-004, USEPA, July 2000. This document shows the maximum MSD, the equations on how to calculate the actual MSD seen in a test, and a table that shows the likely number of replicates that may be needed to meet the MSD requirements. If this requirement is not met, the test is considered invalid and retesting must be performed during the monitoring period. The permittee will be required to submit documentation showing that the appropriate number of replicates was used, and that the proper MSD criterion has been met, with the WET information summary that is submitted to the Division with the WET test results.

b. Altering of Test Methods

The use of alternate testing procedures is not allowed unless specifically authorized in the permit. Alterations that are not already approved, as outlined in the EPA methods, will need to go through the ATP process as outlined in 40 CFR Part 136.4. Note that to use the approved alternate testing procedure, it needs to be demonstrated that the parameter in question caused toxicity.

The currently approved alternative test method is:

- i. Ammonia toxicity based on pH Drift – use of a CO₂ atmosphere will be allowed to control pH drift, and where ammonia toxicity due to pH drift is being identified, as higher pH increases the toxicity of ammonia.

5. Dilution Series

The Division will prescribe the minimum dilution series to be used in the permit. The permittee is free to use additional dilutions however; this information will need to be included in the WET information summary submitted to the Division with the WET test results. At a minimum, the dilution series will contain a control (0% effluent) and 5 effluent dilutions. The effluent dilutions assigned by the Division will typically contain one value at 100% effluent, one value at the IWC, and then bracket the three remaining dilutions around the IWC. These remaining 3 dilutions will typically be calculated based on the following: $(IWC+100)/2$, the $IWC/2$, and the $IWC/4$.

For situations where the IWC=100%, the dilution series will be prescribed as 20, 40, 60, 80 and 100% effluent. Note that where there has been the request for seasonal IWCs, the dilution series for each seasonal IWC will be calculated. Also note that the IWCs will be rounded to the nearest whole number.

6. Reasonable Potential

40 CFR 122.44 and Regulation 61.8(2)(b) contain information on including limitations in permits based on reasonable potential. Note that Regulation 61.8(2)(b) contains the same information and requirements as the 40 CFR citation. Regulation 61.8(2)(b)(i) (B) states: When determining whether a discharge causes, has the reasonable potential to cause, or measurably contributes to an in-stream excursion above a narrative or numeric water quality standard, the Division shall use procedures, including appropriate water quality modeling, which account for existing controls on point and nonpoint sources of pollution, the variability of the pollutant or pollutant parameter in the effluent, the sensitivity of the species to toxicity testing (when evaluating whole effluent toxicity), and where appropriate, the dilution of the effluent in the receiving water.

Regulation 61.8(2)(b)(i) (E) states: Except as provided in this subparagraph, when the Division determines, using the procedures in subsection (b)(i)(B) of this section, toxicity testing data, or other information, that a discharge causes, has the reasonable potential to cause, or measurably contributes to an in-stream excursion above a narrative water quality standard, the permit must contain limitations, which include effluent limits, for whole effluent toxicity. Such limitations to be derived by the Division are based upon the Division's determination of what constitutes an acceptable level of whole effluent toxicity. Limits on whole effluent toxicity are not necessary where the Division demonstrates in the rationale of the permit, using the procedures in subsection (b)(i)(B) of this section, that chemical-specific limits for the effluent are sufficient to attain and maintain applicable numeric and narrative water quality standards.

The fact sheet to the permit (named the rationale in the above regulatory reference) shall contain a discussion of the reasons for including, or not including WET limits or monitoring based on reasonable potential for the effluent to be toxic to aquatic life. For WET testing, reasonable potential determinations will be done on both a qualitative and quantitative basis. The following items, either individually or in combination, may be used to determine RP for a limitation or to determine that "monitoring only" is appropriate:

- Facility type –

Facilities with complex effluent types such as industrial majors or minors, or domestic majors will be given higher consideration for a RP determination. Note that this does not necessarily exclude domestic minor facilities.

- Expected pollutants in the discharge (potential for toxic pollutants, organic chemicals) –

Facilities that have a limited number of toxic pollutants that are adequately controlled through chemical specific effluent limits will have a lower potential for RP (e.g. ammonia and chlorine at domestic minor wastewater treatment facilities). Facilities that have toxic pollutants for which there are no numeric water quality standards, or have a higher number of toxic pollutants and therefore an increased potential for synergistic effects, will have a higher potential for RP.

- Variability of the discharge, in regards to WET test data or other toxic pollutants –

Facilities that have a higher level of variability in WET testing results will have a higher potential for RP. High variability will be determined to be a coefficient of variation (CV) that is greater than or equal to 0.1, and will be determined by dividing the standard deviation of the WET test results by the mean of the WET test results. Note that this will be calculated for the IC₂₅ endpoint only, as the NOEC endpoint by definition, can only be one of the dilutions used in the test, therefore limiting the value of the statistic.

- POTWs required to develop a pretreatment program, or those that have industrial users -

Facilities that have significant industrial users and and/or are required to have a pretreatment program will have higher potential for RP.

- WET testing results less than the IWC determined for the renewal permit -

Since the chronic WET limitation is based on a dual statistical endpoint where both the NOEC and the IC₂₅ need to be less than the IWC to be considered a failure, then if such occurs, there is greater confidence that the effluent was toxic. Therefore, if previous data show test failures at an IWC value lower than the current IWC, then this may be determined to be a strong case for a determination of RP.

- Consideration of improved treatment efficiencies, or upgrades to treatment since previous failures

If a facility has added additional treatment, source control or undergone other activities, which were put into place specifically to (at least in part) lower the toxic characteristic of the effluent, consideration will be given to WET data that was taken after such treatment was put into place. If sufficient data does not exist for such evaluation, RP will continue to be determined solely on the data collected previous to the facility’s activities.

- Multiple failures of 1 of the 2 statistical endpoints –

Although not considered a violation of the permit limitation for WET, facilities that have had multiple instances of failing one of the two statistical endpoints will have a higher potential for RP. Any supporting information submitted by the permittee with the DMRs regarding such failures will be considered. Patterns such as seasonal failures of single endpoints will also have a higher potential for RP.

Examples of how this information may be used are provided in the table below, for three potential POTWs. (+) are shown where specific information may be used as a higher expectation of RP, and a (-) indicates a lower expectation of RP.

	Facility 1	Facility 2	Facility 3
Facility Type	Major POTW (+)	Major POTW (+)	Minor POTW (-)
Expected Pollutants	Ammonia, chlorine, Metals, (+)	Ammonia, chlorine, metals, potential for organics (industrial users) (+)	Ammonia, chlorine, (-)

Pretreatment/ Industrial Users	Have significant industrial users but no pretreatment program (+)	Have significant industrial users and pretreatment program (+)	The facility does not have significant industrial users (-)
Variability of WET test results	CV = 0.12 (+)	CV = 0.07 (-)	Unknown
Previous Violations / WET test results lower than new IWC	No previous violations lowest result is 80% vs a new IWC of 50% (-)	No previous violations, lowest result is 90% vs a new IWC of 85% (-)	Unknown
Treatment Facilities or Upgrades	N/A (no violations)	N/A (no violations)	N/A
Multiple failures of 1 statistical endpoint	No single endpoint failures (-)	Two single endpoint violations, one in the spring, early in the permit term and one in the fall, late in the permit term. (+)	Unknown
Potential Explanation	<p>The facility is a major POTW that has significant industrial users, but does not have the potential for additional parameters to be present in the effluent that are not adequately controlled by a chemical specific effluent limit. This information leans toward having a qualitative determination of RP.</p> <p>However, previous effluent data has high variability, no previous violations or failures of one single endpoint, and the lowest WET test result is 80% vs a new calculated IWC of 50%.</p> <p>The likely result here is no RP for a limit, but based on facility type, and the presence of industrial users, monitoring will be required.</p>	<p>The facility is a major POTW with significant industrial users, and has the potential for a wide variety of pollutants to be present in the effluent.</p> <p>Although the previous effluent data shows low variability, and no violations of the permit limit, the lowest effluent value is not much higher than the new IWC. Additionally, there have been multiple failures of a single endpoint, although this is less significant as they were several years apart and in different seasons.</p> <p>The likely result here is a determination of RP based on the facility type, parameter potential, and that the lowest WET test result is not much higher than the</p>	<p>This facility is a minor POTW without significant industrial users.</p> <p>The facility has not been required to perform WET testing and therefore there are no results to evaluate.</p> <p>Although the facility does not have WET data, the likely result here is that the facility will not be required to have a WET limit or monitoring conditions as the parameters of concern are adequately controlled by chemical specific effluent limitations, and there is little potential for other parameters to be present.</p>

		new IWC.	
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Prior to assigning WET limits and/or monitoring in a permit, the Division may also take into account: WET testing done on native species present in the receiving stream, improved treatment and/or control processes since any prior failures, information on the toxicity of the ambient water, or general consideration of treatment performance. The Division will also take into consideration the results of a TIE/TRE, where the pollutant has been identified and controlled either by a permit limitation for that pollutant, treatment, or other method.

Permits for new facilities may be determined to have reasonable potential based on the pollutants of concern in the discharge, and any WET data submitted with the application. These limits will normally be effective immediately.

Permits for facilities that have not previously had WET limitations or monitoring requirements, have had a switch from acute WET testing to chronic WET testing, or have a more stringent IWC, and are determined to have reasonable potential (e.g., based on pollutants of concern in the discharge and/or any WET data submitted with the application), will include a WET limit but may be eligible for a delayed effective date, to determine if the limitation can be met, as it would be a new limitation in such a case.

Facilities that are new or have not previously had WET limits or monitoring, and have an unknown potential for toxicity, may get monitoring requirements to collect data for a future determination of reasonable potential.

V. AUTOMATIC COMPLIANCE RESPONSE FOR WET TESTING FAILURES

The permittee will be responsible for implementing the following automatic compliance response items, which will be outlined in the permit, when one of the following occurs:

- A routine WET test is failed (both endpoints are less than the IWC)
- Two consecutive WET tests fail one of the statistical endpoints (either the IC25 or the NOEC)
- When notified by the Division after multiple failures (non-consecutive) of one of the statistical endpoints

The permittee may choose either: 1) accelerated testing to determine a pattern of toxicity or spontaneous disappearance or; 2) move directly into a toxicity identification evaluation (TIE) or a toxicity reduction evaluation (TRE). The permittee must provide written notification of the test failure to the Division, along with a statement as to whether accelerated testing or a TIE/TRE is being performed, within 14 calendar days of receiving the test results.

1. Accelerated Testing

Accelerated testing is to be done using the single species found to be more sensitive. WET tests shall be run at least once every 2 weeks (note that only 1 test should be run at a time), for up to 5 tests, until either: 1) 2 consecutive tests fail, or 3 out of 5 tests fail, at which point a pattern of toxicity will have been identified, or 2) 2 consecutive tests pass, or 3 out of 5 tests pass, in which

case no pattern of toxicity is identified. The permittee shall submit all WET summary sheets to the Division after a pattern of toxicity has been confirmed or denied.

Only the IC₂₅ statistical endpoint shall be used for determining a passed or failed test (at the IWC) during accelerated testing, except for the instance where accelerated testing is being completed due to two consecutive failures of a single statistical endpoint, and that endpoint was the NOEC on both occasions. In such a case, the NOEC shall be used to determine a passed or failed accelerated test, and the same dilution series must be used as was used during the failed test.

If a pattern of toxicity is found, then the permittee shall move into a TIE/TRE.

If no pattern of toxicity is found, this is determined to be spontaneous disappearance of toxicity and the permittee shall return to routine WET testing as outlined in the permit. If a pattern of toxicity is not demonstrated but a significant level of erratic toxicity is found, the Division may require an increased frequency of routine monitoring or some modified approach in an attempt to ensure toxicant identification and control. If such an event happens frequently, the Division may require a TIE/TRE.

2. TIE/TRE

A TIE and/or TRE must be performed if a pattern of toxicity is found during accelerated testing, if the permittee elects to skip the accelerated testing and move directly into a TIE/TRE, or at the direction of the Division based on past test results. A TIE is a set of site-specific procedures used to identify the specific chemical(s) causing effluent toxicity. A TRE is a toxicity reduction evaluation where the purpose is to identify corrective actions to reduce the toxicity of an effluent.

Although there are no regulatory requirements on how a TIE/TRE is performed, EPA has a series of guidance documents aimed to assist the permittee in conducting TIEs/TREs. There are also numerous other references for conducting TIEs/TREs available. Some of the EPA guidance documents regarding TIEs/TREs are referenced below.

- Methods for aquatic toxicity identification evaluations: Phase I toxicity characterization procedures. Second edition. EPA/600/6-91/003. 1991c.
- Methods for aquatic toxicity identification evaluations: Phase II toxicity identification procedures. EPA/600/3-88/035. 1989b.
- Methods for aquatic toxicity identification evaluations: Phase III toxicity confirmation procedures. EPA/600/3-88/036. 1989c.
- Toxicity identification evaluation: Characterization of chronically toxic effluents, Phase I. EPA/600/6-91/005F. 1992.
- Methods for aquatic toxicity identification evaluations: Phase II toxicity identification procedures for samples exhibiting acute and chronic toxicity. EPA/600/R-92/080. 1993b.
- Methods for aquatic toxicity identification evaluations: Phase III toxicity identification procedures for acutely and chronically toxic samples. EPA/600/R-92/081. 1993c.

- Toxicity reduction evaluation guidance for municipal wastewater treatment plants. Second Edition. EPA/833/B-99/002. 1999b.
- Clarifications regarding toxicity reduction and identification evaluations in the national pollutant discharge elimination system program. March 27, 2001.

Toxicity Identification Evaluation (TIE)

If the permittee elects to or is required to perform a TIE, the Division recommends that the EPA guidance documents be followed. If another method is to be used (i.e. a laboratory specific method) the TIE procedure should be submitted to the Division prior to initiating the TIE.

The results of the TIE investigation are to be submitted to the Division within 180 days of the demonstration of acute or chronic WET in the routine test, as defined above, or if accelerated testing is performed, the date the pattern of toxicity is demonstrated. A status report is to be provided to the Division monthly during the TIE investigation. The Division may extend the time frame for investigation where reasonable justification exists. An example of reasonable justification may be a situation where the toxicant is sporadic or seasonal in appearance and the recurrence interval is beyond the 180 days.

If the pollutant(s) causing toxicity is/are identified, and is/are controlled by a permit effluent limitation(s), the permit may be modified upon request to adjust permit requirements regarding the automatic compliance response (e.g. if future test failures can reasonably be shown to be caused by the previously identified pollutant, and the permit limit for that pollutant was met, accelerated testing and/or TIE/TREs will not be required), to allow for monitoring only for WET, or potentially to eliminate the WET limit, depending on the potential for other toxic parameters to be present in the effluent.

If the pollutant(s) causing toxicity is/are identified, and is/are not controlled by a permit effluent limitation(s), the Division may develop and add limitations to the permit for these parameters. If there is not a water quality standard for a parameter, the Division will develop a limitation based on available information on the toxicity of that parameter to aquatic life, particularly that present in the receiving stream. The permit may be modified as noted in the above paragraph.

If the pollutant causing toxicity is not able to be identified, or is unable to be specifically identified (e.g. TDS or ionic balance), or is not able to be controlled by an effluent limit, the permittee will be required to perform either item 1 or item 2 below.

- 1) Conduct an investigation which demonstrates actual instream aquatic life conditions upstream and downstream of the discharge, or identify, for Division approval, and conduct an alternative investigation which demonstrates the actual instream impact. This should include WET testing and chemical analyses of the ambient water. Depending on the results of the study, the permittee may also be required to identify the control program necessary to eliminate the toxicity and its cost. Data collected may be presented to the WQCC for consideration at the next appropriate triennial review of the stream standards;
- 2) Move to a TRE by identifying the necessary control program or activity and proceed with elimination of the toxicity so as to meet the WET effluent limit.

If toxicity spontaneously disappears in the midst of a TIE, the Division may require the permittee to conduct accelerated testing to demonstrate that no pattern of toxicity exists, or may amend the permit to require an increased frequency of WET testing for some period of time. If no pattern of toxicity is demonstrated through the accelerated testing or the increased monitoring frequency, the toxicity incident response will be closed and normal WET testing shall resume.

Toxicity Reduction Evaluation

The control program developed during a TRE consists of the measures determined to be the most feasible to eliminate whole effluent toxicity. This may happen through the identification of the toxicant(s) and then a control program aimed specifically at that toxicant(s) or through the identification of more general toxicant treatability processes. Unless otherwise modified in the permit, a control program is to be developed and submitted to the WQCD within 180 days of beginning a TRE.

If toxicity spontaneously disappears in the midst of a TRE, the Division may require the permittee to conduct accelerated testing to demonstrate that no pattern of toxicity exists, or may amend the permit to require an increased frequency for some period of time. If no pattern of toxicity is demonstrated through the accelerated testing or the increased monitoring frequency, the toxicity incident response will be closed and normal WET testing shall resume.

VI. REDUCED MONITORING AND RELIEF FROM WET TESTING

For routine WET testing requirements, reduced monitoring frequencies may be allowed where the permittee has demonstrated compliance with the limitation. Note that in considering whether reduced monitoring frequencies should apply, the Division will look at whether the limitation has been met, the variability of the test results, and may consider other items such as pollutant potential and facility type. Typically, in accordance with the Division's monitoring policy, reductions in monitoring will not be granted during the first permit term where acute or chronic WET testing requirements are required. However, if an effluent can be shown to be chemically consistent, and lacks the potential to have new pollutants introduced, the Division may consider reducing the monitoring frequency after 4 consecutive passed tests, upon an amendment request by the permittee. Note that due to seasonal variability, it may take more than 1 year to show that an effluent is chemically consistent.

If there is a change from acute to chronic WET, the Division will default back to the normal quarterly monitoring frequency, unless specific information exists that shows that the facility will be able to comply with the new limit. A change from chronic to acute WET testing may, depending on site specific conditions, allow for continuation of the same monitoring frequency as this is typically considered a less stringent limit. Note that consideration will also be given to any changes to the facilities treatment processes, source waters or activities, in relation to whether previous data can be used to show compliance with a new or changed WET limit.

If deemed appropriate by the Division, the permit may be modified to revise the ongoing monitoring and toxicity investigation requirements to avoid an unproductive expenditure of the permittee's resources, provided that the underlying obligation to eliminate any continuing exceedance of the toxicity limit shall remain. For example, the permit may be modified to eliminate the automatic compliance response, if it can be shown that a parameter that was determined to be the cause of toxicity during a previous TIE was present at a similar concentration(s) and therefore can reasonably be assumed to have caused the newly observed toxicity. Note that such relief is subject to the

continued compliance with any limitation for such parameter or other permit requirement, and that relief from a TIE does not mean relief from a TRE.

The Division may require additional testing when it concludes that the routine testing frequency is inadequate to properly reflect effluent conditions or the Division feels that routine test violations in combination with automatic compliance response requirement(s) are not creating an adequate incentive for prompt resolution of a serious pollution problem.

VII. DISCHARGE MONITORING REPORTS, LABORATORY RESULTS, and TEST VALIDITY

1. Discharge Monitoring Reports and Laboratory Results

The permittee will be required to submit the results of WET testing data on the DMR reports. The DMRs for quarter monitoring frequencies will have the WET reporting requirement on the DMR for the last month of the monitoring period (if the monitoring frequency is quarterly, this will show up on the DMR in March, June, Sept, Dec). If the permittee does the WET testing earlier in the monitoring period, those results will need to be recorded on the appropriate DMR. The DMR will typically contain three different codes, "P", "S" and "T", with a report function for the "P" and "S" codes, and the limitation presented on the "T" code. These codes are defined as follows:

"P" – results of the IC₂₅ as a report only condition

"S" – results of the NOEC as a report only condition

"T" – reporting of the highest of the values from the "P" and "S" codes for determination of compliance with the limitation

As the limitation is stated as both an IC₂₅ and a NOEC, and both tests must fail to be considered a violation of the permit, the reporting of the highest value between the IC₂₅ and the NOEC for comparison with the limitation, with the reporting requirements for the individual statistical analyses, will provide the Division with a means to determine compliance with the WET limitation to be reported to ICIS. For example:

The IWC = 85%, the results of the WET test are IC₂₅ = 80%, NOEC = 90%

"P" (IC₂₅ report only) 80%

"S" (NOEC report only) 90%

"T" (highest of "P" and "S", limit = to 85%) 90% - Results in a compliant test

The IWC = 85%, the results of the WET test are IC₂₅ = 80%, NOEC = 70%

"P" (IC₂₅ report only) 80%

"S" (NOEC report only) 70%

"T" (highest of "P" and "S", limit = to 85%) 80% - Results in violation of limitation

The permittee must submit, with the appropriate DMR, all laboratory statistical summary sheets, chain of custody forms, and summaries of the determination of test validity. Such information should be submitted for all WET testing done during the monitoring period, including all valid, invalid, and inconclusive tests. Such determinations must conform to the procedures outlined in the appropriate EPA methodology.

2. Test Validity

Test validity may depend on a number of factors and is outlined in the acute and chronic methods. This policy does not alter the determination of a valid or invalid test as outlined in the methods. In general, all test data should be reviewed to verify that the test acceptability criteria (TAC) requirements for a valid test have been met. Any test not meeting the minimum TAC is considered invalid and must be repeated with a newly collected sample (acute method 12.2.3 or chronic method 10.2.3). Deviations from recommended test conditions may or may not invalidate a test result depending on the degree of the departure and the objective of the test (acute method 12.2.4.2 or chronic method 10.2.4.2). Although the two method documents should be consulted for specifics on test validity, some general criteria are reproduced below.

For acute testing, survival in the controls must be 90% or more (acute method 9.16). Events such as excessive within-test variability may invalidate a test dependent on the upper and lower percent minimum significant difference bounds (acute method 12.2.8.1 or chronic method 10.2.8.1). The concentration-response relationship for each multi-concentration test must be reviewed and EPA offers guidance on how to do so. The guidance states that there are three potential results: either the calculated effect concentrations are reliable and should be reported or; that they are anomalous and should be explained or; that the test was inconclusive and the test should be repeated with a newly collected sample (acute method 12.2.6.2 or chronic method 10.2.6.2).

A new WET test should be performed as soon as possible in an attempt to obtain a valid, conclusive test result during the monitoring period. Although inconclusive tests results should be repeated, they will not normally be considered a failure to monitor, providing that the permittee has made a good faith effort to obtain a conclusive test during the monitoring period (e.g. has conducted additional WET testing during the monitoring period that also resulted in an inconclusive test). Note that when an initial WET test result is determined to be inconclusive, was performed at the end of the monitoring period, and did not allow for time for repeated testing, that this will be considered a failure to monitor.

VIII. REQUIRED INFORMATION TO BE SUBMITTED WITH AN APPLICATION

At the time of permit application for a new or renewal permit, permittees (as outlined below) are required to submit the results of a chronic WET test (except as noted in item 5, where an acute test may be substituted using an LC50 = 100% effluent endpoint). The test shall be conducted on a 20, 40, 60, 80 and 100% effluent dilution series and be for both *Ceriodaphnia dubia* and fathead minnows. Where routine testing has been performed during the previous permit term, this will not be required. Permittees subject to testing are:

1. All POTWs with design influent flows equal to or greater than one million gallons per day;
2. All POTWs with approved pretreatment programs or POTWs that are required to develop a pretreatment program;
3. Other POTWs where the variability of the pollutants or pollutant parameters in the effluent (based on chemical-specific information, the type of treatment facility, and types of industrial/pollutant contributions);
4. All industrial facilities.

5. Permittees that have a known dilution ratio of $>10:1$, or who meet one of the exemptions outlined in Part III.2 of this policy, may substitute an acute WET test. Facilities are free to perform both tests.