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Guidance for Conducting Toxicity Testing and TIE/TRE Studies

This document is intended to work with an NPDES permit to provide permittees guidance for whole effluent toxicity (WET) testing and to inform operators what actions are required in case of non-compliance.

1. Test Procedures

Acute and chronic toxicity is a measure of the toxic effect that a wastewater effluent may have on living organisms (i.e., *Pimephales promelas* and *Ceriodaphnia* species). Acute and chronic toxicity analyses are conducted using the EPA approved Whole Effluent Toxicity (WET) test methods set forth in 40 CFR, Part 136 (February 2021 edition). There are separate and distinct test methods for measuring acute and/or chronic toxicity impacts. These test methods establish standardized conditions and require that certain chemical and physical analyses be conducted in conjunction with the toxicity analysis.

2. Results Reporting

Test results are reported in terms of toxic units. Acute toxic units are abbreviated TUa, and are the inverse of the LC₅₀ (i.e., the concentration of effluent that is lethal to 50% of the organisms) expressed as a decimal fraction. Chronic toxic units are abbreviated TUc, and are the inverse of the NOEC (i.e., the highest concentration of effluent at which there is no observed effect on the organisms) with respect to growth or reproductive inhibition) expressed as a decimal fraction. The tables below provide examples of the LC₅₀ and NOEC conversions to toxic units.

LC ₅₀	Decimal Fraction	TUa	NOEC	Decimal Fraction	TUc
0.1 %	0.001	1000	0.1 %	0.001	1000
1%	0.01	100	1%	0.01	100
10%	0.1	10	10%	0.1	10
25%	0.25	4	25%	0.25	4
50%	0.5	2	50%	0.5	2
100%	1	1	100%	1	1

3. Initial Response to Non-Compliance

If the permit limits for toxicity are exceeded, the permittee needs to perform the following actions, unless the Department specifies alternative procedures:

- a. Submit a written non-compliance report (NCR) within 5 days. In the NCR, identify any suspect sources of the toxicity and describe any measures being taken to reduce toxicity.
- b. Conduct follow-up toxicity testing with both organisms within four (4) weeks.

4. Return to Compliance

If the follow-up test results are in compliance with the limits in the permit, the permittee needs to perform the following actions, unless the Department specifies alternative procedures:

- a. Within 30 days submit the results of the follow-up test in a written report to the NDEE. The written report should discuss the effect of the measures taken to reduce toxicity.
- b. The report should also provide the NDEE with a recommendation relative to their success and, if ongoing, the need to continue implementing these measures.
- c. Testing can generally be resumed on the routine schedule established in the permit, unless the NDEE specifies otherwise. The NDEE can require additional follow-up testing on a case-by-case basis (e.g., if there was no apparent reason why toxicity decreased). Any measures taken to reduce toxicity will generally need to be continued as Best Management Practices, unless the NDEE provides a written approval for their discontinuance.

5. Actions to Address Continued Non-Compliance

If the follow-up toxicity test results are not in compliance with the permit limits, the permittee needs to perform Toxicity Identification Evaluations (TIE) and Toxicity Reduction Evaluations (TRE) as specified below, unless the Department specifies alternative procedures. The permittee is responsible for maintaining compliance with the toxicity limits in this permit. The undertaking of the TIE/TRE process does not constitute compliance unless or until compliance with the permit limits is achieved. See explanatory note below concerning Departmental actions to address non-compliance.

- a. Development and implementation of a Toxicity Identification Evaluation (TIE) needs to begin immediately. A summary plan for the initiation of the TIE should be submitted to the NDEE within 30 days of when the follow-up of non-compliant result is received. A meeting with the NDEE to discuss TIE/TRE alternatives within this period is encouraged.
- b. A complete TIE/TRE schedule should be submitted to the NDEE within 90 days. The NDEE will seek clarification on at least some aspects of the schedule, and may request some modification.
- c. The TIE and TRE processes should continue concurrently; i.e., as the TIE process identifies toxicity sources, reasonable measures to reduce the toxicity from these sources should be taken. The TIE/TRE schedule may need to be revised in response to ongoing TIE/TRE activities.
- d. Sometimes it is possible to forego or discontinue the TIE process, and proceed directly with the TRE process. However, before abandoning the TIE process, it is important that:
 - i) the source of the toxicity be known; and
 - ii) NDEE concurs with this approach.

Note: On-going non-compliance with a toxicity limit will typically be addressed in one of two ways depending on whether agreement can be achieved between the NDEE and permittee on the TIE/TRE schedule and procedures. If agreement can be achieved, the NDEE and the permittee may wish to enter into a Consent Order. If agreement cannot be achieved, the NDEE may proceed unilaterally via administrative and/or enforcement actions. In most instances, it is advantageous for all parties if a mutually agreed to TIE/TRE process can be implemented. For that reason, early meetings and discussions with the Department are encouraged.