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GOVERNOR

STATE OF MICHIGAN
DEPARTMENT OF ENVIRONMENTAL QUALITY
LANSING



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To All Interested Parties:

SUBJECT: State of Michigan Requirements for Whole Effluent Toxicity (WET) Test Methods

A) WET Test Methods

The State of Michigan requires the use of the following methods for all WET tests, except when an approved plan requires different methods, or when tests are not being conducted for compliance assessment (e.g., toxicity reduction evaluation tests). Of course, labs may use their own protocols, provided that the protocols meet the requirements of the methods:

Chronic toxicity tests: EPA/600/4-91/002

Acute toxicity tests: EPA/600/4-90/027F

B) Specific WET Test Method and Reporting Requirements

Listed below is a compiled list of specific requirements and guidance for WET test methods and reporting (see below). Failure to observe these requirements may invalidate test data.

1. Consulting laboratories shall initially validate to the Michigan Department of Environmental Quality (MDEQ) their ability to perform each test method to be used prior to MDEQ acceptance of WET data. A submittal of the results of five consecutive acceptable reference toxicant tests and a comprehensive Standard Operating Procedure (SOP) for the method are sufficient for validation. Please also include raw data from at least one set of tests.

Consulting laboratories that have already validated their ability to conduct WET tests using the previous versions of the USEPA WET protocols do not need to revalidate this ability.

2. Synthetic fresh water as described in EPA/600/4-91/002 shall be used as diluent for *Ceriodaphnia dubia* (*C. dubia*) WET tests, unless prior approval to use an alternative dilution water has been obtained from the MDEQ. Synthetic water may be prepared using mineral water or reagent-grade chemicals. We do not require the use of synthetic dilution water for *D. magna* or fathead minnow tests.

Moderately hard synthetic water is recommended, but synthetic water of the same hardness as the receiving water may also be used.

3. For compliance tests, chlorinated samples shall be dechlorinated before testing [≤ 20 micrograms per liter total residual chlorine (TRC)]. Detected levels of chlorine shall be reported. If effluent samples are dechlorinated, dechlorination controls shall be included in the test.

4. We require use of the Orion^R probe or amperometric titration techniques for TRC determination. Other methods do not produce reliable results, and interferences are common.
5. For effluent compliance tests, test solution pH shall be maintained within the range of 6.5 - 9.0 standard units unless the National Pollution Discharge Elimination System (NPDES) permit allows a different range for the effluent, or prior approval is obtained to conduct tests at alternative pH levels.
6. Sufficient ice or other cooling material shall be used to maintain low sample temperatures during holding and shipment ($\leq 6^{\circ}$ Celsius). Increased temperature can affect sample toxicity and may invalidate test results. Please avoid freezing samples. Sample receiving temperature shall be reported for all samples.
7. Chronic tests shall include a 100 percent effluent concentration to allow acute toxicity assessment.
8. We have observed that some chronic toxicity tests do not include effluent concentrations low enough to assess toxicity at the effluent receiving water concentration (RWC). The RWC is a critical point of regulation. Chronic toxicity limits are generally calculated as the value 100/RWC. Tests that do not assess toxicity at or below the RWC are not valid for compliance determination. Test concentration series shall assess toxicity of a test concentration equivalent to, or lower than, the effluent RWC.
9. The time between toxicity test sample collection and first use shall not exceed 36 hours for any sample without MDEQ approval. Please call us if there is a problem with sample holding time.
10. Avoid effluent sample filtration, because filtration can change the toxicity of effluent samples. Routine test sample filtration without cause is not acceptable. Effluent samples should only be filtered if organisms present in the sample could compromise the test results. Test results could potentially be compromised by predators, competitors, parasites, or indigenous daphnids.

If filtration is performed prior to testing, an unfiltered 100 percent effluent treatment shall also be included in the test.
11. Neonates used for toxicity tests must be obtained from individually cultured organisms, and tests must be blocked by female.
12. *C. dubia* brood number shall be tracked daily. Aborted or resorbed eggs shall be noted and reported.
13. Only first- through third-brood live young may be included in reproductive totals for all concentrations and the control in *C. dubia* chronic tests. Dead young, and young from broods released after the third brood, shall not be included in the reproductive totals.
14. *C. dubia* chronic tests are ended when ≥ 60 percent of diluent control animals have produced their third brood. This usually occurs on days six, seven, or eight.

15. The chronic value (ChV), or maximum acceptable toxicant concentration (MATC), shall be reported for all chronic toxicity tests. The MATC is defined as the geometric mean of the no observable effect concentration and lowest observable effect concentration. Inhibition concentration endpoints may be reported in addition to the MATC/ChV values.
16. Acute toxic units (TUa) values (based on mortality) shall be reported for acute toxicity tests.
17. Both chronic toxic units (TUc) (based on MATC/ChV) and TUa (mortality) values shall be reported for all chronic toxicity tests.
18. **If TUa is < 1.0** ($LC_{50} > 100$ percent), please derive and report a numerical value for TUa based on percent mortality in the 100 percent effluent concentration of the toxicity test as follows:

0 TUa if mortality is 0 to 10 percent

TUa = 0.02 x percent mortality if mortality is 10 to 49 percent

Example: Mortality in 100 percent effluent = 25 percent

$TUa = 0.02 \times 25 = 0.5$ TUa

Permittee reports 0.5 TUa on discharge monitoring report.

Report **0 TUc** if no chronic toxicity is detected (MATC/ChV > 100 percent effluent).

19. The results of all effluent toxicity tests, including invalid tests, must be reported. Tests that fail to meet test acceptability criteria shall be repeated, unless the results of the test are sufficient to assess the toxicity of the effluent. We intend to provide an assessment of test acceptability prior to report preparation under these circumstances at the consultant's request. Please call us before submitting questionable data.
20. An unexplainable concentration response often invalidates toxicity test data. Adverse effects should generally increase with increasing effluent concentration. Consult the USEPA guidance¹ for concentration response interpretation. Please call us if the concentration response appears unexplainable.
21. Labs shall provide reference toxicity data for in-house cultures with each test report. For fish purchased from an external source, reference toxicant data may be obtained from a simultaneously-conducted reference test using the same batch of fish, or from a recent reference toxicant test conducted by the source hatchery.
22. The duration of fish and daphnid acute toxicity tests shall be 96 hours and 48 hours, respectively.
23. USEPA guidance¹ recommends reducing the statistical significance rate (alpha) for certain chronic WET data analyses. The lower alpha value (0.01) increases the amount of

¹ USEPA. 2000. Method Guidance and Recommendations for Whole Effluent Toxicity Testing (40 CFR Part 136). EPA 821-B-00-004. USEPA Office of Water, Washington, DC.

adverse effect needed to indicate chronic toxicity relative to the 0.05 alpha originally recommended by the USEPA test methods.

The MDEQ has approved the option to use 0.01 alpha. Alpha remains 0.05 for data that does not meet the USEPA criteria.

24. The MDEQ expects consulting laboratories to implement the Percent Minimum Significant Difference (PMSD) criteria of Section 10.2.8 of the USEPA chronic toxicity manual (EPA-821-R-02-013). In short, the criteria are applied as follows:
- When a test is insensitive (PMSD > 90th percentile), and does not detect toxicity, “a retest must be performed.” The MDEQ intends to use professional judgment in these cases. For example, if a PMSD is > 90th percentile, but performance in the effluent concentrations exceeds control performance, the test may not need to be repeated. Please call us if this situation occurs.
 - When a test is unusually sensitive (PMSD <10th percentile), the 10th percentile must be used as a substitute PMSD: “...a test concentration shall not be considered toxic (i.e., significantly different from the control) if the relative difference from the control is less than the lower PMSD bounds in Table 6.”

We welcome the opportunity to discuss the above requirements, or any other WET-related issues, with all interested parties. If you need further information, please contact me at alexanderc2@michigan.gov.

Sincerely,

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