



JOHN ENGLER, Governor

DEPARTMENT OF ENVIRONMENTAL QUALITY

"Better Service for a Better Environment"

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REPLY TO:

SURFACE WATER QUALITY DIVISION
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February 5, 2002

To All Interested Parties:

State of Michigan Requirements for Whole Effluent Toxicity Test Methods

The State of Michigan requires the use of the following methods for all whole-effluent toxicity (WET) tests, except when an approved biomonitoring 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 (*Ceriodaphnia dubia*, fathead minnows)
ASTM 1193-97 (*Daphnia magna*), as modified by "SWQD Modifications to ASTM E1193-97" (attached)
- acute toxicity tests: EPA/600/4-90/027F

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

1. Laboratories shall validate to 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 (three for ASTM 1193-97) is sufficient for validation. Please also include raw data from at least one set of tests.
2. Biomass (sum of survivor weights/initial # individuals) shall be used as the fathead minnow chronic growth endpoint, except in the rare case when an existing test condition or approved plan specifies the previous chronic method (EPA/600/4-89/001). The 600/4-89/001 method required mean weight (sum of survivor weights/# survivors) to be used as the growth endpoint.
3. Synthetic fresh water as described in EPA/600/4-91/002 shall be used as diluent for *Ceriodaphnia dubia* WET tests, unless prior approval to use an alternative dilution water has been obtained from 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.

4. For compliance tests, chlorinated samples shall be dechlorinated before testing (≤ 20 ug/l total residual chlorine). Detected levels of chlorine shall be reported. If effluent samples are dechlorinated, dechlorination controls should be included in the test.
5. 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.
6. For effluent compliance tests, test solution pH shall be maintained within the range of pH 6.5 - 9.0 unless the NPDES permit allows a different pH range for the effluent, or prior approval is obtained to conduct tests at alternative pH levels.
7. Sufficient ice shall be used to maintain low sample temperatures during holding and shipment ($\leq 4^{\circ}\text{C}$). Increased temperature can affect sample toxicity and may invalidate test results. Please avoid freezing samples. Sample receiving temperature shall be reported for all samples.
8. Chronic tests shall include a 100% effluent concentration to allow acute toxicity assessment.
9. 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 $100/\text{RWC}$. Tests which do not assess toxicity at or below RWC are of very limited value. Test concentration series shall assess toxicity of a test concentration equivalent to, or lower than, the effluent RWC.
10. Avoid effluent sample filtration, because filtration can change the toxicity of the 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% effluent treatment shall also be included in the test.
11. *C. dubia* brood number shall be tracked daily. Aborted or resorbed eggs shall be noted and reported.
12. 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.
13. *C. dubia* chronic tests are ended when $\geq 60\%$ of diluent control animals have produced their third brood. This usually occurs on days 6, 7, or 8.
14. *Daphnia magna* chronic toxicity tests shall be performed according to the ASTM Standard Guide ASTM 1193-97 "Standard Guide for Performing *Daphnia magna* Life-Cycle Toxicity Tests", including the following specific methods:

- At least 10 *D. magna* shall be used per test treatment.
 - Each test chamber shall contain only one *D. magna*.
 - A minimum of three test water samples shall be collected per each test week (minimum of 9 samples).
 - Test solutions shall be renewed daily.
15. In addition to the QA/QC measures described in ASTM Standard E1193-97 "Standard Guide for Conducting *Daphnia magna* Life-Cycle Toxicity Tests, the following sections of EPA/600/4-91/002 are required for the *D. magna* chronic toxicity test method: Sections 6.5, 7, 8, 9, and 10.
 16. Measurements of water quality parameters for the *D. magna* chronic toxicity test shall be made as described in EPA/600/4-91/002, Section 13.10.1. This includes water quality parameter analyses on all new samples. This also includes daily measurements of pH, dissolved oxygen, and temperature on new and old test solutions.
 17. We also require that the statistical analyses described for *Ceriodaphnia dubia* in EPA/600/4-91/002 be used for the *D. magna* chronic test method.
 18. Demonstrations of ongoing laboratory performance for the *D. magna* chronic test method may be made using quarterly (or more frequent) reference toxicant tests, or by conducting reference toxicant tests simultaneous to any regulatory tests.
 19. The chronic value, or MATC, shall be reported for all chronic toxicity tests. The MATC is defined as the geometric mean of the NOEC and LOEC. ICp endpoints may be reported in addition to MATC/ChV values.
 20. TUa values (based on mortality) shall be reported for acute toxicity tests.
 21. Both TUc (based on ChV/MATC) and TUa (mortality) values shall be reported for all chronic toxicity tests.
 22. **If TUa is < 1.0 (LC50 > 100%),** please derive and report a numerical value for TUa based on % mortality in the 100% effluent concentration of the toxicity test as follows:
 - 0 TUa** if mortality is 0-10%
 - TUa = 0.02 x % mortality** if mortality is 10-49%

Example: Mortality in 100% effluent = 25%
 TUa= 0.02 x 25 = 0.5 TUa
 Permittee reports 0.5 TUa on DMR.

Report **0 TUc** if no chronic toxicity is detected (MATC/ChV > 100% effluent).
 23. The results of all effluent toxicity tests shall 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. Please call us before submitting questionable data.

24. An unexplainable dose-response often invalidates toxicity test data. Adverse effects should generally increase with increasing effluent concentration. Consult USEPA guidance¹ for dose-response interpretation. Please call us if dose-response appears unexplainable.
25. 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.
26. The duration of fish and daphnid acute toxicity shall be 96 hours and 48 hours, respectively.
27. USEPA guidance¹ recommends reducing statistical significance rate (alpha) for certain chronic WET data analyses. The lower alpha (0.01) increases the amount of adverse effect needed to indicate chronic toxicity relative to the 0.05 alpha recommended by the promulgated EPA test methods.

The Michigan Department of Environmental Quality has approved the option to use 0.01 alpha. Alpha remains 0.05 for data which do not meet the criteria.

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

We welcome the opportunity to discuss the above requirements, or any other WET-related issues, with all interested parties. Please feel free to e-mail, fax, or call me.

Sincerely,

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WD Modifications to ASTM E1193-97

Test method preferences, additions, and changes shall be applied to ASTM E1193-97 "Standard Guide for Conducting *Daphnia magna* Life-Cycle Toxicity Tests" as follows:

Preferences

At least 10 *D. magna* shall be used per test treatment.

Each test chamber shall contain only one *D. magna*.

A minimum of three test water samples shall be collected per test week (minimum of 9 samples/test).

Additions

1. SOPs for *D. magna* culture and chronic testing methods shall be developed and maintained.
2. The time between toxicity test sample collection and first use shall not exceed 36 hours without Department approval.
3. The initial demonstration of acceptable performance of the test method shall be made with at least three acceptable chronic *D. magna* reference toxicant tests.
4. Demonstrations of ongoing laboratory performance for the test method shall be made using quarterly (or more frequent) reference toxicant tests, or by conducting reference toxicant tests simultaneous to all regulatory tests.
5. All provisions of the following EPA/600/4-91/002 Sections shall be followed: Sections 8.1, 8.2, 8.3 (except that three samples shall be collected **for each week of testing**), 8.5, 8.6, 8.7, 8.8 (applied to *D. magna* as if it were *C. dubia*), 8.10, and Section 10.
6. For compliance tests, chlorinated samples shall be dechlorinated before testing (≤ 20 ug/l total residual chlorine). Detected levels of chlorine shall be reported. If effluent samples are dechlorinated, dechlorination controls shall be included in the test unless the Department approves of alternative methods.
7. The Orion^R probe method or amperometric titration shall be used for total residual chlorine determination.
8. Chronic tests shall include a 100% effluent concentration to allow acute toxicity assessment.
9. The chronic value, or MATC, shall be reported for all tests.
10. Both TUc (based on ChV/MATC) and TUa (based on LC₅₀) values shall be reported for tests.
11. If TUa is < 1.0 (LC₅₀ $> 100\%$), the numerical TUa value shall be derived based on % mortality in the 100% effluent concentration of the toxicity test as follows:
 - TUa=0 if mortality is 0-10%
 - TUa = $0.02 \times \%$ mortality if mortality is 10-49%
Example: Mortality in 100% effluent = 25%
TUa= $0.02 \times 25 = 0.5$ TUa
 - TUc=0 if no chronic toxicity is detected (MATC/ChV $> 100\%$ effluent).

1. The results of all effluent toxicity tests shall be reported. Tests that fail to meet test acceptability criteria shall be repeated unless the Department approves of an alternative.

Changes

1. Hypothesis testing statistical analyses as described in EPA/600/4-91/002, Section 9, for the *C. dubia* test shall be used for data analyses.
2. Toxicity test solutions shall be renewed daily.
3. Measurements of water quality parameters shall be made as described in EPA/600/4-91/002, Section 13.10.1. This includes water quality parameter analyses on all new samples. This also includes daily measurements of pH, dissolved oxygen, and temperature on new and old test solutions as described in the EPA method.

Updated: 3/4/99