

**APPENDIX B of PART II****WHOLE EFFLUENT TOXICITY LIMITS (7-DAY CHRONIC NOEC FRESHWATER)**

*It is unlawful and a violation of this permit for a permittee or his designated agent, to manipulate test samples in any manner, to delay sample shipment, or to terminate or to cause to terminate a toxicity test. Once initiated, all toxicity tests must be completed unless specific authority has been granted by EPA Region 6 or the State NPDES permitting authority.*

**1. SCOPE AND METHODOLOGY**

- a. The permittee shall test the effluent for toxicity in accordance with the provisions in this section.

APPLICABLE TO FINAL OUTFALL(S): 001

REPORTED ON DMR AS FINAL OUTFALL: 001

CRITICAL DILUTION (%): 61%

EFFLUENT DILUTION SERIES (%): 26%, 34%, 46%, 61%, and 81%

COMPOSITE SAMPLE TYPE: Defined at PART I

TEST SPECIES/METHODS: 40 CFR Part 136

Ceriodaphnia dubia chronic static renewal survival and reproduction test, Method 1002.0, EPA-821-R-02-013, or the most recent update thereof. This test should be terminated when 60% of the surviving females in the control produce three broods or at the end of eight days, whichever comes first.

Pimephales promelas (Fathead minnow) chronic static renewal 7-day larval survival and growth test, Method 1000.0, EPA-821-R-02-013, or the most recent update thereof. A minimum of five (5) replicates with eight (8) organisms per replicate must be used in the control and in each effluent dilution of this test.

- b. The NOEC (No Observed Effect Concentration) is herein defined as the greatest effluent dilution at and below which toxicity that is statistically different from the control (0% effluent) at the 95% confidence level does not occur. Chronic lethal test failure is defined as a demonstration of a statistically significant lethal effect at test completion to a test species at or below the critical dilution. Chronic sub-lethal test failure is defined as a demonstration of a statistically significant sub-lethal effect (i.e., growth or reproduction) at test completion to a test species at or below the critical dilution.
- c. The conditions of this item are effective beginning with the effective date of the WET limit. When the testing frequency stated above is less than monthly and the effluent fails the lethal or sub-lethal endpoint at or below the critical dilution, the permittee shall be considered in violation of this permit limit and the frequency for the affected species will increase to monthly until such time compliance with the No Observed Effect Concentration (NOEC) effluent limitation is

demonstrated for a period of three consecutive months, at which time the permittee may return to the testing frequency stated in PART I of this permit. During the period the permittee is out of compliance, test results shall be reported on the DMR for that reporting period. The purpose of additional tests (also referred to as 'retests' or confirmation tests) is to determine the duration of a toxic event. A test that meets all test acceptability criteria and demonstrates significant toxic effects does not need additional confirmation. Such testing cannot confirm or disprove a previous test result.

- d. This permit may be reopened to require chemical specific effluent limits, additional testing, and/or other appropriate actions to address toxicity.

## 2. REQUIRED TOXICITY TESTING CONDITIONS

### a. Test Acceptance

The permittee shall repeat a test, including the control and all effluent dilutions, if the procedures and quality assurance requirements defined in the test methods or in this permit are not satisfied, including the following additional criteria:

- i. The toxicity test control (0% effluent) must have survival equal to or greater than 80%.
- ii. The mean number of Ceriodaphnia dubia neonates produced per surviving female in the control (0% effluent) must be 15 or more.
- iii. 60% of the surviving control females must produce three broods.
- iv. The mean dry weight of surviving Fathead minnow larvae at the end of the 7 days in the control (0% effluent) must be 0.25 mg per larva or greater.
- v. The percent coefficient of variation between replicates shall be 40% or less in the control (0% effluent) for: the young of surviving females in the Ceriodaphnia dubia reproduction test, the growth and survival of the Fathead minnow test.
- vi. The percent coefficient of variation between replicates shall be 40% or less in the critical dilution, unless significant lethal or nonlethal effects are exhibited for: the young of surviving females in the Ceriodaphnia dubia reproduction test; the growth and survival endpoints in the Fathead minnow test.
- vii. A Percent Minimum Significant Difference (PMSD) range of 13 - 47 for Ceriodaphnia dubia reproduction;
- viii. A PMSD range of 12 - 30 for Fathead minnow growth.

Test failure may not be construed or reported as invalid due to a coefficient of variation value of greater than 40%. A repeat test shall be conducted within the required reporting period of any test determined to be invalid.

b. Statistical Interpretation

- i. For the Ceriodaphnia dubia survival test, the statistical analyses used to determine if there is a significant difference between the control and the critical dilution shall be Fisher's Exact Test as described in EPA-821-R-02-013 or the most recent update thereof.
- ii. For the Ceriodaphnia dubia reproduction test and the Fathead minnow larval survival and growth test, the statistical analyses used to determine if there is a significant difference between the control and the critical dilution shall be in accordance with the methods for determining the No Observed Effect Concentration (NOEC) as described in EPA-821-R-02-013, or the most recent update thereof.
- iii. If the conditions of Test Acceptability are met in Item 2.a above and the percent survival of the test organism is equal to or greater than 80% in the critical dilution concentration and all lower dilution concentrations, the test shall be considered to be a passing test, and the permittee shall report a survival NOEC of not less than the critical dilution for the DMR reporting requirements found in Item 3 below.

c. Dilution Water

- i. Dilution water used in the toxicity tests will be receiving water collected as close to the point of discharge as possible but unaffected by the discharge. The permittee shall substitute synthetic dilution water of similar pH, hardness, and alkalinity to the closest downstream perennial water where the receiving stream is classified as intermittent or where the receiving stream has no flow due to zero flow conditions.
- ii. If the receiving water is unsatisfactory as a result of instream toxicity (fails to fulfill the test acceptance criteria of Item 2.a), the permittee may substitute synthetic dilution water for the receiving water in all subsequent tests provided the unacceptable receiving water test met the following stipulations:
  - (A) a synthetic dilution water control which fulfills the test acceptance requirements of Item 2.a was run concurrently with the receiving water control;
  - (B) the test indicating receiving water toxicity has been carried out to completion (i.e., 7 days);

- (C) the permittee includes all test results indicating receiving water toxicity with the full report and information required by Item 3.a below; and
- (D) the synthetic dilution water shall have a pH, hardness, and alkalinity similar to that of the receiving water or closest downstream perennial water not adversely affected by the discharge, provided the magnitude of these parameters will not cause toxicity in the synthetic dilution water.

d. Samples and Composites

- i. The permittee shall collect a minimum of three flow-weighted composite samples from the outfall(s) listed at Item 1.a above.
- ii. The permittee shall collect second and third composite samples for use during 24-hour renewals of each dilution concentration for each test. The permittee must collect the composite samples such that the effluent samples are representative of any periodic episode of chlorination, biocide usage or other potentially toxic substance discharged on an intermittent basis.
- iii. The permittee must collect the composite samples so that the maximum holding time for any effluent sample shall not exceed 72 hours. The permittee must have initiated the toxicity test within 36 hours after the collection of the last portion of the first composite sample. Samples shall be chilled to 6 degrees Centigrade during collection, shipping, and/or storage.
- iv. If the flow from the outfall(s) being tested ceases during the collection of effluent samples, the requirements for the minimum number of effluent samples, the minimum number of effluent portions and the sample holding time are waived during that sampling period. However, the permittee must collect an effluent composite sample volume during the period of discharge that is sufficient to complete the required toxicity tests with daily renewal of effluent. When possible, the effluent samples used for the toxicity tests shall be collected on separate days if the discharge occurs over multiple days. The effluent composite sample collection duration and the static renewal protocol associated with the abbreviated sample collection must be documented in the full report required in Item 3 of this section.
- v. MULTIPLE OUTFALLS: If the provisions of this section are applicable to multiple outfalls, the permittee shall combine the composite effluent samples in proportion to the average flow from the outfalls listed in Item 1.a above for the day the sample was collected. The permittee shall perform the toxicity test on the flow-weighted composite of the outfall samples.

3. REPORTING

- a. The permittee shall prepare a full report of the results of all tests conducted pursuant to this section in accordance with the Report Preparation Section of EPA-821-R-02-013, or the most current publication, for every valid or invalid toxicity test initiated whether carried to completion or not. The permittee shall retain each full report pursuant to the provisions of PART III.C.3 of this permit. The permittee shall submit full reports upon the specific request of the Agency. For any test which fails, is considered invalid or which is terminated early for any reason, the full report must be submitted for agency review.
- b. The permittee shall report the Whole Effluent Toxicity values for the 30-Day Average Minimum and the 7-Day Minimum under Parameter No. 22414 on the DMR for that reporting period in accordance with PART III.D.4 of this permit.

If more than one valid test for a species was performed during the reporting period, the test NOECs will be averaged arithmetically and reported as the DAILY AVERAGE MINIMUM NOEC for that reporting period.

If more than one species is tested during the reporting period, the permittee shall report the lowest 30-Day Average Minimum NOEC and the lowest 7-Day Minimum NOEC for Whole Effluent Toxicity.

A valid test for each species must be reported on the DMR during each reporting period specified in PART I of this permit. Only ONE set of biomonitoring data for each species is to be recorded on the DMR for each reporting period. The data submitted should reflect the LOWEST lethal and sub-lethal effects results for each species during the reporting period. All invalid tests, repeat tests (for invalid tests), and retests (for tests previously failed) performed during the reporting period must be attached to the DMR for EPA review.

- c. The permittee shall submit the results of the valid toxicity test on the DMR for that reporting period in accordance with PART III.D.4 of this permit, as follows below. Submit retest information clearly marked as such with the following month's DMR. Only results of valid tests are to be reported on the DMR.

i. Pimephales promelas (Fathead Minnow)

- A. If the No Observed Effect Concentration (NOEC) for lethal effects is less than the critical dilution, enter a "1"; otherwise, enter a "0" for Parameter No. TLP6C
- B. Report the NOEC value for survival, Parameter No. TOP6C
- C. Report the Lowest Observed Effect Concentration (LOEC) value for survival, Parameter No. TXP6C
- D. Report the NOEC value for growth, Parameter No. TPP6C
- E. Report the LOEC value for growth, Parameter No. TYP6C

- F. If the No Observed Effect Concentration (NOEC) for growth is less than the critical dilution, enter a "1"; otherwise, enter a "0" for Parameter No. TGP6C
- G. Report the highest (critical dilution or control) Coefficient of Variation, Parameter No. TQP6C

ii. Ceriodaphnia dubia

- A. If the NOEC for lethal effects is less than the critical dilution, enter a "1"; otherwise, enter a "0" for Parameter No. TLP3B
- B. Report the NOEC value for survival, Parameter No. TOP3B
- C. Report the LOEC value for survival, Parameter No. TXP3B
- D. Report the NOEC value for reproduction, Parameter No. TPP3B
- E. Report the LOEC value for reproduction, Parameter No. TYP3B
- F. If the No Observed Effect Concentration (NOEC) for reproduction is less than the critical dilution, enter a "1"; otherwise, enter a "0" for Parameter No. TGP3B
- G. Report the higher (critical dilution or control) Coefficient of Variation, Parameter No. TQP3B

4. MONITORING FREQUENCY REDUCTION

This section does not apply to any species for which the permit establishes whole effluent toxicity (WET) limits. For the first five years after the effective date of a WET limit, the minimum monitoring frequency for the affected species is once per quarter.

- a. The permittee may apply for a testing frequency reduction upon the successful completion of the first four consecutive quarters of testing for a test species, with no lethal or sub-lethal effects demonstrated at or below the critical dilution. If granted, the monitoring frequency for that test species may be reduced to not less than once per year for the less sensitive species (usually the Fathead minnow) and not less than twice per year for the more sensitive test species (usually the *Ceriodaphnia dubia*).
- b. CERTIFICATION - The permittee must certify in writing that no test failures have occurred and that all tests meet all test acceptability criteria in item 3.a. above. In addition the permittee must provide a list with each test performed including test initiation date, species, NOECs for lethal and sub-lethal effects and the maximum coefficient of variation for the controls. Upon review and acceptance of this information the agency will issue a letter of confirmation of the monitoring frequency reduction. A copy of the letter will be forwarded to the

agency's Permit Compliance System section to update the permit reporting requirements.

- c. SUB-LETHAL OR SURVIVAL FAILURES - If any test fails the survival or sub-lethal endpoint at any time during the life of this permit, three monthly retests are required and the monitoring frequency for the affected test species shall be increased to once per quarter until the permit is re-issued. Monthly retesting is not required if the permittee is performing a TRE.
- d. This monitoring frequency reduction applies only until the expiration date of this permit, at which time the monitoring frequency for both test species reverts to once per quarter until the permit is re-issued.

Any monitoring frequency reduction granted applies only until the expiration date of this permit, at which time the monitoring frequency for both test species reverts to once per quarter until the permit is re-issued.