Effluent Biotoxicity Testing Protocol for Industrial and Municipal Effluents

NOTICE

The Department's biomonitoring program continues to evolve. As such, this document will be periodically updated to reflect changes in toxicity testing methodologies, toxicity reduction evaluation protocols, and other issues related to the control of toxic discharges.

I. PROGRAM DESCRIPTION

Since 1980, the Maryland Department of the Environment (MDE) has utilized whole effluent toxicity testing to assess acute and chronic toxicity in discharges to Maryland surface waters. In 1987 the emphasis greatly increased with the addition of the State Biomonitoring Laboratory. The current effort relies on toxicity testing of effluents performed by the permittee, MDE, and the United States Environmental Protection Agency (see sections A, B and C below). In addition to these routine toxicity testing efforts, MDE may request dischargers to perform toxicity testing outside of the permit process. All tests consist of separate experiments using both a vertebrate (fish) and an invertebrate (crustacean) as the test species.

A finding of no toxicity in the effluent of a facility does not relieve the permittee from the obligation to provide best available treatment technology or to comply with water quality standards. In all cases, MDE reserves the authority to require additional biotoxicity testing and a toxicity reduction evaluation (TRE). This authority to require biomonitoring appears in COMAR 26.08.03.07 entitled "Control of the Discharge of Toxic Substances to Surfaces Waters". Specific provisions are found in sections A and D.

A. Permit Required Toxicity Testing

Biotoxicity testing is required in new or renewed discharge permits for all major and selected non-major dischargers. Maryland regulation (COMAR 26.08.03.07D(1)) specifically requires the following:

D. Applicability to Dischargers.

(1) Dischargers Required to Conduct Monitoring for Toxic Substances. The Department shall require any permittee who has a discharge that falls into one of the following categories to perform biological or chemical monitoring for toxic substances:

(a) A POTW with a pretreatment program established in accordance with COMAR 26.08.08;

(b) An industrial discharger or POTW treatment plant with a wastewater flow greater than or equal to 1,000,000 gallons per day;

(c) A discharger whose discharge has demonstrated actual or potential toxicity; or

(d) A discharger whose discharge the Department has reason to believe may cause toxicity as determined by an evaluation of manufacturing processes, indirect discharges, treatment processes, effluent or receiving water data, or other relevant information.

NPDES biotoxicity testing requirements for major facilities generally consist of four quarterly tests to be conducted during the first year of the permit (Appendices A & B). Where the discharge flow is less than 10% of the receiving water flow, the permit requirements usually consist of three acute tests and one

chronic test. Where the effluent flow is greater than 10% of the receiving water flow, chronic testing is emphasized. In estuarine waters where the discharge flow exceeds 10% of the receiving water flow, the permittee is required to use estuarine test organisms.

NPDES permit requirements for dischargers of lower concern where there is reason to believe a potential for toxicity exists generally consist of two quarterly tests to be conducted during the first year of the permit (Appendices C & D).

Additional effluent toxicity testing beyond that specifically described in the permit may be required by MDE of dischargers upon findings of toxicity or upon the performance of testing inconsistent with the permittee's approved biomonitoring study plan for that facility. A permittee will be required to repeat the permit required toxicity testing when initial findings of acute toxicity are not confirmed (COMAR 26.08.03.07E(4)f). The reporting of permittee test results must be consistent with MDE's document entitled "Reporting Requirements for Effluent Biomonitoring Data" (Appendix E). A toxicity reduction evaluation (TRE) is required when a review of the data indicates unacceptable toxicity.

The test organisms utilized in permittee toxicity testing are those recognized in federal guidance or local species approved by the Department (Appendix F).

B. MDE Toxicity Testing

Since June 1987, MDE has been performing effluent toxicity testing at a State contracted laboratory. Effluent samples are collected by MDE inspectors and transported to the laboratory for testing. Approximately 12 acute and 4 chronic tests are performed each quarter. The protocols followed by the State laboratory (Fisher et al 1993, Fisher et al 1988) are identified in section V and are consistent with federal biomonitoring guidance. Test organisms are listed in Appendix G.

C. EPA Toxicity Testing

EPA Region III performs chronic toxicity tests on effluent samples from selected Maryland POTWs. Effluent samples are collected by EPA personnel and transported back to the EPA biomonitoring laboratory in Wheeling, West Virginia. Facilities to be tested by EPA are selected by MDE on the basis of the in-stream waste concentration (IWC) of the discharge, permit renewal date and the frequency of previous biotoxicity testing. POTWs with IWCs greater than 10% and relatively little previous biotoxicity testing are given high priority for EPA testing.

EPA's chronic testing of POTWs employs Ceriodaphnia dubia and larval fathead minnows (Pimephales promelas), regardless of the receiving water types. Methods employed for chronic toxicity testing by EPA are consistent with EPA guidance.

II. INTERPRETATION OF BIOTOXICITY MONITORING RESULTS

Acute toxicity is broadly defined as the ability of a substance to cause deleterious effects to living organisms during a short term exposure. In practice, acute toxicity testing of effluents involves the measurement of lethality or immobilization of aquatic organisms exposed to several effluent dilutions for time periods usually lasting up to 48 hours. The results of an acute toxicity test are expressed as an LC50 (effluent concentration at which 50% of the test organisms die during the test) or EC50 (effluent concentration at which 50% of the organisms are killed or disabled during the test). In order to calculate an LC50 (or EC50), at least one of the test concentrations must cause more than 50% mortality (or immobilization) and at least one of the test concentrations must cause less than 50% mortality (or immobilization). The lower the LC50 or EC50, the more toxic the effluent. For example, an LC50 (or EC50) of greater than 100% means that full strength effluent (100%) did not kill (or immobilize) at least half the test organisms. An LC50 (or EC50) of 50% means that half strength effluent (50%) killed (or immobilized) 50% of the test organisms.

Chronic toxicity testing is broadly defined as the ability of a substance to cause deleterious effects to living organisms during a long term exposure. In practice, chronic toxicity testing of effluents usually involves the measurement of survival, growth, reproduction, and hatchability of aquatic organisms exposed to several effluent dilutions for time periods lasting up to 7 days. Generally, the "sub-lethal" endpoints of growth, reproduction, and hatchability are more sensitive indicators of chronic toxicity than survival. Because chronic toxicity tests involve the measurement of more sensitive endpoints over longer exposure periods compared to acute tests, chronic tests are considered to be more sensitive for measuring effluent toxicity.

The results of chronic toxicity testing are generally expressed as the NOEC (highest concentration at which no observable effect occurred), LOEC (the lowest concentration at which an observable effect occurred), Chronic Value (the geometric mean of the NOEC and LOEC) and the IC25 (effluent concentration which causes a 25% reduction in growth or reproduction and survival). In addition to these measures of chronic toxicity, acute toxicity data, in the form of LC50s or EC50s, can be gathered during the first 48 hours of chronic toxicity testing.

A. Acute Toxicity of Effluents

For purposes of determining the acute toxicity of effluents, the following criteria apply.

1. An effluent is considered to be acutely toxic when its 48-hour LC50 or EC50 (as determined from acute or chronic toxicity testing) is 100% or less.

2. An effluent is generally considered not acutely toxic when its 48-hour LC50 or EC50 (as determined from acute or chronic toxicity testing) is greater than 100%.

Upon consistent findings of acute toxicity, a permittee shall be required to conduct a TRE (see section III).

B. Chronic Toxicity of Effluents

For purposes of determining the chronic toxicity of effluents, the following criteria apply.

1. An effluent is considered to be chronically toxic when its IC25 is less than or equal to the in-stream waste concentration.IWC = QD/(QD+QRW)x100 where QRW = 30Q5

2. An effluent is generally considered not chronically toxic when its IC25 is greater than the in-stream waste concentration.

Upon consistent findings of chronic toxicity, a permittee may be required to perform a TRE (see section III).

III. Toxicity Reduction Evaluation (TRE)

When effluent toxicity is confirmed, the discharger is required to perform a TRE. A TRE is an investigation conducted to identify the cause(s) of effluent toxicity or isolate the source(s) and determine the effectiveness of control options, implement the necessary control measures and then confirm the reduction in toxicity (see appendix H). TREs range widely in complexity. They may be as simple as the dechlorination of municipally supplied noncontact cooling water in response to measurements of toxic levels of chlorine. Alternatively, they may involve the performance of an in-depth investigation to determine the source or type of toxicity, evaluate control measures, and implement those selected. Guidance documents covering the various tiers, phases, and other aspects of a TRE are under continuous development by the EPA and its contractors (see section IV).

IV. Permit Limitations

In situations where a discharger is not making satisfactory progress toward the elimination of toxicity, MDE may consider including a specific limitation for effluent toxicity in that permit.

Situations in which a Permit Limitation will be Considered.

When issuing a NPDES permit renewal, MDE will consider the imposition of a permit limitation for effluent toxicity when:

1. A permittee has spent three years performing a TRE, with little or no progress toward identifying or isolating the source of toxicity, or

2. Unacceptable toxicity is confirmed in the effluent of a permittee, who has not made any changes to the facility's processes since satisfactorily completing a TRE and the required confirmatory testing.

V. Relevant Guidance Documents

Fisher, D.J., B.S. Turley, L.T. Yonkos, and G.P. Ziegler 1993. Standard operating procedures for measuring the acute toxicity of effluents and receiving waters to freshwater and saltwater organisms. 1993. The University of Maryland System, Wye Research and Education Center, Queenstown, MD. 79 p.

Fisher, D.J., D.T. Burton, L.W. Hall Jr., R.L. Paulson and C.M. Hersh. 1988. Standard operating procedures for short-term chronic effluent toxicity tests with freshwater and saltwater organisms. 1988. Johns Hopkins University, Applied Physics Laboratory, Aquatic Ecology Section, Shadyside, MD. 94 p.

Maryland Department of the Environment, Water Management Administration. "Reporting Requirements for Effluent Biomonitoring Data".

Weber, C.I. [ed.] 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms (Fourth edition). EPA/600/4-90/027F. USEPA, Office of Research and Development, Washington, DC. 293 p.

Lewis, P.A., W.H. Peltier, T.J. Norberg-King, D.J. Klemm, J.M. Lazorchak, M.A. Heber [eds.] 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms (Third edition). EPA/600/4-91/002. USEPA, Environmental Monitoring Systems Laboratory, Office of Research and Development, Cincinnati, OH. 341 p.

Klemm, D.J., G.E. Morrison, T.J. Norberg-King, W.H. Peltier, M.A. Heber [eds.] 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms (Second edition). EPA/600/4-91/003. USEPA, Environmental Monitoring Systems Laboratory, Office of Research and Development, Cincinnati, OH. 483 p.

Fava, J.A., D. Lindsay, W.H. Clement, R. Clark, G.M. DeGraeve, J.D. Cooney, S. Hansen,

W. Rue, S. Moore, P. Lankford, and K. Dostal 1989. Generalized Methodology for Conducting Industrial Toxicity Reduction Evaluations (TREs). EPA/600/2-88/070. USEPA, Chemicals and Chemical Product Branch, Risk Reduction Engineering Laboratory, Cincinnati, OH.

Botts, J.A., J.W. Braswell, J. Zyman, W.L. Goodfellow, S.B. Moore, D.F. Bishop 1989.

Toxicity Reduction Evaluation Protocol for Municipal Wastewater Treatment Plants. EPA/600/2-88/062. USEPA, Risk Reduction Engineering Laboratory, Office of Research and Development, Cincinnati, OH.

Mount, D.I., L. Anderson-Carnahan 1988. Methods for Aquatic Toxicity Identification

Evaluations - Phase I Toxicity Characterization Procedures. EPA-600/3-88/034. USEPA, National Effluent Toxicity Assessment Center, Duluth, MN.

Durhan, E.J., T.J. Norberg-King, and L.P. Burkhard 1993. Methods for Aquatic Toxicity

Evaluations - Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity. EPA/600/R-92/080. USEPA, National Effluent Toxicity Assessment Center, Duluth, MN.

Mount, D.I., and T.J. Norberg-King 1993. Methods for Aquatic Toxicity Identification

Evaluations - Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity. EPA-600/R-92/081. USEPA, National Effluent Toxicity Assessment Center, Duluth, MN.

Norberg-King, T.J., D.I. Mount, J.R. Amato, D.A. Jensen, J.A. Thompson 1991. Toxicity

Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I. EPA/600/6-91/005. USEPA, Office of Research and Development, Duluth, MN.

Appendix A

BIOMONITORING PROGRAM (Significant concern and effluent flow is greater than 10% of the receiving water low flow)

1. Within three months of the effective date of the permit, the permittee shall submit to the Department for approval a study plan to evaluate wastewater toxicity at Outfall by using biomonitoring. The study plan should include a discussion of:

a. wastewater and production variability

b. sampling & sample handling

c. source & age of test organisms

d. source of dilution water

e. testing procedures/experimental design

f. data analysis

g. quality assurance/quality control

h. report preparation

i. testing schedule

2. The testing program shall consist of definitive quarterly chronic testing for one year. This testing shall be initiated no later than three months following the Department's acceptance of the study plan.

a. Each quarterly testing shall include the Ceriodaphnia survival and reproduction test and the fathead minnow larval survival and growth test.

b. If the receiving water is estuarine the permittee shall substitute estuarine species for those species specified above. Approved estuarine species for chronic testing are sheepshead minnow, inland

silversides, and mysid shrimp. In all cases, testing must include one vertebrate species and one invertebrate species.

3. The samples used for biomonitoring shall be collected at the same time and location as the samples analyzed for the effluent limitations and monitoring requirements for this outfall. For chlorinated effluents, samples shall be collected after dechlorination.

4. The following EPA documents discuss the appropriate methods:

a. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, July, 1994, EPA/600/4-91/003.

b. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, July 1994, EPA/600/4-91/002.

5. Test results shall be submitted to the Department within one month of completion of each set of tests.

6. Test results shall be reported in accordance with MDE/WMA "Reporting Requirements for Effluent Biomonitoring Data."

7. As a minimum, the reported chronic results shall be expressed as NOEC, LOEC, ChV, and IC25.

8. If significant mortality occurs during the first 48 hours of the chronic tests, 48-hour LC50s shall be calculated and reported along with the chronic results.

9. If testing is not performed in accordance with MDE-approved study plan, additional testing may be required by the Department.

10. If the test results indicate that the effluent is toxic, additional biomonitoring or a toxicity reduction evaluation will be required by the Department.

11. If plant processes or operations change so that there is a significant change in the nature of the wastewater, the Department may require the permittee to conduct a new set of tests.

12.* If a significant industrial user locates within the service area so that significant change in the nature of the wastewater might be anticipated, MDE may require the the permittee to conduct a new set of tests.

13. Submit all Biomonitoring related materials to:

Maryland Department of the Environment
Technical and Regulatory Services Administration
Environmental Risk Assessment Program
1800 Washington Blvd.
Baltimore, Maryland 21230

*omit for industrial facilities
Appendix B

BIOMONITORING PROGRAM (Significant concern and effluent flow is less than 10% of the receiving water low flow)

1. Within three months of the effective date of the permit, the permittee shall submit to the Department for approval a study plan to evaluate wastewater toxicity at Outfall by using biomonitoring. The study plan should include a discussion of:
a. wastewater and production variability

b. sampling & sample handling
c. source & age of test organisms
d. source of dilution water
e. testing procedures/experimental design
f. data analysis
g. quality control/quality assurance
h. report preparation
i. testing schedule

2. The testing program shall consist of definitive quarterly testing for one year. Three of the quarters shall have acute testing and one of the quarters shall have chronic testing. This testing shall be initiated no later than three months following the Department's acceptance of the study plan.
a. The acute testing shall consist of 48-hour static renewal tests using fathead minnow and the 48-hour static renewal tests using a daphnid.
b. The chronic testing shall include the Ceriodaphnia survival and reproduction test and the fathead minnow larval survival and growth test.
c. If the receiving water is estuarine, the permittee may elect to substitute estuarine species for those species specified above. Approved estuarine species for acute testing are sheepshead minnows, silversides, grass shrimp, and mysid shrimp. Approved estuarine species for chronic testing are sheepshead minnow, inland silverside, and mysid shrimp. In all cases, testing must include one vertebrate species and one invertebrate species.
d. Acute test results shall be expressed as LC50. Chronic test results shall be expressed as NOEC, LOEC, ChV, and IC25.

3. The samples used for biomonitoring shall be collected at the same time and location as the samples analyzed for the effluent limitations and monitoring requirements for this outfall. For chlorinated effluents, samples shall be collected after dechlorination.

4. The following EPA documents discuss the appropriate methods:
a. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, August, 1993, EPA/600/4-90/027F.
b. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, July 1994, EPA/600/4-91/003.
c. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, July, 1994, EPA/600/4-91/003.

5. Test results shall be submitted to the Department within one month of completion of each set of tests.

6. Test results shall be reported in accordance with MDE/WMA "Reporting Requirements for Effluent Biomonitoring Data".

7. As a minimum, the reported chronic results shall be expressed as NOEC, LOEC, ChV, and IC25.

8. If significant mortality occurs during the first 48 hours of the chronic tests, 48-hour LC50s shall be calculated and reported along with the chronic results.

9. If testing is not performed in accordance with MDE-approved study plan, additional testing may be required by the Department.

10. If the test results indicate that the effluent is toxic, additional biomonitoring or a toxicity reduction evaluation will be required by the Department.

11. If plant processes or operations change so that there is a significant change in the nature of the wastewater, the Department may require the permittee to conduct a new set of tests.

12.* If a significant industrial user locates within the service area so that significant change in the nature of the wastewater might be anticipated, MDE may require the the permittee to conduct a new set of tests.

13. Submit all Biomonitoring related materials to:

Maryland Department of the Environment
Technical and Regulatory Services Administration
Environmental Risk Assessment Program
1800 Washington Blvd.
Baltimore, Maryland 21230

*omit for industrial facilities

Appendix C

BIOMONITORING PROGRAM (Lower concern and effluent flow is greater than 10% of the receiving water low flow)

1. Within three months of the effective date of the permit, the permittee shall submit to the Department for approval a study plan to evaluate wastewater toxicity at Outfall by using biomonitoring. The study plan should include a discussion of:
a. wastewater and production variability
b. sampling & sample handling
c. source & age of test organisms
d. source of dilution water
e. testing procedures/experimental design
f. data analysis
g. quality control/quality assurance
h. report preparation
i. testing schedule

2. The testing program shall consist of two definitive acute testing events, three months apart. This testing shall be initiated no later than three months following the Department's acceptance of the study plan.
a. Each of the two testing events shall include a 48-hour static renewal test using fathead minnow and a 48-hour static renewal test using a daphnid species.
b. If the receiving water is estuarine the permittee shall substitute estuarine species for those species specified above. Approved estuarine species for acute testing are sheepshead minnows, silversides, grass shrimp and mysid shrimp. In all cases, testing must include one vertebrate species and one invertebrate species.

3. The samples used for biomonitoring shall be collected at the same time and location as the samples analyzed for the effluent limitations and monitoring requirements for this outfall. For chlorinated effluents, samples shall be collected after dechlorination.

4. Testing shall be conducted in accordance with the procedures described in Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, August 1993, EPA/600/4-90/027F.

5. Test results shall be submitted to the Department within one month of completion of each set of tests.

6. Test results shall be reported in accordance with MDE/WMA "Reporting Requirements for Effluent Biomonitoring Data".

7. If testing is not performed in accordance with MDE-approved study plan, additional testing may be required by the Department.

8. If the test results indicate that the effluent is toxic, additional biomonitoring or a toxicity reduction evaluation will be required by the Department.

9. If plant processes or operations change so that there is a significant change in the nature of the wastewater, the Department may require the permittee to conduct a new set of tests.

10.* If a significant industrial user locates within the service area so that significant change in the nature of the wastewater might be anticipated, MDE may require the the permittee to conduct a new set of tests.

11. Submit all Biomonitoring related materials to:

Maryland Department of the Environment
Technical and Regulatory Services Administration
Environmental Risk Assessment Program
1800 Washington Blvd.
Baltimore, Maryland 21230

*omit for industrial facilities

Appendix D

BIOMONITORING PROGRAM (Lower concern and effluent flow is less than 10% of the receiving water low flow)

1. Within three months of the effective date of the permit, the permittee shall submit to the Department for approval a study plan to evaluate wastewater toxicity at Outfall by using biomonitoring. The study plan should include a discussion of:
a. wastewater and production variability
b. sampling & sample handling
c. source & age of test organisms
d. source of dilution water
e. testing procedures/experimental design
f. data analysis
g. quality control/quality assurance
h. report preparation
i. testing schedule

2. The testing program shall consist of two definitive acute testing events, three months apart. This testing shall be initiated no later than three months following the Department's acceptance of the study plan.
a. Each of the two testing events shall include a 48-hour static renewal test using fathead minnow and a 48-hour static renewal test using a daphnid species.
b. If the receiving water is estuarine the permittee may substitute estuarine species for those species specified above. Approved estuarine species for acute testing are sheepshead minnows, silversides, grass shrimp, and mysid shrimp. In all cases, testing must include one vertebrate species and one invertebrate species.

3. The samples used for biomonitoring shall be collected at the same time and location as the samples analyzed for the effluent limitations and monitoring requirements for this outfall. For chlorinated effluents, samples shall be collected after dechlorination.

4. Testing shall be conducted in accordance with the procedures described in Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, August 1993, EPA/600/4-90/027F.

5. Test results shall be submitted to the Department within one month of completion of each set of tests.

6. Test results shall be reported in accordance with MDE/WMA "Reporting Requirements for Effluent Biomonitoring Data".

7. If testing is not performed in accordance with MDE-approved study plan, additional testing may be required by the Department.

8. If the test results indicate that the effluent is toxic, additional biomonitoring or a toxicity reduction evaluation will be required by the Department.

9. If plant processes or operations change so that there is a significant change in the nature of the wastewater, the Department may require the permittee to conduct a new set of tests.

10.* If a significant industrial user locates within the service area so that significant change in the nature of the wastewater might be anticipated, MDE may require the the permittee to conduct a new set of tests.

11. Submit all Biomonitoring related materials to:

Maryland Department of the Environment
Technical and Regulatory Services Administration
Environmental Risk Assessment Program
1800 Washington Blvd.
Baltimore, Maryland 21230

*omit for industrial facilities

Appendix E

REPORTING REQUIREMENTS FOR EFFLUENT BIOMONITORING DATA

MARYLAND DEPARTMENT OF THE ENVIRONMENT

BACKGROUND

The Maryland Department of the Environment has compiled the following guidelines for reporting toxicity data from biomonitoring tests. These guidelines were formulated in an effort to standardize evaluations of toxicity data submitted to the Department.

BIOMONITORING REPORTING REQUIREMENTS

The results from biomonitoring toxicity tests shall be reported in a concise, easily understood manner. Each test report, in addition to an overall summary of the results, shall include the following documentation.

1. Chain of Custody Forms: A chain of custody form should accompany each individual sample collected. Each form shall include the following information.

o Facility name
o Sample collection date, time, and location (start and finish)
o Sampling Method (grab or composite)
o Volume of sample
o Type of test (Acute or Chronic)
o Sampler's signature and date
o Description of sample storage during transportation
o The signatures of all persons receiving custody of sample prior to use in testing, dates and times of receipt
o Comments (as appropriate)

2. Effluent Quality Measurements: These data shall be reported for each effluent sample either at the time of collection or upon receipt by the toxicity testing laboratory.

Date and time of measurements Conductivity and Salinity
Temperature Hardness
pH Alkalinity
Dissolved Oxygen Visual Description
Total Residual Chlorine*(TRC) Comments (as appropriate)

** If the TRC exceeds 0.02 mg/l, the samples are dechlorinated in the laboratory, prior to their use in toxicity tests.*

3. Toxicity Test Data:

A. Dilution Water.

(1) Source of the dilution water
(2) Manipulation steps (if any)
B. Test Organisms.
(1) Source of the test organisms
(2) Age of test organisms
(3) Any acclimation steps
(4) Disease treatment (if applicable)
(5) Reference toxicant test data*
(a) Reference toxicant identity.
(b) Test date(s)
(c) Test results (48-hr LC50 with 95% confidence limits for acute tests; NOEC, LOEC, ChV & IC25 for chronic tests)
**When in-house organisms are used, monthly test data from the previous 5 months shall be reported. When organisms from an outside source are used, reference toxicant data from a test performed concurrently with the effluent test shall be reported, unless the test organism supplier provides control chart data from at least the last five monthly toxicity tests.*
C. Effluent Toxicity Tests. The organisms utilized shall be clearly idenitified in the reporting of the following information for each effluent toxicity test.
(1) Test results.
(a) For both acute and chronic tests, the LC50 value, with 95% confidence limits, from the first 48 hours of the test.
(b) For chronic tests, the values for NOEC, LOEC, ChV, AND IC25 (based on biomass with 95% confidence limits).
(2) Water quality measurements.
(a) Daily measurements (before and after renewal) of temperature, DO*, and pH for all dilutions.
(b) Daily measurements of conductivity, alkalinity, and hardness for 100% and 0% dilutions.
(c) A summary (mean and range) of the data described in (a) and (b) above.
**If DO is below 40% saturation (3.3 mg/l at 25oC), samples are to be aerated gently before toxicity testing. The report shall indicate if aeration is necessary.*
(3) Initial test measurements.
(a) Number of replicates.
(b) Number of organisms in each replicate.
(c) For chronic tests, in which growth is measured, initial organism weights for each dilution.
(d) Volume of solution and the size of test chambers.
(e) Daily diet or lack of feeding.
(4) Daily mortality data, and for chronic reproduction tests, daily brood production.
(5) For chronic growth tests, final weight data for all organisms remaining at test conclusion.
(6) Summarized mortality, and for chronic tests, growth and reproduction data.
(7) Statistical calculations, including tests on assumptions (e.g., normality, homogeneity of variance). The statistical method and data used shall be clearly identified.
(8) Any test method deviations.
(9) Relevant observations on test organisms or conditions.

EFFLUENT TOXICITY TEST PROCEDURES GUIDANCE

On October 16, 1995, the EPA published its final rule in the Federal Register establishing whole effluent toxicity test methods at 40 CFR Part 136. These test methods are described in the following manuals. All WET testing required to be conducted for discharge permits issued under the National Pollutant Discharge Elimination System must conform to these methods.

EPA Effluent Toxicity Test Manuals:

Weber, C.I. [ed.] 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms (Fourth edition). EPA/600/4-90/027F. USEPA, Office of Research and Development, Washington, DC. 293 p.

Lewis, P.A., W.H. Peltier, T.J. Norberg-King, D.J. Klemm, J.M. Lazorchak, M.A. Heber [eds.] 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms (Third edition). EPA/600/4-91/002. USEPA, Environmental Monitoring Systems Laboratory, Office of Research and Development, Cincinnati, OH. 341 p.
Klemm, D.J., G.E. Morrison, T.J. Norberg-King, W.H. Peltier, M.A. Heber [eds.] 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms (Second edition). EPA/600/4-91/003. USEPA, Environmental Monitoring Systems Laboratory, Office of Research and Development, Cincinnati, OH. 483 p.

Appendix F - Biotoxicity Tests Employed by Permittees

freshwaterthe Department may approve, upon request, the use of additional locally important test species

acute - 48 hour static renewal assays for lethality or immobility utilizing:

fathead minnow (Pimephales promelas) and Daphnia magna, Daphnia pulex, or Ceriodaphnia dubia

chronic - Ceriodaphnia dubia survival & reproduction

larval fathead minnows (Pimephales promelas) survival & growth

estuarine/marine2

acute - 48 hour static renewal assays for lethality or immobility utilizing:

sheepshead minnows (Cyprinodon variegatus) or inland silversides (Menidia menidia or Menidia beryllina)

and grass shrimp (Palaemonetes spp.) or mysid shrimp (Mysidopsis bahia, M. bigelowi, or Neomysis

americana)

chronic - sheepshead minnows (Cyprinodon variegatus) larval survival & growth

inland silversides (Menidia beryllina) larval survival & growth

mysid shrimp (Mysidopsis bahia) survival, growth & fecundity

Appendix G - Tests Employed by State Biomonitoring Laboratory
freshwater

acute - 48 hour static renewal assays for lethality or immobility utilizing:

Daphnia magna or Ceriodaphnia dubia and juvenile fathead minnows,(Pimephales promelas)

chronic - Ceriodaphnia dubia survival & reproduction

larval fathead minnows (Pimephales promelas) survival & growth

estuarine/marine

acute - 48 hour static renewal assays for lethality or immobility utilizing:

Mysidopsis bahia, M. bigelowi, or Neomysis americana and juvenile sheepshead minnows, (Cyprinodon

variegatus)

chronic - Mysidopsis bahia, M. bigelowi or

Neomysis americana survival, growth & reproduction

larval sheepshead minnows, (Cyprinodon variegatus) survival & growth

Appendix H TOXICITY REDUCTION EVALUATION

The permittee shall conduct a Toxicity Reduction Evaluation (TRE) when a review of toxicity test data by the Department indicates unacceptable acute or chronic effluent toxicity. A TRE is an investigation conducted to identify the causative agents of effluent toxicity, isolate the source(s), determine the effectiveness of control options, implement the necessary control measures and then confirm the reduction in toxicity.

1. Within 90 days of notification by the Department that a TRE is required, the permittee shall submit a plan of study and schedule for conducting a TRE. The permittee shall conduct the TRE study consistent with the submitted plan and schedule.

for industrials: 2. This plan should follow the framework presented in Generalized Methods for Conducting Industrial Toxicity Reduction Evaluations (EPA/600/2-88/070).

for municipals: 2. This plan should follow the framework presented in Toxicity Reduction Evaluation Protocol for Municipal Wastewater Treatment Plants (EPA/600/2-88/062).

3. Beginning 60 days from the date of the Department's acceptance of the TRE study plan and every 60 days thereafter, the permittee shall submit progress reports including all relevant test data to the Department. This shall continue until completion of the toxicity reduction confirmation.

4. Within 60 days of completion of the toxicity identification, or the source identification phase of the TRE, the permittee shall submit to the Department a plan and schedule for implementing those measures necessary to eliminate acute toxicity and/or reduce chronic toxicity to acceptable levels. The implementation of these measures shall begin immediately upon submission of this plan.

5. Within 60 days of completing the implementation of the control measures to eliminate or reduce toxicity, the permittee shall submit to the Department for approval a study plan to confirm the elimination or reduction of toxicity by using biomonitoring.

6. If, for any reason, the implemented measures do not result in compliance with the Department's toxicity limitations, the permittee shall continue the TRE.

7. Submit all TRE-related materials to:

Maryland Department of the Environment
Technical and Regulatory Services Administration
Environmental Risk Assessment Program
1800 Washington Blvd.
Baltimore, Maryland 21230