

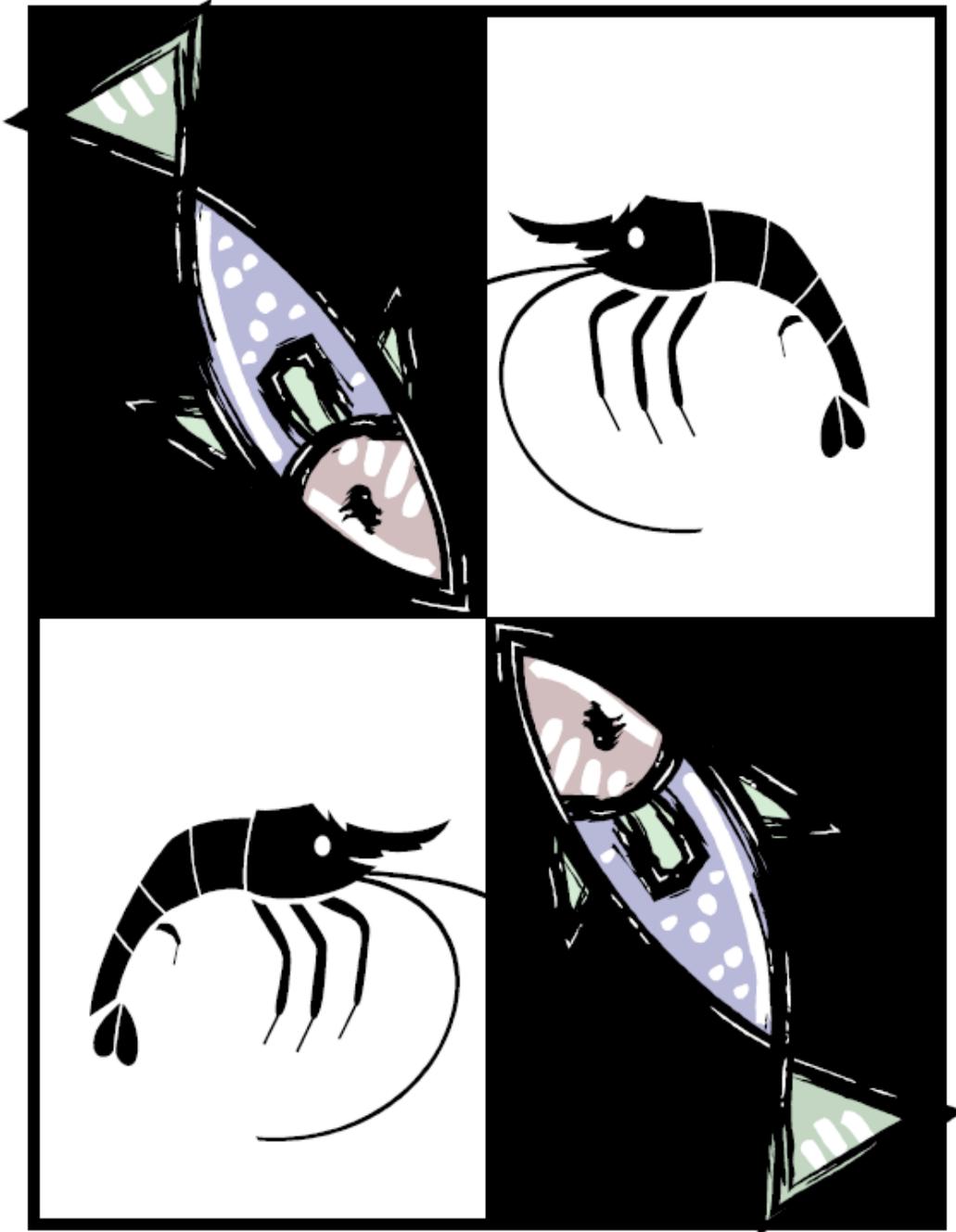
# MARYLAND DEPARTMENT OF THE ENVIRONMENT

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410-537-3000 • 1-800-633-6101 • <http://www.mde.state.md.us>

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## WASTEWATER PERMITS PROGRAM

# EFFLUENT BIOTOXICITY TESTING PROTOCOL FOR INDUSTRIAL AND MUNICIPAL EFFLUENTS



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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 (WET) 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. 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 Surface 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.

Maryland regulation (COMAR 26.08.03.07D(2)) specifically requires the following:

- (2) NPDES Permit Monitoring Requirements.

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- (a) A discharger identified in §D(1) of this regulation shall have requirements for toxic substance monitoring included in its permit at the time of permit issuance or reissuance.
- (b) Modifications to these requirements may be allowed on a case-by-case basis if the:
  - (i) Specific conditions of the discharge suggest that a full scale toxics monitoring program is not necessary; or
  - (ii) Characteristics of the receiving water indicate that a full scale toxics monitoring program is not needed.
- (c) Data submitted under any previous toxic substance monitoring program may be used to satisfy these requirements if the data is indicative of the current process and treatment conditions.
- (d) Any toxic substance monitoring, including test protocols, shall be approved by the Department before initiation of the testing. All data generated shall be within the quality assurance and quality control specifications of the test protocol.
- (e) Measurements below the minimum level may be reported as BML (below minimum level).
- (f) If the Department determines through the monitoring described in §D(1) of this regulation, that a discharge causes or has the potential to cause the discharge of toxic substances or an impact on surface waters, the Department may modify the discharge permit to require the discharger to collect data to verify or rule out the existence of an impact from a toxic substance.

NPDES biotoxicity testing requirements for major facilities generally consist of four quarterly tests to be conducted during the first year of the permit for industrial facilities. As required by 40 CFR 122.21(j)(5)(iv), municipal facilities must submit (A) results of a minimum of four quarterly tests for a year, from the year preceding the permit application; or (B) results from four tests performed at least annually in the four and one half year period prior to the application (Appendices A & B). The Department has chosen option B as the standard permit requirement. 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 acute tests to be conducted during the first year of the permit (Appendices C & D). Chronic, instead of acute, tests may be required in sensitive discharge situations such as discharges to intermittent streams.

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

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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).

## 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 LC<sub>50</sub> (effluent concentration at which 50% of the test organisms die during the test) or EC<sub>50</sub> (effluent concentration at which 50% of the organisms are killed or disabled during the test). In order to calculate an LC<sub>50</sub> (or EC<sub>50</sub>), 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 LC<sub>50</sub> or EC<sub>50</sub>, the more toxic the effluent. For example, an LC<sub>50</sub> (or EC<sub>50</sub>) of greater than 100% means that full strength effluent (100%) did not kill (or immobilize) at least half the test organisms. An LC<sub>50</sub> (or EC<sub>50</sub>) 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 IC<sub>25</sub> (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 LC<sub>50</sub>s or EC<sub>50</sub>s, 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.

Name of Guidance: *Effluent Biotoxicity Testing Protocol For Industrial And Municipal Effluents*  
Last Revision: 12/14/2012

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1. An effluent is considered to be acutely toxic when its 48-hour LC<sub>50</sub> or EC<sub>50</sub> (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 LC<sub>50</sub> or EC<sub>50</sub> (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 IC<sub>25</sub> is less than or equal to the in-stream waste concentration.<sup>1</sup>
2. An effluent is generally considered not chronically toxic when its IC<sub>25</sub> is greater than the in-stream waste concentration.

Upon consistent findings of chronic toxicity, a permittee shall 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 V).

## IV. Permit Limitations and Compliance Schedule

MDE will include a specific limitation for effluent toxicity and a compliance schedule for the elimination of the effluent toxicity in the facility's discharge permit as indicated below:

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$${}^1\text{IWC} = Q_D / (Q_D + Q_{RW}) \times 100 \text{ where } Q_{RW} = 30Q_5$$

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## **Situations in which a Permit Limitation and Compliance Schedule will be included.**

When issuing a NPDES permit renewal, MDE will include a permit limitation for effluent toxicity when toxicity testing demonstrates a reasonable potential for the discharge to cause or contribute to a violation of water quality standards. Reasonable potential is determined by the Department as at least one test from all current test results showing toxicity as defined in Sections II. A & B, unless there are a sufficient number of tests over time that provide for a statistical basis for concluding no reasonable potential. A compliance schedule could be considered, if necessary, which would outline the activities needed to eliminate the toxicity. During the compliance schedule period, the permittee is required to conduct a TRE as specified in Section III above, unless the basis of the schedule is to implement significant treatment system upgrades or major process modifications which are expected to address the toxicity. If the results of the TRE identify the chemical specific parameter(s) causing the toxicity, the whole effluent toxicity permit limit could be replaced with the chemical specific effluent limitation(s).

As described in the “Determination of discharge permit WET limitations” section below, the determination of discharge permit limitations may incorporate dilution resulting from mixing zone allowances in accordance with COMAR 26.08.02.05. However, when determining whether a TRE must take place to eliminate the toxicity under COMAR 26.08.03.07.E(4)(e), dilution is not considered when determining if an effluent is acutely toxic.

As indicted in I.A above, 40 CFR 122.21(j)(5)(iv) requires that municipal facilities must submit (A) results of a minimum of four quarterly tests for a year, from the year preceding the permit application; or (B) results from four tests performed at least annually in the four and one half year period prior to the application. Permit limitations expressed in toxic units and a compliance schedule will be included in a municipal facility’s discharge permit when any of the four 40 CFR 122.21(j)(5)(iv) required tests show toxicity. A component of the compliance schedule will require eighteen months of quarterly whole effluent toxicity testing resulting in six individual test that are the same type of test that determined the original toxicity. If none of the six tests show toxicity, the permittee may request a permit modification to remove the permit limit and the compliance schedule. If any of the six tests are toxic, the permit limit will go into effect and a TRE will begin to discover the source of the toxicity and explore solutions to remove that toxicity.

To address federal NPDES requirements for WET testing and limits, MDE shall implement permit limits in a new or renewal permit when a WET test result shows reasonable potential for toxicity unless it can be demonstrated that the source of toxicity has been eliminated, inappropriate test procedures were utilized, or the source has been controlled via a chemical specific permit limitation. Where reasonable potential has been assumed based on one test result, the permit shall include a WET limit effective within the term of the permit unless the effluent shows no toxicity in six follow-up quarterly tests.

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## Determination of discharge permit WET limitations

The determination of discharge permit limits may incorporate dilution resulting from mixing zone allowances in accordance with COMAR 26.08.02.05.

### Acute Conditions

To protect aquatic life against acute effects, the ambient toxicity should be less than 1.0 acute toxic unit ( $TU_a$ ) where a  $TU_a$  is defined as 100 divided by the  $LC_{50}$  value resulting from the first 48 hours of a valid acute or chronic toxicity test.

### Chronic Conditions

To protect aquatic life against chronic effects, the effluent's  $IC_{25}$  shall be greater than the in-stream waste concentration (IWC).

Using the formula for IWC shown in footnote 1 and the requirement that the effluent's  $IC_{25}$  shall be greater than the in-stream waste concentration (IWC) the below relationship for allowable effluent toxicity can be expressed in chronic toxic units ( $TU_c$ ) where a  $TU_c$  is defined as 100 divided by the  $IC_{25}$  value resulting from a valid chronic toxicity test.

For effluent not to be chronically toxic

$$IC_{25} > IWC$$

$$100/TU_c > IWC$$

$$100/[(TU_c)(IWC)] > 1$$

$$100/[\{TU_c\}\{Q_D/(Q_D + Q_{RW})\}\{100\}] > 1$$

$$\text{Therefore Allowable Effluent Chronic Toxicity} = TU_c < (Q_D + Q_{RW})/Q_D$$

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Example WET Limit Calculations - for discharge situations when 1/3 of the receiving stream flow is the limiting factor for determination of the mixing zone for the effluent.

### Acute WET Limit

Using the below mass balance equation

$$C_R = [(C_D)(Q_D) + (C_{RW})(Q_{RW})]/[Q_D + Q_{RW}]$$

Where:

Facility Flow =  $Q_D = 6.8 \text{ MGD} = 10.52 \text{ cfs}$

Facility Toxicity =  $C_D$

Upstream Receiving Stream 7Q10 flow =  $Q_{RW} = 45.81 \text{ cfs}$

Allowable Acute Mixing Zone flow =  $(1/3)(Q_{RW}) = (1/3)(45.81 \text{ cfs}) = 15.27 \text{ cfs}$

Assumed Upstream Receiving Stream Toxicity =  $C_{RW} = 0 \text{ TU}_a$

Downstream Receiving Stream Toxicity =  $C_R$

Allowable  $C_R < 1.0 \text{ TU}_a$

Assuming Allowable In-Stream Toxicity =  $0.9999 \text{ TU}_a$

$$0.9999 \text{ TU}_a = [(C_D)(Q_D) + (C_{RW})(Q_{RW})]/[Q_D + Q_{RW}]$$

$$0.9999 \text{ TU}_a = [(C_D)(10.52 \text{ cfs}) + (0 \text{ TU}_a)(15.27 \text{ cfs})]/[10.52 \text{ cfs} + 15.27 \text{ cfs}]$$

Permit Acute WET Limit =  $C_D < 2.45 \text{ TU}_a$

### Chronic WET Limit

Using the below equation for allowable effluent chronic toxicity

$$\text{Allowable Effluent Chronic Toxicity} = \text{TU}_c < (Q_D + Q_{RW})/Q_D$$

Where:

Facility Flow =  $Q_D = 6.8 \text{ MGD} = 10.52 \text{ cfs}$

Facility Toxicity =  $C_D$

Upstream Receiving Stream 30Q5 flow =  $Q_{RW} = 23.75 \text{ cfs}$

Assumed Upstream Receiving Stream Toxicity =  $C_{RW} = 0 \text{ TU}_c$

$$\text{Permit Chronic WET Limit} = C_D = \text{TU}_c < (Q_D + Q_{RW})/Q_D$$

$$\text{Permit Chronic WET Limit} = C_D = \text{TU}_c < (10.52 \text{ cfs} + 23.75 \text{ cfs})/10.52 \text{ cfs}$$

$$\text{Permit Chronic WET Limit} = C_D < 3.26 \text{ TU}_c$$

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## V. Relevant Guidance Documents

Maryland Department of the Environment, Water Management Administration. "Reporting Requirements for Effluent Biomonitoring Data." 3/21/03

Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition, EPA-821-R-02-012, October 2002

Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms Fourth Edition, EPA-821-R-02-013, October, 2002

Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms Third Edition, EPA-821-R-02-014, October 2002

Generalized Methodology for Conducting Industrial Toxicity Reduction Evaluations (TREs). EPA/600/2-88/070. USEPA, March 1989

Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants. EPA/833B-99/002. USEPA, Office of Wastewater Management, Washington DC

Methods for Aquatic Toxicity Identification Evaluations - Phase I Toxicity Characterization Procedures. EPA-600/6-91/003. USEPA, Second Edition, February 1991

Methods for Aquatic Toxicity Identification Evaluations - Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity. EPA/600/R-92/080. USEPA, September 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, September 1993

Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I. EPA/600/6-91/005. USEPA, June 1991

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## 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. For industrial facilities:

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.

For municipal facilities:

The testing program shall consist of four quarters of definitive annual chronic testing. The testing events shall be conducted annually during January or February of each of the first four years after approval of the study plan. This testing shall be initiated no later than the January or February following the Department's acceptance of the study plan.

For Freshwater Receiving Stream

Each annual testing event shall include the Ceriodaphnia survival and reproduction test and the fathead minnow larval survival and growth test.

For Estuarine Receiving Stream

Testing shall include the sheepshead minnow (*Cyprinodon variegatus*) or inland silverside (*Menidia beryllina*) larval survival and growth tests and mysid shrimp (*Americamysis bahia* AKA *Mysidopsis bahia*) survival, growth, and fecundity tests. Testing must include one vertebrate species and one invertebrate species. Test results shall be expressed as NOEC, LOEC, ChV, and IC<sub>25</sub>.

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. The permittee shall collect 24-hour flow-proportioned composite samples unless the

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Department has given prior approval of an alternative sampling type.

4. The following EPA document discusses the appropriate methods:

## For Freshwater Receiving Stream

Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms Fourth Edition, EPA-821-R-02-013, October, 2002

## For Estuarine Receiving Stream

Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms Third Edition, EPA-821-R-02-014, October 2002

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," 3/21/03.
7. As a minimum, the reported chronic results shall be expressed as NOEC, LOEC, ChV, and IC<sub>25</sub>.
8. If a 50% mortality or greater occurs in one or more effluent concentrations during the first 48 hours of the chronic tests, 48-hour LC<sub>50</sub>s 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 of any two consecutive valid toxicity tests show acute or chronic toxicity (LC<sub>50</sub> equal to or less than 100% for acute tests and an IC<sub>25</sub> equal to or less than the in-stream waste concentration for chronic tests), the permittee shall repeat the test within 30 days to confirm the findings of acute or chronic toxicity. Intermittent toxicity or other concerns may require additional testing or limits. If acute and/or chronic toxicity is confirmed, the permittee shall:
  - a. Eliminate the source of toxicity through operational changes as soon as possible but in any case not longer than within three months, or
  - b. Perform a TRE. If the permittee repeats the toxicity testing as stated above and the results of the repeat test do not confirm the acute or chronic toxicity, the Department will require the permittee to repeat the toxicity testing as

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stated above to reconfirm a finding of no acute or chronic toxicity. After reconfirmation, the permittee shall complete any remaining quarterly testing required.

11. If the permittee completes a TRE in accordance with II.E.10.b and unacceptable toxicity is confirmed, a Whole Effluent Toxicity (WET) permit limit and a compliance schedule will be required.
12. To address federal NPDES requirements for WET testing and limits, MDE shall implement permit limits in a new or renewal permit when a WET test result shows reasonable potential for toxicity unless it can be demonstrated that the source of toxicity has been eliminated, inappropriate test procedures were utilized, or the source has been controlled via a chemical specific permit limitation. Where reasonable potential has been assumed based on one test result, the permit shall include a WET limit effective within three years unless the effluent shows no toxicity in six follow-up quarterly tests. The permit may be modified to remove the WET limit if the six follow-up quarterly tests show no toxicity.
13. 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.
- \*14. 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 permittee to conduct a new set of tests.
15. Submit all Biomonitoring related materials to:

Maryland Department of the Environment  
Water Management Administration  
Compliance Program  
1800 Washington Blvd., Suite 420  
Baltimore, MD 21230-1708

\*omit for industrial facilities

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## 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. For industrial facilities:

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.

#### For municipal facilities:

The testing program shall consist of definitive testing for four annual testing events. Three of the events shall have acute testing and one of the events shall have chronic testing. The testing events shall be conducted annually during January or February of each of the first four years after approval of the study plan. One of these first two testing events shall include the chronic tests. This testing shall be initiated no later than January or February following the Department's acceptance of the study plan.

#### For Freshwater Receiving Stream

- 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. Acute test results shall be expressed as LC<sub>50</sub>. Chronic test results shall be expressed as NOEC, LOEC, ChV, and IC<sub>25</sub>.

#### For Estuarine Receiving Stream

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- a. The acute testing shall consist of 48-hour static renewal tests using either sheepshead minnows (*Cyprinodon variegatus*), silversides (*Menidia beryllina*, *Menidia menidia*, *Menidia peninsulae*) and mysid shrimp (*Americamysis bahia* A.K.A. *Mysidopsis bahia*). Testing must include one vertebrate species and one invertebrate species
  - b. The chronic testing shall include the sheepshead minnow (*Cyprinodon variegatus*) or inland silverside (*Menidia beryllina*) larval survival and growth tests and mysid shrimp (*Americamysis bahia* AKA *Mysidopsis bahia*) survival, growth, and fecundity tests. Testing must include one vertebrate species and one invertebrate species
  - c. Acute test results shall be expressed as LC<sub>50</sub>. Chronic test results shall be expressed as NOEC, LOEC, ChV, and IC<sub>25</sub>.
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. The permittee shall collect 24-hour flow-proportioned composite samples unless the Department has given prior approval of an alternative sampling type.
  4. The following EPA documents discuss the appropriate methods:

## For Freshwater Receiving Stream

- a. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition, EPA-821-R-02-012, October 2002
- b. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms Fourth Edition, EPA-821-R-02-013, October, 2002

## For Estuarine Receiving Stream

- a. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition, EPA-821-R-02-012, October 2002
  - b. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms Third Edition, EPA-821-R-02-014, October 2002
5. Test results shall be submitted to the Department within one month of completion of each set of tests.

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6. Test results shall be reported in accordance with MDE/WMA "Reporting Requirements for Effluent Biomonitoring Data," 3/21/03.
7. As a minimum, the reported chronic results shall be expressed as NOEC, LOEC, ChV, and IC<sub>25</sub>.
8. If a 50% mortality or greater occurs in one or more effluent concentrations during the first 48 hours of the chronic tests, 48-hour LC<sub>50</sub>s 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 of any two consecutive valid toxicity tests show acute or chronic toxicity (LC<sub>50</sub> equal to or less than 100% for acute tests and an IC<sub>25</sub> equal to or less than the in-stream waste concentration for chronic tests), the permittee shall repeat the test within 30 days to confirm the findings of acute or chronic toxicity. Intermittent toxicity or other concerns may require additional testing or limits. If acute and/or chronic toxicity is confirmed, the permittee shall:
  - a. Eliminate the source of toxicity through operational changes as soon as possible but in any case not longer than within three months, or
  - b. Perform a TRE. If the permittee repeats the toxicity testing as stated above and the results of the repeat test do not confirm the acute or chronic toxicity, the Department will require the permittee to repeat the toxicity testing as stated above to reconfirm a finding of no acute or chronic toxicity. After reconfirmation, the permittee shall complete any remaining quarterly testing required.
11. If the permittee completes a TRE in accordance with II.E.10.b and unacceptable toxicity is confirmed, a Whole Effluent Toxicity (WET) permit limit and a compliance schedule will be required.
12. To address federal NPDES requirements for WET testing and limits, MDE shall implement permit limits in a new or renewal permit when a WET test result shows reasonable potential for toxicity unless it can be demonstrated that the source of toxicity has been eliminated, inappropriate test procedures were utilized, or the source has been controlled via a chemical specific permit limitation. Where reasonable potential has been assumed based on one test result, the permit shall include a WET limit effective within three years unless the effluent shows no toxicity in six follow-up quarterly tests. The permit may be modified to remove the WET limit if the six follow-up quarterly tests show no toxicity.

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13. 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.
- \*14. 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 permittee to conduct a new set of tests.
15. Submit all Biomonitoring related materials to:

Maryland Department of the Environment  
Water Management Administration  
Compliance Program  
1800 Washington Blvd., Suite 420  
Baltimore, MD 21230-1708

\*omit for industrial facilities

# MARYLAND DEPARTMENT OF THE ENVIRONMENT

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## 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.

#### For Freshwater Receiving Stream

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.

#### For Estuarine Receiving Stream

- a. The testing shall consist of 48-hour static renewal tests using either sheepshead minnows (*Cyprinodon variegatus*), silversides (*Menidia beryllina*, *Menidia menidia*, *Menidia peninsulae*) and mysid shrimp (*Americamysis bahia* A.K.A. *Mysidopsis bahia*). Testing must include one vertebrate species and one invertebrate species
- b. Test results shall be expressed as LC<sub>50</sub>.
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. The permittee shall collect 24-hour flow-proportioned composite samples unless the Department has given prior approval of an alternative sampling type.
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

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Marine Organisms, Fifth Edition, EPA-821-R-02-012, October 2002

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," 3/21/03.
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 of any two consecutive valid toxicity tests conducted within any 12-month period show acute toxicity (LC<sub>50</sub> equal to or less than 100%) the permittee shall repeat the test within 30 days to confirm the findings of acute toxicity. If acute toxicity is confirmed, the permittee shall:
  - a. Eliminate the source of toxicity through operational changes as soon as possible but in any case not longer than within three months, or
  - b. Perform a TRE. If the permittee repeats the toxicity testing as stated above and the results of the repeat test do not confirm the acute toxicity, the Department will require the permittee to repeat the toxicity testing as stated above to reconfirm a finding of no acute toxicity. After reconfirmation, the permittee shall complete any remaining quarterly testing required.
9. If the permittee completes a TRE in accordance with II.E.8.b and unacceptable toxicity is confirmed, a Whole Effluent Toxicity (WET) permit limit and a compliance schedule will be required.
10. To address federal NPDES requirements for WET testing and limits, MDE shall implement permit limits in a new or renewal permit when a WET test result shows reasonable potential for toxicity unless it can be demonstrated that the source of toxicity has been eliminated, inappropriate test procedures were utilized, or the source has been controlled via a chemical specific permit limitation. Where reasonable potential has been assumed based on one test result, the permit shall include a WET limit effective within three years unless the effluent shows no toxicity in six follow-up quarterly tests. The permit may be modified to remove the WET limit if the six follow-up quarterly tests show no toxicity.
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 permittee to conduct a new set of tests.

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13. Submit all Biomonitoring related materials to:

Maryland Department of the Environment  
Water Management Administration  
Compliance Program  
1800 Washington Blvd., Suite 420  
Baltimore, MD 21230-1708

\*omit for industrial facilities

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## 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.

#### For Freshwater Receiving Stream

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.

#### For Estuarine Receiving Stream

- 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. The permittee may substitute 48-hour static renewal tests using either sheepshead minnows (*Cyprinodon variegatus*), silversides (*Menidia beryllina*, *Menidia menidia*, *Menidia peninsulae*) and mysid shrimp (*Americamysis bahia* A.K.A. *Mysidopsis bahia*) for the above tests. Testing must include one vertebrate species and one invertebrate species
  - c. Test results shall be expressed as LC<sub>50</sub>
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. The permittee shall collect 24-hour flow-proportioned composite samples unless the Department has given prior approval of an alternative sampling type.

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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, Fifth Edition, EPA-821-R-02-012, October 2002
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," 3/21/03.
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 of any two consecutive valid toxicity tests conducted within any 12-month period show acute toxicity ( $LC_{50}$  equal to or less than 100%), the permittee shall repeat the test within 30 days to confirm the findings of acute toxicity. If acute toxicity is confirmed, the permittee shall:
  - a. Eliminate the source of toxicity through operational changes as soon as possible but in any case not longer than within three months, or
  - b. Perform a TRE. If the permittee repeats the toxicity testing as stated above and the results of the repeat test do not confirm the acute toxicity, the Department will require the permittee to repeat the toxicity testing as stated above to reconfirm a finding of no acute toxicity. After reconfirmation, the permittee shall complete any remaining quarterly testing required.
9. If the permittee completes a TRE in accordance with II.E.8.b and unacceptable toxicity is confirmed, a Whole Effluent Toxicity (WET) permit limit and a compliance schedule will be required.
10. To address federal NPDES requirements for WET testing and limits, MDE shall implement permit limits in a new or renewal permit when a WET test result shows reasonable potential for toxicity unless it can be demonstrated that the source of toxicity has been eliminated, inappropriate test procedures were utilized, or the source has been controlled via a chemical specific permit limitation. Where reasonable potential has been assumed based on one test result, the permit shall include a WET limit effective within three years unless the effluent shows no toxicity in six follow-up quarterly tests. The permit may be modified to remove the WET limit if the six follow-up quarterly tests show no toxicity.
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.

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- \*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 permittee to conduct a new set of tests.
13. Submit all Biomonitoring related materials to:

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\*omit for industrial facilities

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## 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.*

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## 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 LC<sub>50</sub> with 95% confidence limits for acute tests; NOEC, LOEC, ChV & IC<sub>25</sub> 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. Regardless of the source of test organisms (in-house cultures or purchased from external suppliers), the testing laboratory must perform at least one acceptable reference toxicant test per month for each toxicity test method conducted in that month. If a test method is conducted only monthly, or less frequently, a reference toxicant test must be performed concurrently with each effluent toxicity test.*

### C. Effluent Toxicity Tests. The organisms utilized shall be clearly identified in the reporting of the following information for each effluent toxicity test.

- (1) Test results.
  - (a) For both acute and chronic tests, the LC<sub>50</sub> value, with 95% confidence limits, from the first 48 hours of the test.
  - (b) For chronic tests, the values for NOEC, LOEC, ChV, AND IC<sub>25</sub> (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 25°C), 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.

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- (b) Number of organisms in each replicate.
- (c) Volume of solution and the size of test chambers.
- (d) 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:

Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition, EPA-821-R-02-012, October 2002

Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms Fourth Edition, EPA-821-R-02-013, October, 2002

Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms Third Edition, EPA-821-R-02-014, October 2002

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## Appendix F – Whole Effluent Toxicity Tests to be Employed by Permittees

Test methods utilized by permittees for whole effluent toxicity testing must conform to the test methods found in *Table IA—List of Approved Biological Methods for Wastewater and Sewage Sludge* found in the latest edition of 40 CFR Part 136

### freshwater<sup>2</sup>

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

fathead minnow (*Pimephales promelas*), Bannerfin shiner (*Cyprinella leedsi*), Rainbow Trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*) and *Daphnia magna*, *Daphnia pulex*, or *Ceriodaphnia dubia*

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:

sheepshead minnows (*Cyprinodon variegatus*), inland silversides (*Menidia beryllina*), Atlantic silverside (*Menidia menidia*), tidewater silverside (*Menidia peninsulae*) mysid shrimp (*Americamysis bahia*, formerly *Mysidopsis bahia*)  
chronic - sheepshead minnows (*Cyprinodon variegatus*) larval survival & growth  
inland silversides (*Menidia beryllina*) larval survival & growth  
mysid shrimp (*Americamysis bahia*, formerly *Mysidopsis bahia*) survival, growth & fecundity

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## Appendix G 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 for approval by the Department a plan of study, schedule and completion date 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) March 1989.

for municipals: 2. This plan should follow the framework presented in Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants (EPA/833B-99/002) August 1999.

Additional Guidance documents on the TRE process are shown below:

Methods for Aquatic Toxicity Identification Evaluations Phase I Toxicity Characterization Procedures Second Edition United States Environmental Protection Agency Office of Research and Development Washington, DC 20460  
EPA/600/6-9 1/003 February 1991

Methods for Aquatic Toxicity Identification Evaluations Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity, United States Environmental Protection Agency Office of Research and Development EPA/600/R-92/080 September 1993  
Washington DC 20460

Methods for Aquatic Toxicity Identification Evaluations Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity, United States Environmental Protection Agency Office of Research and Development Washington DC 20460 EPA /600/R-92/08 1 September 1993

Clarifications Regarding Toxicity Reduction and Identification Evaluations in the National Pollutant Discharge Elimination System Program, March 27, 2001, U.S. Environmental Protection Agency, Office of Wastewater Management , Office of Regulatory Enforcement, Washington, DC 20460

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

Name of Guidance: Effluent Biotoxicity Testing Protocol For Industrial And Municipal Effluents  
Last Revision: 12/14/2012

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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, schedule and completion date for implementing those measures necessary to eliminate acute toxicity, an LC<sub>50</sub> greater than 100%, and/or eliminate chronic toxicity, an IC<sub>25</sub> greater than the in-stream waste concentration (IWC). 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 and a Whole Effluent Toxicity (WET) permit limit and a compliance schedule will be required.
7. Submit all TRE-related materials to:

Maryland Department of the Environment  
Water Management Administration  
Compliance Program  
1800 Washington Blvd., Suite 420  
Baltimore, MD 21230-1708