NPDES Whole Effluent Toxicity Testing, Monitoring and Reporting

This guidance is intended to promote compliance and enhance program efficiency and effectiveness. This is not intended to, nor does it, constitute rulemaking by EPA and may not be relied upon to create a right or a benefit, substantive or procedural, enforceable at law or in equity, by any person. This document was prepared for NPDES Permittees to: (1) clarify Whole Effluent Toxicity (WET) testing, monitoring and reporting requirements; (2) provide guidance; and (3) provide a list of EPA contacts available to answer questions.

TIPS:

1. **NPDES Permit Requirements**
   The sampling location, sample type, test frequency, test species, monitoring period, and reporting requirements are specified in Part I (and ATTACHMENTS) of the NPDES Permit. Read the NPDES Permit carefully. Permittees and analytical laboratories must adhere to Permit requirements and test protocols. The Permittee is responsible for data quality, data integrity and NPDES reporting. EPA recommends that the Permittee provide its testing laboratory with a copy of the entire NPDES Permit (i.e., Part I and ATTACHMENTS, and Part II "General Conditions") and any subsequent modifications together with any alternate dilution water authorization letters. Mistakes have been made in the past that could have been avoided if the bioassay laboratory had a copy of these documents.

2. **WET Tests Data Quality and Reporting**
   Carefully review bioassay test results and be sure that the data are valid (i.e., the minimum test requirements, test review requirements and test acceptability criteria (TAC) are met for EPA's standard and EPA-New England protocol) and are correctly reported on the DMR.

3. **WET Test Scheduling**
   Laboratories have scheduled WET tests using test organisms that are at or near the oldest acceptable age at test start. If this is done and there is a delay in sample delivery, the test organisms may be too old for use in the bioassay test when the sample arrives. This could create some scheduling difficulties or could require a contingency plan that includes a secondary emergency source of test organisms. It is suggested that Permittees ask whether laboratories have contingency plans for such situations.

GUIDANCE:

4. **WET Guidelines and Methods Manuals**

Guidelines Establishing Test Procedures for the Analysis of Pollutants; Whole Effluent Toxicity Test Methods; Final Rule (Federal Register: November 19, 2002, Volume 67, Number 223, Rules and Regulations pp. 69951-69972)

The most current methods manuals, posted at Web address www.epa.gov/waterscience/WET/, are as follows:

a. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition, October 2002, EPA-821-R-02-012;
b. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms, Fourth Edition, October 2002, EPA-821-R-02-013;
c. Short-Term Methods for Estimating Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, Third Edition, October 2002, EPA-821-R-02-014; and
d. Standard Methods for the Examination of Water and Wastewater, 21st Edition, 2005.

5. **WET Monitoring and Reporting**

EPA rejects WET test reports that do not follow Permit requirements, applicable protocols, and meet all minimum criteria for acceptability and variability of test results, and requires tests to be repeated until valid results are obtained. Results, valid or otherwise, must be submitted by the date specified in Part I of the NPDES Permit even if the test has to be repeated. Therefore, EPA recommends that sampling and testing be initiated early in the monitoring period prescribed by the Permit.

If a valid WET test is not completed by the reporting deadline, the Permittee must report the invalid test using the proper code on the DMR; the code is "H." The cover letter must explain the monitoring and reporting violation and indicate when the test will be repeated. A corrected DMR must be resubmitted once valid data are available, and the entire report submitted as required by the Permit. The report shall include, among other things, bench sheets to document that there was an invalid test and that the test was repeated.

6. **Sample Dechlorination**

The total residual chlorine concentration of the discharge sample shall be measured and, if detected, the sample shall be dechlorinated in the laboratory prior to WET testing in accordance with Standard Methods for the Examination of Water and Wastewater, 21st Edition, 2005 (see also Section VI, Region I Protocol). The total residual chlorine concentration of the discharge sample

must be reported and the dechlorination method described. When the sample is dechlorinated in the laboratory, an additional thiosulfate control (with the maximum amount of thiosulfate in the lab control or the receiving water control) must also be run. This information must also be included in the report.

7. **Sample Hold Time**
   Sample hold time must be consistent with that specified by test protocol. The holding times for the initial use of original or renewal sample is less than **24 hours** for on-site tests and less than **36 hours** for off-site tests as specified in the protocols unless a waiver is obtained in writing from EPA. In isolated cases where the test cannot be started within 36 hours of sample collection, data must be submitted to EPA and the State to demonstrate that the effluent toxicity of a sample is not reduced by extending the holding time beyond 36 hours. Subsequent to initial use of the original or renewal sample, samples may be used for test renewal at 24, 48 and 72 hours.

8. **Salinity Adjustment of the Effluent Sample**
   The Region's test protocols require the use of sea salts for salinity adjustment in every case.

9. **Age of the Test Organisms**
   The protocols specify what the age of the test organism must be at test initiation. Evidence to verify test organism age must be included in each report.

10. **Raw Data and Bench Sheets**
    Raw data and bench sheets must be included in the full report.

11. **Report Integrity and DMR Accuracy**
    WET test data summary tables must be consistent with the report text, data analyses, bench sheets; and DMRs. Report integrity and DMR accuracy are crucial, and are the responsibility of the Permittee.

12. **Data Analyses**
    Flow charts in the EPA acute and chronic WET test manuals must be followed so that the correct analyses are performed. Statistical program printouts and graphical displays (e.g. NOEC and LC50 calculations, etc.) must be submitted.

13. **Chronic Ceriodaphnia dubia Survival and Reproduction Test**
    The duration of the chronic Ceriodaphnia dubia survival and reproduction test must not exceed **eight** days. The minimum acceptability criteria for each test is measured and documented for all test controls. Offspring from the fourth or higher broods must not be included with test results. (See EPA-821-R-02-013, October 2002, p. 161.)

14. **Document Ongoing Laboratory Performance**
    As part of an in-house Quality Assurance program, each laboratory must perform reference toxicant tests on the test organisms it uses and must analyze the data

for the reported test endpoints. Reference toxicant testing must be performed monthly, or concurrently depending on test frequency, for each test endpoint, in accordance with the EPA Methods Manual. Reference toxicity tests are to be performed and interpreted according to the referenced EPA Method Manuals. (See EPA-821-R-02-013, Section 4.16.1, p. 15.) Reference toxicity test results and applicable control charts must be included in every report.

In the case where a reference toxicity test is performed concurrently with an effluent or receiving water test and the reference toxicity test results fall slightly outside the control limits established by the laboratory for the test endpoint and the primary test meets the test acceptability criteria, the primary test will be considered "conditionally" acceptable. However, if the results of a concurrently run reference toxicity test fall well outside the established upper control limits, the primary test will be considered unacceptable and must be repeated immediately. (See EPA-821-R-02-013, Section 4.16).

15. **Sampling Methods, Holding Times, and Preservation Techniques**
All sampling methods, holding times and preservation techniques must be consistent with 40 C.F.R. Parts 122 and 136. Note that EPA-approved test methods require that samples collected for metals analyses be preserved immediately after collection.

16. **Dilution Water**
The objective of the WET test is to estimate the toxicity of the effluent in uncontaminated receiving water. Ideally, a grab sample of receiving water must be collected immediately upstream and outside of the influence of the outfall for use as dilution water in the tests.

17. **Alternate Dilution Water**
EPA-New England has adopted a **species-specific, self-implementing policy** for switching to alternate dilution water use in WET tests where the receiving water is documented to be toxic or unreliable. The policy authorizes alternate dilution water use in the following two cases:
(1) when a WET test is repeated due to site water toxicity; and
(2) in future WET tests where there are two recent documented incidents of site water toxicity associated with a particular test species. The details of EPA-New England's species-specific, self-implementing policy is provided below.

Case (1): EPA-New England authorizes the use of an alternate dilution water for any WET test repeated due to site water toxicity. Additionally:

- The test must be repeated during the monitoring period specified by the Permit.
- The selected alternate dilution water must have characteristics such as hardness similar to those of the receiving water, and not produce a toxic response.
- A receiving water control must be run in alternate dilution water tests.
- A complete WET test report must be submitted as required by the Permit.

- If the retest documents that the receiving water controls met the TAC, receiving water must be used as diluent in future WET tests.
- If the receiving water controls of the retest failed to meet the TAC, an alternate dilution water may be used in future WET tests using that test organism only after the Permittee submits a written request to EPA and receives written authorization from EPA. (See Case (2) below.)

Case (2): Before an alternate dilution water is used in future WET tests, the Permittee must submit a notification letter to EPA of species-specific, site water toxicity. The notification letter shall be sent electronically to the NPDES Applications Coordinator in EPA Water Division (WD) at the following email address:

R1NPDESReporting@epa.gov

The letter must include:

1. WET data documenting the two recent incidents of site water toxicity to a test species;
2. Information on the alternate dilution water selected for future WET tests including hardness data and a comparison to the receiving water chemistry; and
3. A list of the controls (e.g., site water control, alternate dilution water control, laboratory culture water control, thiosulfate control) that will be run in future WET tests.

Then, EPA-New England will respond in writing to authorize or to deny the use of alternate dilution water in future WET tests. When EPA-New England authorizes the use of an alternate dilution water in future WET tests, it is for the duration of the life of the Permit. At a minimum, EPA will review alternate dilution water authorizations during Permit reissuance.

EPA reserves the right to revoke this guidance at any time and may immediately require the Permittee to use site water as diluent as EPA deems necessary. Such a determination will be provided in writing to the Permittee.

18. **Site Water Controls in Alternate Dilution Water Tests**
Alternate dilution water WET tests shall be run with a minimum of two controls; a site water control and a toxic free alternate dilution water control. Additional controls such as a laboratory culture control or a thiosulfate control must also be run, if necessary. Chemical data of the receiving water and dilution water samples must be included in the report.

19. **Use of Control Data**
When performing statistical analyses, the dilution water control, whether synthetic alternate dilution water or receiving water, must be used for data comparison.

In alternate dilution water tests, the receiving water control results are "report only" data.

If an alternate dilution water control, the thiosulfate control or the lab culture water control fail to meet the minimum TAC, the toxicity test must be repeated using a fresh sample.

20. **Test Results Review**
Toxicity test controls must meet the minimum test acceptability criteria. Additionally, WET test results are reviewed as follows:

a. Concentration-Response Relationship
The WET data concentration-response relationship is reviewed, and Hypothesis Testing and Point Estimate techniques are used to determine test endpoints. A dose-response review must be performed according to Section 10.2.6 of EPA-821-R-02-013 (for freshwater tests) or Section 10.2.6. of EPA-821-R-02-014 (for marine tests) to support the reported test endpoint values and to evaluate the reliability of the WET test results. In most cases, the review will draw in one of the following three conclusions: (1) Results are reliable and reportable; (2) Results are anomalous and require explanation; or (3) Results are inconclusive and a retest with a fresh sample is required.

b. Test Variability
The within-test variability must be evaluated to determine test sensitivity which is a required part of the chronic WET test review. This review is only applicable to the sub-lethal test endpoints such as growth and reproduction that were determined using hypothesis testing. The test sensitivity evaluation is done by examining the calculated Percent Minimum Significant Difference (PMSD).

The PMSD is calculated for test endpoints which was determined using parametric statistical analysis techniques. For cases where a NOEC was determined using non-parametric technique, the PMSD is only calculated to determine test variability and is calculated using a comparable,

parametric statistical analysis technique. As a final step in the evaluation, the calculated PMSD is compared to the upper and lower PMSD bounds shown for freshwater tests in Table 6 of EPA-821-R-02-013, Section 10.2.8.3, p. 52, and for marine tests in Table 6 of EPA-821-R-02-014, Section 10.2.8.3., p. 54.

1.) If the PMSD exceeds the upper bound test variability criterion of Table 6, the test results are considered too highly variable to determine the WET of the discharge at the permitted receiving water concentration (RWC). If the test results indicate that the discharge is not toxic at the RWC, then the test is considered insufficiently sensitive and must be repeated using fresh samples. If the test results indicate that the discharge is toxic at the RWC, the results are considered acceptable and the test does not have to be repeated.

2.) If the PMSD falls below the lower bound test variability criterion of Table 6, the test is highly sensitive, and the percent relative difference (PRD) between the control and each concentration must be calculated and compared to the lower PMSD boundary. If the PRD for the concentration falls below the lower bound, the difference is considered statistically insignificant. If the PRD for the concentration is above the lower bound, then the concentration is considered statistically significant. (See Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the NPDES Program, EPA 833-R-00-003, June 2002, Section 6.4.2.)

3.) When PMSDs fall within the upper and lower bounds of Table 6, the sub-lethal test endpoint determinations shall be reported.

21. **Sign and Certify Each WET Report**

Under 40 C.F.R. §122.41(k), each WET test report submitted to the EPA shall be signed and certified by a person described below or by a duly authorized representative of that person in accordance with 40 C.F.R. §122.22(b)-(d):

(1) for a corporation, by a responsible corporate officer;
(2) for a partnership or sole proprietorship, by a general partner or the proprietor, respectively; and
(3) for a municipality, State, Federal or other public agency, the principal executive officer or ranking elected official.

The Permittee is responsible for the data quality that it reports to EPA. When a report is signed and certified, it documents that the NPDES Permittee is certain that the WET test data submitted meet the Permit requirements for testing and reporting. Please include the following certification statement of 40 C.F.R. §122.22(d) in every report:

WHOLE EFFLUENT TOXICITY TEST REPORT CERTIFICATION (Permittee)

I certify under penalty of law that this document and all ATTACHMENTS were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations.

Executed on _____ _____
[Date] [Authorized Signature]

_____
[Print or Type Name and Title]

_____
[Print or Type the Permittee's Name]

_____
[Print or Type the NPDES Permit No.]

Since the WET test and report check is complicated, you may wish to have your WET laboratory certify the validity of the WET test data and report accuracy to you. Suggested language is given below. Please note that this does not relieve the Permittee from its responsibility to sign and certify the report under 40 C.F.R. §122.41(k).

WHOLE EFFLUENT TOXICITY TEST REPORT CERTIFICATION (Bioassay Laboratory)

I certify under penalty of law that this document and all ATTACHMENTS were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations.

Executed on _____ _____
[Date] [Authorized Signature]

_____
[Print or Type Name and Title]

_____
[Print or Type Name of Bioassay Laboratory]

22. Telephone Contacts

If you have questions, please contact:

Solanch Pastrana-Del Valle, **ECA**D at (617) 918-1746, "pastrana-del-valle.solanch@epa.gov", or
Janet Deshais, **W**ater Division at 617-918-1667, "deshais.janet@epa.gov", or
Jack Paar, **NE** Regional **L**aboratory & **A**pplied Science Division at (617) 918-8604, "paar.jack@epa.gov".