

# EXECUTIVE SUMMARY

## Background

The Federal Water Pollution Control Act, commonly known as the Clean Water Act, was enacted in 1972 with the objective of “*restoring the chemical, physical, and biological integrity of the Nation’s waters.*” Among the U.S. Environmental Protection Agency’s (EPA’s) efforts toward this objective is the National Pollutant Discharge Elimination System (NPDES) program. This program is designed to control toxic discharges, implement water quality standards, and restore waters to “fishable and swimmable” conditions. Point sources that discharge pollutants must do so under the terms and conditions of an NPDES permit. One approach EPA employs to control toxic pollutants under the NPDES permits program is using whole effluent toxicity (WET) controls.

EPA is issuing this document to both address questions raised on WET test method variability and to satisfy a requirement of a July 1998 settlement agreement with litigants for the Western Coalition of Arid States (WestCAS) and Edison Electric Institute et al. This document was developed by an EPA workgroup consisting of EPA’s Office of Water’s (OW) Headquarters, Office of Enforcement and Compliance Assurance, Office of Research and Development, and Regional staff. The document was externally peer reviewed in accordance with EPA’s peer review guidelines. The document addresses WET test method variability by identifying the potential sources of variance associated with WET testing, discusses how to minimize it and, finally, describes how to address it within the NPDES permitting program. The document cites both Agency and external ongoing research on this topic and scientific findings, particularly technical information that support efforts to minimize WET test result variability.

While the document provides recommendations on how to reduce or minimize WET test variability, the document does not supersede current Agency guidance, policy, or regulation, including EPA’s promulgated test methods (40 CFR Part 136), which remain in effect. EPA expects that implementation of the NPDES program and NPDES permits will continue to comply with regulatory requirements and follow applicable EPA guidance and policy.

## Why WET Testing?

Whole effluent toxicity is the aggregate toxic effect of an aqueous sample (e.g., effluent, receiving water) measured directly by an aquatic toxicity test. Aquatic toxicity tests are laboratory experiments that measure the biological effect (e.g., growth, survival, and reproduction) of effluents or receiving waters on aquatic organisms. In aquatic toxicity tests, organisms of a particular species are held in test chambers and exposed to different concentrations of an aqueous sample, for example, a reference toxicant, an effluent, or a receiving water, and observations are made at predetermined exposure periods. At the end of the test, the responses of test organisms are used to estimate the effects of the toxicant or effluent.

Whole effluent toxicity test results are an integral tool in the assessment of water quality. For the protection of aquatic life, the integrated strategy includes the use of three control approaches: the chemical-specific control approach, the WET control approach, and the biological criteria/bioassessment/bioassay approach. The primary advantage of using WET controls over individual, chemical-specific controls is that WET integrates the effects of all chemical(s) in the aqueous sample. Reliance solely on chemical-specific numeric criteria or biological criteria would result in only a partially effective State toxics control program. These toxicity tests therefore must be performed using best laboratory practices, and every effort must be made to enhance repeatability of the test method. This document presents EPA’s approaches to achieve the goals listed below.

## Effect of This Guidance

This document clarifies several issues regarding WET variability and reaffirms EPA's guidance in the *Technical Support Document for Water Quality-Based Toxics Control* (TSD, USEPA 1991a). This document provides NPDES regulatory authorities and all stakeholders, including permittees, with guidance and recommendations on how to address WET variability. EPA's recommendations and conclusions are detailed in Chapter 7, and Appendix C provides sample NPDES permit language reflecting these recommendations.

The most significant recommendation is to use and report the values for the percent minimum significant difference (PMSD) with all WET data results. The minimum significant difference (MSD) represents the smallest difference between the control mean and a treatment mean that leads to the statistical rejection of the null hypothesis (i.e., no toxicity) at each concentration of the WET test dilution series. The MSD provides an indication of within-test variability and test method sensitivity. Using this information, the regulatory authority and permittees can better evaluate WET test results.

This document makes several other recommendations, such as continue to use the TSD statistical approach without adjusting for test method variability, obtain sufficient representative effluent samples, verify effluent toxicity data against reference toxicant data, maintain clear communication between the regulatory authority and permittee, and maintain good laboratory checks and certification programs.

## Three Goals of This Document

This document describes three goals EPA has defined to address issues surrounding WET variability. In addition, the document is intended to satisfy the requirements of a settlement agreement to resolve litigation over rulemaking to standardize WET testing procedures.

1. Quantify the variability of promulgated test methods and report a coefficient of variation (CV) as a measure of test method variability (see Chapter 3 and Appendix A).
2. Evaluate the statistical methods described in the *Technical Support Document for Water Quality-Based Toxics Control* (TSD) for determining the need for and deriving WET permit conditions (see Chapter 6 and Appendix G).
3. Suggest guidance for regulatory authorities on approaches to address and minimize test method variability (Chapter 6). In addition, the document is intended to provide guidance to regulatory authorities, permittees, and testing laboratories on conducting the biological and statistical methods and evaluating test effect concentrations (Chapter 5).

## Data Evaluated

EPA assembled a comprehensive data base to examine variability in the WET test methods from the EPA Regions, several States, and private laboratories, which represent a widespread sampling of typical laboratories and laboratory practices. EPA applied several criteria to the data before they were accepted, including detailed sample information, strict adherence to published EPA WET test methods, and test acceptability criteria (TAC). The resulting data base contains data from 75 laboratories for 23 methods for tests concluded between 1988 and 1999.

## Approach Taken To Evaluate Test Method Variability

The variability that EPA is assessing is associated with replicate tests using reference toxicants and WET testing methods within analytical laboratories. The focus of this guidance is *not* to quantify test variability between laboratories or to quantify the total variability of WET tests conducted on effluents. Rather, the purpose is to quantify method variability within laboratories (repeatability) to enable NPDES

programs to distinguish between variability caused by the testing method and variability associated with toxicity of multiple effluent samples taken from the same facility.

To quantify test method variability within and between laboratories using this data base, EPA examined two key parameters: (1) the effect concentrations [effect concentration (EC25), lethal concentration (LC50), no observed effect concentration (NOEC)] estimated by the test, which are used to derive WET permit limits and evaluate self-monitoring data with those limits; and (2) the minimum significant difference (MSD), which summarizes the variability of organism responses at each test concentration within an individual test. The MSD represents the smallest difference that can be distinguished between the response of the control organisms and the response of the organisms exposed to the aqueous sample. The MSD provides an indication of within-test variability and test method sensitivity.

## **Principal Conclusions**

The principal conclusions of this document follow.

### ***Evaluation of Test Method Variability***

- Comparisons of WET method precision with method precision for analytes commonly limited in NPDES permits clearly demonstrate that the variability of the promulgated WET methods is within the range of variability experienced in other types of analyses. Several independent researchers and studies also have concluded that method performance improves when prescribed methods are followed closely by experienced analysts (Section 4.3).
- This document provides interim CVs for promulgated WET methods in Appendix A, Tables A-1 (acute methods) and A-2 (chronic methods), pending completion of between-laboratory studies, which may affect these interim CV estimates.

### ***Evaluation of Approach To Incorporate Test Method Variability***

- EPA's TSD presents guidance for developing effluent limits that appropriately protect water quality, regarding both effluent variability and analytical variability, provided that the WET criteria and waste load allocation (WLA) are derived correctly (Section 6 and Appendix G).
- EPA's analysis of data gathered in the development of this document indicates that the TSD approach appropriately accounts for both effluent variability and method variability. EPA does not believe a reasonable alternative approach is available to determine a factor that would discount the effects of method variability using the TSD procedures, because the approach would not ensure adequate protection of water quality (Section 6.1.1 and Appendix G).

### ***Development of Guidance to Regulatory Authorities***

- EPA recommends that regulatory authorities implement the statistical approach as described in the TSD to evaluate effluent for reasonable potential and to derive WET limits or monitoring triggers (Section 6.1 and Appendix G).
- EPA recommends that regulatory authorities calculate the facility-specific CVs using point estimate techniques to determine the need for and derive a permit limit for WET, even if self-monitoring data are to be determined using hypothesis testing techniques, for example, to determine a "no effect" concentration ("NOEC"). This document describes such facility-specific calculations (Section 3.4.1 and 6.2).

## Additional Recommendations and Guidance

This document also provides recommendations and guidance on minimizing variability in three specific areas in order to generate sound WET test results: (1) obtaining a representative effluent sample; (2) conducting the toxicity tests properly to generate the biological endpoints; and (3) conducting the appropriate statistical analysis to obtain defensible effect concentrations (EC25, LC50, NOEC). If these recommendations are addressed, the reliability of the test endpoint values should improve.

- **Regulatory Authorities:** Design a sampling program that collects representative effluent samples to fully characterize effluent variability for a specific facility over time (Sections 6.1.3 and 6.2).
- **Regulatory Authorities:** Ensure proper application of WET statistical procedures and test methods (Sections 5.2 through 5.5).
- **Regulatory Authorities:** Incorporate both the upper and lower bounds using the percent minimum significant difference (PMSD) to control and to minimize within-test method variability and increase test sensitivity. To achieve the PMSD upper bound, either the replication should increase or within-test method variability should decrease, or both (Section 6.4 and Table 3-6).
- **Testing Laboratories:** Encourage WET testing laboratories to maintain control charts for PMSD and the control mean and report the PMSD with all WET test results (Section 5.3.1.1).
- **Regulatory Authorities:** Participate in the National Environment Laboratory Accreditation Program and routine performance audit inspections to evaluate laboratory performance (Section 5.3.1.1).
- **Regulatory Authorities:** Incorporate EPA's guidance on error rate assumption adjustments, concentration-response relationships, confidence intervals, acceptable dilution waters, how to block by parentage for the chronic *Ceriodaphnia dubia* test, and control of pH drift (USEPA 2000a).