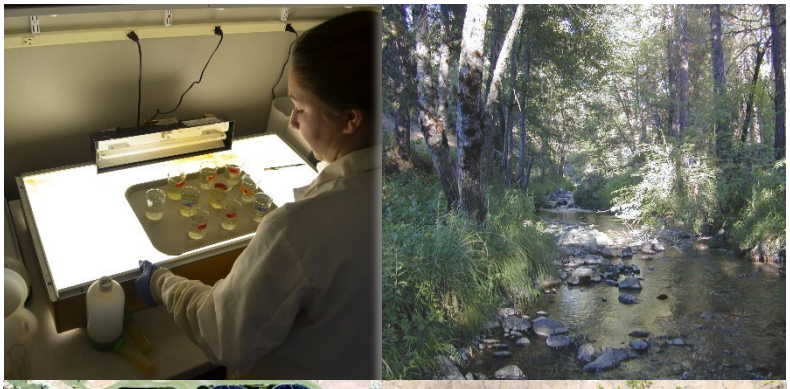
U.S. Environmental Protection Agency

National Pollutant Discharge Elimination System Whole Effluent Toxicity Permit Writers' Manual



Office of Wastewater Management

Water Permits Division

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United States
Environmental Protection Agency

Office of Water
Washington, DC

United States
Environmental Protection Agency

National Pollutant Discharge Elimination System
Whole Effluent Toxicity Permit Writers' Manual

This guidance was developed by staff within the U.S. Environmental Protection Agency's (EPA's) Office of Wastewater Management and addresses Whole Effluent Toxicity (WET) in wastewater discharge permits under the National Pollutant Discharge Elimination System (NPDES). NPDES permit development is governed by existing requirements of the Clean Water Act (CWA) and the EPA NPDES implementing regulations. CWA provisions and regulations contain legally binding requirements. This document does not substitute for those provisions or regulations. Recommendations in this guidance are not binding; the NPDES permitting authority may consider other approaches consistent with the CWA and EPA regulations. When EPA makes a permitting decision, it will make each decision on a case-by-case basis and will be guided by the applicable requirements of the CWA and implementing regulations, taking into account comments and information presented at that time by interested persons regarding the appropriateness of applying these recommendations to the situation. This guidance incorporates, and does not modify, existing EPA policy and guidance on the use of WET in NPDES permits. EPA may change this guidance in the future.

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Acronyms and Abbreviations

°C	degrees Celsius
7Q10	lowest consecutive 7-day average stream flow during any 10-year period
ACR	acute-to-chronic ratio
AML	average monthly limit
AO	administrative order
ATE	acute toxicity endpoint
ATP	alternate test procedure
AWL	average weekly limit
BPJ	best professional judgment
CCC	Criterion Continuous Concentration
CD	critical dilution
C_e	concentration of pollutant in effluent
CFR	Code of Federal Regulations
cfs	cubic feet per second
CMC	criterion maximum concentration
C_r	concentration in receiving water
CRR	concentration-response relationship
CTE	chronic toxicity endpoint
CV	coefficient of variation
CWA	Clean Water Act
DMR	discharge monitoring report
DMR-QA	discharge monitoring report-quality assurance
EC_{50}	effluent concentration causing 50% effect
EDTA	ethylenediaminetetraacetic acid
EPA	U.S. Environmental Protection Agency
FR	Federal Register
HSB	hypersaline brine
IC_{25}	25% inhibition concentration
IC_{50}	50% Inhibition Concentration
ICIS	Integrated Compliance Information System
ICIS-NPDES	Integrated Compliance Information System for NPDES
IP	implementation procedure
IWC	in-stream waste concentration
LC_{50}	50% lethal concentration
LOAEC	lowest observed adverse effect concentration
LOEC	lowest observed effect concentration
LTA	long-term average
LTA_a	long-term average acute
$LTA_{a,c}$	acute to chronic long-term average, expressed in chronic units
LTA_c	long-term average chronic
MDL	maximum daily limit
mgd	million gallons per day
MSD	minimum significant difference
NOAEC	no observed adverse effect concentration
NOEC	no observed effect concentration
NOV	notice of violation

NPDES	National Pollutant Discharge Elimination System
OECA	Office of Enforcement and Compliance Assurance
OWM	Office of Wastewater Management
PMSD	percent minimum significant difference
POTWs	publicly owned treatment works
ppt	parts per thousand
QA	quality assurance
QC	quality control
Q_e	effluent flow
RP	reasonable potential
RMD	regulatory management decision
RPA	reasonable potential analysis
RPM	rotations per minute
RPMF	reasonable potential multiplying factor
RWC	receiving water concentration
SD	standard deviation
SNC	significant noncompliance
TAC	Test Acceptability Criteria
TBEL	technology-based effluent limit
TIE	toxicity identification evaluation
TMDL	total maximum daily load
TRC	total residual chlorine
TRE	toxicity reduction evaluation
TSD	EPA's 1991 Technical Support Document for Water Quality-based Toxics Control
TST	test of significant toxicity
TU	toxic unit
TU_a	toxic unit-acute
TU_c	toxic unit-chronic
WET	whole effluent toxicity
WLA	wasteload allocation
WLA_a	wasteload allocation-acute
WLA_c	wasteload allocation-chronic
$WLA_{a,c}$	wasteload allocation-acute from chronic
WQBEL	water quality-based effluent limit
WQC	water quality criteria
WQP	Water Quality Portal
WQS	water quality standards
YCT	yeast, Cerophyll, and TetraMin®
ZID	zone of initial dilution


Ceriodaphnia dubia

1 Introduction to the Manual

This section provides a brief discussion of the regulatory and technical basis for the U.S. Environmental Protection Agency's (EPA's) National Pollutant Discharge Elimination System (NPDES) Whole Effluent Toxicity (WET) program, describes the purpose of this National Pollutant Discharge Elimination System Whole Effluent Toxicity Permit Writers' Manual (hereafter referred to as "the manual") and what it covers, and provides additional resources for use when implementing the NPDES WET program.

The Clean Water Act (CWA) was enacted in 1972 with the objective of "restoring the chemical, physical, and **biological integrity** of the Nation's waters" (emphasis added). Among EPA's efforts towards achieving that objective has been implementing the NPDES permit program. The NPDES permit program addresses water pollution by regulating point sources that discharge pollutants to waters of the United States. It is designed to control pollutants, including toxics, in permitted discharges, implement aquatic life protection water quality standards (WQS), and restore and maintain the "fishable and swimmable" designated beneficial uses in waters of the United States.

In the early 1980s, chemical, biological, and toxicological data indicated that, despite efforts to control pollutants through technology-based effluent limits (TBELs), some NPDES permittees were discharging effluents with sufficient toxicity to result in adverse impacts on aquatic life. Further reductions in toxic discharges were necessary to achieve compliance with states', territories', or authorized Tribes' aquatic life protection WQS that prohibit the discharge of toxic pollutants in toxic amounts or otherwise provide for the maintenance and propagation of an aquatic life community.

In response, EPA developed a policy to reduce or eliminate toxic discharges in toxic amounts. The Policy for the Development of Water Quality-Based Permit Limitations for Toxic Pollutants (49 FR 9016, March 9, 1984) introduced EPA's integrated toxics control program under the NPDES program. This policy consists of both chemical-specific and biological analytical methods for the assessment and reduction of toxic discharges. In 1989, EPA promulgated regulations in Title 40 of the Code of Federal Regulations (CFR) § 122.44(d) providing that NPDES permits are to include conditions more stringent than TBELs requirements, when necessary, to achieve WQS including applicable narrative criteria for water quality. Permit conditions established under 40 CFR § 122.44(d) are referred to as water quality-based effluent limits (WQBELs). The NPDES regulations provide that:

- Permits must specify monitoring requirements, including type, intervals, and frequency sufficient to yield data representative of the monitored activity [40 CFR § 122.48(b)];
- When the NPDES authority determines that a discharge causes, has the reasonable potential to cause, or contributes to an in-stream excursion above the numeric criterion for WET, the permit must contain effluent limits for WET [40 CFR § 122.44(d)(1)(iv)]; and
- When the NPDES authority determines that a discharge causes, has the reasonable potential to cause, or contributes to an in-stream excursion above a narrative criterion within an applicable

state's, territory's, or authorized Tribe's WQS, the permit must contain effluent limits for WET [40 CFR § 122.44(d)(1)(v)]. WET limits are not necessary if the NPDES permit authority determines that chemical-specific limits for the effluent are sufficient to attain and maintain applicable numeric and narrative state WQS [40 CFR § 122.44(d)(1)(v)].

EPA developed the Technical Support Document for Water Quality-based Toxics Control (TSD) to support implementation of EPA's NPDES regulations (USEPA 1991a). The TSD provides guidance on water quality NPDES implementation issues, including for chemical and biological approaches.

EPA's approved toxicity tests as specified in 40 CFR Part 136, Table 1A are used to evaluate reasonable potential (RP) for levels of toxicity above the state's, territory's, or authorized Tribe's aquatic life protection criteria that are part of WET WQS (88 FR 29496, May 5, 2023) and for establishing and determining compliance with the appropriate NPDES WET permit limit. EPA has recommended WET water quality criteria (WQC), which are discussed in Section 5 of this manual. WET test results also are considered for purposes of enforcement determinations in which NPDES WET limits have been exceeded or other permit limitations and/or requirements are not met under the NPDES permit program.

On July 7, 1994, EPA issued a national NPDES WET policy on NPDES effluent limitations for the protection of aquatic life (USEPA 1994). This EPA policy contains eight statements of policy that reaffirm EPA's strong continuing commitment to the existing CWA provisions and water quality permit regulations. EPA's (1994) statements of policy include information based on WET controls; evaluating RP for WET [40 CFR § 122.44(d)(1)]; setting NPDES WET limits, monitoring requirements, and compliance schedules where appropriate; special considerations regarding ammonia and chlorine with regard to WET testing; and applicability of WET controls for publicly owned treatment works (POTWs).

This manual reiterates EPA's eight statements of policy and refers to other EPA guidance and regulatory provisions that provide further clarification for NPDES authorized permit authorities (i.e., EPA regions and states, territories, and authorized Tribes). When issuing or reissuing an NPDES permit, the NPDES permit authority evaluates whether a discharge causes, has a reasonable potential to cause, or contributes to an in-stream excursion above a numeric or narrative criterion within an applicable state's, territory's, or authorized Tribe's WQS and, where appropriate, establishes permit limits for WET [40 CFR § 122.44(d)(1)(iii-v)] for lethal and/or sublethal aquatic life toxic effects. On October 26, 1995, EPA promulgated toxicity test methods and added them to the list of analytical methods approved under Section 304(h) of the CWA [40 CFR Part 136] for use under the NPDES program. EPA's toxicity test methods were subsequently challenged, and, under a settlement agreement, EPA conducted an interlaboratory variability study, which evaluated EPA's toxicity test methods (USEPA 2001a, 2001b). In addition, as part of the settlement agreement, EPA issued two documents in 2000—an NPDES WET test method variability document, Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System (USEPA 2000b), and an EPA toxicity test methods document, Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing [40 CFR Part 136; (USEPA 2000a)]. In November 2002, EPA ratified most of the challenged toxicity test methods based on the results of its interlaboratory variability study (67 FR 69951, November 19, 2002). This action also revised some of the EPA toxicity test methods to improve performance and increase statistical confidence in the toxicity test results.

In 2010, EPA issued guidance regarding the Test of Significant Toxicity (TST), which is another statistical approach that the NPDES permit writer can use to analyze valid toxicity data for industrial, municipal,

and ambient samples. EPA provided two companion TST documents: National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document, written for the NPDES permit writer for implementing the TST statistical approach under the NPDES WET program (USEPA 2010b), and National Pollutant Discharge Elimination System Test of Significant Toxicity Technical Document (USEPA 2010c), which is a more technically detailed document that provides an in-depth statistically detailed discussion of the design of the TST statistical approach. The TST statistical approach can be used to evaluate RP for both acute and short-term chronic valid toxicity data, expressing NPDES WET limits, and determining compliance with NPDES WET limits based on the TST statistical approach.

1.1 Purpose of EPA's NPDES WET Permit Writers' Manual

The manual provides the NPDES permit writer with information and resources that support writing NPDES permit WET requirements. The manual was designed for the NPDES permit writer, whether new to or experienced with the NPDES program, or anyone else interested in learning about the regulatory, programmatic, and technical aspects of developing and implementing WET provisions in an NPDES permit. It summarizes recommendations and requirements for developing NPDES WET permit requirements based on EPA guidance, policy, and regulations, as well as the CWA. It provides recommendations and requirements for appropriate implementation approaches and describes relevant technical and programmatic details based on EPA guidance or policy for developing NPDES WET permit requirements. While EPA's NPDES policy and guidance for WET are applicable in many cases, EPA recognizes that each NPDES permit authority will tailor specific aspects of their NPDES WET requirements and implementation procedures (IPs) to address site-specific circumstances (Appendix A).

The manual is not intended to be a stand-alone reference document. Rather, it builds upon the NPDES Permit Writers' Manual (USEPA 2010a) and should be integrated into, as necessary, EPA's, states', territories', and authorized Tribes' regulations, policy, and guidance applicable to specific types of dischargers and site-specific circumstances (USEPA 2020a). The manual cites and references those resources throughout the text and provides hyperlinks to them in Section 8 for easy access. Appendix B provides a glossary.

1.2 What It Covers

This manual discusses many of the facets of applying WET in NPDES permits in the following sections:

Section 1. Introduction to the Manual: An overview of the NPDES WET program based on the CWA, EPA regulations, policy, and guidance. In addition, it covers the purpose of the document, as well as what the document covers and some additional WET resources.

Section 2. NPDES WET Program Background: An overview of how and why WET requirements are used in NPDES permits.

Section 3. Toxicity Testing: A brief description of EPA's different toxicity test methods and statistical approaches for analyzing valid toxicity test data used in the NPDES permit program.

Section 4. NPDES Permit Conditions for WET Monitoring: Overview of NPDES permit compliance monitoring and assessment conditions, including, but not limited to, dilution water, toxicity test concentration series, types of toxicity tests, quality assurance (QA) and quality control (QC), reviewing WET toxicity test reports, statistical test endpoints, and WET diagnostic

approaches (e.g., toxicity reduction evaluations [TREs] and toxicity identification evaluations [TIEs]).

Section 5. Reasonable Potential Analysis for Evaluating Need for NPDES WET Permit Limits: A review of EPA's regulations, guidance, and approaches used to conduct reasonable potential analyses (RPAs) for WET.

Section 6. Developing NPDES WET Permit Limits: A brief description of the recommended steps necessary to develop WET permit limits.

Section 7. Evaluating NPDES Compliance for WET and Enforcement Considerations: An overview of the required and recommended information to include in a WET test laboratory report as well as the required and recommended approaches for reporting of WET test results to the NPDES permit authority. A brief overview of recommendations for reviewing WET monitoring reports by both the permittee and the NPDES permit authority and a review of EPA's NPDES WET enforcement policies and procedures.

Section 8. References

1.3 Additional WET Resources

NPDES permit writers can use these EPA online resources to assist them in implementing WET in NPDES permits:

- NPDES Permit Limits - Whole Effluent Toxicity
  - https://www.epa.gov/npdes/permit-limits-whole-effluent-toxicity-wet
- Whole Effluent Toxicity Test Methods
  - https://www.epa.gov/cwa-methods/whole-effluent-toxicity-methods
- EPA West Coast Short-term Chronic Marine and Estuarine Whole Effluent Toxicity Test Methods
  - https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NERL&dirEntryId=46584
- NPDES Training–Whole Effluent Toxicity Training and Videos
  - https://www.epa.gov/npdes/npdes-training
- EPA HQ NPDES Whole Effluent Toxicity Spreadsheet
  - https://www.epa.gov/npdes/whole-effluent-toxicity-wet-npdes-spreadsheet

2 NPDES WET Program Background

This section provides background information on the purpose and importance of the NPDES WET program. Along with other goals, CWA Section 101(a)(2) and (3) states that:

> It is the national goal that wherever attainable, an interim goal of water quality which provides for the protection and propagation of fish, shellfish, and wildlife and provides recreation in and on the water be achieved by July 1, 1983.
>
> It is the national policy that the discharge of toxic pollutants in toxic amounts be prohibited.

The CWA "*biological integrity*" objective provision provides support to EPA's NPDES WET program. The CWA goals of "*protection and propagation*" and the CWA's national policy that the "*discharge of pollutants in toxic amounts be prohibited*" provide a basis for EPA's acute and short-term chronic endpoints, including chronic sublethal (e.g., growth, reproduction), and implementation of WET endpoints under the NPDES WET program. EPA has pursued the CWA national goal by implementing the water quality standards program and the NPDES permit program. These programs have adopted an "integrated" strategy of water quality-based toxics control that includes three approaches: chemical-specific testing, toxicity testing (e.g., WET testing), and biological criteria / bioassessment as discussed in EPA's TSD (Sec 1.5.3). CWA Section 301 prohibits the discharge of any pollutant into waters of the United States unless authorized by another provision of the Act, including pursuant to an NPDES permit issued under CWA Section 402 (67 FR 69952; November 19, 2002). Toxicity testing and NPDES WET permit requirements, in addition to chemical-specific controls and biological monitoring, are used to implement the goals of the CWA, including protection of aquatic organisms in receiving waters.

States, territories, and authorized Tribes are encouraged to define their numeric or narrative WQS to include chemical-specific criteria, criteria for WET, and biological criteria. Some states, territories, and authorized Tribes have provided numeric criteria for WET (e.g., 0.3 toxic unit-acute [TU_a] and 1.0 toxic unit-chronic [TU_c]), while others have relied on narrative criteria (e.g., no toxics in toxic amounts). In NPDES permits, WQBELs help achieve and maintain these WQS.

2.1 Integrated Approach to Controlling Toxics

EPA's integrated strategy for water quality-based toxics control uses a three-part approach consisting of chemical-specific, WET, and bioassessment evaluations as a means of protecting aquatic life (USEPA 1991b).[1] Agency policies and guidance further clarify and recommend that states, territories, and authorized Tribes use chemical-specific, toxicity, and biological measurements and criteria to monitor and protect designated uses for receiving waters (USEPA 1997).

EPA's independent application policy addresses how these three assessment approaches should be used to make water quality management decisions based on the assessment approach that provides the most

[1] Surface toxics control regulation, 54 FR 23868, June 2, 1989.

protection to aquatic life and aquatic life uses (USEPA 1997). The chemical-specific approach to aquatic life toxics control relies on numeric WQC in states', territories', and authorized Tribes' WQS and interpretations of their narrative WQC to assess and control specific toxicants individually. The whole effluent toxicity approach to toxics control involves the use of toxicity tests and WQC for the parameter "toxicity" to assess and control the aggregate toxicity of effluents. Bioassessments for states', territories', and authorized Tribes' biocriteria are "post-impact" assays of possible impacts on aquatic life communities. Each of these three approaches has its own limitations and advantages, and thus, the exclusive use of one approach alone might not adequately comply with the CWA requirement to protect aquatic life. Reliance solely on chemical-specific numeric criteria or bioassessments may result in an ineffective toxics control program.

The advantages and disadvantages of each approach, independent applicability, and how the integrated approach creates an effective toxics control program are outlined in EPA's 1997 memorandum for implementing EPA's 1989 regulation (see also USEPA 1991b).

Toxicity tests (e.g., WET tests) measure the aggregate toxic effect of an aqueous sample (e.g., a reference toxicant, an effluent, or an ambient sample from a receiving water) on an aquatic species. The two primary advantages of using WET permit controls over individual, chemical-specific permit controls are (1) WET tests evaluate the total effects (additive and synergistic) of all chemicals in the aqueous sample; and (2) while EPA has established aquatic life criteria for a relatively small number of chemical-specific pollutants (61), EPA's toxicity tests can measure toxicity caused by other compounds for which EPA has not yet established chemical-specific numeric criteria to protect aquatic life or approved parameter-specific analytical test methods. The primary advantage of WET permit controls over the bioassessment approach is that bioassessments evaluate impacts on the aquatic community that have already occurred, while NPDES WET requirements can be used to indicate possible toxicity (predictive), which can be used towards the development of permit controls that could prevent adverse impacts.


Pimephales promelas

3 Toxicity Testing

This section provides a summary of the applied science of aquatic toxicology as it pertains to EPA's NPDES WET program and EPA's toxicity tests at 40 CFR 136, Table 1A and based on EPA final toxicity test method guidance (i.e., EPA 1995 short-term chronic marine and estuarine west coast toxicity test methods).

Aquatic toxicity tests are laboratory bioassays that measure biological effects (e.g., growth, survival, or reproduction) of an aqueous sample on EPA toxicity test species of aquatic organisms. Organisms of a particular species and age are held in test chambers and exposed to different concentrations of an aqueous sample. Observations are then made and recorded on laboratory data sheets for predetermined toxicity test exposure periods established under EPA's toxicity test methods. At the end of the toxicity test, the responses of the test organisms are assessed. The results of the toxicity test are used to estimate possible adverse effects of an aqueous sample (e.g., ambient or effluent) on toxicity test species used as surrogates for the aquatic organisms in receiving waters.

3.1 Toxicity Test Methods Used in the NPDES Permit Program

40 CFR § 122.41(j)(4) and 40 CFR § 136.1(a) provide that monitoring of NPDES permit applications and reports required by NPDES permits must be conducted according to the EPA test procedures approved under 40 CFR Part 136. 40 CFR Part 136 recognizes three classes of test procedures:

- EPA test procedures under 40 CFR Part 136
- EPA test procedures not identified under 40 CFR Part 136 but allowed for use in NPDES permits under EPA regulations in 40 CFR § 122.21(j)(5)(viii)
- Alternate test procedures approved by EPA under either 40 CFR § 136.4 or 40 CFR § 136.5

3.1.1 EPA-Approved Toxicity Test Methods in 40 CFR Part 136

In 1995, EPA published its final guidelines adding WET testing to the list of EPA-approved methods in 40 CFR § 136.3, Table 1A under the CWA (60 FR 53529; October 16, 1995). The aquatic toxicity test methods were designed specifically for measuring acute and short-term chronic toxicity of effluents and receiving water. These test methods employed a suite of standardized freshwater, marine, and estuarine plants, invertebrates, and vertebrates to estimate acute and short-term chronic toxicity of effluents and ambient waters. The following toxicity test method manuals provide the specific procedures for conducting the EPA-approved toxicity tests:

- Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (USEPA 2002a)
- Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition (USEPA 2002b)
- Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, Third Edition (USEPA 2002c).

Accordingly, using these toxicity test methods and adhering to the required toxicity test procedures specified therein are necessary when implementing WET under the NPDES program (Table 3-1).

Table 3-1. *EPA-approved acute and short-term chronic toxicity test methods listed in 40 CFR § 136.3, Table 1A (USEPA 2002a, 2002b, 2002c).*

Promulgated Test Method Number	Matrix	Test Description	Endpoint(s)
2000.0	Freshwater	Fathead minnow, *Pimephales promelas*, acute toxicity test	Mortality
2002.0	Freshwater	Water flea, *Ceriodaphnia dubia* acute toxicity test	Mortality
2004.0	Estuarine/Marine	Sheepshead minnow, *Cyprinodon variegatus*, acute toxicity test	Mortality
2006.0	Estuarine/Marine	Silverside, *Menidia beryllina, Menidia menidia,* and *Menidia peninsulae*, acute toxicity test	Mortality
2007.0	Estuarine/Marine	Mysid, *Americamysis bahia* (formerly *Mysidopsis bahia*), acute toxicity test	Mortality
2019.0	Freshwater	Rainbow trout, *Oncorhynchus mykiss*, and brook trout, *Salvelinus fontinalis*, acute toxicity test	Mortality
2021.0	Freshwater	Water flea, *Daphnia pulex* and *Daphnia magna*, acute toxicity test	Mortality
1000.0	Freshwater	Fathead minnow, *Pimephales promelas*, larval survival and growth short-term chronic toxicity test	Survival and growth (weight)
1001.0	Freshwater	Fathead minnow, *Pimephales promelas*, embryo-larval survival and teratogenicity short-term chronic toxicity test	Combined mortality (dead and deformed organisms)
1002.0	Freshwater	Daphnid, *Ceriodaphnia dubia*, survival and reproduction short-term chronic toxicity test	Survival and reproduction
1003.0	Freshwater	Green alga, *Raphidocelis subcapitata* (formerly *Selenastrum capricornutum*), growth short-term chronic toxicity test	Growth (cell concentration)
1004.0	Estuarine/Marine	Sheepshead minnow, *Cyprinodon variegatus*, larval survival and growth short-term chronic toxicity test	Survival and growth (weight)
1005.0	Estuarine/Marine	Sheepshead minnow, *Cyprinodon variegatus*, embryo-larval survival and teratogenicity short-term chronic toxicity test	Percent hatch; percent larvae dead or with debilitating morphological and/or behavior abnormalities (e.g., gross deformities; curved spine; disoriented behavior, abnormal swimming behavior; surviving normal larvae from original embryos)
1006.0	Estuarine/Marine	Inland silverside, *Menidia beryllina*, larval survival and growth short-term chronic toxicity test	Survival and growth (weight)
1007.0	Estuarine/Marine	Mysid, *Americamysis bahia* (formerly *Mysidopsis bahia*), survival, growth, and fecundity short-term chronic toxicity test	Survival and growth; egg development
1008.0	Estuarine/Marine	Sea urchin, *Arbacia punctulata*, fertilization short-term chronic toxicity test	Fertilization of sea urchin eggs
1009.0	Estuarine/Marine	Red macroalga, *Champia parvula*, sexual reproduction short-term chronic toxicity test	Reduction in cystocarp production compared to controls

Note: Words such as "must" or "shall" in these toxicity test methods manuals indicate a required test procedure. When these toxicity test method manuals use terms such as "may" or "should," it is a recommended procedure or condition that the NPDES permit writer or laboratory can take into consideration (USEPA 1997).

3.1.2 EPA-Approved Toxicity Test Methods Not in 40 CFR Part 136

There may be permitted discharges that require limitations using toxicity test procedures not yet promulgated under 40 CFR Part 136, if the toxicity test species listed in Table 1A will not be protective of the applicable WET WQS and receiving water's designated use. Under 40 CFR § 122.41(j)(4) and 122.44(i)(1)(iv)(B), permit writers may include, through permit proceedings, limitations that require the use of toxicity test procedures that are not promulgated in 40 CFR Part 136. EPA also may include such limitations in accordance with the provisions prescribed at 40 CFR § 401.13, ''Test Procedures for Measurements.'' Permits may include, for example, effluent limitations for WET using standardized testing procedures other than those in 40 CFR Part 136 that are EPA-approved for use in NPDES permits (see Section 3.1.3). In such cases, using the particular test species and toxicity test protocol would remain subject to challenge on a case-by-case basis in NPDES permit proceedings. Unless, however, a NPDES state, territory, or authorized Tribe conducted a rulemaking to standardize a particular toxicity testing procedure to be applicable within its NPDES permits.

In Alaska, California, Hawaii, Oregon, Washington, and the Pacific Island territories, POTWs are exempt from the EPA requirement to use the EPA-promulgated short-term chronic marine toxicity test methods for NPDES permit application data and instead can use "alternative guidance as directed by the NPDES permit authority" as specified in 40 CFR § 122.21(j)(5)(viii). Additionally, 40 CFR § 122.21(g)(7)(i) and 40 CFR § 122.44(i)(1)(iv)(B) allow for use of toxicity test methods not approved under 40 CFR Part 136 when testing methods are not listed in Table 1A in 40 CFR Part 136, such as for toxicity testing of West Coast species. *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms* (USEPA 1995b) are an example of alternative EPA toxicity test methods for West Coast ecosystems in the U.S. Tables 3-2 and 3-3 provide a list of alternative EPA West Coast toxicity test methods.

3.1.3 EPA Guidance on Non-Promulgated Toxicity Test Methods

Additionally, use of any test species or test conditions other than those described in EPA's 2002 toxicity test methods manuals (USEPA 2002a, 2002b, 2002c) and referenced in Table 1A in 40 CFR § 136.3, or the 1995 West Coast toxicity test methods [40 CFR § 122.21(j)(5)(viii); (USEPA 1995b)] (see Section 3.1.2) is considered a major modification to the toxicity test method and subject to application and approval through EPA's alternate test procedures (ATPs). Under 40 CFR § 136.4, an ATP can be approved by EPA HQ's Office of Science and Technology for nation-wide application, and, under 40 CFR § 136.5, an ATP can be approved by an EPA region for limited use (e.g., for a specific discharger or the states, territories, and authorized Tribes within the respective EPA region).

For Pacific coastal waters, for which states, territories, or authorized Tribes have developed culturing and toxicity testing methods for indigenous species other than EPA's West Coast short-term chronic marine toxicity test methods, data comparing the sensitivity of the substitute species and one or more of the recommended species should be obtained in side-by-side toxicity tests with reference toxicants and/or effluents to determine if the test species selected are at least as sensitive as the recommended test species (USEPA 1995b).

Table 3-2. *EPA 1995 West Coast short-term chronic marine and estuarine toxicity test methods (USEPA 1995b)*.*

Matrix	Test Type	Endpoint(s)
Estuarine/Marine	Topsmelt, *Atherinops affinis*, 7-d larval growth and survival test method	Survival and growth
Estuarine/Marine	Mysid, *Holmesimysis costata*, Survival and growth test method	Survival and growth
Estuarine/Marine	Pacific oyster, *Crassostrea gigas*, and Mussel, *Mytilus sp.*, Embryo-larval development test method	Survival and normal shell development
Estuarine/Marine	Red abalone, *Haliotis rufescens*, Larval development test method	Normal shell development
Estuarine/Marine	Purple urchin, *Strongylocentrotus purpuratus*, and sand dollar, *Dendraster excentricus*, Larval development test method	Normal development, mortality can be included
Estuarine/Marine	Purple Urchin, *Strongylocentrotus purpuratus*, and Sand Dollar, *Dendraster excentricus*, Fertilization Test Method	Fertilization of eggs
Estuarine/Marine	Giant Kelp, *Macrocystis pyrifera*, Germination and Germ-Tube Growth Test Method	Germination and germ-tube length

Note: * - Federal regulations exempt POTWs in certain West Coast states from using 40 CFR Part 136 methods for permit applications [40 CFR § 122.21(j)(5)(viii)] and allow for use of alternate methods for industrial permit applications and monitoring [40 CFR § 122.21(g)(7)(i); 40 CFR § 122.44(i)(1)(iv)(B)].

Table 3-3. *Additional EPA-approved marine and estuarine acute toxicity test method (USEPA 2002a)**

Matrix	Test Type	Endpoint(s)
Estuarine/Marine	West Coast mysid, *Holmesimysis costata*, Acute toxicity test	Mortality

Note: * - This method is specific to Pacific Coast waters and is not listed in 40 CFR § 136.3 for nationwide use. This method had been proposed but not approved in 40 CFR § 136.3.

3.2 Types of Toxicity Tests

There are two types of toxicity tests: acute and short-term chronic. An "acute toxicity test" is usually conducted over a short period of time, generally 96 hours or less, and the biological test endpoint measured is mortality or lethality (USEPA 2002a). The statistical test endpoint for an acute toxicity test is often expressed as the percent of aqueous sample that is lethal to 50% of the exposed test organisms (LC_{50}) or a no observed adverse effect concentration (NOAEC). A "short-term chronic toxicity test" is usually conducted during a critical life phase of the organism and the biological test endpoints measured are mortality or immobility along with sublethal effects (e.g., growth, reproduction, development, or fertilization). A short-term chronic test can occur over a matter of minutes, hours, or days (e.g., 40 minutes, 48 hours, or 7 days), depending on the species tested and biological test endpoint measured (USEPA 2002b, 2002c; USEPA 1995b). The statistical test endpoint for a short-term chronic toxicity test is often expressed as an IC_{25} (i.e., the percent of aqueous sample that is lethal or sublethal to 25% of the exposed test organisms) or no observed effect concentration (NOEC).

3.2.1 Acute vs. Chronic Toxicity Testing in NPDES Permit Monitoring

The decision as to whether an acute or chronic toxicity test should be required for a permitted discharge is dependent on whether the applicable WET WQS has criteria for acute, chronic, or both acute and chronic toxicity. After identifying the applicable WET WQS, the NPDES permit authority should consider which toxicity test would yield data representative of the permitted discharge [40 CFR § 122.48(b)] after taking into consideration any authorized mixing within the receiving water (USEPA 1991a). The choice to conduct acute or chronic toxicity is dependent on the applicable WET WQS, the test type that would yield data representative of the permitted discharge and may depend on the background receiving water flow and the discharge flow as discussed below.

EPA recommends that an NPDES discharger conduct acute toxicity testing if the dilution of the effluent is greater than 1,000 parts receiving water to one part effluent (1,000:1) or if the in-stream waste concentration (IWC) is less than or equal to 0.1% effluent at the edge of the mixing zone in the receiving water [40 CFR § 122.21(j)(5)(v)(A)].

EPA recommends that an NPDES discharger conduct either acute or chronic toxicity testing if the dilution of the effluent falls between 100:1 and 1,000:1 (IWC greater than 0.1 but less than or equal to 1.0% effluent) when fully mixed [40 CFR § 122.21(j)(5)(v)(B)], and chronic toxicity testing if the dilution of the effluent falls below 100:1 (IWC greater than or equal to 1.0% effluent) when fully mixed [40 CFR § 122.21(j)(5)(v)(C); (USEPA 1991a)].

Although EPA's toxicity test methods (USEPA 1995a, 2002b, 2002c) indicate that daily observations in a chronic test make it possible to also calculate acute toxicity endpoints, EPA does not recommend acquiring acute toxicity endpoints from chronic tests due to differences in the toxicity test design (e.g., number of test organisms and replicates) and test conditions of the acute versus chronic tests (e.g., residual chronic test food in test chambers; versus acute test organisms are fed prior to test chamber renewals).

3.2.2 Choice of Test Species Used for Toxicity Testing

For initial NPDES characterization, EPA recommends that, if acute testing is necessary, at least two EPA-approved toxicity test species that are representative of different trophic levels—such as a vertebrate (e.g., a fish) and an invertebrate (e.g., water flea)—should be used to evaluate the acute toxicity of a minimum of three aqueous sample events using EPA toxicity tests (USEPA 2010a).

For the initial NPDES characterization, EPA recommends that, if chronic testing is necessary, three EPA-approved test species—an invertebrate, a vertebrate, and a plant or algae—should be used to evaluate the short-term chronic toxicity of a minimum of three sampling events using EPA toxicity tests (USEPA 2010a). Species listed in Tables 3-1, 3-2, and 3-3 are EPA-approved toxicity test species for acute and short-term chronic toxicity testing under the NPDES program.

For some types of toxicity tests (e.g., freshwater acute invertebrate), there is a choice of an approved test species. Where a choice of test species is available for a given type of toxicity test, the NPDES permit writer should consider the relative test species sensitivity to the pollutants in the effluent or the ambient sample [40 CFR § 122.44(d)(1)(ii); (MacKnight 2011)]. Once the most sensitive test species is determined for that sample source, that species may be used moving forward in routine toxicity monitoring unless changes occur to the effluent or ambient waters that might impact toxicity. In conjunction with the promulgated test species required to be used in NPDES permits under 40 CFR Part 136, the permit writer may consider including additional toxicity tests that use non-promulgated or

non-approved EPA test species to assess potential adverse impacts on threatened or endangered species.

3.2.3 Freshwater vs. Saltwater Toxicity Tests

EPA recommends that the test species used should be determined by the receiving water type (USEPA 2002a, 2002b, 2002c; 1995a). The estuarine and marine toxicity test methods identify a salinity of 1.0 part per thousand (ppt) as the point at which salinity begins to exert a toxic effect on freshwater species. Therefore, EPA generally recommends that freshwater test species be used in toxicity testing when the receiving water salinity is less than 1.0 ppt and that an estuarine or marine test species be used when the receiving water salinity equals or exceeds 1.0 ppt (USEPA 1991a; USEPA 2002a, 2002b, 2002c).

3.2.3.1 Saline NPDES Effluent Discharges to Saltwater

Dissolved salts in the effluent may or may not be the same as those present in the receiving water. Also, the proportion of dissolved salts in the effluent might be different from that of the salts in the receiving water. Toxicity testing can determine if salts in the effluent contribute to toxicity in the receiving water (USEPA 1991a). In this case, the NPDES permit writer should consider using estuarine or marine toxicity test species in NPDES permits.

3.2.3.2 Saline NPDES Effluent Discharges to Freshwater

In this case, the receiving water is freshwater and the biota in the receiving water are freshwater species. Therefore, to support a state's, territory's, or authorized Tribe's aquatic life protection criteria for this situation, freshwater toxicity test species should be included as an NPDES permit toxicity testing requirement (USEPA 1991a).

3.2.3.3 Freshwater NPDES Effluent Discharges to Saltwater

In this instance, the lack of dissolved salts in the NPDES permitted facility's effluent can cause an effect on the test organisms in an estuarine/marine toxicity test, particularly as the effluent test concentrations approach 100% (USEPA 1991a). In this case, the permit writer, using their best professional judgment
(BPJ) depending on the permitting situation, can select either:

- a freshwater species for toxicity tests using freshwater dilution water, or
- a saltwater species for toxicity tests using artificially salted effluent that matches the salinity of the receiving water under the critical NPDES permit condition.

Most NPDES industrial and municipal effluents entering marine and estuarine systems have little measurable salinity. If the permit writer determines it is necessary to increase the salinity of the effluent before testing using marine or estuarine toxicity test methods, two salt sources are available to adjust salinities: artificial sea salts and hypersaline brine (HSB) derived from natural seawater. Use of artificial sea salts is necessary only when high effluent test concentrations preclude salinity adjustment by HSB alone. Procedures for making HSB are provided in EPA's saltwater toxicity test methods (USEPA 2002c; USEPA 1995b). The use of HSB will limit the ability to test high effluent concentrations because of the dilution of the effluent with the HSB.

3.3 Recommended Statistical Approaches for Analyzing Toxicity Test Data

After toxicity tests are conducted, the data must be analyzed to determine whether a statistically significant difference exists in the biological response (e.g., survival, reproduction, or growth) measured in the test organisms exposed to the aqueous sample (e.g., effluent, ambient) tested compared to the

control. The statistical analysis helps to determine if a permit limit is needed to meet WET WQS or whether the established permit WET limits are being met. This section discusses statistical approaches outlined in EPA's TSD, toxicity test methods (USEPA 1991a; USEPA 2002a, 22002b, 2002c; USEPA 1995b), and the Test of Significant Toxicity (TST) statistical approach (USEPA 2010b, 2010c). The permit writer should review and follow the NPDES permit authority's policies and WET IPs. Additional statistical review steps are discussed in Section 3.4.

3.3.1 Point Estimates According to EPA's Technical Support Document

The point estimate statistical approach, which is outlined in EPA's TSD and included in EPA's toxicity test methods manual as a recommended statistical approach (USEPA 1991a, USEPA 2002a, 2002b, 2002c; USEPA 1995b), determines the effluent concentration at which a particular measured percent effect occurs. For example, if the desired statistical endpoint is the LC_{50} using the point estimation approach, the effluent concentration that should result in a 50% effect on organism survival is extrapolated from the observations made in all the effluent concentrations tested. The identified point estimate effluent concentration is then compared to the permittee's IWC to determine whether the effluent sample is toxic. The concentration that is determined to cause a particular level of response does not have to be one of the effluent concentrations tested as it can be extrapolated from the data.

Figure 3-1 illustrates the response observed in each of the effluent test concentrations and the control treatment. The effluent test concentrations in this example are a control treatment, or 0% effluent, and 6.25%, 12.5%, 25%, 50%, and 100% effluent. The concentrations from 0% to 100% effluent have been plotted on a log scale on the y-axis with corresponding percent mortality on the x-axis. For analysis using a point estimate approach, data are represented on a log scale so the data points can be graphed in a linear fashion. If the data were not represented on a log scale, they would appear as a curve.

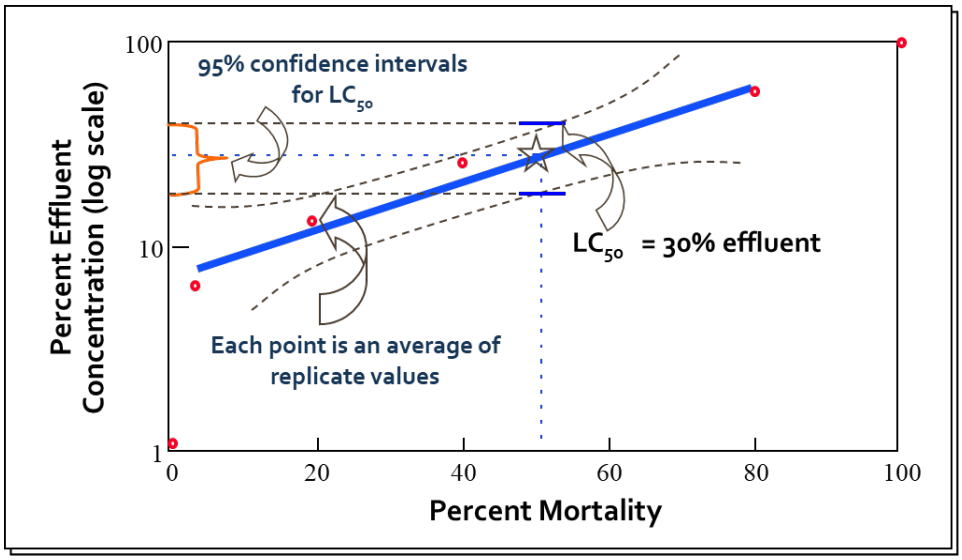


Figure 3-1. *Point estimates used to determine the percent effluent that causes a predetermined percent effect in the biological test endpoint (e.g., mortality).*

Point estimation of WET data, such as percent mortality, can be readily analyzed if the data are presented as a straight line. The test organism response in the control treatment, or 0% effluent, was 0% mortality, while there was 100% mortality observed in the 100% effluent test concentration. The dotted

lines within the graph indicate the 50% mortality threshold, which, when extrapolated from the line to the y-axis, is approximately 30% effluent.

If the toxicity test demonstrates a sample concentration at or below the critical concentration or IWC (e.g., 12.5% effluent when the critical concentration or IWC is 20%) elicited a percent effect greater than the predefined percentage (e.g., 50% mortality for acute or 25% reduction in growth for chronic sublethal biological endpoints), then the sample is declared toxic, and the test result is a "fail." The percent effect for a toxicity test is the difference between the mean biological response of the control concentration (e.g., lethality or reproduction) and the mean biological response of the critical concentration or IWC, divided by the mean biological response of the control concentration. The percent effect is calculated using the following formula:

$$Percent\ Effect = \frac{(Mean\ Control\ Response - Mean\ Response\ at\ IWC)}{Mean\ Control\ Response} * 100$$

The percent effect does not reflect the amount of variability among replicates in the control or test concentration of a toxicity test as calculated by the 95% confidence intervals. Thus, the point estimate statistical approach does not consider the variability surrounding the point estimate to determine the statistical result. Whether a toxicity test result is "pass" or "fail" using the point estimate statistical approach is determined based only on whether the percent effect observed at the critical concentration or IWC is less than (i.e., test is a "pass") or greater than or equal to (i.e., test is a "fail") the predefined percentage (e.g., 50% mortality [LC_{50}] or 25% for sublethal endpoints [IC_{25}]). Using the point estimate analysis, however, provides 95% confidence limits around the point estimate endpoint and states may use this as part of their review of toxicity test data. The 95% confidence intervals in this example are relatively small, 20%–40%, indicating reasonable confidence in the LC_{50} estimate for this WET test. This analysis indicates that one can be 95% confident that the LC_{50} for organism mortality in this test lies between 20% and 40% effluent.

3.3.2 Hypothesis Testing According to EPA's Technical Support Document

Another statistical approach included as a recommended option in the TSD, and toxicity test methods manuals provides a NOAEC or NOEC endpoint (USEPA 1991a; USEPA 2002a, 2002b, 2002c; USEPA 1995b). This approach examines a null hypothesis in which the organism response in the sample is equal to or better than the organism response in the control to determine whether the null hypothesis should be rejected. This hypothesis testing statistical approach determines the highest effluent test concentration in a multi-concentration toxicity test that elicits a biological response statistically equal to the control response (i.e., NOAEC or NOEC). The hypothesis testing statistical approach identifies the lowest test concentration in the multi-concentration toxicity test that elicits a biological response that is statistically significantly less than the control (i.e., lowest observed adverse effect concentration [LOAEC] or lowest observed effect concentration [LOEC]), which indicates that there is an observed statistically significant impact on the test organisms, such as increased mortality or decreased growth (Figure 3-2). The multi-concentration hypothesis testing statistical approach is dependent on the test concentrations series used in the toxicity test; and the NOAEC/NOEC and the LOAEC/LOEC can be only one of the concentrations tested. A significant difference (i.e., lower organism response) between the critical test concentration or IWC and the control results in the null hypothesis being rejected and, therefore, the sample is declared toxic, and the test result being declared a "fail." If the multi-concentration hypothesis statistical approach analysis does not indicate a significant difference between the control and the sample, then the null hypothesis cannot be rejected, which is interpreted as a "pass" using the multi-concentration hypothesis testing statistical approach.

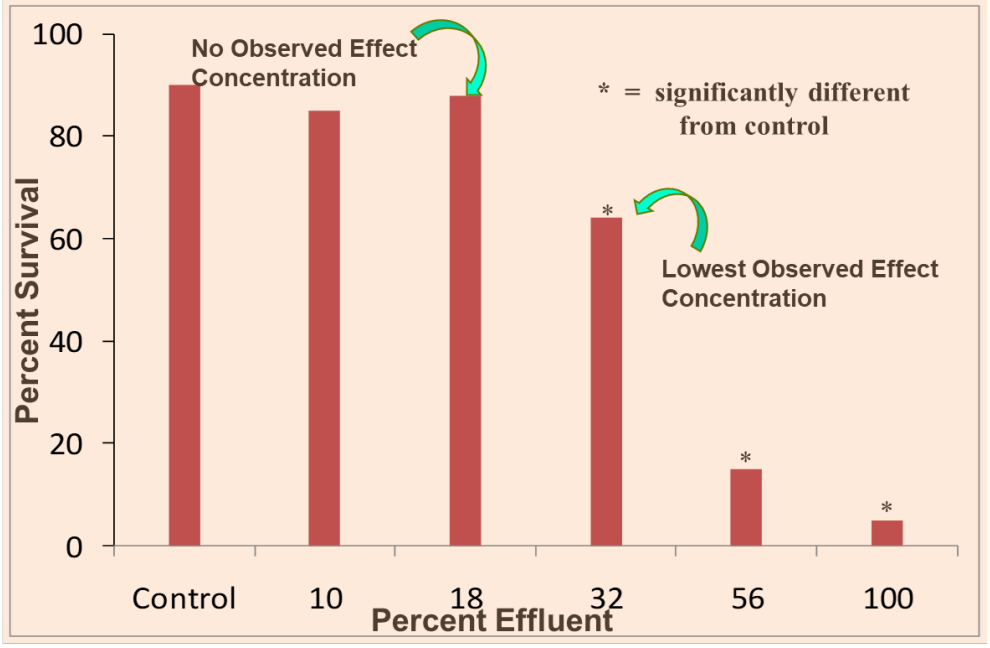


Figure 3-2. *Hypothesis testing used to determine whether each concentration used in a toxicity test is significantly different from the control.*

3.3.3 Hypothesis Testing According to EPA's Test of Significant Toxicity

Another option for analyzing valid toxicity test data and assessing both RP and permit compliance is the TST statistical approach. The TST is a hypothesis testing statistical approach developed by EPA building on the 2000 Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications (hereafter the WET Variability guidance) and the 1991 TSD (USEPA 1991a; USEPA 2000b; USEPA 2010a, 2010b). Use of the TST statistical approach is consistent with EPA's toxicity test methods in 40 CFR Part 136 and the NPDES regulations, which do not require the use of any particular statistical approach for analyzing toxicity test data. The TST does not supersede the statistical analysis approaches recommended in EPA's TSD, but rather is another optional statistical approach that can be used.

The TST statistical approach is designed to compare the biological response from the control concentration calculated as the critical t-value with the biological results of the compliance test concentration (e.g., IWC) or calculated t-value. The results of the TST evaluation indicate a pass when the calculated t-value is greater than the critical t-value and a fail when the calculated t-value is less than the critical t-value. A t-value is simply the calculated difference in units of standard error. Under the TST hypothesis testing statistical approach, the null hypothesis has been restated to reflect that the organism response in the effluent at the critical concentration or IWC is significantly different from the controls at a fixed fraction (b) of the control response (e.g., 0.80 of the control mean response for acute test endpoints and 0.75 of the control mean response for short-term chronic test endpoints). The b values represent regulatory management decisions (RMDs) established by EPA in the development of the TST statistical approach (Table 3-4). Rejecting the TST's hypothesis indicates that the sample at the critical concentration or IWC is not toxic or is bioequivalent to the control. The TST approach can be used to analyze any toxicity test biological endpoint, for freshwater, marine, or estuarine acute and short-term chronic toxicity tests, including for short-term chronic sublethal biological test endpoints, such as reproduction or growth.

Table 3-4. *Summary of b and RMD values used in the Test Significant Toxicity analysis of toxicity test data.*

EPA Toxicity Test Method	*b* Value	Regulatory Management Decision
Acute Toxicity Test Methods		
Pimephales promelas (fathead minnow), *Oncorhynchus mykiss* (rainbow trout), *Salvelinus fontinalis* (brook trout), *Cyprinodon variegatus* (sheepshead minnow), *Atherinops affinis* (topsmelt fish), and *Menidia beryllina* (inland silverside) acute survival	0.80	20%
Ceriodaphnia dubia, *Daphnia magna*, *Daphnia pulex* (water flea), and *Americamysis bahia* (mysid shrimp, formerly *Mysidopsis bahia*) acute survival	0.80	20%
Short-Term Chronic Freshwater Toxicity Test Methods		
Ceriodaphnia dubia (water flea) reproduction	0.75	25%
Pimephales promelas (fathead minnow) survival and growth	0.75	25%
Raphidocelis subcapitata (formerly *Selenastrum capricornutum*) (green algae) growth	0.75	25%
Short-Term Chronic East Coast Marine Toxicity Test Methods		
Americamysis bahia (mysid shrimp, formerly *Mysidopsis bahia*) survival and growth	0.75	25%
Arbacia punctulata (sea urchin) fertilization	0.75	25%
Cyprinodon variegatus (sheepshead minnow) and *Menidia beryllina* (inland silverside) survival and growth	0.75	25%
Short-Term Chronic West Coast Toxicity Test Methods		
Dendraster excentricus (sand dollar) and *Strongylocentrotus purpuratus* (sea urchin) fertilization	0.75	25%
Atherinops affinis (topsmelt fish) survival and growth	0.75	25%
Haliotis rufescens (red abalone), *Crassostrea gigas* (oyster), *Dendraster excentricus* (sand dollar), *Strongylocentrotus purpuratus* (sea urchin), and *Mytilus* sp. (mussel) larval development	0.75	25%
Macrocystis pyrifera (giant kelp) germination and germ-tube length	0.75	25%

The TST relies on two properties of the data—the average values in the control (critical t-value) and the IWC (calculated t-value), and the variability observed among replicates within the IWC and the control. Whether the IWC is considered toxic depends on both data properties. If a sample causes an effect that exceeds the RMD for what is an unacceptable effect (e.g., greater than or equal to 25%), the alpha error rate established using the TST is designed to declare that the sample is toxic (i.e., the test is identified as a "fail") regardless of within-test performance (Figure 3-3). If within-test variability is decreased, a higher probability of rejecting the null hypothesis using the TST exists and, therefore, declaring the sample not toxic as long as the percent effect in the sample is not greater than or equal to the RMD (e.g., 25%) as compared to the control. Both toxicity test design and laboratory performance of the toxicity test method affect within-test variability. Generally, within-test variability can be decreased by using more replicates in a toxicity test and by improving laboratory performance of the toxicity test method.

The TST approach was designed to declare very small observed effects at the IWC in a toxicity test (i.e., less than or equal to 10% effect) not toxic (i.e., the test is identified as a "pass"), even with moderately high within-test variability, because this effect is not considered biologically significant (Figure 3-3). As within-test precision increases and the average percent effect is between 10% and 25%, a sample is more likely to be declared not toxic using the TST if the observed effect is below the RMD (e.g., 25%) for what is considered an unacceptable effect (Table 3-4). High within-test variability in the control and/or the IWC is more likely to result in identifying the test as a "fail," when the average percent effect in the sample is between 10% and 25% (Figure 3-3). Better within-test precision becomes more important as the observed effect of a sample approaches the RMD for unacceptable toxicity (Figure 3-3).

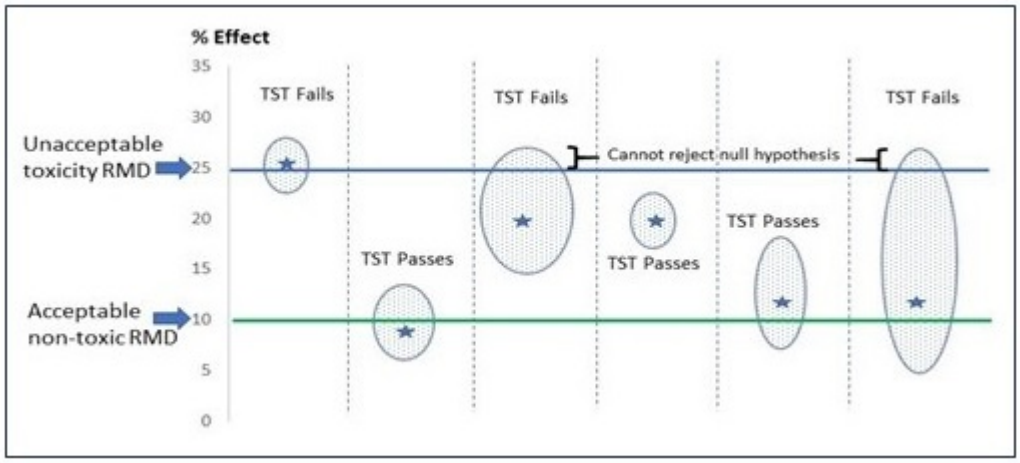


Figure 3-3. *Probable Test of Significant Toxicity results (i.e., pass or fail) for different tests (X-axis) using a short-term chronic toxicity test method depending on within-test variability observed in each test. Blue star = mean percent effect at IWC; oval cloud = within-test variability demonstrated by the length of the cloud. Width of each cloud has no special meaning in this illustration.*

3.4 Statistical Approach Review Steps

The permittee and/or their laboratory completes a test review on each toxicity test following EPA's Toxicity Test Review guidance in the EPA toxicity test method manuals (see Section 6 in each manual). The NPDES permit authority reviews the WET test data to ensure the evaluation conducted by the permittee and/or lab was completed accurately (USEPA 2002b, 2000c).

3.4.1 Review Steps for Technical Support Document Statistical Approaches

When using multi-concentration testing, EPA's toxicity test method review steps include the review of the concentration-response relationship (CRR) for each toxicity test (USEPA 2000a, 2002a, 2002b, 2002c). There is no requirement that a specific CRR be established as part of the valid toxicity test requirements, but certain steps may need to be taken based on the specific CRR observed as described in the EPA Method Guidance and Recommendations for WET Testing (USEPA 2000a) (Figure 3-4).

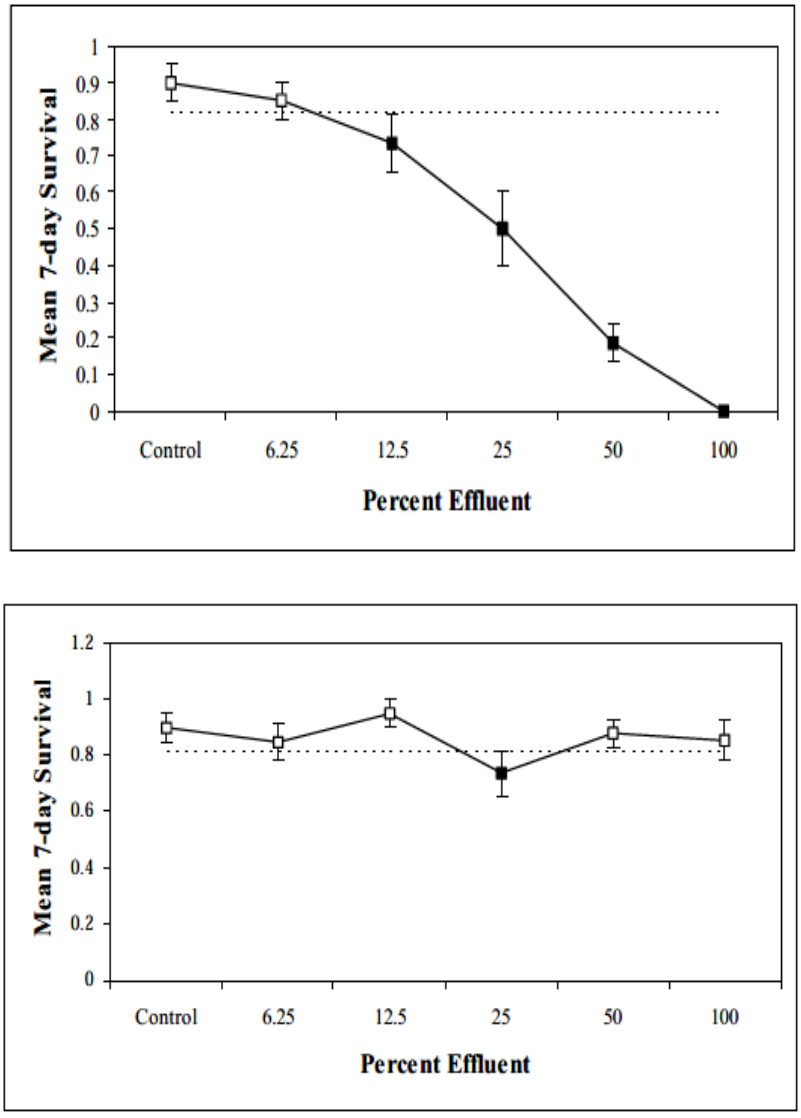


Figure 3-4. *Example concentration-response relationships that might be observed in toxicity testing (USEPA 2000a). The top graph represents a classic concentration response-relationship (CRR) that depicts increased mortality with increased sample concentration, in this case, effluent. The bottom graph illustrates an interrupted CRR, in which a single effluent concentration was identified as significantly different (25%).*

The promulgated toxicity test methods in 40 CFR Part 136, Table 1A state that the within-test variability is reviewed, and the variability criteria applied when NPDES permits specify hypothesis testing endpoints (e.g., NOEC and LOEC) using short-term chronic sublethal toxicity methods 1000.0, 1002.0, 1003.0, 1006.0, or 1007.0, as described in Table 3-5 and Figure 3-5 [see Section 10.2.8.2 of EPA's toxicity test

methods (USEPA 2002b, 2002c) (Table 3-5). The permit should include a requirement that the within-test variability be reviewed by the permittee/laboratory, but a specific PMSD is not required and a PMSD below, within or above the PMSD bounds are acceptable depending on the data. EPA recommends that the NPDES permit authority reviews the within-test variability, too. The within-test variability is reviewed based on the calculation of the percent minimum significant difference (PMSD) for the toxicity test (USEPA 2002b, 2002c). The PMSD is calculated by first calculating the minimum significant difference (MSD) using the following formula (USEPA 2002b, Appendix C; 2000c):

$$MSD = ds_w * \sqrt{\left(\frac{1}{n_1}\right) + \left(\frac{1}{n}\right)}$$

where:

d = critical value for the Dunnett's procedure
s_w = the square root of the error mean squares
n_1 = the number of replicates in the control treatment
n = the number of replicates per concentration, assuming an equal number at all other concentrations

Then to calculate the PMSD, use the following formula:

$$PMSD = 100 * \frac{MSD}{Control\ Mean}$$

Specific actions are required if the PMSD for a toxicity test exceeds the lower or upper bounds established by EPA for each specific toxicity test method, as illustrated in Figure 3-5.

Table 3-5. *Percent minimum significant difference bounds for short-term chronic sublethal test endpoints under EPA's promulgated toxicity test methods.*

EPA Toxicity Test Method	Biological Test Endpoint with Calculated PMSD Bounds	Lower PMSD Bound	Upper PMSD Bound
Method 1000.0, *Pimephales promelas* Larval Survival and Growth Test[a]	Growth	12	30
Method 1002.0, *Ceriodaphnia dubia* Survival and Reproduction Test*	Reproduction	13	47
Method 1003.0, *Raphidocelis subcapitata* (formerly *Selenastrum capricornutum*) Growth Test*	Growth	9.1	29
Method 1006.0, *Menidia beryllina* Larval Survival and Growth Test**	Growth	11	28
Method 1007.0, *Americamysis bahia* (formerly *Mysidopsis bahia*) Survival, Growth, and Fecundity Test**	Growth	11	37

Notes: * USEPA 2002b.
** USEPA 2002c.

Following are the five potential outcomes of the PMSD review summarized in Figure 3-5:

1. PMSD is within the bounds and effect at the IWC is not significant: toxicity test is acceptable, and monitoring is continued based on permit conditions and requirements.
2. PMSD exceeds the upper bound and effect at the IWC is significant: the permittee needs to conduct additional toxicity tests with new samples.

3. PMSD exceeds the upper bound and effect at the IWC is not significant: the permittee must conduct the toxicity test again with a new sample.
4. PMSD is within the bounds and effect at the IWC is significant: the permittee needs to conduct additional toxicity tests with new samples, and
5. PMSD is below the lower bound and effect at the IWC is significant: toxicity test is acceptable.

3.4.2 Review Steps for TST Statistical Approach

The TST does not include any additional review steps besides verifying that the toxicity data analyzed is valid based on meeting all of EPA's toxicity test method TACs.

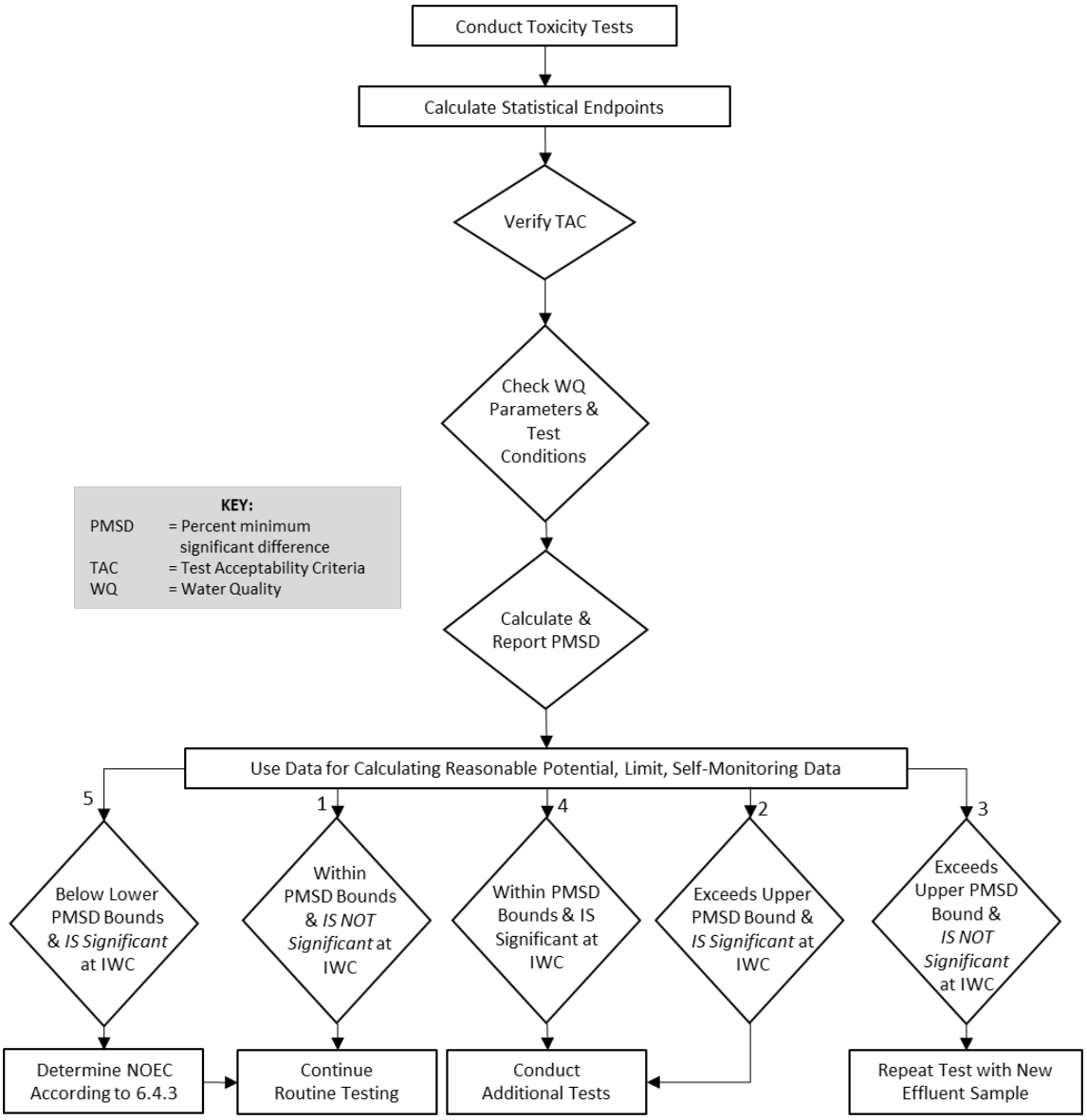
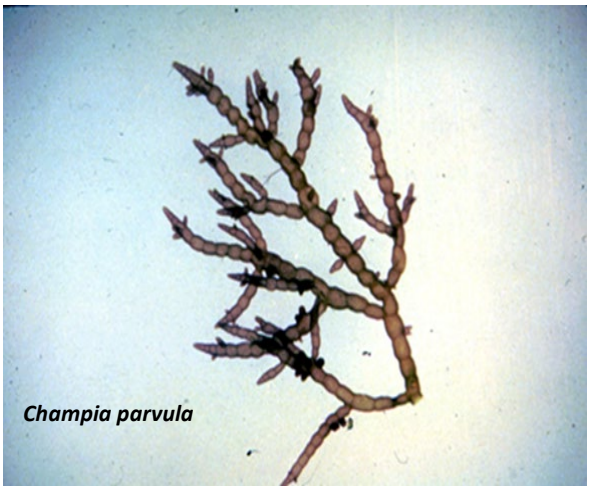


Figure 3-5. *Decision tree for WET PMSD review (USEPA 2000b, Section 6.4.3).*


Champia parvula

4 NPDES Permit Conditions for WET Monitoring

This section describes the monitoring conditions for WET that a permit writer establishes in an NPDES permit. This includes requirements for the permittee to conduct self-monitoring of permitted discharges and internal operations (as applicable) and report the analytical results to the NPDES permit authority with the information necessary to evaluate discharge characteristics and compliance status. A monitoring frequency that is representative of the permitted discharge and a reporting approach that is consistent with the current EPA Integrated Compliance Information System (ICIS) codes can establish an ongoing record of the permittee's compliance status and, if violations are detected, create a basis for any necessary enforcement actions. The monitoring and reporting conditions section of an NPDES permit generally includes specific requirements related to monitoring location and frequency, sample collection and handling, EPA toxicity test method to be used, permit limits or triggers, and reporting and recordkeeping requirements. See Table 4-1 for a list of NPDES permit WET provisions and associated examples.

States, territories, or authorized Tribes should consider several factors when developing toxicity testing permit requirements, including:

- Federal regulations such as the requirement for WET limits when RP is demonstrated [40 CFR § 122.44(d)(1)(iii – v)], representative sampling and monitoring, [40 CFR § 122.48(b)] and reporting requirements
- Applicable WQS, such as receiving stream use designations and criteria
- NPDES permit authority policies and IPs, such as processes for evaluating RP and EPA toxicity testing-specific information (e.g., toxicity test concentration series, data interpretation, and statistical approaches protective of applicable WET WQS)
- Case-specific considerations for the permitted discharge, such as receiving water body impairment or total maximum daily load (TMDL) status, freshwater versus marine receiving water, continuous versus intermittent effluent discharges, permittee's compliance history, test species sensitivity to a particular effluent, and accounting for effluent variability [40 CFR § 122.44(d)(1)(ii)]

NPDES permit authorities may recommend other permit conditions not specifically required by the regulations, where the regulations allow for that discretion. For example, under the authority to specify required monitoring intervals and frequency in 40 CFR § 122.48, NPDES permit authorities may recommend a "step-wise approach" in permits, such as accelerated toxicity testing when the monitoring requirement in the permit identifies toxicity that is a violation of the permit WET limit or if there is an in-stream excursion of an applicable WET WQS when the permit does not include a WET limit. NPDES permit authorities may develop written IPs or guidance that provides direction to the permit writer when making these types of permit development decisions and the supporting rationale, as appropriate.

Table 4-1. *NPDES permit WET provisions and example conditions.*

NPDES Permit WET Provisions	Examples of NPDES Permit Condition*
WET Limit or WET Monitoring Requirements	WET monitoring requirements or limits (see Section 6 of this manual).
Toxicity Test Type	Acute or chronic and marine or freshwater with reference to specific toxicity test(s) and appropriate toxicity method manual(s) (see Section 3 of this manual).
Toxicity Test Species	Invertebrate, vertebrate, plant (e.g., algae) (see Section 3 of this manual).
Type of Effluent Sample	Grab or composite (duration and minimum number of subsamples for composites).
Dilution Water	Uncontaminated receiving water or lab synthetic of similar pH and hardness.
In-Stream Waste Concentration	Greater than 1%–100% (based on the specific discharge and receiving water flows).
Toxicity Test Concentration Series	Specified directly, such as "0, 6.25%, 12.5%, 25%, 50%, and 100%," or indirectly, such as "IWC bracketed by two lower and two higher concentrations."
Test Acceptability Criteria for EPA Toxicity Tests	All criteria required by the toxicity test methodology, plus any additional requirements established by the NPDES permit authority.
Other Criteria for Valid Toxicity Test	See EPA toxicity methods manuals (2002a, 2000b, 2000c; 1995b) and Appendix C of this manual for examples of criteria for valid toxicity tests.
Statistical Analysis Test Endpoint	LC_{50}, NOEC, IC_{25}, and pass/fail.
Toxicity Monitoring Frequency	Monthly, quarterly, annual, etc. that is representative of the permitted discharge [see EPA's TSD (USEPA 1991a) and 40 CFR § 122.48(b)].
Accelerated Toxicity Testing Requirements	Requirement to conduct additional toxicity tests (e.g., six) at certain intervals (e.g., 14 days) for the affected species, after exceeding an NPDES permit WET limit or WET monitoring trigger.
Toxicity Reduction Evaluation Requirements	Mandatory initiation of toxicity reduction evaluation study, which might include a toxicity identification evaluation and reporting for failure of any additional toxicity test in the accelerated testing mode and not meeting the permit's toxicity reduction evaluation schedule requirements.
Compliance Schedule	Schedule to attain compliance with permit limit [40 CFR § 122.47].
NPDES Reporting Requirements	Requirement to electronically submit discharge monitoring reports (DMRs) and any other requirements specified by the permit writer, such as submission of laboratory reports as an attachment to the DMR.

Note: * Appropriate requirement to be determined by the permit writer and specified in the permit on a case-by-case basis.

These NPDES implementation decisions should be explained and documented clearly in the permit fact sheet addressed in 40 CFR § 124.56 to inform the permittee and the public of the basis for the permit decisions and requirements, including for WET (USEPA 1994). NPDES permit authorities should strive to maintain staff with the level of expertise necessary for thorough WET data review when performing RP evaluations, compliance monitoring review, TREs and TIEs for WET, and laboratory testing oversight, as necessary.

The following sections provide an overview of the considerations involved in determining representative monitoring, appropriate reporting, and recordkeeping requirements and how to properly incorporate the key requirements into an NPDES permit.

4.1 NPDES Toxicity Monitoring

Toxicity testing, including the use of WET monitoring, is performed to evaluate toxicity in effluent, support toxicity identification evaluations (TIEs) and toxicity reduction evaluations (TREs), determine compliance with effluent limitations established in NPDES permits (see Section 6), establish a basis for enforcement actions, and evaluate receiving water toxicity. EPA regulations requiring the establishment of monitoring and reporting conditions in NPDES permits are in 40 CFR § 122.44(i) and 122.48. Regulations in 40 CFR § 122.44(i) require permittees to monitor pollutant mass (or other applicable unit of measure) and effluent volume and to provide other measurements (as appropriate) using the EPA toxicity test methods established in 40 CFR Part 136. 40 CFR § 122.44(i) also establishes that NPDES permits must require permittees to monitor for all limited pollutants and report data at least once per year. EPA provides that all permits must specify requirements for proper use, maintenance, and installation of monitoring equipment or analytical methods (including biological monitoring methods such as toxicity testing, when appropriate). NPDES permits also must specify the monitoring type, intervals, and frequency sufficient to yield data that are representative of the monitored activity [40 CFR § 122.48(a), (b)]. The following sections focus on developing permit monitoring conditions that properly address these regulatory requirements.

4.2 Establishing NPDES Permit Conditions for WET Monitoring, Assessment, and Compliance

WET monitoring conditions in NPDES permits should specify parameters addressing the following factors (see Table 4-1 for these provisions and examples of each):

- Type of toxicity test (see Section 3)
- Type of effluent sample
- Toxicity test species sensitivity (see Section 3)
- Dilution water selection
- In-stream waste concentration
- Toxicity test concentration series
- Test acceptability criteria
- Other criteria for valid toxicity test
- Statistical analysis test endpoint
- Monitoring frequency
- Accelerated toxicity testing
- Toxicity Reduction Evaluation provision

- Compliance schedule
- Reporting and recordkeeping requirements

4.2.1 Effluent Sampling

Effluent sample collection is an important part of the NPDES permit compliance monitoring process [40 CFR § 122.41(j)(1); 40 CFR § 122.21(g)(7)(i)]. Without proper effluent sample collection procedures, the results of toxicity monitoring (e.g., WET tests) are neither useful nor valid. Obtaining samples that are representative of the permitted discharge and maintaining their composition integrity relative to the effluent during sampling and handling are critical. EPA toxicity test methods have been standardized and validated, but toxicity test results are only as good as the sampling and sample preservation procedures used when collecting (permittee or designee) or storing (laboratory). Once the sample is collected, the constituents of the sample must stay in the same condition until the sample is analyzed. The length of time the chemical constituents in the sample will remain stable is related to their chemical characters and the preservation method used (e.g., temperature, exposure to light, holding time, and shipping). The permit writer should specify on a case-by-case basis whether a grab or composite sample is to be taken based on the type of effluent to be sampled as well as requirements related to sample handling.

4.2.1.1 Effluent Sample Collect on Procedure

The NPDES permit writer should specify the sample collection procedure for all parameters required to be monitored in the permit, including WET. The sample collection procedure and location should be determined by the permit writer based on the characteristics of the NPDES permitted discharge. The two most frequently used sampling procedures involve composite sampling and grab sampling (USEPA 2010a). As reviewed in the next two sections, determination of the most appropriate sampling procedure to require in the permit depends upon the potential toxicity of the effluent and characteristics of the treatment process. Additional sample collection and storage requirements are identified as part of EPA's toxicity test methods (USEPA 2002a, 2002b, c2002; USEPA 1995b) incorporated by reference in 40 CFR Part 136. The tables in Appendix C summarize many of these important sample collection and storage requirements, including sample type, holding times, and sample volume. Other sample collection and storage requirements, included in EPA's toxicity test methods but not in the tables in Appendix C, include requirements for sample temperature and shipping and receiving of the sample by the laboratory (e.g., recording sample temperature and starting test within 36 hours of completion of sample collection) (USEPA 2002a, 2002b, 2002c; USEPA 1995b).

It is important to note that NPDES effluent monitoring that is representative of the discharge should include consideration of any chemicals added during the facility's treatment process, including chlorine or other disinfectants, so the facility's final effluent discharge is used for WET testing required by the permit. If chlorine is used to disinfect the permitted discharge(s), EPA requires that the total residual chlorine (TRC) in the effluent sample be measured immediately (e.g., within 15 minutes) following sample collection. TRC is then measured again upon arrival at the toxicity testing laboratory prior to WET testing (USEPA 2002a, 2002b, 2002c; USEPA 1995b). Regardless of whether TRC is observed in the effluent sample, the effluent sample should be evaluated for WET without adjusting for TRC. The laboratory should record the effluent TRC concentration in the WET test reporting documentation.

4.2.1.2 Composite Sampling

A composite sample is defined as a sample formed by mixing discrete samples taken at periodic points in time or a continuous proportion of the flow. The number of discrete samples that make up the composite should depend upon the variability of pollutant concentration and flow.

Composite samples represent the average characteristics of the waste stream during the period of sample collection. For most NPDES discharges, a 24-hour composite (time composite) sample is typically appropriate for toxicity testing. Since the sample is collected over a much longer period than grab samples, 24-hour composite samples may more readily catch any toxicity spikes, but the compositing process may tend to dilute the toxicity, resulting in a misleading measure of the maximum toxicity of the effluent. Composited samples are, therefore, more appropriate for short-term chronic tests in which the peak toxicity of short duration is of lesser concern or for acute tests in which the discharge is not highly variable (USEPA 1991a). For some discharges, a flow-weighted composite, such as stormwater discharges where flows change during a discharge event, might be more appropriate than a time-weighted composite sample. In a flow-weighted composite, the volume of sample collected at a certain period is based on the flow instead of on a fixed volume, whereas in a time-weighted composite, a fixed volume is collected at a prescribed time to make up the composite (e.g., 100 milliliters [mL] per hour for 24 hours for a 2.4 L composite).

4.2.1.3 Grab Sampling

Grab samples are individual samples collected at a specific time, not to exceed fifteen minutes, and are representative of the conditions at the time the sample is collected (USEPA 1991a). The collection of a grab sample is appropriate when a sample is needed to:

- Represent an effluent that does not discharge on a continuous basis,
- Provide information about instantaneous concentrations of pollutants at a specific time,
- Allow collection of a variable sample volume, and
- Corroborate composite samples

Based on the NPDES permit application and any other additional information provided by the permittee, the permit writer will determine if grab samples are appropriate for toxicity monitoring and will identify in the permit what sampling procedures must be used. If the chemical characteristics and potential toxicity of the effluent are known to be variable over short periods of time (i.e., hours) due to the type of process and/or treatment used, grab samples collected during the peaks of potential effluent toxicity provide a measure of maximum toxic effect. Collection of grab samples may also be appropriate if there is little dispersion or mixing of the effluent in the receiving water. Under certain discharge conditions, particularly where a discharger is lacking a diffuser, tidal receiving waters or receiving waters with a high velocity may not disperse the effluent as quickly, thus leading to longer duration of exposure to higher concentrations of effluent. In tidal waters, the effluent tends to move upstream and downstream with the tide and not get fully mixed. In areas with high velocity waters, the effluent plume may be pushed near shore and not get mixed into the receiving water for some time. Grab samples may be appropriate in these instances. Grab samples are also appropriate for batch wastewater treatment facilities or permitted discharges that are known to be highly variable in chemical composition over a day due to their input characteristics. Sample volume depends on the type and number of analyses to be performed.

4.2.2 Type of Effluent

The permit writer should consider whether the permitted discharge is continuous or intermittent when determining sampling requirements.

4.2.2.1 Continuous Discharges

Generally, continuous discharges occur constantly or near-constantly [40 CFR 122.2]. The appropriate sampling approach for continuously discharged effluent depends on whether the effluent is retained in the wastewater treatment facility prior to discharge, and if so, the length of the retention time. The retention time of the effluent in the wastewater treatment facility may be estimated based on the volume of the retention basin and rate of wastewater inflow. The calculated retention time, however, may be much longer than the actual time and a more accurate estimate of the retention time can be obtained by carrying out a dye study (USEPA 2002a).

If the facility discharge is continuous, but the estimated retention time within the wastewater treatment unit is less than 14 days and the variability of the effluent toxicity is unknown, EPA's acute toxicity test methods manual recommends that, at a minimum, four grab samples or four composite samples are collected over a 24-hour period and used for WET testing (USEPA 2002a). For example, a grab sample taken every six hours (for a total of four samples) and each sample used for a separate toxicity test—or four successive 6-hour composite samples taken—and each used in a separate WET test (USEPA 2002a).

If the estimated retention time of a continuously discharged effluent is longer than 14 days, or if it can be demonstrated that the wastewater does not vary more than 10% in WET over a 24-hour period, regardless of retention time, EPA recommends a single grab sample collected for a single WET test as sufficiently representative of the effluent (USEPA 2002a).

For chronic toxicity testing with continuous discharges, EPA recommends using 24-hour composite samples (USEPA 2002b, 2002c).

4.2.2.2 Intermttent Dscharges

Intermittent discharges are more periodic, occurring at frequencies such as several hours per day, month, or year, and may result in smaller volumes of effluent discharged annually than continuous discharges. An intermittent discharge does not meet the definition of continuous discharge as defined in 40 CFR 122.2. Toxic impacts on aquatic life (acute or chronic) may be related to intermittent discharges regardless of the frequency of discharge. Pollutant loading from intermittent discharges and their possible impact(s) on water quality may be difficult to detect during periods when no discharge is occurring, but the potential for negative impacts on water quality remains (e.g., resuspension of effluent-related constitutes deposited in the sediment), regardless of effluent discharge frequency (USEPA 1991a). Examples of intermittent discharges include, but are not limited to:

- Effluent continuously discharged during a single 8-hour work shift and discontinued after the shift, or two successive 8-hour work shifts and discontinued after the second shift;
- Wastewater retained during an 8-hour work shift and is then treated and released as a batch NPDES discharge; and
- Wastewater discharged to an estuary only during an outgoing tide, usually during the four hours following slack high tide (i.e., the four hours following high tide).

If the facility discharge is intermittent, for acute testing EPA recommends that a grab sample (i.e., instantaneous sample) be collected midway during the discharge period specific to the type of discharge (USEPA 2002a). For chronic toxicity testing with intermittent discharges, EPA recommends using a composite sample collected over a 24-hour period. The 24-hour period may include multiple intermittent discharges (USEPA 2002b, 2002c).

4.2.3 Dilution Water

EPA's toxicity test methods allow the permit writer to choose whether reconstituted laboratory water or receiving water is required to be used as the dilution water during the toxicity test. NPDES permit authority policies and procedures may lay out specific requirements for the permit writer. Generally, the type of dilution water used in effluent toxicity tests will depend largely on the objectives of the toxicity test.

If the objective of the toxicity test is to estimate the absolute toxicity of the effluent, reconstituted (standard) laboratory dilution water is used. EPA describes procedures for making reconstituted freshwater or saltwater dilution water of varying hardness or salinity, respectively, in the toxicity test methods (USEPA 2002a, 2002b, 2002c; USEPA 1995b). The level of hardness or salinity to be used by the toxicity testing laboratory (e.g., soft, moderately hard, hard, or very-hard water for freshwater testing or estuarine/marine salinity for saltwater testing) should be specified in the NPDES permit and should be based on known natural hardness or salinity ranges for the receiving water. EPA recommends that laboratories use standard dilution water having approximately the same characteristics (e.g., hardness and/or salinity) as the receiving water. If the toxicity test organisms selected have been cultured in water that is a very different hardness or salinity than the toxicity test dilution water, a second set of controls, using laboratory culture water, should be included in the toxicity test.

If the objective of the toxicity test is to estimate the toxicity of the effluent in receiving water, the test may be conducted using dilution water consisting of a single grab sample of receiving water collected either upstream and outside the influence of the outfall or with other uncontaminated natural water. It should be demonstrated that the receiving water used as dilution water in WET testing is not toxic to the selected toxicity test organisms and is representative of the receiving water characteristics at the point of the NPDES discharge. If the test is conducted using receiving water or other natural water, a second set of controls, using laboratory water, should be included in the toxicity test. These secondary controls are only used to demonstrate that the test organisms met the applicable EPA test method TACs. If the receiving water is demonstrated to be toxic, testing should be conducting using laboratory water.

Because laboratory water eliminates any potential interference from background pollutants that might be present in the receiving water, (i.e., stimulation resulting from nutrients causing reduced toxicity in the tests that would not occur in the receiving water body), it is often used by NPDES permit authorities for WET compliance monitoring in NPDES permits. The use of laboratory water as dilution water will evaluate the toxicity of the effluent only and not account for any additive, mitigating, or synergistic effects in the receiving water. Therefore, the ambient toxicity of the receiving water should be considered as part of the calculation of the permit limitation (see Section 6). Seasonal variations in the quality of surface waters may affect effluent toxicity and the laboratory water should be adjusted accordingly. Therefore, the hardness of fresh receiving water and the salinity of saline receiving water samples should be measured before each use.

4.2.4 In-Stream Waste Concentration

IWC is the concentration of a toxicant or effluent in the receiving water after mixing. It is the inverse of the dilution factor (or the available dilution in the receiving water based on facility design flow and receiving water low flow or a portion of the receiving water low flow based on applicable implementation guidance) and sometimes is referred to as the "receiving water concentration" (RWC) or "critical dilution" (CD). The IWC should be documented in the NPDES permit or the fact sheet. The IWC is used where a mixing zone or dilution allowance is allowed in the state's, territory's, or Tribe's WQS (USEPA 2014). If a mixing zone or dilution allowance is not allowed in the applicable WQS or implementing regulations, then the IWC would be 100% effluent and the WET criterion will need to be met at the point of discharge, commonly referred to as the end-of-pipe (USEPA 2014). To calculate the IWC, the permit writer needs to know the facility design flow and the critical receiving water low flow. The choice of critical receiving water low flow (e.g., the lowest consecutive 7-day average stream flow during any 10-year period [7Q10] or the lowest 30-day stream flow during any 5-year period [30Q5]) is dependent on the NPDES permit authority and the amount allowed for mixing zone or dilution allowance. It should be documented in the NPDES permit. Using these two terms, the IWC is calculated using the following formula:

$$IWC\ (\%\ effluent) = \frac{Design\ Flow\ (mgd)}{Design\ Flow\ (mgd) + Receiving\ Water\ Low\ Flow (mgd)} * 100$$

For example, if the facility design flow of a wastewater treatment plant is 3.25 million gallons per day (mgd) and the receiving water flow is 4.45 mgd, to calculate the IWC, the facility's design flow, is used as the numerator, divided by the sum of the critical receiving water low flow, which for this example is 4.45 mgd, and the facility's design flow (which is provided in the NPDES permit application), which in this example, was 3.25 mgd, the denominator. This division yields an IWC value of 42%. Thus, the IWC in this example is 42% effluent.

$$42\%\ effluent = \frac{3.25\ mgd}{3.25\ mgd + 4.45\ mgd} * 100$$

4.2.5 Toxicity Test Concentration Series

The toxicity test concentration series used in a toxicity test can influence the sensitivity and precision of statistical toxicity test endpoints, such as LC_{50}, IC_{25}, and NOEC. Therefore, the effluent test concentrations used in a toxicity test should be selected carefully considering the IWC. The toxicity test concentration series to be used in the toxicity tests should bracket the IWC and should generally consist of two test concentrations above the IWC, the IWC itself, and two test concentrations below the IWC. EPA recommends that the effluent toxicity test concentration series should either be specified in the permit (e.g., 0%, 6.25%, 12.5%, 25%, 50%, and 100% effluent) or the requirements for the toxicity test concentration series selected be included in the permit (e.g., control, the IWC, two toxicity test concentrations lower than the IWC, and two toxicity test concentrations higher than the IWC). For facilities that have a permitted IWC less than or equal to 50% effluent, the effluent toxicity test concentration series could be 0.25 times the IWC for the lowest effluent toxicity test concentration, 0.5 times the IWC, the IWC, 1.5 times the IWC, and 2 times the IWC, plus a control treatment. For facilities that have an IWC higher than 50% effluent, the effluent toxicity test concentration series should be adjusted accordingly. For facilities that have an IWC at or near 100%, it might be appropriate to use a

general toxicity test concentration series, such as 6.25%, 12.5%, 25%, 50%, 100% effluent, and a control treatment (Figure 4-1).

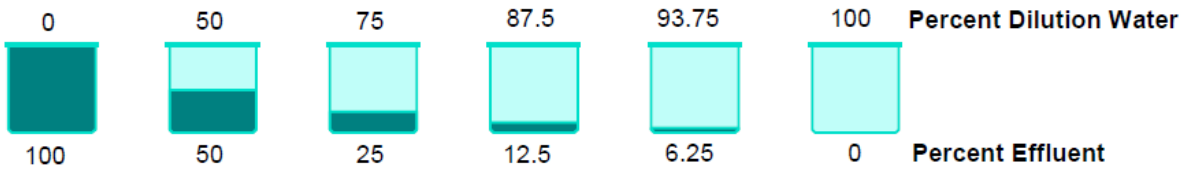


Figure 4-1. *Example test concentration series used in NPDES WET testing for facilities that have an in-stream waste concentration at or near 100%.*

The effluent test concentration series should not use closely spaced effluent test concentrations. EPA recommends using a dilution factor greater than or equal to 0.5. If too small a dilution factor is used the precision of the statistical test endpoint will be compromised and there will be low confidence in the toxicity test endpoint reported. For example, if the IWC is 80% effluent, a test concentration series of a control treatment, 70%, 75%, 80%, 85%, 90% effluent would be too closely spaced.

For those facilities with an IWC less than 1%, EPA's TSD recommends acute effluent toxicity tests, and the recommended concentration series is dependent on whether a zone of initial dilution (ZID) is allowed in the applicable WQS (USEPA 1991a). If a ZID is allowed under the applicable WQS, the recommended concentration series may only need to include a high-test concentration of two-to-three times the IWC (e.g., IWC = 0.5%, maximum tested concentration of 2%). The recommended concentration series may still be IWC/4, IWC/2, IWC, 2xIWC, and 4xIWC (e.g., for an IWC of 0.5%, the concentration series maybe 0.125%, 0.25%, 0.5%, 1.0%, and 2.0% effluent). Whereas, if a ZID is not applicable and the WQS needs to be met at the end-of-pipe, the concentration series should encompass concentrations up to 100% effluent (e.g., 0.125%, 0.25%, 0.5%, 10%, and 100% effluent).

4.2.6 Test Acceptability Criteria

EPA toxicity test methods include prescribed toxicity test conditions, some of which are required and must be used while others are recommended and should be used. The "must" test conditions are required to be met for toxicity test results to be considered valid toxicity test data. For example, test acceptability criteria (TAC) are specified provisions that are required for control performance of lethal and sublethal test endpoints as well as other toxicity test conditions, including the minimum number of test replicates, minimum number of toxicity test concentrations, and toxicity test water temperature. They are required to be met, and, if they are not met, the toxicity test is invalid, and a new toxicity test must be conducted with a new sample or samples (Appendix C, Tables C-1–C-4).

For both freshwater and marine acute toxicity tests, one control performance TAC requirement is that there can be no more than 10% mortality of the control test organisms (i.e., 90% or greater survival of the control test organisms must be demonstrated) (Appendix C, Table C-2). Therefore, if an acute toxicity test indicates less than 90% survival in the control, the toxicity test is invalid and cannot be used for NPDES permit reasonable potential analysis (RPA) or compliance purposes. Another new toxicity test must be initiated using a new sample or samples.

Freshwater chronic toxicity tests have TAC for survival, reproduction, biomass, and/or cell density (Appendix C, Table C-2). East Coast marine chronic toxicity tests have TAC for survival, growth, fecundity, reproduction, and fertilization (Appendix C, Table C-3); while West Coast marine chronic toxicity tests have TAC for survival, growth, development, fertilization, length, and germination (Appendix C, Table C-4).

4.2.7 Other Criteria for Valid Toxicity Tests

EPA's toxicity test methods prescribe that; effluent samples must be first used in toxicity testing within 36 hours of sample collection unless the NPDES permit authority authorizes a longer time not exceeding 72 hours of sample collection. If the permittee is using an onsite laboratory, it is recommended that the effluent samples first be used in toxicity testing within 24 hours of sample collection (Section 8.5.4 in USEPA 2002a, 2002b, 2002c). If needed, however, the initial effluent samples taken and used in the toxicity test may be used later for the toxicity test renewals each day for a chronic test. Effluent samples should be stored at less than six degrees Celsius (°C) when being held prior to testing, including during sample collection and transit from the permittee's facility to the laboratory. For several chronic toxicity tests, multiple effluent samples are collected and used in toxicity testing, each of which should have a completed chain-of-custody form that documents the temperature of the sample at time of receipt. Completed chain-of-custody forms document the history of the sample, including the collection process, timing, shipping, and receipt by the laboratory.

Additionally, many aspects of toxicity testing can influence the quality of the data collected and any requirements related to them should be included in the permit (USEPA 2002a, Section 4; 2002b, Section 4; 2002c, Section 4; 1995b). These include the following:

- Sample collection and handling
- Source, age, and condition of toxicity test organisms
- Condition of equipment
- Appropriate toxicity test conditions
- Instrument calibration
- Adequate treatment replication within the toxicity test
- Reference toxicants
- Recordkeeping
- Data evaluation
- Laboratory experience and level of proven toxicity test performance competence

4.2.8 Statistical Analysis Test Endpoints

The permit writer should specify in the permit the statistical test endpoint to be used to assess NPDES WET compliance with the permit. NPDES permit authority IPs may provide guidance on which statistical approach to use for analyzing valid toxicity test results (see Section 3.3 for additional details on statistical approaches and Section 3.4 for details on review steps for each statistical approach).

EPA's recommended statistical test endpoints for acute toxicity tests include the effluent toxicity test concentration that is lethal to 50% of the test organisms (LC_{50}) and the highest effluent toxicity test concentration at which survival is not significantly lower than the control (e.g., NOAEC). Additionally, a pass/fail test using EPA's TST statistical approach also may be used.

EPA's chronic toxicity test statistical endpoints that are used in the NPDES WET program include the NOEC, the 25% inhibition concentration (IC_{25}), and a pass/fail test using EPA's TST statistical approach. The TST statistical approach also can be used to evaluate whether the biological response at the critical concentration or IWC is significantly different (i.e., worse) than the toxicity test control treatment (USEPA 2010b, 2010c). In NPDES permits, an effluent sample is considered toxic (i.e., noncompliant with the permit WET limit, exceeding a trigger, or otherwise found to cause an excursion of applicable WET WQS) if the NOEC is less than the permitted IWC.

EPA's Office of Wastewater Management (OWM) developed a publicly available statistics spreadsheet tool that analyzes toxicity test data using EPA's recommended statistical approaches, including the TSD hypothesis test (i.e., NOAEC and NOEC/LOEC), the point estimate (i.e., LC_{50} and IC_{25}), and the TST. The NPDES permit writer can use this tool to check WET test endpoint results submitted by a permittee. EPA's statistics spreadsheet tool can be found at https://www.epa.gov/npdes/whole-effluent-toxicity-wet-npdes-spreadsheet.

4.2.9 WET Test Monitoring Frequency

Once the need for an NPDES WET limit or monitoring requirement has been determined (see Section 5), the frequency of WET testing must be determined. The frequency for monitoring pollutants or pollutant parameters such as WET should be determined on a case-by-case basis and be representative of the permitted discharge(s). Decisions for setting the monitoring frequency should be documented in the NPDES permit fact sheet [40 CFR § 124.56].

NPDES WET monitoring should be sufficiently frequent to properly characterize the toxicity to be considered representative of the permitted effluent. Some NPDES permit authorities have their own recommended sampling guidelines that can help the permit writer determine an appropriate monitoring frequency. The intent is to establish a frequency of monitoring that will optimize detecting noncompliance events without requiring unnecessary monitoring (Table 4-2). In general, EPA recommends monthly WET testing for high-flow effluent discharges (e.g., more than one mgd) and quarterly WET testing for NPDES discharges with less flow (USEPA 2010d).

Table 4-2. *Likelihood of detecting at least one toxic event using the number of observations (n) for specified true probability of occurrence.*

Number of Observations, *n*	True Probability of Occurrence *		
	10%	20%	30%
1	0.10	0.20	0.30
2	0.19	0.36	0.51
3	0.27	0.49	0.66
4	0.34	0.59	0.76
5	0.41	0.67	0.83
6	0.47	0.75	0.88
8	0.57	0.83	0.94
10	0.65	0.89	0.97
12	0.72	0.93	0.99
16	0.81	0.97	0.99
20	0.88	0.99	0.99

Note: * Assumes (i) negligible serial correlation among observations, and (ii) true probability of occurrence remains the same over time. Probability of occurrence is stated as a percentage of the possible independent sampling events.

Additional factors to consider when determining the appropriate effluent WET test monitoring frequency of effluents include:

- Whether the effluent is discharged intermittently, such as wastewater treatment discharges released into the receiving stream periodically rather than continuously,
- The NPDES facility's compliance record for other NPDES permit conditions, and
- The degree of effluent variability in terms of other water quality parameters monitored in their permit or discharge flow rates.

If the effluent is discharged intermittently, the timing and frequency of NPDES WET monitoring should be specified accordingly. If the facility has a history of NPDES permit noncompliance or the discharge is highly variable due to the facility's operations or type of wastewater treatment used, then frequent monitoring, such as monthly NPDES WET testing, may be warranted.

As discussed in the EPA NPDES Permit Writers' Manual (USEPA 2010a), establishing a monitoring frequency requires the permit writer to estimate the variability of toxicity in the permitted effluent or the ambient sample from a receiving water. A highly variable NPDES discharge should require more frequent monitoring than a discharge that is relatively consistent over time (particularly in terms of flow or observed toxicity). In addition to the estimated sample variability, other factors that should be considered include the types of treatment processes; environmental significance and nature of the pollutants or pollutant parameters in the effluent; past compliance record/history; cost of monitoring relative to permitted dischargers capabilities; number of monthly samples used in developing the NPDES permit limit; and, for intermittent dischargers, the frequency of the permitted discharge (USEPA 1991a). NPDES permit authorities should consider the risks associated with infrequent WET monitoring. Table 4-2 provides information on the likelihood of detecting at least one toxic event based on a specified true probability of occurrence. The true probability of occurrence also can be understood as the true percentage of time the discharge is actually toxic (Table 4-2; Figure 4-2). Increased monitoring frequency will always increase the potential for capturing toxicity, even when it is only present for a short period of time. For example, suppose the discharge is toxic 20% of the time (e.g., probability of occurrence is 20%) (Table 4-2; Figure 4-2). Then, if toxicity testing is performed once per quarter for a year (n = 4), the probability that, in one-year, at least one of the four toxicity tests will demonstrate toxicity is 0.59 (Table 4-2; Figure 4-2). The same would apply to monitoring once per year for four years (n = 4). Increasing the monitoring frequency, however, increases the likelihood of capturing toxicity in a WET test. For example, if the discharge is toxic 20% of the time, quarterly monitoring for three years (n = 12) would be expected to capture toxicity with high probability (0.93).

An NPDES permit writer also may establish a monitoring schedule that reduces or increases the monitoring frequency during a permit cycle. Monitoring schedules that reduce monitoring over time might be appropriate for NPDES discharges for which the initial monitoring shows compliance with NPDES permit effluent limits for WET, does not exceed WET triggers, or otherwise demonstrates the discharge does not cause an excursion of WET WQS. The permit writer could establish a monitoring frequency with a permit clause that would allow a decrease in the toxicity testing frequency after a certain number of tests. For example, the permit could include a requirement for at least 20 observations (e.g., independent toxicity test results) within a 4-year period that are measured and are

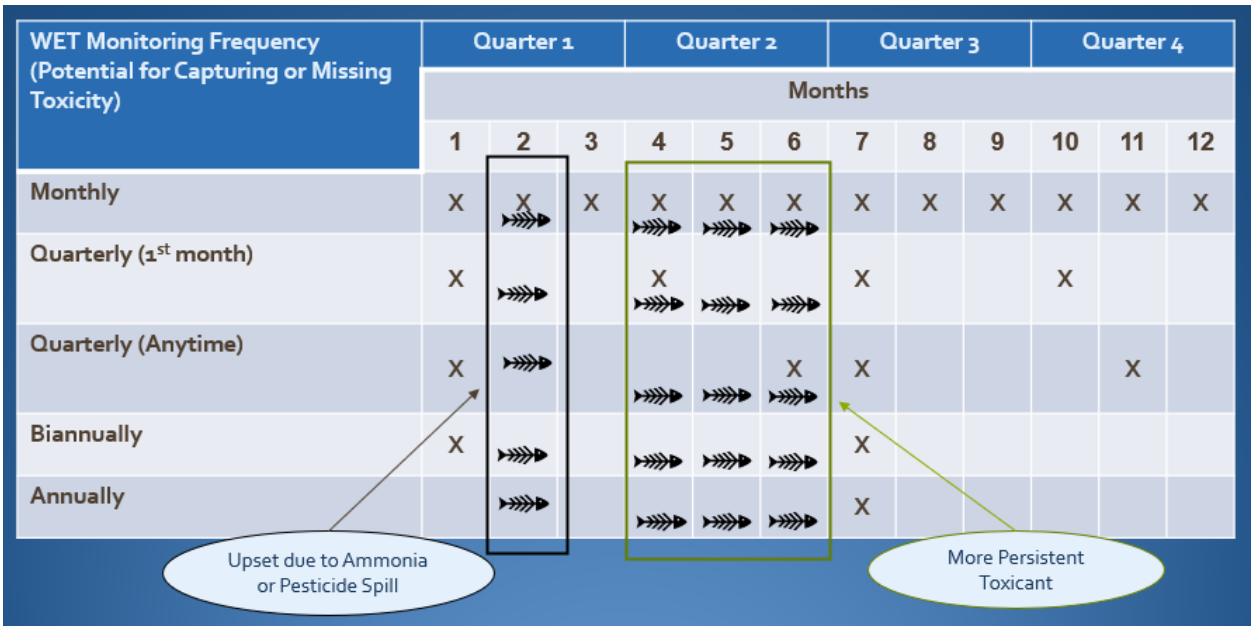


Figure 4-2. *Example of how various selected WET monitoring frequencies can support the potential for capturing or missing possible toxicity of effluent discharges or in ambient samples from receiving waters. The black box represents a short-term toxic event possibly from ammonia or a pesticide spill, which might not be captured in toxicity monitoring depending on sampling frequency. The green box represents a more persistent toxicant (e.g., water treatment chemical) that has a higher probability of being captured in toxicity monitoring.*

not toxic per the permit requirements (i.e., results are below the NPDES effluent WET limit or the numeric monitoring triggers as specified in the permit), then the permittee might be able to reduce testing frequency (Table 4-2).

If a facility expects any changes in its discharge composition, discharge flow, or the facility's treatment, the facility's discharge should be assessed for the continued assumption of no toxicity at the higher level of toxicity testing frequency to obtain a data set (e.g., n = 20) based on the treatment changes before consideration of a reduced monitoring frequency. Whether the NPDES-permitted facility is a POTW, or an industrial facility, should also be considered. By the nature of indirect discharges to a POTW, POTWs often discharge varying concentrations and types of toxicants, which can change readily because of their chemical composition or exposure to other factors, including light, temperature, and other chemicals in the effluent. Therefore, POTW permits should include a carefully selected toxicity monitoring frequency along with the appropriate toxicity test type and test species to assess the continued toxic impact potential from effluent exposure. A minimal level of WET testing frequency might be appropriate for POTWs with no industrial discharges to the POTW and those with a small effluent discharge volume relative to the volume of the receiving water body.

4.2.10 NPDES Accelerated WET Testing

NPDES permit conditions should specify follow-up or accelerated WET testing requirements if a WET test result indicates noncompliance with the NPDES permit's WET limits, exceedance of a WET trigger, or an excursion of applicable WET WQS. Accelerated WET monitoring requirements are common NPDES permit conditions that can vary among NPDES permit authorities. For example, permits can include a requirement for conducting more frequent WET testing over a short period, generally every two weeks, to determine if toxicity is considered to be persistent. If the results generated in accelerated WET testing

do not show toxicity, the permit may allow for a return to the previous WET monitoring frequency schedule. If toxicity is shown in the accelerated WET testing data, the initiation of the toxicity reduction evaluation (TRE) process is recommended as a follow-up option and any requirements related to this should be specified in the permit (see Section 4.2.11). If the permit contains a reopener clause, the NPDES permit authority may reopen the permit to add a permit limitation for WET.

The number of toxicity tests and the duration of toxicity testing included in the permit's accelerated monitoring requirements should be adequate to establish the presence of continued toxicity (e.g., at least six additional toxicity tests to be conducted at 14-day intervals). EPA recommends that the permit include a trigger for accelerated testing following notification of unacceptable toxicity measured in a valid toxicity test (USEPA 2010d). Initially, if the receiving water was used as the dilution water and is suspected to be toxic, the permit should direct the permittee to conduct a follow-up WET test using laboratory water with a similar pH and hardness as the dilution water. If the follow-up WET test using laboratory water does not show toxicity, the permit should allow the permittee to return to a normal monitoring frequency but should continue to use laboratory water as the dilution water. When the receiving water is not suspected to be the cause or contributor to the failed WET test, EPA's recommendation of a minimum of six additional toxicity tests is based on the probability of encountering at least one exceedance of permit requirements (e.g., permit WET limits or triggers) assuming that the effluent is toxic, but at an unknown level of toxic impact on aquatic life (Table 4-2).

4.2.11 Toxicity Reduction and Toxicity Identification Evaluations

TREs and TIEs are recommended procedures used in the NPDES permit program to enable permittees to identify and reduce toxicity observed using toxicity tests. This section discusses what should be included in the permit regarding TREs/TIEs. Additionally, EPA's TRE and TIE procedures manuals are available at the following website: https://www.epa.gov/npdes/permit-limits-whole-effluent-toxicity-wet (USEPA 1989a, 1989b, 1991c, 1992, 1993a, 1993b, 1996, 1999).

The NPDES permit should specify when a TRE is required, such as when the accelerated WET testing data indicate that the effluent is toxic at a level that would result in an excursion of the applicable WET WQS (Figure 4-3). The permit should also include what is required when a TRE is triggered.

Additionally, TIEs can be a useful part of the TRE in helping identify the cause of toxicity and, therefore, increase the permittee's ability to control it. Some TREs, however, can be resolved early on through a review of facility information and performance evaluation, and, in those cases, a TIE might not be necessary. The permit should include details related to TIEs when they are a necessary step in resolving toxicity, which might include submission of a plan by the permittee specifying when a TIE would be conducted and who would conduct it, if necessary. Unless revised in writing by the NPDES permit authority, EPA recommends that permits include the following requirements when a TRE is triggered. Any such requirements included by the NPDES permit authority should be clearly specified in the permit:

- Notice of TRE study implementation to be submitted to the NPDES permit authority within 10 days of activation of this TRE trigger.
- A TRE schedule and TRE action plan to be submitted to the NPDES permit authority within 60 days of the initiation of the TRE.
- The initial term of the TRE should be no longer than 24 months as follows: The "TRE initiation date" should be the date of the toxicity test that confirms toxicity is initiated and the "TRE

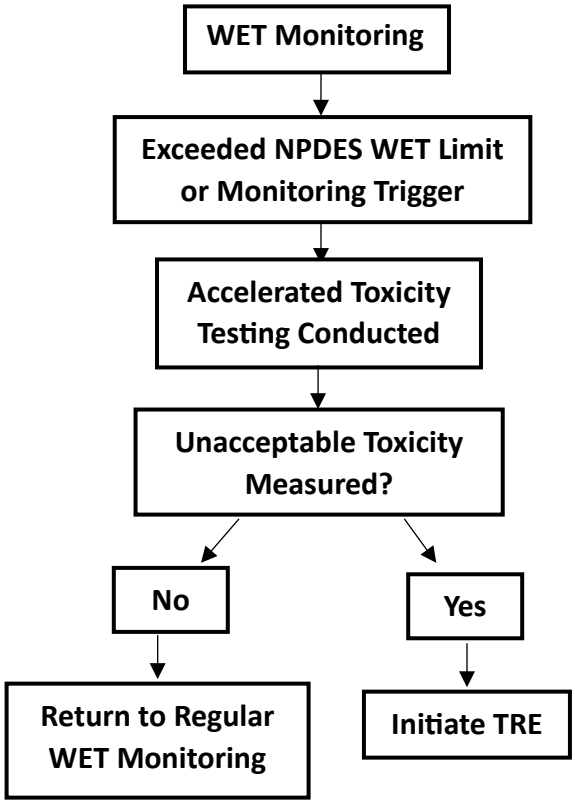


Figure 4-3. *TRE trigger sequence flowchart.*

termination date" is the date corrective actions to resolve toxicity are to be identified and be no more than 24 months from the TRE initiation date. There are circumstances that could extend this recommended schedule, including intermittent toxicity or seasonal toxicity.

- A quarterly TRE progress report should be submitted with the discharge monitoring report (DMR) to the NPDES permit authority at the end of each quarter, based on the TRE initiation date. The progress report should list all activities and findings related to resolving toxicity, including all WET and chemical test data. The data summaries of the TRE also should be provided in a tabulated format with explanations of the procedures used and the recorded findings from the study.
- Any exceedance of an NPDES WET monitoring trigger or permit limit during the implementation of a TRE should be reported within five working days to the NPDES permit authority. A final TRE report should be submitted to the NPDES permit authority within 45 days of the TRE termination date and should summarize the TRE activities and findings, propose the corrective action(s) to be taken, and propose a schedule to complete any identified corrective action(s).
- The minimum monitoring frequency for the affected test species should be noted in the TRE work plan. The NPDES permit authority, however, might recommend additional toxicity testing, which might include streamlined toxicity tests using a single test concentration of the sample compared against the control to identify toxic samples for further investigation as part of the

iterative process used in a TRE. This iterative process could include using toxicity tests and chemical analysis of portions of effluent treated in the TRE and identified to be toxic.

- All samples used for toxicity testing during the TRE should be analyzed for any toxicant identified as being a potential source of toxicity. If later toxicity testing determines the toxicant to be a probable source of toxicity, the analysis may be discontinued when all the findings and analytical results are clearly documented in the quarterly TRE progress report. The objective of this testing is to ascertain whether the same level of toxicity occurs when the suspected toxicant level varies, indicating the potential for more than one source of toxicity. This information might lead to finding additional toxicants or confirming or eliminating the suspected toxicant and possibly its source.
- Where toxicity is intermittent, the NPDES permit authority may include additional requirements based on BPJ.
- TRE triggers and the actions that follow are the initial recommended responses to the confirmation of a demonstrated toxicity above the NPDES WET limit or WET numeric monitoring trigger. Those actions do not constitute a compliance order, nor do they preclude a possible enforcement action.

A TRE also might lead to an additional NPDES permit control, such as a WET permit limit, a chemical-specific permit limit, or a compliance requirement to reduce or eliminate toxicity.

In addition to NPDES permit conditions, several other mechanisms are available that the NPDES permit authority can use to require a permittee to conduct a TRE. For example, the NPDES permit authority can require a TRE through a CWA Section 308 letter, a CWA Section 309 administrative order (AO), or as part of any consent decree requirements.

4.2.12 Compliance Schedules

When allowed under federal, state, territory, and authorized Tribal law, and when appropriate [40 CFR § 122.47], NPDES permits may contain schedules for compliance with WET effluent limitations (USEPA 2007). Compliance schedules may be included, where appropriate, to allow the permittee to conduct a TRE and attain compliance with NPDES WET limits.

The CWA establishes a deadline of no later than July 1, 1977, for compliance with effluent limitations developed to meet states', territories', and authorized Tribes' WQS. NPDES permits may contain schedules of compliance beyond July 1, 1977, to meet WQBELs if two requirements are met:

1. The permit effluent limitation must be based either on a post-July 1, 1977, state's, territory's, or authorized Tribe's WQS or a new or revised interpretation of a pre-July 1, 1977, state's, territory's, or Tribe's WQS; and
2. The applicable state's, territory's, and Tribe's WQS must explicitly authorize schedules of compliance.[2]

[2] Section 131.15 provides that while compliance schedule authorizing provisions can be codified in a state's, territory's or authorized Tribe's water quality standards or implementing regulations, EPA will take action to approve that provision as a water quality standard under CWA Section 303(c). If a state, territory, or authorized Tribe has already adopted an authorizing provision that is consistent with the CWA, it need not readopt the provisions for purposes of satisfying the final rule. Instead, the state, territory or authorized Tribe can submit the provision to EPA with an Attorney General or appropriate tribal legal authority certification. Moreover, consistent

40 CFR § 122.47 also governs compliance schedules in NPDES permits. The regulation authorizes, where appropriate, schedules requiring compliance with effluent limitations as soon as possible and no later than the applicable CWA statutory deadline. The regulation imposes certain restrictions on allowing schedules of compliance for new sources, new dischargers, and recommencing dischargers. The regulation establishes requirements for interim dates for certain schedules of compliance and for permittee reporting. Any compliance schedules developed for WET limitations must also satisfy 40 CFR § 122.47, if applicable. Thus, to decide whether to allow a compliance schedule in an NPDES permit for effluent limitations to control WET, the NPDES permit authority must answer these questions:

1. **Was the applicable water quality criterion promulgated or interpreted after July 1, 1977?**

   Most NPDES permit authorities established effluent limitations to control WET based on the state's, territory's or authorized Tribe's narrative WQC. Most of the narrative quality criteria for toxicity were adopted before July 1, 1977. Where this is the case, the NPDES permit authority can allow a schedule of compliance in the NPDES permit only if the state, territory, or authorized Tribe has made a new or revised interpretation of the applicable narrative water quality criterion after July 1, 1977. If the NPDES permit authority establishes an effluent limitation to control WET based on a numeric water quality criterion for WET, it is more likely that the criterion is a post-July 1, 1977, criterion.

2. **Has a compliance schedule authorizing provision been approved by EPA as part of the state's, territory's, or authorized Tribe's applicable water quality standards?**

   Where EPA has approved an explicit statement authorizing compliance schedules as part of a state's, territory's, or authorized Tribe's applicable water quality standards, permit writers may include compliance schedules for WET.

3. **Do other relevant provisions of federal, state's, territory's, or authorized Tribe's law or policy allow the schedule of compliance?**

   Here, for example, the NPDES permit authority should consider whether allowing a schedule of compliance for the specific discharge meets the requirements in 40 CFR § 122.47, if applicable, or any other requirements of state, territory, or authorized Tribal law.

If the NPDES permit authority answers "yes" to each of these questions, it might allow a schedule of compliance in the NPDES permit. The NPDES permit authority is not compelled to establish a schedule of compliance in the NPDES permit, however, even though they have the authority to do so. The NPDES permit authority should impose a schedule of compliance only if appropriate under the specific conditions of the permitted discharge. Consistent with 40 CFR § 122.47, EPA and NPDES states, territories or authorized Tribes should require compliance with states', territories' and authorized Tribes' WQS as soon as possible to ensure the permittee complies with the CWA.

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with 40 CFR § 131.21(c), if any permit compliance schedule authorizing provision that was adopted, effective, and submitted to EPA before May 30, 2000, is applicable for purposes of 40 CFR § 131.15. See 80 Federal Register 51020, 51041 (August 21, 2015).

4.3 Quality Assurance and Quality Control

4.3.1 Laboratory Performance

It is the laboratory's responsibility to demonstrate and maintain its ability to generate consistent, precise toxicity test results with reference toxicants (e.g., sodium chloride and copper) before it performs toxicity tests with effluents for NPDES permit purposes or ambient samples. The NPDES permit writer should include requirements related to reference toxicant tests in the permit. According to the EPA toxicity test methods manuals (USEPA 2002a, 2002b, 2002c; 1995b), regardless of the source of test organisms (in-house cultures or purchased from external suppliers), the laboratory must perform at least one acceptable reference toxicant test per month for each toxicity test method conducted in that month. If a toxicity test method is conducted once monthly or less frequently, a reference toxicant test must be performed concurrently with each NPDES effluent toxicity test. Organisms cultured in-house are required to be used in a reference toxicant test monthly; whereas organisms purchased from external suppliers are required to be used in a reference toxicant test for each batch of organisms.

For a given toxicity test method, successive tests must be performed with the same reference toxicant, at the same test concentrations, in the same dilution water, and using the same data analysis approach. Each of the laboratory's reference toxicity results should reflect good toxicity test endpoint repeatability. Many NPDES permit authorities include a requirement in their permits for the laboratory's reference toxicant control chart to be submitted to the NPDES permit authority for review along with the toxicity test conditions and results (Figure 4-4).

Reference toxicant toxicity test results should not be used as a de facto criterion for rejection of individual sample toxicity tests. Reference toxicant toxicity testing is used for evaluating the health and sensitivity of test organisms over time and for documenting initial and ongoing laboratory performance. While reference toxicant test results should not be used as a de facto criterion for test rejection, sample toxicity test results should be reviewed and interpreted considering reference toxicant toxicity test results. The following describes EPA's recommendations on the interpretation of reference toxicant toxicity tests, however, NPDES permit authorities may use other reference toxicity test data review and interpretation approaches. EPA recommends that reference toxicant toxicity tests should be reviewed to consider the degree to which the reference toxicant toxicity test results are outside the range of control chart limits (see Section 4 – Quality Assurance in each of the WET Methods Manuals; USEPA 2002a, Section 4; 2002b, Section 4; 2002c, Section 4; 1995b). In addition, the reviewer should examine whether the deviation indicates increased test organism sensitivity (i.e., lower than the lower bound [mean minus two standard deviations (SDs)]) or decreased test organism sensitivity (i.e., higher than the upper bound [mean plus 2SDs]) (Figure 4-4).

4.3.2 Reducing Toxicity Test Variability

NPDES permit authorities should encourage and work with permittees to select a laboratory with qualified staff who follow EPA's toxicity test methodology [40 CFR Part 136] and that produces quality test results in a timely and consistent manner. EPA has developed guidance for laboratories, permittees, and regulatory authorities on considering variability in toxicity testing, including both analytic and effluent variability (USEPA 2000b) and has identified three critical areas that can minimize toxicity test method variability: (1) obtaining a representative effluent sample for the monitored activity, (2) conducting the toxicity tests properly to generate biological test endpoints, and (3) calculating the appropriate statistical test endpoints to optimize confidence in the measured toxicity effect

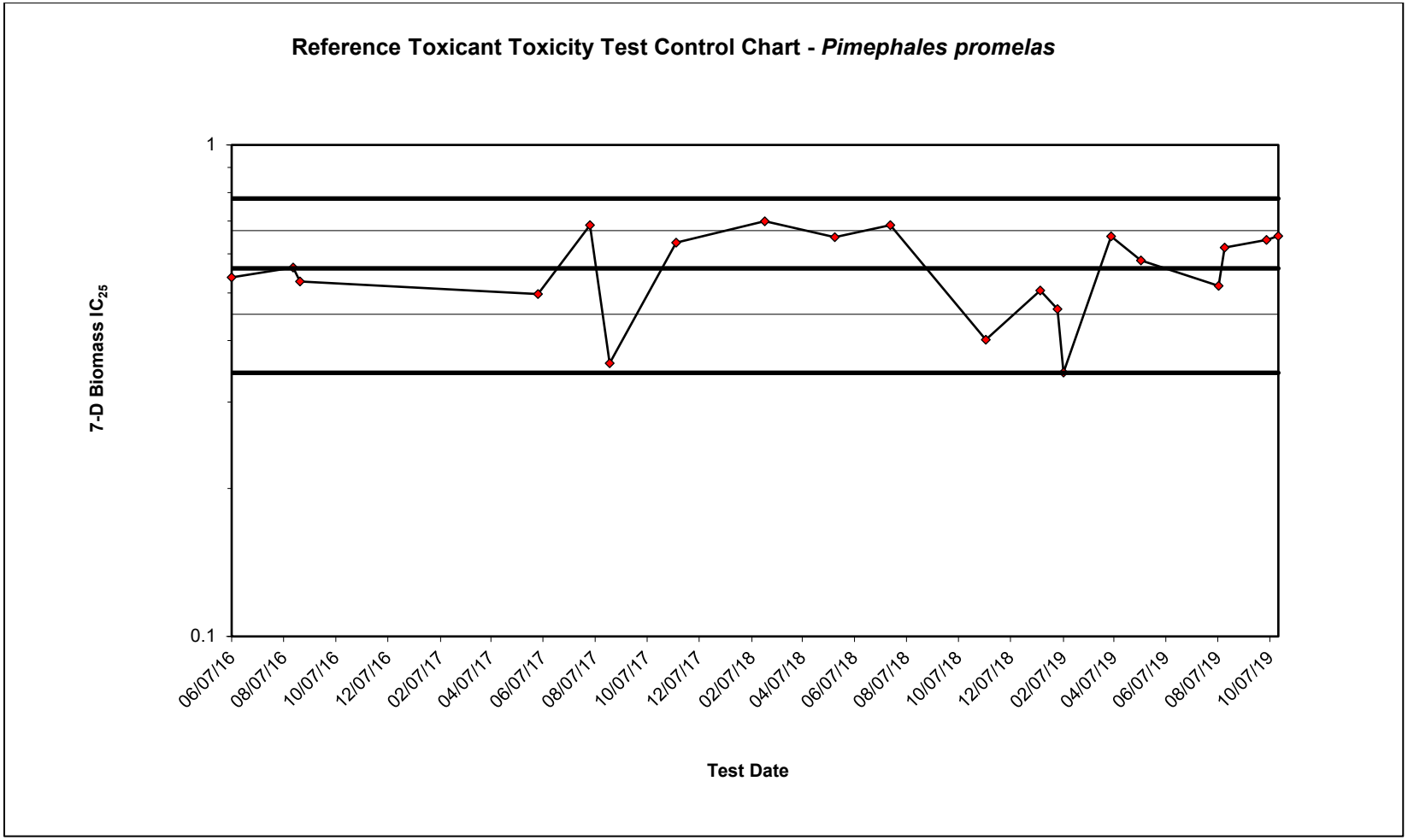


Figure 4-4. *Example reference toxicant toxicity test control chart illustrating the log of the IC_{25} over time for the freshwater fathead minnow (Pimephales promelas). Each data point represents the 7-day biomass IC_{25} measured in the test conducted. EPA's 2002 toxicity test methods require laboratories to chart the 20 most recent data points and develop upper and lower bounds. The upper and lower bounds represent the mean ±1 or ±2 SD. Plotted data that fall above the upper bound (mean + 2SD) indicate the organisms are more tolerant than is typically observed at this laboratory. Plotted data that fall below the lower bound (mean – 2SD) indicate that the organisms are more sensitive than is typically observed at this laboratory.*

concentration. EPA's WET Variability guidance also provides specific guidance for the testing laboratories (USEPA 2000b, Section 7.2.1) on how to improve laboratory performance and reduce within-test variability. Some specific procedures laboratories should consider for improving within-test precision are:

- Using increased feeding rates within acceptable protocol guidelines, which might improve control sublethal endpoint precision in chronic toxicity tests (e.g., reproduction in *Ceriodaphnia dubia* chronic toxicity tests or growth/biomass in fish chronic toxicity tests).
- Training and evaluating staff to ensure they can consistently achieve adequate within-test precision (e.g., control coefficient of variation [CV] for a given toxicity test endpoint at or below the national 50th percentile). EPA has quantified the within-test variability (expressed as both SD and CV) and mean control responses for many EPA toxicity test methods on a national basis (USEPA 2010c; Appendix D).
- Making good use of reviewing laboratory QC control charts, including:
    - Tracking and evaluating control response, including mean, SD, and CV values over time.
    - Calculating its long-term control 50th and 90th percentiles for tracking their performance instead of using the national percentiles for tracking and evaluation purposes if laboratory performance might be better than indicated in the national percentiles.

4.3.3 Reporting WET Data

The regulations in 40 CFR § 122.41(l)(4)(i) provide that permits require monitoring results to be reported on a DMR. As of December 21, 2016, all reports and forms required by a permit must be submitted electronically by the permittee either through NetDMR (see EPA's NetDMR web page), which flows data into the Integrated Compliance Information System for NPDES (ICIS-NPDES), or a similar state tool unless the permittee has obtained a waiver from electronic reporting (see EPA's NPDES eReporting – Information for Permittees and Other Regulated Entities web page) or the permit specifies otherwise. NPDES permit authorities may require WET test laboratory reports to be submitted as an attachment to the DMR.

The NPDES permit is to specify, directly or by reference to an NPDES permit authority's implementation document, the minimum content that must be included in DMRs and any additional required WET data reports. In addition to monitoring results, EPA recommends that NPDES permits include a requirement to submit WET testing laboratory reports (USEPA 2002a, 2002b, 2002c; USEPA 1995b) that include the recommended contents in the *WET Laboratory Report Recommended Contents* text box.

EPA also recommends that permits include a requirement for permittees to report any known problems with QA at the time of DMR submission, if not before, and that the permit specify that, in these cases, the toxicity test must be conducted again with a new sample (USEPA 1991a; USEPA 2002a, 2002b, 2002c; USEPA 1995b).

The NPDES permit writer should coordinate with the staff responsible for entering ICIS-NPDES codes for their NPDES permit authority and work with them to resolve any coding issues. EPA recommends that, where possible, parameters and associated units used in a permit correspond to existing codes within ICIS-NPDES. A parameter request form, including for WET parameters, can be submitted to EPA for review, however, and added to the ICIS-NPDES database, if appropriate.

4.3.4 Recordkeeping

NPDES permits must require permittees to retain records of all monitoring information, including all calibration and maintenance records, copies of all reports required by the permit, and records of all data used to complete the application for this permit, for a period of at least three years from the date of the sample, measurement, report, or application [40 CFR § 122.41(j)(2)]. Given the complexities of WET testing, EPA recommends that the NPDES permit specifies the documentation and records retention requirements associated with WET test data used in NPDES permits. If the permit does not require that laboratory reports be submitted with the DMR, the permit should clarify that laboratory reports are retained as a recordkeeping requirement.

WET Laboratory Report Recommended Contents	
General Information	
• NPDES permit number • Facility's wastewater discharge location(s) • Name of laboratory conducting the toxicity testing • Objective of toxicity test(s)–compliance, monitoring	• Toxicity testing requirements of the NPDES permit • Name of receiving water body • Phone number and address of facility
Description of Effluent Sample(s) Used in Toxicity Testing	
• Sampling point • Sample collection method • Average daily discharge on sample collection date(s) • Sample temperature(s) when received at the laboratory	• Sample collection dates and times • Physiochemical data collected with the sample • Time(s) from sample collection to arrival at lab and to test setup
Description of Dilution Water Used in Toxicity Testing	
• Dilution water source • Collection date(s) and time(s)	• Physicochemical characteristics • Any applicable pretreatment
Description of Toxicity Test Conditions Used	
• Toxicity test method(s) used (year, source) • Deviation(s) from reference toxicity test method, if any, and the reason(s) • Date and time toxicity test(s) terminated • Volume of solution used per chamber • Number of replicate test chambers per concentration • Test temperature (mean and range) • Feeding frequency, and amount and type of food, if any	• Endpoint(s) of toxicity test • Date and time toxicity test(s) started • Type and volume of toxicity test(s) chambers • Number of toxicity test organisms per test chamber • Acclimation of toxicity test organisms (temperature mean and range) • Whether aeration was needed • Whether (and how) pH control measures were implemented
Description of Test Organisms Used in Toxicity Testing	
• Scientific name and how determined • Life stage • Source • Taxonomic key used for species identification	• Age • Mean length and weight (where applicable) • Diseases and treatment (where applicable)
Description of Toxicity Test Results	
• Raw toxicity data in tabular form, including daily records of affected toxicity test organisms in each toxicity test concentration (including controls) and replicate, and in graphical form (plots of toxicity data) • Statistical approaches used to calculate test endpoints • Quality assurance data	• Table of LC_{50}, NOECs, IC_{25}, IC_{50}, etc. (as required in the applicable NPDES permit) • Summary table of physical and chemical data measured during the test • Percent minimum significant difference (PMSD) calculated for chronic sublethal test endpoints
Quality Assurance and Quality Control	
• Reference toxicant used and source • Dilution water used in reference toxicant test(s) • PMSD calculated for chronic sublethal test endpoints determined by hypothesis testing in reference toxicant test(s)	• Date and time of most recent reference toxicant test, test results, and current control chart • Results (NOEC or, where applicable, LOEC, LC_{50}, EC_{50}, IC_{25} and/or IC_{50}) • Physical and chemical analytical methods used to measure water quality

Note: EC_{50} = effluent concentration causing 50% effect.

5 Reasonable Potential Analysis for Evaluating Need for NPDES WET Permit Limits

This section presents EPA's interpretation and application of regulations under CWA 40 CFR § 122.44(d)(1) for evaluating whether a discharge causes, has the reasonable potential to cause, or contributes to an in-stream excursion above a WET criterion to determine if an NPDES WET limit is needed. Where appropriate, 40 CFR Part 132 Appendix F Procedure 6 is applicable to use for Great Lake states.

5.1 WET Water Quality Standards

WQS for states, territories, and authorized Tribes provide the foundation for water quality-based pollution control programs. The purpose of NPDES WET limits and monitoring requirements is to implement applicable numeric or narrative WQC established to protect the designated uses of a water body. This section briefly describes the WQS that are the basis for WET WQBELs in NPDES permits.

WQS are provisions of state, territory, or authorized Tribal (or, in certain instances, federal) law that define the water quality goals of a water body, or portion thereof, by designating the use or uses to be made of the water body and by setting criteria necessary to protect those uses. States, territories, and authorized Tribes adopt WQS to protect public health or welfare, enhance the quality of water, and serve the purposes of the CWA (see Section 2). The adopted WQS serve the dual purposes of establishing the water quality goals for a specific water body and serving as the regulatory basis for the establishment of water quality-based treatment controls and strategies beyond the TBEL levels of treatment required by CWA Sections 301(b) and 306 [40 CFR § 131.2].

WQC are elements of states', territories', and authorized Tribes' WQS expressed as either constituent concentration levels or narrative statements representing a quality of water that supports a particular use. When criteria are met, water quality will generally protect the designated use [40 CFR § 131.3(b)]. States, territories, and authorized Tribes have adopted a variety of criteria expressed as constituent concentration levels (or numeric criteria) for various pollutants or pollutant parameters for the protection of aquatic life, including WET. All states, territories, and authorized Tribes, however, have at least adopted criteria expressed as narrative statements (or narrative criteria) for WET. These narrative criteria, often referred to as "free-from" (or, in the case of toxicity, "no toxics in toxic amounts") criteria, are an effective tool for controlling the discharge of pollutants if numeric criteria are not available. Narrative criteria can be interpreted as a numeric expression (e.g., 0.3 toxic unit-acute [TU_a] and 1.0 toxic unit-chronic [TU_c]) for implementing in NPDES permits and can be a basis for establishing WET controls specified in the NPDES permit regulations in 40 CFR § 122.44(d)(1).

Section 304(a) criteria are developed by EPA under authority of Section 304(a) of the CWA based on the latest scientific information on the relationship that a constituent concentration has on a particular aquatic species and/or human health. This information is issued periodically to the states, territories, and authorized Tribes as guidance for use in developing criteria. In adopting criteria to protect their designated uses, states, territories, and authorized Tribes may establish criteria based on (1) Section

304(a) guidance, (2) Section 304(a) guidance modified to reflect site-specific conditions, or (3) other scientifically defensible methods.

Although EPA has not published recommended numeric WQC for WET under Section 304(a), it has provided general guidance on appropriate NPDES WET limits. The TSD recommends for most water bodies 0.3 TU_a as an acute criterion and 1.0 TU_c as a chronic criterion (USEPA 1991a). EPA recommends the acute WET WQC or criterion maximum concentration (CMC) of 0.3 TU_a because it adjusts an LC_{50} (50% mortality) to an LC_1, or 1% mortality, which is almost no acute toxicity (USEPA 1991a). As noted in the TSD (USEPA 1991a, Section 2.3.3), the factor of 0.3 TU_a was found to include 91% of the observed LC_1 to LC_{50} ratios in 496 effluent toxicity tests. As a result, EPA recommended that 0.3 TU_a be used as EPA's acute criterion. EPA also recommends that 1.0 TU_c be used as the chronic criterion. The acute toxicity test has an upper sensitivity level of 100-percent effluent, or 1.0 TU_a, when using an LC_{50} as the test endpoint. If less than 50 percent of the test organisms die at 100-percent effluent an alternative test endpoint for evaluating reasonable potential or establishing a WET limit, such as one specified in terms of a NOAEC (e.g., "no significant difference in acute toxicity between 100 percent effluent sample and the control") is an appropriate application of the 0.3 TU_a at the end of the pipe (USEPA 1991a, Section 5.7.4). This is the most sensitive application of an acute test and could be used for determining reasonable potential or in establishing a WET limit.

For chronic protection, the criterion continuous concentration (CCC) should be set at 1.0 TU_c for the most sensitive of at least three EPA approved toxicity test method species. A CCC of 1.0 TU_c should be applied at the edge of the mixing zone to prevent chronic toxicity in the receiving water outside the mixing zone (Figure 5-1).

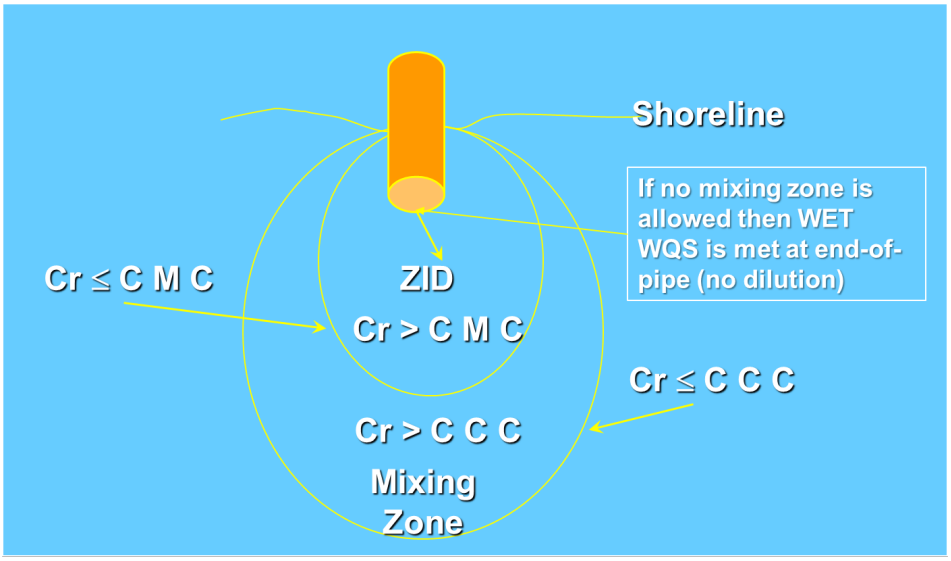
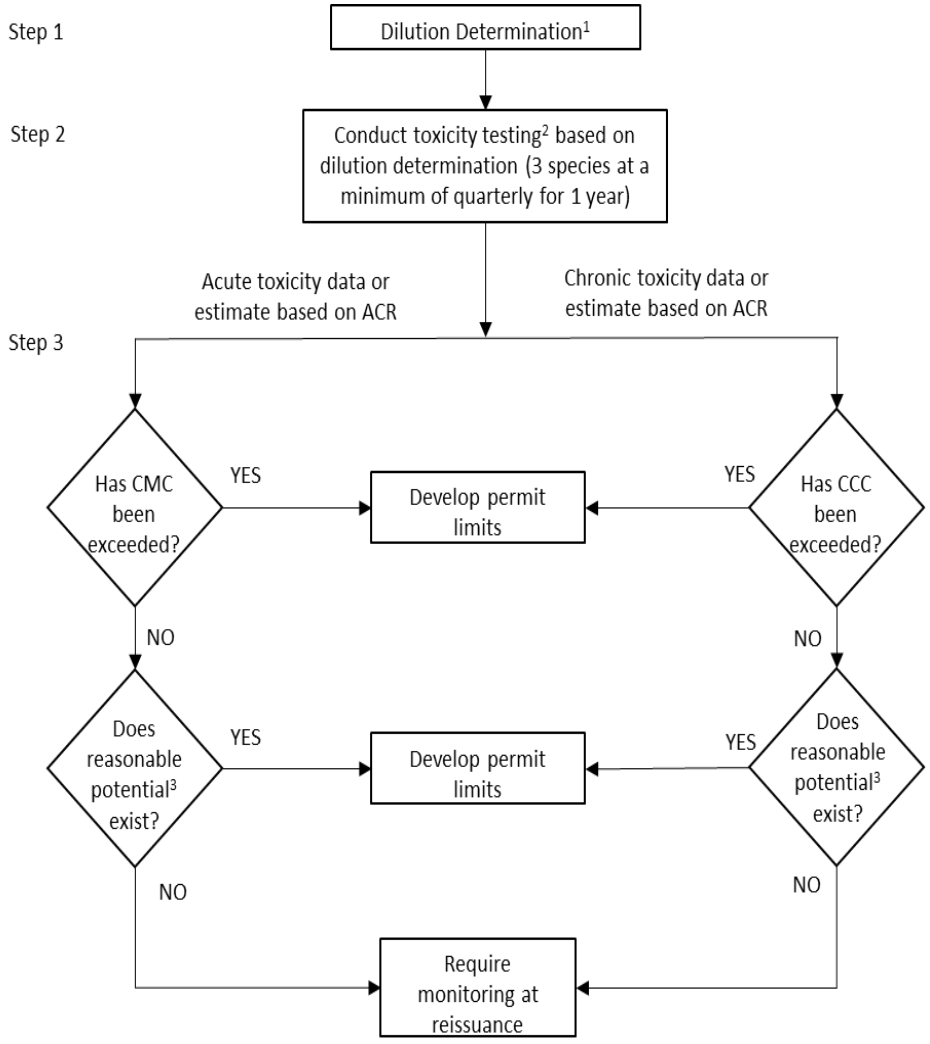


Figure 5-1. *Example of mixing zones that might be applicable to NPDES WET permit limits depending on the state's, territory's, or authorized Tribe's water quality standards. Cr = concentration in receiving water.*

Federal regulations in 40 CFR § 122.44(d)(1)(i) establish different approaches to implementing WQC for toxicity in NPDES permits, depending on whether the criterion is expressed in a numeric or narrative form. If a state, territory, or authorized Tribe has not adopted numeric criteria for WET, EPA expects the NPDES permit authority to *interpret* the state's, territory's, or authorized Tribe's narrative criterion so that

the appropriate effluent limits, including any necessary toxicity numeric limits, can be established. NPDES permit authorities should identify the approach they intend to use in regulating toxics based on narrative criteria and describe how their toxics control program will protect aquatic life and maintain the designated use [40 CFR § 131.11(a)(2)]. The implementation procedures when using WET narrative criteria should specify for the permit writer what to include in the permit. For example, the implementation procedures, at a minimum, should indicate which toxicity testing method to use, the duration of the toxicity tests (acute or chronic), the toxicity test species to use (most sensitive), the frequency of toxicity testing required, and the numeric benchmarks used to interpret the narrative criteria (Figure 5-2). The TSD provides a basis for establishing criteria for aquatic toxicity (USEPA 1991a). Criteria consist of three components: magnitude, duration, and frequency.



Notes:
[1] Dilution determinations should be performed for critical flows and any applicable mixing zones.
[2] Toxicity testing recommendations
a. Dilution > 1000:1: acute testing, check CMC only.
b. 100:1 < Dilution < 1000:1: acute or chronic testing, check CMC and CCC with data or ACR.
c. Dilution < 100:1: conduct chronic testing, check CCC with data and CMC using acute data or ACR.
[3] Reasonable potential: Use procedures in Box 3-3 of EPA's TSD (USEPA 1991a)

Figure 5-2. *Decision tree for effluent characterization for WET as outlined in EPA's Technical Support Document (USEPA 1991a). ACR = acute-to-chronic ratio.*

Magnitude refers to the concentration of the pollutant. EPA's recommended magnitudes for WET are as follows:

- For acute protection, a CMC should be specified to protect against acute (short-term) effects. The CMC should be set at 0.3 TU_a to the most sensitive of at least three EPA toxicity test species for acute tests (e.g., one fish and two invertebrates, or two fish and one invertebrate).
- For chronic protection, a CCC should be specified to protect against chronic (long-term) effects. The CCC should be set at 1.0 TU_c to the most sensitive of at least three EPA toxicity test species for chronic tests (fish, invertebrate, and plant).

Duration is the period of time (averaging period) over which the in-stream concentration is averaged for comparison with criteria concentrations. This specification limits the length of time that in-stream concentrations may exceed the criteria concentrations. EPA's recommended duration for aquatic life criteria, including toxicity, are:

- For acute criteria, an averaging period of one-hour to be representative of fast-acting toxicants. The one-hour acute averaging period was derived primarily from data on response time for toxicity to ammonia, a fast-acting toxicant. The one-hour average exposure should not exceed the CMC. Scientifically justifiable alternative (site-specific) averaging periods can be derived (USEPA 1991a, p. 35).
- For chronic criteria, an averaging period of four-days. That is, the four-day average exposure should not exceed the CCC. Different chronic averaging periods could be derived, depending on the nature of the pollutant and the toxic endpoint of concern (e.g., the rate of uptake and accumulation and the mode of action). EPA selected the four-day averaging period based on the shortest duration in which chronic effects are sometimes observed for certain species and toxics, and thus should be fully protective even for the fastest acting toxics (USEPA 1991a, p. 35).

Frequency is how often the criteria can be exceeded and still be protective to allow the aquatic community sufficient time to recover from excursions of aquatic life criteria. For frequency, neither acute nor chronic criteria should be exceeded for the above durations more than once every three years on the average. Based on site-specific considerations, states, territories, and authorized Tribes may allow for a different frequency (USEPA 1991a, p. 36).

5.2 Evaluating Reasonable Potential for WET

EPA's NPDES regulations in 40 CFR § 122.44(d)(1)(i) require that an NPDES permit must include an effluent limit for any pollutant that is or may be discharged at a level that "will cause, have a reasonable potential to cause, or contribute to an excursion above any" state's, territory's, or authorized Tribe's WQS, including narrative WQC.

In determining the need for an effluent limit, the permit writer must consider existing controls on other point and nonpoint sources, the variability of the pollutant or pollutant parameter in the discharge, the sensitivity of the EPA toxicity test species selected (for WET), and, where appropriate and allowed in the state's, territory's, or authorized Tribe's WQS or regulations, the dilution of the discharge in the receiving water [40 CFR § 122.44(d)(1)(ii)]. When RP is demonstrated based on a numeric toxicity criterion using the RP consideration requirements for WET outlined in 40 CFR § 122.44(d)(1)(ii), toxicity data, or other information (see Section 5.2.3), the permit must contain effluent limits for WET [40 CFR § 122.44(d)(1)(iv)]. Similarly, when the NPDES permit authority determines that a discharge causes, has

the reasonable potential to cause, or contributes to an excursion above a narrative criterion within an applicable state's, territory's, or authorized Tribe's WQS, the permit must contain effluent limits for WET unless the NPDES permit authority demonstrates in the fact sheet or statement of basis of the NPDES permit using the RP WET requirements in 40 CFR § 122.44(d)(1)(ii), that chemical-specific limits for the effluent are sufficient to attain and maintain applicable numeric and narrative WQC [40 CFR § 122.44(d)(1)(v)]. Regardless of the attainment status of the water body, the permit must include appropriately derived limits to protect designated uses if warranted by an RP evaluation. When the NPDES permit authority determines that the discharge does not cause or does not have the reasonable potential to cause, or contribute, to an excursion of a numeric or narrative toxicity criterion, a permit limit may not be necessary, but the NPDES permit authority may include routine monitoring using WET testing.

EPA has recommended two approaches to evaluating WET RP when valid, representative data are available. The first approach is the TSD's approach to evaluating WET RP (USEPA 1991a). The second approach incorporates steps for evaluating WET RP for data analyzed using the TST statistical approach (based on a minimum of four valid WET tests). Section 5.3 discusses the approaches to evaluating WET RP when valid data are available as well as approaches recommended in EPA's TSD to evaluating WET RP when valid WET data are not available.

In addition to EPA's recommended approaches for evaluating WET RP [40 CFR § 122.44(d)(1)], NPDES permit authorities may develop their own approaches for the process as long as they are consistent with the statutory and regulatory requirements, including ensuring that the permit includes limits as stringent as necessary to meet WET WQS under CWA Section 301(b)(1)(C).

5.2.1 Characterizing Effluent Quality with Respect to WET

When determining the need for a WQBEL, a permit writer should use any available effluent and receiving water data as well as other information pertaining to the discharge and receiving water (e.g., type of industry, existing TBELs, compliance history, or stream surveys). The permit writer might already have data available from DMRs, the permit application, or other previous monitoring or could decide to work with the permittee to generate data before permit issuance or as a condition of the new permit. EPA recommends that monitoring data be generated before effluent limitation development whenever possible. WET data supports WET RP evaluations for the following reasons:

- The presence or absence of effluent toxicity can be more clearly demonstrated.
- Effluent characterization is established, so possible effluent variability is more clearly evaluated.
- If data indicates that the effluent is toxic, a TRE can be initiated by the permittee to discover the source(s) of toxicity and take steps to control, reduce, or eliminate the toxicity, thus possibly eliminating the need for an NPDES WET limit because either the toxicity has been completely resolved or can be controlled using a chemical specific limit in the permit. If the WET limit is not needed, it should be removed through a reopener provision included in the permit at issuance, or not included upon permit reissuance (and document the basis in the permit fact sheet). Removal of the WET limit based on new information that the toxicity has been resolved or can

be controlled using a chemical specific limit may be justified under the anti-backsliding exception at CWA Section 402(o)(2)(B)(i).[3]

EPA's NPDES regulations for permit applications contain requirements concerning submission of WET data for industrial applicants and for POTWs [40 CFR § 122.21(g)(11); 40 CFR § 122.21(j)(5)]. Additionally, if the permittee monitors any pollutant more frequently than required by the permit using EPA's toxicity test methods, the results of that monitoring shall be included in the calculation and reporting of the data submitted in the DMR [40 CFR § 122.41(l)(4)(ii)]. Application and monitoring data submitted by the permittee shall be representative of the permitted discharge [40 CFR § 122.41(j)(1); 40 CFR § 122.48(b)]. Monitoring should begin far enough in advance of permit development to allow sufficient time to conduct WET analyses, including any necessary f]llow-up toxicity tests if the results of any toxicity test are found to be invalid based on EPA's toxicity test method TAC (see Section 5.2.2). Where data are generated as a condition of a permit that does not contain a WQBEL for WET, it might be appropriate for the permit writer to include a reopener clause in the permit to allow the incorporation of a WQBEL if the monitoring data indicate that one is required (e.g., RP exists).

Only valid data (see Section 5.2.2) and data that is representative of the permitted facility discharge should be used to evaluate the need for a WQBEL for WET (USEPA 1991a). When a permittee has a limited amount of valid WET data in advance of NPDES permit issuance or modification, the NPDES permit authority is encouraged to gather additional valid WET data from the permittee, if possible. The use of data sets of 10 data points or more decreases the uncertainty associated with small sample sizes and removes reliance on the recommended default assumptions about the possible effluent variability associated with reliance on fewer than 10 data points. If no valid WET data are available, RP evaluations can still be done based on facility and receiving water characteristics, or other information (see Section 5.2.3).

5.2.2 Determining Validity of WET Data

A valid WET test is one performed consistently with the EPA toxicity test methods specified in 40 CFR Part 136 and associated EPA toxicity test methods manuals (USEPA 2002a, 2002b, 2002c; 1995b). See Section 5.2.3 for additional details. A valid toxicity test must meet all applicable TAC specified in EPA's toxicity test methods and meet any additional QA/QC and toxicity testing requirements established in the NPDES permit. An example of a QA/QC control would be the chain of custody that accompanies a collected sample and the sample handling records.

The NPDES permit authority may require permittees to submit additional information if the results of toxicity tests are determined to be invalid. If the permittee is concerned about any WET test data or suspects that any data are not valid, the permittee should notify the NPDES permit authority as soon as possible and indicate why the permittee considers the data to be invalid. If the NPDES permit authority agrees with the permittee's determination that the toxicity data are not valid, the NPDES permit authority should then document in the permit fact sheet why the data are not valid and should not be used in evaluating RP [40 CFR § 124.56].

The permit should include a statement requiring the permittee to collect another effluent sample and conduct another toxicity test with the newly collected effluent sample within a reasonable time and if

[3] Chapter 7 in EPA's NPDES Permit Writer's Manual (USEPA 2010a) provides an overview and discussion of the CWA Section 402(o) and EPA's regulation [40 CFR 122.44(l)] on anti-backsliding.

possible, within the monitoring period if a toxicity test result is determined to be invalid. The permittee should consider scheduling toxicity tests early enough in the reporting period to allow for additional follow-up toxicity tests when a scheduled toxicity test does not meet EPA's TAC or is otherwise determined to be invalid.

5.2.3 Evaluating WET Data

The *Recommended Steps for Evaluating WET Data* text box provides a guide for the NPDES permit writer to use in evaluating and reviewing toxicity test results (USEPA 2002a, 2002b, 2002c; 1995b). This information should be used as a checklist for individual toxicity tests and does not cover the full range of QC practices necessary for successful completion of toxicity test data analysis. A full range of QC practices for WET data review can be found in EPA publications by type of toxicity test method:

- Acute toxicity test methods (USEPA 2002a, Ch. 12)
- Freshwater short-term chronic toxicity test methods (USEPA 2002b, Ch. 10)
- Estuarine/marine short-term chronic toxicity test methods (USEPA 2002c, Ch. 10)
- West Coast short-term chronic marine and estuarine toxicity test methods (USEPA 1995b, Ch. 4).

Additionally, NPDES permit authorities may have detailed guidance in their NPDES WET IPs on QC practices.

5.2.4 Determining Effluent Representativeness of WET Data

Effluent data used as the basis for effluent characterization should be representative of the monitored activity (i.e., the discharge under current conditions with current treatment and management practices at the facility). Representative WET data are those WET test results that appropriately characterize the permittee's effluent with respect to ongoing facility operations, including the discharge of toxic pollutants, which can vary (e.g., seasonal use of biocides, quarterly cleaning of industrial equipment, and so forth). The NPDES permit authority determines whether available effluent data are representative of the current operating conditions at the NPDES permitted facility [40 CFR § 122.41(j)(1); 40 CFR § 122.48(b)]. For example, data obtained prior to significant modifications to the facility's treatment process, pretreatment, or pollution prevention steps may no longer be representative of the facility's discharge. When data are no longer representative of the current facility discharge, the NPDES permit authority may decide not to use such data in the RP evaluation. Where data are not used because it is no longer representative of the monitored activity, the NPDES permit authority should document the rationale for their decision in the NPDES permit fact sheet or statement of basis. In this case, the RP evaluation should be based on data produced after the facility modification(s), or, if there are no data generated, RP should still be evaluated based on other available facility information using the permit writer's BPJ (see Section 5.3.3).

5.3 Approaches for Evaluating Reasonable Potential for WET

Sections 5.3.1 and 5.3.2 summarize EPA's recommendations for evaluating WET RP using RP approaches based on TSD[4] and TST, respectively, when valid WET test data are available. The NPDES permit authority, however, may select an alternative approach to evaluate WET RP. For example, the NPDES permit authority may opt to use a stochastic dilution model that incorporates both ambient dilution and effluent variability rather than using a steady-state dilution model with a statistically defined maximum

[4] 40 CFR Part 132 Appendix F Procedure 6 outlines the reasonable potential approach for Great Lakes states.

Recommended Steps for Evaluating WET Data

Compare Toxicity Test Conditions to Permit Requirements:

1. Examine the toxicity test results to verify that the laboratory is using the EPA toxicity test method and test concentration series required in the NPDES permit. The test concentration series being tested should include the IWC.
2. Evaluate the toxicity test results against the NPDES permit requirements for WET to determine whether the limit or numeric monitoring trigger is being achieved. For example, where an NPDES WET limit or numeric monitoring trigger is expressed in terms of TUs, then the value is expressed as a value "not to be exceeded." Where an NPDES WET limit or numeric monitoring trigger is expressed in terms of "% effluent at the IWC," the value is expressed as a value that the % effluent must be at or above.

Note: For a new discharger that does not yet have NPDES WET permit requirements, application data can be reviewed to determine if they were gathered using appropriate EPA toxicity test methods. The test review steps below would apply to dischargers with permit requirements, as well as new dischargers.

Review Testing Procedures:

Test review is an important part of the overall quality assurance program and is necessary for ensuring that all toxicity test results are reported accurately. Test review should be conducted on each toxicity test by both the permittee and their testing laboratory as well as the NPDES permit authority.

Note: See the chapters on test review in the EPA toxicity test methods manuals for the specified toxicity test method (USEPA 2002a, 2002b, 2002c; 1995b).

1. Examine the results to verify the sample was maintained at the proper temperature from time of collection to arrival at the testing laboratory. Also, determine whether the sample meets the EPA toxicity test initiation and renewal holding time requirements.
2. Evaluate the toxicity test results for the effluent sample to verify that the laboratory met EPA's TAC as specified in the toxicity test method with respect to lethal and sublethal biological endpoints. See the individual "Summary of Test Conditions and TAC" section in each toxicity test method manual. All invalid toxicity tests must be conducted again with a newly collected sample, as specified in the NPDES permit (USEPA 2002a, 2002b, 2002c; 1995b).
3. Examine the "Summary of Test Conditions and TAC" section for the specific toxicity test method to determine whether the other required and recommended toxicity test conditions were met. Below is a single example for (a) a required toxicity test condition and (b) a recommended toxicity test condition that would be specific to the particular toxicity test method listed in the NPDES permit.
    a. Did the laboratory conduct the toxicity test using the required EPA toxicity test conditions? Some of the toxicity test conditions listed are specified as "required" and, therefore, the condition must be met. For example, did the toxicity test use the required minimum number of replicates, number of test organisms, toxicity test type (e.g., acute, chronic, static, static-renewal), test duration, and so forth? All required toxicity test conditions must be met, or the toxicity test is considered invalid and must be conducted again with a newly collected sample.
    b. Did the laboratory conduct the toxicity test using the recommended EPA toxicity test conditions? Some of the test conditions listed are specified as "recommended" and, therefore, these should be met in the WET test or documented why they were not met in the WET data report. For example, the fathead minnow short-term chronic test method specifies a recommended number of toxicity test organisms per test chamber (e.g., 10 larvae per test chamber). A toxicity testing laboratory can use fewer than the recommended number as long as other requirements are met (e.g., required minimum of 40 larvae per concentration). It may also be appropriate for a laboratory to use more than the recommended number of toxicity test organisms per chamber if, for example, the loading capacity is maintained.
4. Examine the statistical results to verify the EPA-recommended flowcharts for analyzing WET data using the appropriate statistical procedure, such as point estimates (e.g., IC_{25}), hypothesis testing (e.g., NOEC), or TST, were followed (USEPA 2002a, 2002b, 2002c; 1995b). Any deviation from the EPA-recommended flowcharts should be noted in the data report.
5. When using multi-concentration toxicity testing, the test CRR must be reviewed to verify that calculated toxicity test results (e.g., NOEC, IC_{25}) are interpreted appropriately. All WET test results from multi-concentration tests reported under the NPDES program must be reviewed and reported as required in the EPA Quality Assurance section for each respective toxicity test method listed in 40 CFR Part 136, Table 1A (USEPA 2002a, 2002b, 2002c) and in EPA's West Coast short-term chronic marine and estuarine toxicity test methods manual (USEPA 1995b).
6. Test review of a given effluent or receiving water toxicity test should include review of the associated reference toxicant toxicity test and current control chart. Were reference toxicant test results consistent with upper and lower bounds established in the laboratory's control chart and evaluated to determine appropriate corrective action?
7. The within-test variability of individual toxicity tests must be reviewed and may include an evaluation of the PMSD where applicable. See EPA's chapters on quality assurance in the toxicity test methods manuals (USEPA 2002a, 2002b, 2002c; 1995b). When NPDES permits require short-term chronic sublethal hypothesis test endpoints from EPA toxicity test methods 1000.0, 1002.0, 1003.0, 1006.0, and 1007.0 (e.g., growth or reproduction NOECs and LOECs), within-test variability using the PMSD must be reviewed and variability criteria (e.g., upper and lower PMSD criteria) must be applied as described in the manuals in the section on test review (USEPA 2002a, 2002b, 2002c; 1995b).

effluent concentration, as recommended in the TSD. Section 5.3.3 discusses how to evaluate WET RP based on recommendations from EPA's TSD when valid WET data are not available. Regardless of which approach the NPDES authority selects, its RP evaluations must satisfy all requirements in 40 CFR § 122.44(d)(1)(ii) and permits must include limits necessary to meet applicable WQS, as required in 40 CFR § 122.44(d)(1)(i) and under CWA Section 301(b)(1)(C).

EPA recommends that NPDES permit authorities incorporate the statistical approach to be used to analyze valid toxicity data in their NPDES IPs and consistently analyze all WET test data for evaluating RP and NPDES WET permit limit compliance using the selected statistical approach.

5.3.1 EPA's 1991 Technical Support Document Approach to Evaluating Reasonable Potential with WET Data

EPA's TSD provides detailed recommendations on how to evaluate RP for WET, including when the RP evaluation is based on sparse data sets or in the absence of data (USEPA 1991a, Ch. 3). Facility-specific toxicity effluent monitoring data should be used, where available, to predict the potential toxicity at the IWC for WET, which is then compared to the applicable WET WQS.

Although EPA has recommended WET criteria, RP for WET should be based on states', territories', and authorized Tribes' WQS and IPs, including interpretations of narrative criteria, where available.

EPA's TSD provides a recommended approach for evaluating RP for WET, which is similar to the RP procedure recommended for chemicals. See the *Recommended Steps to Evaluate NPDES WET Reasonable Potential Following EPA's 1991 TSD* text box for an example of the process. A more detailed description of the five main steps is provided below.

EPA TSD-Based RP Evalua on

Step 1: Review data generated from WET tests using EPA's 2002 promulgated toxicity test methods [40 CFR Part 136] or EPA's 1995 approved West Coast short-term chronic marine and estuarine toxicity test methods to determine if the data is valid based on EPA's toxicity test methods' TAC and converting each test endpoint value to toxic units (TUs) (Step 1 in the *Recommended Steps to Evaluate NPDES WET Reasonable Potential Under EPA's 1991 TSD* text box). In reviewing the valid WET test data, the number of tests, referred to as "n", is identified, as well as the maximum toxicity value observed, which is expressed as TUs. Using TUs rather than the statistical endpoint value (e.g., LC_{50} or IC_{25}) makes the level of toxicity more intuitive because, as the TUs increase, the toxicity magnitude increases directly. This means that as the TUs increase, a smaller percentage of the effluent will result in an impact on aquatic life. TUs are defined as:

$$TU_a = 100/LC_{50}$$
$$TU_c = 100/IC_{25}$$

Under the recommended approach in the TSD, the TU values of the generated WET test data are not averaged, because averaging could lower the maximum toxicity value and, therefore, might not accurately determine whether there is a possible excursion of the state's, territory's, or Tribe's WET WQS. For the RP analysis, the maximum toxicity value, or TU, is selected.

Monitoring requirements based on the most sensitive EPA toxicity test species should be conducted so the collected data are representative of the permitted discharge and the effluent's variability can be determined (see Section 4.3.2). Also, the effluent dilution with the receiving water is considered when

Recommended Steps to Evaluate NPDES WET Reasonable Potential Following EPA's 1991 TSD

Step 1 – Review valid WET data to identify the number of tests and maximum observed toxicity value (i.e., TU).
Step 2 – Calculate facility-specific CV if sufficient WET data exists.
Step 3 – Using Table 5-1 in this manual taken from EPA's TSD, identify the RPMF.
Step 4 – Using the available WET data, determine the acute and/or chronic WET RP value.
Step 5 – Determine the appropriate acute-to-chronic ratio (ACR) for converting acute to chronic or chronic to acute when both acute or chronic WET data are available. When only acute or chronic WET data are available, a default 10:1 ACR is recommended.
Step 6 – Determine the acute or chronic WET RP value.
Step 7 – Calculate the maximum allowable TU_a or TU_c for the permittee. In this step, any potential background toxicity can be accounted for using the TSD-recommended mass balance equation. Often acute is determined at the end-of-the-pip and no dilution allowance is considered for the acute WET RP value.
Step 8 – Examine whether the maximum toxicity values demonstrate an excursion of a WET criterion factoring in dilution (if allowed).

Example of NPDES Reasonable Potential Evaluation Using WET Data

A municipal discharger has an IWC of 42% effluent. The dilution series used during chronic WET testing was 0%, 10.5%, 21%, 42%, 84%, and 100% effluent. Below is a list of the submitted WET data for use when evaluating RP. In this example, background toxicity has been determined to be 0.

WET Test Date	*C. dubia* Reproduction IC_{25}	Toxicity Units (TU_c)
January 2020	66	1.5
February 2020	58	1.7
March 2020	81	1.2
April 2020	70	1.4
May 2020	50	2.0
June 2020	55	1.8
July 2020	46	2.2
August 2020	59	1.7
September 2020	44	2.3
October 2020	48	2.1
Mean Toxicity Units		1.8
Standard Deviation of Toxicity Units		0.34
Coefficient of Variation		0.19

Step 1 – Determine the number of WET tests and maximum observed TU_c.
- Maximum TU_c = 2.3 in September 2020.
- Number (*n*) of WET tests conducted is 10.

Step 2 – Determine the appropriate CV based on *n*.
- The permittee conducted 10 WET tests, thus the facility-specific CV should be calculated and used.
- CV is determined by the standard deviation divided by the mean. The facility-specific CV is 0.34/1.8 = 0.19.

Step 3 – Using the CV and *n* as well as Table 5-1 in this manual, select the appropriate RPMF based on the CV and number of samples.
- Using a CV of 0.19 and an *n* of 10, the RPMF is 1.2. See Table 5-1 in this manual.

Step 4 – Determine the chronic TU_c WET RP value.
- The chronic WET RP value is determined by multiplying the maximum TU (2.3) by the RPMF (1.2) for a value of 2.8 TU_c.

Step 5 – Determine the appropriate ACR.
- The permittee did not conduct acute testing thus based on the TSD (USEPA 1991a), the recommended ACR to use is 10:1.

Step 6 – Determine the acute TU_a WET RP value.
- The acute WET RP value, TU_a, is determined by dividing the maximum TU_c (2.3) by the ACR (10) and then multiplying by the RPMF (1.2) for an acute WET RP value of of 0.28 TU_a.

Step 7 – Calculate the maximum allowable TU_c for this permittee.
- Based on an IWC of 42% effluent, the maximum allowable TU_c is 100/42 or 2.4 TU_c. If background toxicity is determined to exist, use the full mass balance equation as recommended in the TSD to account for background toxicity. Since acute toxicity in this example is determined at the end-of-the-pipe, no dilution allowance is considered for the acute WET RP value.

Step 8 – Determine whether permittee has RP to cause an excursion of the chronic or acute criterion.
- The chronic WET RP value, 2.8 TU_c, is divided by the maximum allowable chronic TU_c, 2.4 for a value of 1.2TU_c. Since 1.2 TU_c is greater than the chronic WET criterion of 1.0 TU_c, this permittee has demonstrated RP for an excursion of the chronic WET criterion.
- The acute WET RP value, 0.28 TU_a, is compared to the acute WET criterion, 0.3 TU_a. Since 0.28 TU_a is less than 0.3 TU_a, this permittee does not have RP for an excursion of the acute WET criterion.

establishing the monitoring requirements. EPA's TSD provides recommendations on dilution ratios for acute and chronic toxicity (see Section 3.2.1).

WET data could be obtained from the following possible sources:

- DMR reported data
- NPDES permit application data
- Data acquired using the information gathering authority under CWA Section 308 or the state's, territory's, or authorized Tribe's equivalent authority to request WET monitoring data be collected by the discharger

Step 2: Use all the valid WET test data generated to determine the CV (Step 2 in text box example). If there are fewer than 10 valid WET test data points for the selected EPA toxicity test type (i.e., acute or chronic) and species (e.g., *Ceriodaphnia dubia* or *Pimephales promelas*), EPA recommends using the default CV of 0.6[5]. When there are 10 or more valid WET test data points, a calculated, facility-specific CV should be used. The facility CV is calculated by first calculating both the mean and the SD of the valid TU data by test endpoint. After calculating the mean and the SD these are used to calculate the facility specific CV by dividing the SD by the mean of the valid WET data set:

$$Coefficient\ of\ Variation\ (CV) = \frac{Standard\ Deviation\ (SD)}{Mean}$$

Step 3. Identify the RPMF based on the recommended 95% confidence level and 95% probability level using Table 5-1 of this manual (Step 3 in text box example). In addition, EPA's TSD (1991a) provides the 99% confidence level and 99% probability level. The table uses the number of WET test data points (referred to as the "number of samples", or *n*) and the selected CV, which is calculated based on actual data where there are 10 or more data points or EPA's recommended default CV of 0.6—to identify the RPMF. The RPMF accounts for the unknown variability inherent in the discharged effluent and the uncertainty due to small data sets. As the sample size increases, the statistical robustness of the sample population also increases, thus the uncertainty decreases. With decreased variability of the sample population, the CV also decreases. Together these factors influence the RPMF. The RPMF is higher as the number of valid WET test data points decreases and CV increases and is lower as the number of valid toxicity test points increases and CV decreases.

Step 4. Calculate the statistically estimated maximum toxicity value (in TUs) using the RPMF and the maximum observed toxicity value (Step 4 in text box example). The estimated maximum toxicity value (RP value) is calculated using the following formula:

$$RP\ Value = Maximum\ TU * RPMF$$

Like other toxicants, only the maximum WET TU value is used, and data are not averaged to appropriately evaluate possible excursions of WET WQS (USEPA 2013). Additionally, RP is evaluated for each WET test result for each species independently and the toxicity test results of different test species are not combined. This recommended approach supports adequate evaluation of possible detrimental toxic impacts on each population represented by the surrogate toxicity test species. Also, when the TSD's recommended approach is being used to evaluate RP, the TUs must be based on point estimate test endpoints (e.g., IC_{25}) and not hypothesis test endpoints (e.g., NOEC). This is because the point estimate test endpoints provide a definitive

[5] 40 CFR Part 132 Appendix F Procedure 6 outlines the reasonable potential approach for Great Lakes states.

Table 5-1. *Reasonable potential multiplying factors: 95% confidence level and 95% probability basis (USEPA 1991a, p. 54).*

Number of Samples	Coefficient of Variation																			
	0.1	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**	**1.1**	**1.2**	**1.3**	**1.4**	**1.5**	**1.6**	**1.7**	**1.8**	**1.9**	**2.0**
1	1.4	1.9	2.6	3.6	4.7	6.2	8.0	10.1	12.6	15.5	18.7	22.3	26.4	30.8	35.6	40.7	46.2	52.1	58.4	64.9
2	1.3	1.6	2.0	2.5	3.1	3.8	4.6	5.4	6.4	7.4	8.5	9.7	10.9	12.2	13.6	15.0	16.4	17.9	19.5	21.1
3	1.2	1.5	1.8	2.1	2.5	3.0	3.5	4.0	4.6	5.2	5.8	6.5	7.2	7.9	8.6	9.3	10.0	10.8	11.5	12.3
4	1.2	1.4	1.7	1.9	2.2	2.6	2.9	3.3	3.7	4.2	4.6	5.0	5.5	6.0	6.4	6.9	7.4	7.8	8.3	8.8
5	1.2	1.4	1.6	1.8	2.1	2.3	2.6	2.9	3.2	3.6	3.9	4.2	4.5	4.9	5.2	5.6	5.9	6.2	6.6	6.9
6	1.1	1.3	1.5	1.7	1.9	2.1	2.4	2.6	2.9	3.1	3.4	3.7	3.9	4.2	4.5	4.7	5.0	5.2	5.5	5.7
7	1.1	1.3	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.1	3.3	3.5	3.7	3.9	4.1	4.3	4.5	4.7	4.9
8	1.1	1.3	1.4	1.6	1.7	1.9	2.1	2.3	2.4	2.6	2.8	3.0	3.2	3.3	3.5	3.7	3.9	4.0	4.2	4.3
9	1.1	1.2	1.4	1.5	1.7	1.8	2.0	2.1	2.3	2.4	2.6	2.8	2.9	3.1	3.2	3.4	3.5	3.6	3.8	3.9
10	1.1	1.2	1.3	1.5	1.6	1.7	1.9	2.0	2.2	2.3	2.4	2.6	2.7	2.8	3.0	3.1	3.2	3.3	3.4	3.6
11	1.1	1.2	1.3	1.4	1.6	1.7	1.8	1.9	2.1	2.2	2.3	2.4	2.5	2.7	2.8	2.9	3.0	3.1	3.2	3.3
12	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.0
13	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.5	2.6	2.7	2.8	2.9
14	1.1	1.2	1.2	1.4	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.3	2.4	2.5	2.6	2.6	2.7
15	1.1	1.2	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.8	1.9	2.0	2.1	2.2	2.2	2.3	2.4	2.4	2.5	2.5
16	1.1	1.1	1.2	1.3	1.4	1.5	1.6	1.6	1.7	1.8	1.9	1.9	2.0	2.1	2.1	2.2	2.3	2.3	2.4	2.4
17	1.1	1.1	1.2	1.3	1.4	1.4	1.5	1.6	1.7	1.7	1.8	1.9	1.9	2.0	2.0	2.1	2.2	2.2	2.3	2.3
18	1.1	1.1	1.2	1.3	1.3	1.4	1.5	1.6	1.6	1.7	1.7	1.8	1.9	1.9	2.0	2.0	2.1	2.1	2.2	2.2
19	1.1	1.1	1.2	1.3	1.3	1.4	1.5	1.5	1.6	1.6	1.7	1.8	1.8	1.9	1.9	2.0	2.0	2.0	2.1	2.1
20	1.1	1.1	1.2	1.2	1.3	1.4	1.4	1.5	1.5	1.6	1.7	1.7	1.8	1.8	1.8	1.9	1.9	2.0	2.0	2.0

value while the hypothesis test endpoints are a value over a range of dilutions and could result in a zero value with respect to variability.

An acute-to-chronic ratio (ACR) is used to extrapolate acute or chronic WET endpoints when only acute or chronic WET data are available. The ACR expresses the relationship between the concentration of WET or a toxicant causing acute toxicity to a species (expressed as an acute toxicity endpoint (ATE) such as LC_{50}) and the concentration of WET or a toxicant causing chronic toxicity to the same species (expressed as a chronic toxicity endpoint (CTE) such as a NOEC or equivalent) (USEPA 1991a) (Step 5 in text box example). The ACR is calculated using the following formula:

$$ACR = \frac{ATE}{CTE} \; or \; \frac{LC_{50}}{NOEC}$$

The ACR is used to determine maximum acute or chronic value by either dividing the maximum observed chronic value to get the corresponding maximum acute value or multiplying the maximum observed acute value to get the corresponding maximum chronic value (Step 6 in text box example). To use a calculated facility-specific ACR, both acute and chronic valid toxicity data from tests conducted concurrently would be required, which may not be available, so the EPA recommended default ACR of 10:1 or 10 may be used.

5.3.2 Approach for Evaluating Reasonable Potential Using EPA's 2010 Test of Significant Toxicity

In addition to the recommended TSD approach for evaluating RP with valid toxicity test data, EPA also developed a recommended approach for evaluating NPDES RP when analyzing valid WET data using the TST. The TST statistical approach can be used with a toxicity test data set of at least four valid toxicity data points (for either acute or chronic). If there are not at least four valid toxicity test data points, the EPA TSD approach for evaluating RP should be used. EPA's 2010 TST documents include an NPDES implementation document for NPDES permit writers and a companion document, which provides more technical and statistically detailed information on the design of the TST statistical approach (USEPA 2010b, 2010c).

EPA's 2010 TST statistical approach is optional and can be used for interpreting valid toxicity data. The TST approach can also be used for evaluating WET RP for both acute and chronic toxicity data. The TST statistical approach for evaluating RP is based on a comparison of valid toxicity test results of the critical concentration or IWC, the point of NPDES regulation (e.g., IWC), against the toxicity test control treatment to determine if there is a significant difference (i.e., worse) in the biological response observed between the two toxicity test concentrations. The publicly available statistics spreadsheet tool, discussed in Section 4.2.8, analyzes valid toxicity test data using EPA's recommended statistical approaches, including the TST. The NPDES permit writer can use this tool to evaluate RP using valid toxicity data analyzed using the TST statistical approach. EPA's statistics spreadsheet tool is available at https://www.epa.gov/npdes/whole-effluent-toxicity-wet-npdes-spreadsheet.

Following are the EPA-recommended four steps for evaluating WET RP when using the TST approach:

- Step One: Review the WET test data generated using EPA's toxicity test methods to determine its validity based on EPA's toxicity test method TAC.
- Step Two: Determine the TST endpoint with respect to compliance (e.g., pass/fail) of each toxicity test.
- Step Three: Calculate the mean effect threshold observed at the critical concentration or IWC for each toxicity test.
- Step Four: Evaluate the individual test mean effect threshold to determine if it demonstrates RP.

A detailed outline of the recommended approach for determining RP when toxicity data are analyzed using the TST can be found in EPA's 2010 NPDES TST implementation document (USEPA 2010b, Sec. 4.1). The *Recommended Steps to Evaluate NPDES WET Reasonable Potential When Using EPA's TST* text box summarizes the recommended RP evaluation approach and presents examples using that approach.

For conducting RP evaluations when using the TST statistical approach to analyze valid toxicity test data, EPA recommends that NPDES permit authorities use all valid toxicity test data generated during the current permit term and any additional valid toxicity test data submitted as part of the NPDES permit renewal application. In evaluating RP when using the TST statistical approach, there should be a minimum of four valid toxicity tests to address effluent representativeness. For more information on effluent representativeness, see EPA's TSD (USEPA 1991a, p. 57). EPA also recommends that NPDES permit authorities request that their permittees provide the actual toxicity test endpoint responses for the control (i.e., control mean) and critical concentration (i.e., IWC mean) for each toxicity test conducted to make it easier for the permit writer to find the necessary toxicity test results for evaluating RP. Toxicity test data analyzed according to the TST statistical approach using the IWC and control test concentrations are then used for the RP evaluation using the TST-based RP approach. For data sets with fewer than four valid toxicity test data points, RP should be evaluated using EPA's TSD RP approach because it addresses small toxicity test data sets by incorporating an RP multiplying factor to account for effluent variability in small toxicity test data sets (USEPA 1991a, p. 54).[6] If toxicity test data are available and EPA's recommended TST statistical approach indicates that the IWC is toxic in any toxicity test, then an excursion of the applicable WQS has been demonstrated [40 CFR § 122.44(d)(1)(i)]. Similar to the TSD-based RP approach, the RP evaluation applied when using EPA's recommended TST statistical approach can establish the existence of the "potential to cause or contribute to toxicity" or RP for toxicity even when no toxicity tests have been declared unacceptably toxic using the TST.

Under EPA's recommended TST statistical approach, if all WET tests indicate "pass" using the TST, the percent effect is calculated for each toxicity test and test species. The percent effect is calculated using the following formula:

$$Percent\ Effect = \frac{(Mean\ Control\ Response - Mean\ Response\ at\ IWC)}{Mean\ Control\ Response} * 100$$

Under the TST statistical approach, any percent effect greater than 10% mean effect threshold indicates RP for that toxicity test species. Conversely, when all tests result in a percent effect less than or equal to 10% mean effect threshold, RP has not been demonstrated.

[6] Based on EPA's toxicity testing methods (USEPA 2002a, b, c), a multi-concentration toxicity test is required to be conducted for NPDES permits. Therefore, even if the test control and IWC data are analyzed using the TST (USEPA 2010b, c), the multi-concentration data can be used to determine the TSD-based statistical endpoints (e.g., LC_{50}, IC_{25}, or NOEC) and used in the TSD-based RP analysis if fewer than four tests were conducted. In the event that WET testing is conducted in the future using only the control treatment and the IWC test concentration (e.g., if a WET ATP is approved to limit the testing to only these two toxicity test concentrations) and fewer than four toxicity tests are conducted, EPA recommends using the TSD's recommendations for conducting RP without data in addition to examining the TST results for existing exceedances (i.e., the "cause" portion of RP).

Recommended Steps to Evaluate NPDES WET Reasonable Potential When Using EPA's TST

Step 1 – Review toxicity test data to determine if the data meets EPA's toxicity test method TAC and to identify the number of toxicity tests.

Step 2 – Determine the TST endpoint with respect to compliance (e.g., pass/fail) of each toxicity test. If any TST endpoint indicated a fail or a significantly different response at the IWC test concentration when compared to the toxicity test control, this is considered "cause" and RP has been demonstrated.

Step 3 – If all TST endpoints indicated pass or no significantly different response at the IWC test concentration when compared to the toxicity test control, calculate the percent effect observed at the critical concentration or IWC for each toxicity test. The percent effect at the IWC is calculated using the following formula:

*Percent Effect = [(Mean Control Response – Mean Response at IWC)/Mean Control Response]*100*

Step 4 – Evaluate the individual toxicity test percent effect. If any toxicity test has a percent effect greater than the mean effect threshold of 10% at the critical concentration or IWC, then RP has been demonstrated.

Examples of NPDES Reasonable Potential Evaluation Using WET Data Analyzed Using the TST

Example 1: A municipal discharger has an IWC of 42% effluent. The dilution series used during chronic WET testing was 0%, 10.5%, 21%, 42%, 84%, and 100% effluent. Below is a list of the submitted WET data for use when determining RP.

WET Test Date	*C. dubia* Reproduction TST Pass/Fail	Percent Effect at IWC (%)	Demonstrated RP?
January 2023	Pass	9.5	No
April 2023	Pass	1.3	No
July 2023	Pass	5.9	No
October 2023	Pass	-8.4	No

Step 1 – Determine the number of toxicity tests analyzed using the TST.

- Number (*n*) of WET tests conducted is 4.

Step 2 – Determine the TST endpoint for each toxicity test.

- The permittee conducted four WET tests, and all tests were deemed to pass or not to be significantly different at the critical concentration or IWC.

Step 3 – Calculate the percent effect at the IWC for each toxicity test.

- The percent effect in % reproduction ranged from -8.4% (or more reproduction in the IWC than in the controls) to 9.5%.

Step 4 – Evaluate the percent effect and determine whether the permittee has RP to have an excursion of the chronic criterion.

- The percent effect calculated were less than or equal to 10%, thus this permittee does not have RP and does not need an NPDES WET permit limit.

Example 2: A municipal discharger has an IWC of 55% effluent. The dilution series used during chronic WET testing was 0%, 13.5%, 27.5%, 55%, 80%, and 100% effluent. Below is a list of the submitted WET data for use when determining RP.

WET Test Date	*C. dubia* Reproduction TST Pass/Fail	Percent Effect at IWC (%)	Demonstrated RP?
January 2023	Pass	1.4	No
April 2023	Pass	12.3	**Yes**
July 2023	Pass	8.9	No
October 2023	Pass	-5.4	No

Step 1 – Determine the number of toxicity tests analyzed using the TST.

- Number (*n*) of WET tests conducted is 4.

Step 2 – Determine the TST endpoint for each toxicity test.

- The permittee conducted four WET tests, and all toxicity tests were deemed to pass or to not be significantly different at the critical concentration or IWC.

Step 3 – Calculate the percent effect at the IWC for each toxicity test.

- The percent effect in % reproduction ranged from -5.4% (or more reproduction in the IWC than in the controls) to 12.3%.

Step 4 – Evaluate the percent effect and determine whether the permittee has RP to have an excursion of the chronic criterion.

- The percent effect calculated for April 2023 was 12.3%, which is greater than mean effect threshold of 10%, thus this permittee has demonstrated RP and needs an NPDES permit WET limit.

5.3.3 EPA's 1991 Technical Support Document for Evaluating Reasonable Potential without NPDES WET Data

To develop an NPDES permit that complies with CWA Section 301(b)(1)(C), which requires permits to include limits necessary to meet WQS, NPDES permit authorities should conduct RP evaluations for pollutants of concern, including when data are not available. Since WET is a pollutant parameter, RP should be conducted for WET as it is for any other parameter. Given this, NPDES permit authority IPs might allow, or even require, a permit writer to evaluate RP through a qualitative evaluation process without using available facility-specific effluent monitoring data or when such data are not available. If, based on information as outlined in this section and following all requirements of 40 CFR § 122.44(d)(1)(ii), an NPDES permit authority determines that a facility causes, has the reasonable potential to cause, or contributes to an excursion of applicable WQS (e.g., past compliance history or documented information on the facility's operation known to cause toxicity), the NPDES permit authority may decide to develop and impose an NPDES WET limit prior to the generation of analytical results on effluent samples. If the NPDES permit writer determines NPDES permit limits are needed using a qualitative evaluation, they should present a clear rationale and adequate justification in the permit fact sheet for the approach [40 CFR § 124.56]. The NPDES permit authority should obtain facility-specific WET monitoring data before permit reissuance either through permit requirements or the information gathering authority under CWA Section 308 or similar authority under the state's, territory's, or authorized Tribe's law.

When determining whether a discharge causes, has the reasonable potential to cause, or contributes to an in-stream excursion of a narrative or numeric WQC for individual toxics or toxicity where facility-specific effluent monitoring data are not available, the NPDES permit authority can use a variety of other factors and information to evaluate RP. Recommended factors include the following, which are described in detail in the TSD (USEPA 1991a, Sec. 3.2):

- Available dilution (if a mixing zone is allowed in WQS or permit regulations)
- Existing controls on point and nonpoint sources of pollution
- Type of industry (raw materials used, products produced, best management practices, control equipment, and treatment efficiency)
- Type of POTW (type of pretreatment program that is in place, type of indirect discharges that come into the wastewater facility, types, and frequency of industrial loadings, and other WQBELs in place)
- Existing data on toxic pollutants in the discharge and species sensitivities to those pollutants
- Variability of the permitted facility discharge
- History of compliance problems and toxic impact
- Type of receiving water and its designated use
- Receiving water characteristics and/or presence of sensitive, rare, threatened, or endangered species
- The applicable WET criteria (EPA's recommended WET criteria or the state's, territory's, or authorized Tribe's WET criteria)
- Whether any available stream bioassessment data indicate impacts on aquatic life
- Whether the receiving water body is listed on EPA's Section 303(d) impaired waters list under the CWA and results of state Section 305(b) monitoring of the receiving water under the CWA

The presence of a combination of factors, such as low available dilution, reference quality receiving waters, poor compliance record for any parameter, and multiple industrial and municipal discharges in close proximity, could constitute a high priority for effluent limits for WET (i.e., based on cause, reasonable potential to cause, or contributes to in-stream excursions of WET WQS).

After evaluating all available information characterizing the nature of the discharge without effluent monitoring data for the pollutant of concern, if the permit writer is not able to determine whether the discharge causes, has the reasonable potential to cause, or contributes to an excursion above WQC, they may determine that effluent monitoring should be required to gather additional data. The permit writer might work with the permittee to obtain data before permit issuance if sufficient time exists or could require the monitoring as a condition of the newly issued or reissued permit. The permit writer might also include a clause in the permit that would allow the NPDES permit authority to reopen the permit and impose an effluent limitation if the required monitoring establishes that discharge causes, has the potential to cause, or contributes to an excursion above a WQC.

5.4 Outcomes Based on a Reasonable Potential Evaluation

This section discusses the outcomes of the RPA and EPA's recommended NPDES permit conditions for when WET RP has been demonstrated (Section 5.4.1) and for when WET RP has not been demonstrated (Section 5.4.2).

One of the following three outcomes will be reached when evaluating WET RP:

- RP exists: Facility has demonstrated it causes an excursion above WQC for WET and an NPDES WET permit limitation is required.
- RP exists: Facility discharge has reasonable potential to cause or contribute to an excursion above WQC for WET and an NPDES WET permit limitation is required.
- No RP exists: Facility discharge does not cause or have reasonable potential to cause or contribute to an excursion above WQC for WET, and an NPDES WET limitation is not required because there is no RP demonstrated. In this situation, it might be appropriate for the permit writer to establish monitoring requirements for WET in the permit. Monitoring triggers (discussed in Section 4) can also be incorporated into the permit with follow-up steps to address toxicity (e.g., accelerated monitoring or TRE) if NPDES WET monitoring triggers are exceeded.

5.4.1 NPDES Permit Conditions When Reasonable Potential for WET Has Been Demonstrated

If the NPDES permit authority determines that the facility's discharge causes, has the potential to cause, or contributes to an in-stream excursion above a narrative or numeric WQC for aquatic life (i.e., toxicity), it must establish a WQBEL, such as an NPDES WET limit, in the permit [40 CFR § 122.44(d)(1)(i) through (v)].

If the state, territory, or authorized Tribe has narrative toxicity criteria, the source(s) of toxicity are identified and controlled, and the NPDES permit authority can demonstrate in the fact sheet that an NPDES chemical-specific limit is sufficient to attain and maintain the applicable WQS, the NPDES permit authority can establish appropriate chemical-specific permit limits in lieu of an NPDES WET limit [40 CFR § 122.44(d)(1)(v)]. This EPA NPDES regulatory provision would only apply when the permittee has identified and confirmed the chemical(s) resulting in an in-stream excursion above the narrative WET WQC and they are controlled for toxicity using chemical-specific limits. In addition to the chemical-specific permit limit, the permit writer should consider continued WET monitoring and toxicity testing

triggers in case of a recurrence of an unacceptable level of toxicity resulting in an in-stream excursion of the state's, territory's, or authorized Tribe's WET WQS.

When WET RP has been demonstrated, the permit writer should consider the following in establishing permit conditions (see Section 4 of this manual for more on permit conditions):

- An NPDES WET limit must be included in the permit when WET reasonable potential has been demonstrated. However, when the WET criterion is narrative, a chemical limit can be used instead of a WET limit if the chemical limit controls or eliminates the toxicity such that there is no longer an excursion of the applicable WET WQS. WET monitoring requirements (including numeric monitoring triggers) and compliance monitoring requirements should be included in the permit. A compliance schedule may be included (if appropriate and compliance schedules are allowed under the applicable state's, territory's, or authorized Tribe's WQS) to provide a schedule to allow a permittee to attain compliance with a permit limit [40 CFR § 122.47 and USEPA 2007].
- Accelerated WET testing requirements based on an exceedance of the NPDES WET limit or WET numeric monitoring trigger could be included. EPA recommends including a permit requirement for accelerated toxicity monitoring as a first response to an exceedance of an NPDES WET effluent limit or numeric WET monitoring trigger (USEPA 2010d). If a toxicity test result is higher than the NPDES WET limit or monitoring trigger, EPA recommends an accelerated monitoring schedule of six additional toxicity tests conducted biweekly over a 12-week period, beginning within two weeks of the first exceedance of an NPDES WET limit or a numeric WET monitoring trigger.
- TRE NPDES permit triggers could be included based on an exceedance of the NPDES WET limit or numeric WET monitoring trigger and any toxicity observed in accelerated toxicity tests. In response to continued toxicity, the permit could require that, if the results of any one of the additional toxicity tests exceeds the NPDES WET limit or WET numeric monitoring trigger, the permittee would implement corrective actions identified in the TRE plan in the permit.
- The permit could include a permit reopener provision that stipulates that the permit may be reopened to modify or add permit conditions (in this case, NPDES WET permit conditions) if new or additional information or factors are obtained and provided to the NPDES permit authority [40 CFR § 122.44(b) and (c); 40 CFR § 122.62(a)(7)].
- The permit could include requirements for an NPDES WET trigger, WET monitoring, and a permit reopener for NPDES WET limits when a chemical-specific limit is used in lieu of an NPDES WET limit (where WET criteria are narrative) but excursions of the WET WQS may still occur.

5.4.2 NPDES Permit Conditions When Reasonable Potential for WET Has Not Been Demonstrated

If the NPDES permit authority determines that a discharge from a facility does not cause or have the reasonable potential to cause or contribute to an in-stream excursion above a narrative or numeric WQC for aquatic toxicity, then a WQBEL for WET is not necessary. Even where RP does not exist, however, it may be appropriate for the permit to include regular WET monitoring that is representative of the monitored activity or discharge. Regular monitoring is especially important for POTWs that might have unforeseen toxicity related to indirect discharges to the POTW, including new industrial users or an industrial facility adding processes or chemicals not previously introduced into their system.

Additionally, if WET monitoring requirements are included in the permit, the permit should also include accelerated toxicity testing requirements based on an exceedance of a WET trigger specified in the permit as well as a TRE trigger if toxicity is observed in accelerated toxicity tests. Numeric WET triggers can be developed in the same way NPDES WET limits are calculated (see Section 6).

In addition to appropriate monitoring requirements, the NPDES permit authority should include a reopener clause in the permit to allow for possible permit modifications based on new or additional information previously not available at permit application [40 CFR § 122.62(a)(7); (USEPA 1991a)]. For example, the inclusion of a reopener clause authorizes the NPDES permit authority to reopen the permit and establish additional permit conditions based on monitoring results or other new factors that indicate the effluent may cause, have the reasonable potential to cause, or contributes to an in-stream excursion above the applicable state's, territory's, or authorized Tribe's WQS. When permits are reopened in this manner, NPDES permit authorities may impose WQBELs for WET and/or require a permittee to perform a TRE.

In summary, when RP has not been demonstrated, conditions in the permit may, as appropriate, include the following (see Section 4 of this manual for more on permit conditions):

- Routine WET monitoring, using the most sensitive EPA toxicity test species (see Section 3), that adequately characterizes the facility discharge to determine whether excursions of the WQS are occurring and to support an RP evaluation at permit reissuance, along with a numeric WET trigger.
- Requirements for accelerated WET testing and a TRE based on the exceedance of the permit-specified WET numeric monitoring trigger.
- A permit reopener clause to allow the NPDES permit authority to open the permit and modify it to include NPDES WET limits or TRE requirements, should RP be demonstrated in future toxicity testing.

6 Developing NPDES WET Permit Limits

This section describes EPA's recommendations regarding development of NPDES WET permit limits based on EPA's TSD and how to express those limits in the permit based on the statistical approach selected. This section also discusses considerations for development of limits in low flow dilution situations. EPA has developed guidance on the steps for developing WQBELs for all pollutants or pollutant parameters with WQC, including for WET (USEPA 1991a). When deriving WQBELs for NPDES permits, the permit writer needs to examine the state's, territory's, or authorized Tribe's WQC for aquatic life, mixing zone policy, and permit limit derivation steps. The objective when deriving WQBELs for WET should be to establish permit limitations that, after considering effluent variability and receiving water conditions, including available dilution and background toxicity, require facility performance levels with a low statistical probability of resulting in an excursion of the applicable WQS under most foreseeable conditions, including during critical low-flow conditions in the receiving water (USEPA 2010a). The *Recommended Steps in Developing WQBELs for WET* text box outlines recommendations from the TSD for developing WQBELs for WET or WET numeric monitoring triggers.

In general, WQBELs are developed using wasteload allocations (WLAs) based on both acute and chronic criteria, which are then translated into effluent limitations. The goal of the permit writer is to derive effluent limitations that are enforceable, adequately account for effluent variability, consider available receiving water dilution, protect against acute and/or chronic impacts, account for compliance monitoring sampling frequency, and ensure attainment of the WLA and WQS. In developing WQBELs, the permit writer develops limitations that require a facility to perform in such a way that the toxicity of the effluent or the concentration of the pollutant of concern in the effluent discharged is nearly always below the WLA. To accomplish that goal, EPA has developed a statistical permit limitation derivation procedure to translate WLAs into effluent limitations for pollutants with effluent concentration measurements that tend to follow a lognormal distribution. Some states have adopted procedures based on, but not identical to, EPA's guidance that also provide defensible, enforceable, and protective WQBELs. The permit writer should always use the procedures adopted by their NPDES permit authority.

Section 5.4.1 of the TSD provides general guidance for NPDES permit authorities in developing and implementing WQBELs for acute or chronic toxicity (USEPA 1991a). The recommended approach involves calculating a targeted long-term average (LTA) performance level for the discharge for both an acute and a chronic criterion. Under this approach, the NPDES permit limit is derived from whichever LTA performance level is more protective. The recommended steps for deriving permit limits for WET in the TSD are similar to those for any other pollutant parameter with the following adjustments:

- An equivalent chronic waste load allocation (WLA_c) is calculated based on an acute LTA performance level. The LTA performance level for WET is calculated based on TUs so it is protective of both the WLA_as and WLA_cs.
- An acute waste load allocation in chronic TUs or $WLA_{a,c}$ is calculated by multiplying the WLA_a by an ACR. This conversion of the WLA_a is done so that the acute toxicity effects can be compared to the chronic toxicity effects. Ideally, the ACR is based on the actual ratio of acute and chronic

Recommended Steps in Developing WQBELs for WET

Step 1 – Determine the acute and chronic downstream flows.
Step 2 – Determine the acute WLA (WLA_a).
Step 3 – Determine the appropriate acute-to-chronic ratio (ACR).
Step 4 – Calculate the acute WLA in chronic TUs ($WLA_{a,c}$).
Step 5 – Determine the chronic WLA (WLA_c).
Step 6 – Determine the percent probability and CV, then, based on these items, select the acute and chronic multipliers from EPA's TSD tables.
Step 7 – Calculate the long-term average acute in chronic units ($LTA_{a,c}$) and long-term average chronic (LTA_c).
Step 8 – Determine the more limiting LTA.
Step 9 – Determine the percentile probability for calculating the maximum daily limit (MDL) and average monthly limit (AML). **Step 10 –** Based on a sample size default assumption of *n*=4, select the MDL and AML multipliers from the EPA's TSD tables. **Step 11 –** Calculate the MDL and AML.

Example of NPDES WET Permit Limit Development

	Upstream Concentration* (C_u)	Downstream Concentration = Aquatic Life Criteria (C_d)	Effluent Flow (Q_e)	Upstream Flow** (Q_u)
Acute	0 TU_a	0.3 TU_a	116.5 cfs	38 cfs
Chronic	0 TU_c	1.0 TU_c	116.5 cfs	50 cfs

Notes: cfs = cubic feet per second.
* For this example, ambient toxicity testing was conducted upstream and was found to be no toxicity or zero (0).
** This should be based on NPDES permit authority IPs. It could be a 7Q10 or other flow value or only a percentage of the total upstream flow.

Detailed Recommended Steps in Developing WQBELs for WET

Step 1 – Determine the acute and chronic downstream flows.
- Acute Q_e + Acute Q_u = 116.5 cubic feet per second (cfs) + 38 cfs = 154.5 cfs.
- Chronic Q_e + Chronic Q_u = 116.5 cfs + 50 cfs = 166.5 cfs.

Step 2 – Determine the acute WLA (WLA_a).
- C_e or Concentration of pollutant in effluent = $WLA_a = (Q_dC_d - Q_uC_u)/Q_e$
- $WLA_a = [(154.5\ cfs)(0.3\ TU_a) - (38\ cfs)(0\ TU_a)]/116.5\ cfs = 0.40\ TU_a$

Step 3 – Determine the appropriate ACR.
- This ratio should optimally be based on effluent data but can be estimated as 10:1 (USEPA 1991a).

Step 4 – Calculate the acute WLA in chronic TUs ($WLA_{a,c}$).
- $WLA_{a,c}$ = acute WLA * ACR = 0.40 TU_a * 10 = 4.0 $TU_{a,c}$

Step 5 – Determine the chronic WLA (WLA_c).
- C_e or Concentration of pollutant in effluent = $WLA_c = (Q_dC_d - Q_uC_u)/Q_e$
- $WLA_c = [(166.5\ cfs)(1.0\ TU_c) - (50\ cfs)(0\ TU_a)]/116.5\ cfs = 1.43\ TU_c$

Step 6 – Determine the percentile probability, CV, and acute and chronic multipliers.
- EPA recommends the 99th percentile probability and the use of a CV = 0.6 (USEPA 1991a).
- Using the acute and chronic tables in the TSD (USEPA 1991a, p. 102), the acute multiplier = 0.321 and the chronic multiplier = 0.527.

Step 7 – Calculate the $LTA_{a,c}$ and LTA_c.
- $LTA_{a,c} = WLA_{a,c} * e^{(0.5\sigma^2 - z\sigma)} = 4.0\ TU_{a,c} * 0.321 = 1.28\ TU_{a,c}$
- $LTA_c = WLA_c * e^{(0.5\sigma_4^2 - z\sigma_4)} = 1.43\ TU_{a,c} * 0.527 = 0.75\ TU_c$

Step 8 – Determine the more limiting LTA.
- LTA_c or 0.75 TU_c is less than the $LTA_{a,c}$ or 1.28 $TU_{a,c}$; thus, LTA_c or 0.7 TU_c is more limiting.

Step 9 – Determine the percentile probability for calculating the MDL and AML.
- EPA recommends the 99th percentile for the MDL and the 95th percentile for the AML.

Step 10 – Determine the MDL and AML multipliers.
- Assuming a sample size of *n* = 4 (USEPA 1991a pp. 107–110), the MDL multiplier is 3.11 and the AML multiplier is 1.55 (USEPA 1991a, p. 103).

Step 11 – Calculate the MDL and AML.
- MDL = Limiting LTA (LTA_c in this example) * $e^{(z\sigma - 0.5\sigma^2)} = 0.75\ TU_c * 3.11 = 2.3\ TU_c$
- AML = Limiting LTA (LTA_c in this example) * $e^{(z\sigma_n - 0.5\sigma_n^2)} = 0.75\ TU_c * 1.55 = 1.2\ TU_c$

toxicity units from valid WET test data for the permitted facility discharge. If a site-specific ACR is not available, EPA recommends a default ACR of 10:1 (USEPA 1991a).

- Before calculating the LTA, the WLA_c is calculated in TU_c. To calculate the AML, the TSD recommends a default minimum number of samples (*n*) of four be used even when fewer than four sampling events per month will be required in the permit, which is typically the case for WET (USEPA 1991a).

6.1 EPA's Recommendation for Deriving NPDES WET Permit Limits for Low-Flow Dilution Situations

Under some NPDES discharge scenarios (e.g., low-flow receiving water body or absence of a mixing zone/dilution policy approved by EPA under CWA Section 303(c)), mixing zones/dilution might not be authorized by the state, territory, or authorized Tribe. Additionally, some NPDES permit authorities under their WQS or IPs, may choose to use only a certain percentage of the receiving water flow for determining the allowed dilution. The permit writer should carefully review the NPDES permit authority's mixing zone/dilution policies before developing NPDES WET permit limits.

Currently, NPDES permit authorities use different approaches for expressing WQBELs for regulating toxicity under low-flow discharge scenarios. EPA continues to recommend that numeric WQBELs for chemicals and WET be established using statistical approaches outlined in the TSD or the TST (USEPA 1991a, USEPA 2010b). The TSD provides guidance related to implementation considerations unique to WET when a mixing zone/dilution allowance is not authorized for an NPDES discharge (USEPA 1991a).

EPA recommends that for situations for which no mixing zone or dilution allowance exists, the AML should be set to the criteria value of 1.0 TU_c. In this situation, MDLs could be set in accordance with statistical approaches (USEPA 1991a). For example, when using the 99th percentile to calculate the LTA, an n = 4, and an effluent CV of 0.6, EPA recommends the 99th percentile for the MDL for chronic WET, which is 1.6 TU_c, and the AML is 1.0 TU_c (USEPA 1991a, Appendices E and F).

Some NPDES permit authorities establish WET limits as an LC_{50} greater than or equal to 100% effluent at the end of the pipe, but this approach might not be appropriate for effluent-dominated and/or low-flow receiving waters. The toxicity of an effluent depends on the magnitude (i.e., the pollutant concentration), duration of pollutant exposure, and frequency of the exposure, especially in effluent-dominated waters. For example, an effluent that has an LC_{50} = 100% effluent contains enough toxicity to be lethal to up to 50% of the test organisms. If the effluent is discharged to a low-flow receiving water body that provides no more than a threefold dilution at the critical flow, significant mortality can occur in the receiving water (USEPA 1991a). EPA recommends that all WET limits be set following the statistical permit limit derivation procedures discussed in the TSD or using the TST. The permit writer should review the NPDES permit authority IPs for information on the approach used by their state, territory, or Tribe.

6.2 Expression of NPDES WET Permit Limits

The NPDES regulations in 40 CFR § 122.45(d) require that permit limits for continuous discharges be expressed, unless impracticable, as both an AML and an MDL for discharges other than POTWs. POTW NPDES limits are expressed as an average weekly limit (AWL) and AML. The MDL is the highest allowable facility discharge of a pollutant measured during a calendar day or 24-hour period representing a calendar day. The AML is the highest allowable value of the average of daily discharges obtained over a

calendar month. The AWL is the highest allowable value for the average of daily discharges obtained over a calendar week (USEPA 1991a).

In the TSD, EPA recommends establishing an MDL, rather than an AWL, for discharges of toxic pollutants from POTWs (USEPA 1991a). That approach is appropriate for at least two reasons. First, the basis for the AWL for POTWs is the secondary treatment requirements and is not related to the need to ensure attainment of WQS. Second, an AWL, which could be the average of up to seven daily facility discharges, could average-out peak toxic concentrations and, therefore, the facility discharge's potential for causing acute toxic effects might be missed. An MDL would be more likely to identify potential acutely toxic impacts. Chapter 5 of the TSD includes recommended statistical tools for calculating MDLs and AMLs from the LTA value (USEPA 1991a).

The permit should contain a notation indicating that the MDL is signifying the maximum test result for that month unless otherwise specified by the state's, territory's, or authorized Tribe's WQS. The AML is the highest allowable value for the average of daily facility discharge obtained over a calendar month. For WET, this translates into the average of the toxicity test result(s) over a 30-day period. Note that federal regulations in 40 CFR § 122.41(l)(4)(iii) specify that the calculation for all limitations that require averaging of measurements shall use an arithmetic mean (i.e., average), unless otherwise specified in the NPDES permit. For cases in which little dilution is available or where a state's, territory's, or authorized Tribe's WQS do not allow mixing zones, the AML should be expressed as a monthly median limit (USEPA 2010d).

6.2.1 Expression of WET Permit Limits Using EPA's Technical Support Document Approach

With the TSD approach, NPDES WET permit limits would be expressed in terms of percent effluent or TUs. For example, a permit might indicate that there can be no toxicity at or below the IWC (percent effluent [e.g., LC_{50} greater then IWC and IWC = 50% or NOEC greater than or equal to the IWC]) or that there can be no more than the calculated TU at the IWC (e.g., IWC = 50% would be 2.0 TU [100/50 = 2]) (see Section 5.3.1).

As discussed in Section 6.2, some NPDES permit authorities establish WET limits as an LC_{50} greater than or equal to 100% effluent at the end of the pipe, but this approach may not be appropriate for effluent-dominated and/or low-flow receiving waters. Furthermore, a permit limit for WET expressed as an LC_{50} might not ensure protection against possible chronic toxic effects in the receiving water body. Chronic effects could occur if the dilution in the receiving water multiplied by the ACR is greater than 100% (USEPA 1991a). In contrast, NPDES WET limits set using this approach in high receiving water flow conditions might be overly restrictive. Because of this, EPA recommends that the statistical derivation steps discussed in the TSD are followed when developing WQBELs for WET (USEPA 1991a) (see the *Recommended Steps in Developing WQBELs for WET* text box).

6.2.2 Expression of WET Permit Limits Using EPA's Test of Significant Toxicity Approach

With the TST approach, the MDL, AML, or other appropriate permit limit for WET would be expressed as "no significant toxicity of the effluent at the IWC using the TST statistical approach." A valid toxicity test result of pass indicates the calculated t value is greater than the critical t value (see Section 3.3.3 of this manual for discussion of TST) (USEPA 2010b). A valid toxicity test result of fail indicates the calculated t value is less than the critical t value (USEPA 2010b).

7 Evaluating NPDES Compliance for WET and Enforcement Considerations

This section describes EPA's recommendations regarding compliance monitoring and enforcement for NPDES WET permit requirements, including DMRs and other self-monitoring reports, evaluation of WET test data, the DMR-Quality Assurance study program, noncompliance with WET permit conditions, and WET enforcement procedures. While the information in this section goes beyond what a permit writer must include in a permit regarding WET, it is important for the permit writer to be familiar with how WET compliance and enforcement is conducted. This allows the permit writer to better understand how to write enforceable permits and use compliance monitoring and enforcement data for permit reissuance, as well as better coordinate with enforcement personnel, when appropriate.

Chapter 8 in the EPA Office of Compliance's NPDES Compliance Inspection Manual describes the objectives for compliance monitoring activities, such as inspections, audits, and records review, for WET data (USEPA 2017). These objectives may include:

- Documenting the presence or absence of effluent toxicity based on valid WET data;
- Assessing compliance with the conditions and limits in the NPDES permit;
- Assessing a permittee's laboratory WET test performance, including reference toxicant testing and other WET QA/QC requirements;
- Evaluating the quality of self-monitoring data; and
- Assessing the adequacy of self-monitoring procedures.

Based on these evaluations, the permittee can be required to perform a TRE or TIE. Inspectors are encouraged to coordinate with the permit writer if they identify language in a permit that could be clarified and/or strengthened.

The NPDES Compliance Inspection Manual provides examples of procedures and records that might be reviewed during an inspection (USEPA 2017), including:

- The NPDES permit;
- WET test results from the last three years;
- Effluent sample collection and chain-of-custody procedures for WET testing; and
- Permittee sampling logs that should include the date, time, type of sample taken, and the sampler's name.

Compliance inspectors, including EPA or NPDES permit authority personnel, also should review the following:

- WET test data interpretations
- Calculations
- WET test CRR based on multiple concentration WET tests
- Whether the WET tests meet all of EPA's toxicity test mandatory TAC

- The PMSD evaluation of WET test variability

Many of the considerations for evaluating WET data when conducting an RPA for evaluating whether WET permit limits are needed are also applicable to evaluating WET data for compliance purposes. Section 5 of this manual provides an in-depth discussion of reviewing and evaluating WET data and factors, such as EPA's toxicity test mandatory TAC, which impact the quality of WET data. Individual NPDES permit authorities may have additional detailed guidance to assist permit and compliance personnel with WET data review.

7.1 Discharge Monitoring Reports and Other Self-Monitoring Reports

Self-monitoring reports provide much of the compliance data used by the NPDES permit authority in reviewing permittee compliance. These reports include DMRs (see Section 4.3.3) and reports of progress on compliance schedules (USEPA 1991a). DMRs contain information on the analytical results of permittee self-monitoring based on the detailed requirements (e.g., parameter, sampling method, frequency of analysis, and location) specified in the permit. ICIS-NPDES will automatically "flag" violations of permit limitations based on DMR data submitted, including for WET, as well as other violations of compliance schedules and reporting requirements entered into the system.

7.2 Evaluating the Quality of WET Data

Evaluation of QA information (e.g., laboratory bench sheets, chain-of-custody forms, and reference toxicant data) can be useful in detecting problems with the quality of the sample analysis. Permits may require permittees to submit QA forms, laboratory reports, and/or other information related to QA procedures as attachments to their DMRs or otherwise submitted to the NPDES permit authority. In addition, permits should contain a permit condition that requires the permittee to retain records of all monitoring information, including all equipment calibration and maintenance records, for a period of at least 3 years from the collection date of the sample, measurement, report, or application [40 CFR § 122.41(j)(2)]. With respect to toxicity testing, these records would include information demonstrating compliance with QA procedures as specified in the permit, including those established in EPA's toxicity test methods required by the permit.

Permittees should report any known problems with QA to the NPDES permit authority at the time of DMR submission, if not before, and the permit should specify that, in these cases, the toxicity test must be repeated with a new sample (USEPA 1991a; USEPA 2002a, 2002b, 2002c; USEPA 1995b).

Permittees are required to follow the instructions for reporting results and include a signed certification statement in accordance with 40 CFR § 122.22 (USEPA 1991a; USEPA 2000c).[7]

Review of WET laboratory data reports is an important part of the NPDES permit authority's responsibilities to ensure that toxicity test results are reported accurately [40 CFR § 122.41(j)(5)], including:

[7] EPA has clarified that the purpose of the certification requirement is primarily to ensure that the individual submitting the information certifies the veracity of statements made in the forms and acknowledges liability for false statements. The certification of WET test results does not necessarily indicate that the WET results meet all EPA toxicity test method TAC (USEPA 2000c). Therefore, it is important for permit and compliance staff to review and verify whether EPA's toxicity test method TAC were met independent of the certification requirement.

- **Sample collection and handling:** For example, chain-of-custody forms must be reviewed to verify that samples were tested within allowable sample holding times and held at appropriate temperatures prior to testing (USEPA 2002a, 2002b, 2002c; USEPA 1995b).
- **TAC have been met:** Toxicity test data must be reviewed to verify that EPA's toxicity test method TAC have been met for a toxicity test to be considered valid (see Appendix C of this manual); USEPA 2002a, 2002b, 2002c; USEPA 1995b). Any toxicity test not meeting the minimum TAC is considered invalid under EPA's toxicity test methods (USEPA 2002a, 2002b, 2002c; USEPA 1995b). All invalid toxicity tests must be conducted again with a newly collected sample (USEPA 2002a, 2002b, 2002c; USEPA 1995b).
- **Toxicity test conditions:** Physical and chemical measurements taken during the toxicity test (e.g., temperature, pH, and dissolved oxygen) are reviewed and compared to specified ranges.
- **Statistical approaches and calculations:** The statistical approaches used for analyzing toxicity test data should be reviewed to verify that EPA's recommended flowcharts for each respective statistical analysis approach were followed (USEPA 2002a, 2002b, 2002c; USEPA 1995b). Some deviations from the flowcharts may be appropriate, but the laboratory must document the deviation and provide the rationale for why the deviation was used (USEPA 2002a, b2002, 2002c; USEPA 1995b).
- **Review the CRR in the toxicity test:** The CRR generated for each multi-concentration toxicity test must be reviewed (USEPA 2002a, 2002b, 2002c; USEPA 1995b). EPA's toxicity test methods document provides guidance on evaluating CRRs to assist in determining the validity of WET test results when using EPA's toxicity test methods recommended statistical approaches (USEPA 2000a; USEPA 2002a, 2002b, 2002c; USEPA 1995b). Toxicity tests that exhibit unexpected CRRs may indicate a need for further investigation and possibly the need to conduct the toxicity test again with a newly collected sample (USEPA 2000a). Section 3.4 Statistical Approach Review Steps includes more details on the CRR review.
- **Reference toxicant test results:** The reviewer should verify that a QC reference toxicant test was conducted according to the specified frequency (e.g., monthly) required by the NPDES permit authority or as recommended by the EPA toxicity test method.
- **Within-test variability in toxicity test organism response:** Excessive within-test variability may invalidate a toxicity test result and the need to conduct the toxicity test again with a newly collected sample. For evaluating within-test variability using NOEC as the test endpoint, the reviewer should consult EPA guidance on upper and lower PMSD bounds (USEPA 2000b). EPA has developed upper and lower bounds for the PMSD to verify that only appropriate within-test variability is observed (see Section 3.4 and Table 3-5 of this manual) (USEPA 2002a, 2002b, 2002c).

In addition, there may be an NPDES permit authority's QA data form that should be considered. Once the NPDES permit authority's QA data form has been completed by the permittee, it is reviewed by the NPDES permit authority and then included in the compliance file (USEPA 1991a, App. B-5).

7.3 Discharge Monitoring Report Quality Assurance Study Program

Under its CWA Section 308 authority, EPA has required many NPDES permittees to participate in the annual Discharge Monitoring Report Quality Assurance (DMR-QA) study program (USEPA 2017). CWA Section 308 also grants NPDES-authorized states, territories, and authorized Tribes the authority to require their permittees to participate in the program. The DMR-QA study program evaluates the ability of laboratories serving NPDES permittees to analyze and report accurate self-monitoring data (USEPA 2017). The program is intended to improve overall laboratory analytical performance for self-monitoring data (USEPA 1991a). In the DMR-QA program, permittees are required annually to have their laboratory

or contract laboratory analyze unknown samples with constituents that can be found in their industrial or municipal wastewaters. Permittees are expected to use the same personnel and methods employed for reporting NPDES data to analyze the samples. EPA's Office of Enforcement and Compliance Assurance (OECA) compiles the results of the annual DMR-QA study.

The results of the study are compared to the known content of the sample, and an evaluation of the reported data is sent to the permittees. The results of a DMR-QA study assign a grade of "acceptable" or "not acceptable" to the laboratory's performance of EPA toxicity tests. NPDES permit authorities conduct follow-up investigations to address poor or incomplete DMR-QA study results, failure to participate in the DMR-QA program, and late submittal of DMR-QA results.

7.4 Compliance Inspections of NPDES Facilities and WET Laboratories

Compliance officials may conduct inspections of NPDES facilities and WET laboratories to verify permittee compliance with permit conditions and QA procedures. Inspections may include reviewing records, inspecting treatment facilities, assessing progress with NPDES permit compliance schedules, evaluating laboratory facilities and performance, collecting samples for analysis, and splitting samples taken by the permittee for concurrent analyses. EPA has defined several types of inspections based on the tasks included in the NPDES Compliance Inspection Manual (USEPA 2017). NPDES permit authorities should inspect all major NPDES permittees annually regardless of compliance status. Non-sampling inspections (which are generally less resource-intensive) are encouraged for routine evaluation of permittee performance. On-site inspections involving sampling are encouraged to address NPDES permit and enforcement priorities.

Inspections that focus on toxics control can provide useful information for water quality assessment and NPDES permit reissuance in addition to compliance data. Procedures for inspecting facilities with toxicity testing requirements and measuring effluent toxicity are detailed in the NPDES Compliance Inspection Manual (USEPA 2017, Ch. 8). The inspector should understand the permittee's WET testing requirements so the appropriate inspection objectives can be met. Objectives may include the following:

- Assessing compliance with NPDES permit conditions.
- Assessing NPDES permit conditions for clear and inclusive language.
- Considering overall laboratory WET test performance (reference toxicants and other WET QA/QC requirements), especially EPA's mandatory toxicity test method TAC.
- Evaluating quality of self-monitoring data.
- Assessing adequacy of self-monitoring procedures.
- Documenting presence or absence of toxic conditions.
- Identifying the need to perform a TRE and/or TIE.

7.5 Noncompliance with NPDES WET Permit Conditions

Examples of noncompliance that might be identified during a compliance inspection include:

- Exceedances of NPDES WET permit limits;
- WET tests not performed as required by the permit;
- Samples that are not representative of the effluent discharge;
- WET test data from the laboratory incorrectly reported to the permittee or on the DMR; and

- Deviations from NPDES permit requirements, including any deviations (including toxicity test method requirements) from the EPA toxicity test methods required in the permit.

EPA's enforcement response to any CWA violation is case-specific, based on consideration of several factors, including type, frequency, and magnitude of the violation; potential environmental or human health impacts; and compliance history of the facility. WET violations are automatically flagged in ICIS-NPDES as they would be for any other parameter. However, WET parameters, like some chemical parameters, are not included in EPA's Significant Non-Compliance (SNC) criteria [40 CFR § 123.45]. This means they are not automatically labeled with the status of Significant/Category I noncompliance, which is a designation intended to indicate whether violations or noncompliance events at a given facility may pose a more severe level of concern for the environment or program integrity. Because WET violations are not automatically flagged in this way good communication between the compliance and permit staff is important in helping to identify and evaluating NPDES WET violations.

Intermittent violations of NPDES WET limits, with potential or known impacts, should be addressed by the permittee and should be reviewed by the NPDES permit authority to determine an appropriate response. In some instances, however, there may be extended periods of noncompliance that might require an enforcement response as well as incentives to resolve. Repeated, intermittent noncompliance might require an extended period of evaluation and problem-solving by the permittee through the implementation of a TRE and perhaps a TIE. The NPDES permit authority should acknowledge and accommodate attempts to resolve toxicity violations, when appropriate.

Incentives for the permittee to resolve noncompliance might include a reasonable, mutually agreed-upon schedule, with an end date by which the permittee must implement corrective measures to resolve the toxicity and return to compliance.

The NPDES permit authority retains its authority to assess penalties for a permittee's failure to move decisively to correct permit violations.

The NPDES permit authority can encourage compliance with NPDES WET permit requirements by suggesting that the permittee increase its WET test monitoring frequency to help the permittee determine the source of toxicity and, ultimately, to select and implement a remedy to achieve compliance with permit requirements. If the permittee is not responsive, the NPDES permit authority can require the permittee to conduct additional WET tests and a TRE through an information collection request issued under CWA Section 308 or as a provision in an enforcement action. If requested, the NPDES permit authority should provide feedback to the permittee on the proposed steps to implement a TRE. The TRE plan should include a reasonable amount of time for the TRE activities to occur and a reasonable schedule with specified reporting requirements to verify that the permittee has completed the identified follow-up actions to control, reduce, or eliminate the source(s) of toxicity and achieve compliance with the NPDES permit.

7.6 EPA Enforcement Procedures for NPDES WET Requirements

7.6.1 EPA WET Basic Permit Principles and Enforcement Strategy

The Whole Effluent Toxicity Basic Permitting Principles and Enforcement Strategy memorandum discusses in detail key EPA permit and WET enforcement principles (USEPA 1989b). The strategy was developed by a work group comprised of EPA headquarters and regional staff and state personnel with

the goal of promoting national consistency in the development of NPDES WET permit requirements and in the enforcement of those requirements.

Following are two of the key permit principles included in the 1989 strategy document:

- NPDES permits must be protective of the state's, territory's, or authorized Tribe's WQS.
- Permits should be clearly written to ensure enforceability. Therefore, all NPDES permits should have WET limits and requirements included, where necessary, to comply with the state's, territory's, or authorized Tribe's WET WQS (USEPA 1989b).

As outlined in the 1989 strategy:

- The permittee is responsible for attaining, monitoring, and maintaining compliance with their NPDES permit, which must include limits to comply with state's, territory's, or authorized Tribe's WET WQS and ultimately, with the CWA.
- The NPDES permit authority evaluates the permittee's compliance status by reviewing all available information, such as self-monitoring results reported on DMRs, inspection results, citizen complaints, and other information.
- The NPDES permit authority identifies violations of NPDES WET permit requirements, such as effluent limit exceedances and failure to monitor or report monitoring results and, if applicable, establishes compliance schedules and determines an appropriate enforcement response.

Requiring a TRE may be an appropriate response to WET violations if it is included in an enforcement action that includes implementing requirements to control, reduce, or eliminate the source of toxicity and includes a final compliance date.

7.6.2 EPA NPDES Enforcement Management System

The 1989 EPA NPDES Enforcement Management System (EMS) Guide (USEPA 1989c) is EPA's NPDES compliance and enforcement guidance (USEPA 1989c). It establishes a framework upon which to build the management of a national enforcement program, describes the key principles of EPA's compliance and enforcement program, and includes enforcement response recommendations for different types of violations.

The EMS Guide provides information on NPDES WET violations in the enforcement response guidelines. EPA treats WET like any other pollutant parameter by considering all case-specific facts related to a noncompliance event, including the magnitude, frequency, and duration of a violation; associated environmental harm; and the compliance history of the permitted facility.

7.6.3 Types of NPDES Enforcement Actions

EPA uses enforcement discretion in determining an appropriate enforcement response to NPDES violations. The response should reflect the nature and severity of the NPDES violations. EPA recommends an escalating response to continuing NPDES permit violations. Examples of possible enforcement escalating responses include a letter of violation (LOV) or a notice of violation (NOV) and an administrative penalty order (APO).

7.6.3.1 Notice of Violation

A single or infrequent violation of an NPDES WET limit causing no known harm may be appropriately addressed by an informal action, such as an LOV or NOV, or by a formal action that does not include a penalty, such as an AO.

7.6.3.2 Administrative Penalty Order

A stronger response, such as an AO along with an APO or a civil judicial action with a penalty might be appropriate for continuing NPDES violations or for violations causing harm, such as a significant fish kill.

7.6.4 Factors to Consider When Determining an Appropriate Response to Noncompliance of an NPDES Permit Requirement

EPA's OWM and OECA issued a joint memorandum in 1995 regarding EPA's recommendations for enforcement of a single NPDES WET permit exceedance (USEPA 1995c). This memorandum reiterated EPA's existing guidance, including the following:

- EPA considers case-specific circumstances of violations and uses enforcement discretion to determine an appropriate response.
- EPA recommends an escalating response to continuing violations.
- EPA guidance does not typically recommend that the initial response to a single exceedance of an NPDES WET limit causing no known harm, be a formal enforcement action with a civil penalty.

The memorandum also reaffirmed the OWM's commitment to providing technical support to NPDES-permitted facilities with inconclusive TREs.

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APPENDIX A

List of Federal Statutes, EPA Final Regulations, and Guidance by NPDES WET Topic

List of Federal Statutes, EPA Final Regulations, and Guidance by NPDES WET Topic

Topic	Subtopic	Final Regulations and Issued Guidance on Topic	Section/Page Number of Referenced Document
NPDES Regulatory Framework for WET	Why WET?	(1) CWA (2) USEPA 1991a (3) USEPA 1991b (4) 40 CFR § 122.2 (5) 40 CFR § 136.3 (6) 60 FR 53529; 1995a (7) USEPA 2002a (8) USEPA 2002b (9) USEPA 2002c (10) USEPA 1995b	(1) Section 101(a)(2) and (3), Section 301, Section 402 (2) Section 1.5 "Integration of the Whole Effluent, Chemical-specific, and Bioassessment Approaches" pp. 20 – 23 (3) Section "Independent Application" p. 13 – 14 (4) 40 CFR § 122.2 WET definition p. 166 (5) 40 CFR § 136.3(a); Section III (Background), Subsection A (Regulatory History) pp. 69953 – 69954 – Table 1A (6) Table 1A – List of Approved Biological Methods pp. 53542 (7) Section 1.1 p. 1 (8) Section 1.8 p. 1 (9) Section 1.8 p. 1 (10) Section 1.8 p. 3
EPA Toxicity Test Methods	Toxicity Test Methods to Be Used Under NPDES Program	(1) 40 CFR § 136.3 (2) 40 CFR § 122.21 (3) 40 CFR § 122.44 (4) USEPA 2002a (5) USEPA 2002b (6) USEPA 2002c (7) USEPA 1995b (8) USEPA 1997	(1) 40 CFR § 136.3(a); Section III (Background), Subsection A (Regulatory History) pp. 69953 – 69954 136.4, 136.5 – Table 1A (2) 40 CFR § 122.21(j)(5)(vii) – WET test summaries must be provided for each WET test conducted; 40 CFR § 122.21(g)(7)(i) – when quantitative data are required, applicant must collect a sample of effluent and use methods approved under 40 CFR Part 136 (3) 40 CFR § 122.44(i)(1)(iv)(B) – if no approved methods under 40 CFR Part 136 or 40 CFR Chapter 1, subchapters N or O, analysis procedures should be specified in the permit (4) Section 2 "Types of Tests", pp. 2-4 (5) Section 2.2 "Types of Tests," pp. 5-6 (6) Section 2.2 "Types of Tests," PP. 5-6 (7) Section 2.2 "Types of Tests," pp. 8-9 (8) P.1.
	Types of Toxicity Tests	(1) USEPA 2002a (2) USEPA 2002b (3) USEPA 2002c (4) USEPA 1995b (5) 40 CFR § 122.21 (6) 40 CFR § 122.21 (7) 40 CFR § 122.21 (8) USEPA 1991a (9) USEPA 2010a (10) USEPA 1991a	(1) Section 2 'Types of Tests', pp.2-4 (2) Section 2.2 "Types of Tests," pp. 5-6 (3) Section 2.2 "Types of Tests," pp. 5-6 (4) Section 2.2 "Types of Tests," pp. 8-9 (5) 40 CFR § 122.21(j)(5)(v)(A) – acute testing at greater than 1000:1 mixing zone (6) 40 CFR § 122.21(j)(5)(v)(B) – acute or chronic testing between 1000:1 and 100:1 mixing zone (7) 40 CFR § 122.21(j)(5)(v)(C) – chronic testing at less than 100:1 mixing zone (8) Section 5.2.3 Expression of Permit Limits, pp. 96

Topic	Subtopic	Final Regulations and Issued Guidance on Topic	Section/Page Number of Referenced Document
			(9) Chapter 6, "Water Quality-Based Effluent Limitations" (10) Section 3.3.6, p. 61 Freshwater or marine test organisms
	Non-Promulgated Toxicity Test Species or Alternate Test Species	(1) 40 CFR § 136.3 (2) 40 CFR § 122.21 (3) 40 CFR § 122.44 (4) 40 CFR § 136.4 (5) 40 CFR § 136.5	(1) 40 CFR § 136.3(a) – Table 1A – List of Approved Biological Methods for Wastewater and Sewage Sludge (2) 40 CFR § 122.21(j)(5)(vii) – WET test summaries must be provided for each WET test conducted; 40 CFR § 122.21(g)(7)(i) – when quantitative data are required, applicant must collect a sample of effluent and use methods approved under 40 CFR Part 136 (3) 40 CFR § 122.44(i)(1)(iv)(B) – if no approved methods under 40 CFR Part 136 or 40 CFR Chapter 1, subchapters N or O, analysis procedures should be specified in the permit. (4) 40 CFR § 136.4 Application for and approval of alternate test procedures for nationwide use. (5) 40 CFR § 136.5 Approval of alternate test procedures for regional limited use.
	Choice of Toxicity Test Species	(1) 40 CFR § 136.3 (2) USEPA 2002a (3) USEPA 2002b (4) USEPA 2002c (5) USEPA 1995a (6) USEPA 1995b (7) USEPA 2017	(1) 40 CFR § 136.3(a) – Table 1A (2) Section 6 "Test Organisms", pp. 26-31 (3) Section 6 "Test Organisms", pp. 22-25 (4) Section 6 "Test Organisms", pp.22-25 (5) Section 3, Subsection D "Test Species Selection", pp. 14-16 (6) Section 6 "Test Organisms", pp. 30-35 (7) Chapter 8, Subsection B "Requirements of WET Testing", pp. 154-156
	Species Sensitivity	(1) 40 CFR § 136.3 (2) MacKnight 2011	(1) 40 CFR § 136.3(a), Table 1A (2) Final full paragraph on p. 3
	Acute Freshwater Toxicity Test Duration	(1) USEPA 2002a (2) USEPA 1995b	(1) Section 9.15 "Test Duration," pp. 50-66 (2) Section E.5. "Test Conditions," p. 20
	Static vs Static Renewal vs Flow-Through Acute Toxicity Tests	(1) USEPA 2002a (2) USEPA 2002b (3) USEPA 1995b	(1) Section 9 "Acute Toxicity Test Procedures", pp. 43-45 (2) Section 2.4 "Advantages and Disadvantages of Toxicity Test Types," p. 6 (3) Section 2.4 "Advantages and Disadvantages of Toxicity Test Types," pp. 9-10

Topic	Subtopic	Final Regulations and Issued Guidance on Topic	Section/Page Number of Referenced Document
	20 vs 25 °C Test Temperature for Acute Toxicity Tests	(1) USEPA 2002a	(1) Section 4.6 "Test Conditions," p. 8. Section 9.12 "Test Temperature," p. 46. Section 9.17 "Summary of Test Conditions for the Principal Test Organisms," pp. 50-56
	Acute vs Chronic Toxicity Tests	(1) USEPA 2002b (2) USEPA 2002c (3) USEPA 1995b (4) USEPA 1991a	(1) Section 2.1 "Introduction," pp. 3-5. Section 2.2 "Types of Tests," pp. 5-6 (2) Section 2.1 "Introduction," pp. 3-5. Section 2.2 "Types of Tests," pp. 5-6 (3) Section 2.1 "Introduction" pp. 4-8; Section 2.2. "Types of Tests," pp. 8-9 (4) Section 3.3.3; p. 58; Section 5.4.1; p. 98
NPDES Effluent Sample Collection	Grab or Composite Samples	(1) 40 CFR § 122.21 (2) USEPA 2002a (3) USEPA 2002b (4) USEPA 2002c (5) USEPA 1995b (6) USEPA 2017 (7) USEPA 2010a	(1) 40 CFR § 122.21(j)(7)(i) Composite sampling unless specified otherwise in 40 CFR Part 136 (2) Section 8.1.2 p. 37; Section 8.2 "Effluent Sample Types", pp. 37-38 (3) Section 8.1.2 p. 30; Section 8.2 "Effluent Sample Types" pp. 30-31 (4) Section 8.1.2 p. 31; Section 8.2 "Effluent Sample Types" pp. 31-32 (5) Section 8.1.2 p. 43; Section 8.2 "Effluent Sample Types" pp. 43-44 (6) Section B "Sampling Procedures and Techniques," pp. 101-103. (7) Section 8.1.4 "Sample Collection," p. 8-7.
	Batch Treatment Systems	(1) USEPA 2002a (2) USEPA 2002b (3) USEPA 2002c (4) USEPA 1995b	(1) Section 8, "Effluent and Receiving Water Sampling and Sample Handling and Sample Preparation for Toxicity Tests", pp. 37-40 (2) Section 8, "Effluent and Receiving Water Sampling and Sample Handling and Sample Preparation for Toxicity Tests", pp. 30-31 (3) Section 8, "Effluent and Receiving Water Sampling and Sample Handling and Sample Preparation for Toxicity Tests", pp. 31-32 (4) Section 8, "Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests", pp. 43-54
	Municipal Effluents	(1) USEPA 2002a (2) USEPA 2002b (3) USEPA 2002c (4) USEPA 1995b	(1) Section 8, "Effluent and Receiving Water Sampling and Sample Handling and Sample Preparation for Toxicity Tests", pp. 37-40

Topic	Subtopic	Final Regulations and Issued Guidance on Topic	Section/Page Number of Referenced Document
			(2) Section 8, "Effluent and Receiving Water Sampling and Sample Handling and Sample Preparation for Toxicity Tests", pp. 30-31 (3) Section 8, "Effluent and Receiving Water Sampling and Sample Handling and Sample Preparation for Toxicity Tests", pp. 31-32 (4) Section 8, "Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests", pp. 43-54
	Industrial Effluents	(1) USEPA 1991a (2) USEPA 1982	(1) Chapter 7 "Case Examples", pp. 129-139 (2) Chapter 6 "Sampling Industrial Wastewaters", pp. 180-187
	Intermittent Discharges (e.g., Mine Discharges)	(1) USEPA 2002a (2) USEPA 2002b (3) USEPA 2002c (4) USEPA 1995b	(1) Section 8.3.4.2 "Intermittent Discharges," pp. 37-38 (2) Section 8.3.4.2 "Intermittent Discharges," p. 31 (3) Section 8.3.4.2 "Intermittent Discharges," p. 32 (4) Section 8.3.4.2 "Intermittent Discharges," p. 45
Reasonable Potential (RP)	Why do RP?	(1) 40 CFR § 122.44 (2) 40 CFR § 122.44 (3) 40 CFR § 122.44 (4) 40 CFR § 132	(1) 40 CFR § 122.44(d)(1)(iii) - Need to have NPDES WET limit if discharge causes, has potential to reasonable potential to cause, or contributes to an in-stream excursion above the numeric criterion for WET. (2) 40 CFR § 122.44(d)(1)(i) – Limits must control all pollutants or pollutant parameters. (3) 40 CFR § 122.44(d)(1)(ii) – Must use procedures which account for existing controls on point and nonpoint sources of pollution, the variability of the pollutant or pollutant parameter, the sensitivity of the species to toxicity testing, and where appropriate, the dilution of the effluent in the receiving water. (4) 40 CFR § 132 – Appendix F Procedure 6 discusses the procedures to be used by Great Lake states to evaluate WET reasonable potential.
	Reasonable Potential Analysis (RPA) when there is no WET Data	(1) OWM NPDES WET Training Course (2) USEPA 1991a (3) USEPA 2010b (4) USEPA 1994 (5) 40 CFR § 122.44 (6) 40 CFR § 122.44 (7) 40 CFR § 122.44 (8) 40 CFR § 122.44 (9) 40 CFR § 122.44 (10) 40 CFR § 122.47 (11) 40 CFR § 122.47 (12) 40 CFR § 122.21	(1) Module 5: Determining WET Reasonable Potential for NPDES Permits (2) Section 3 "Effluent Characterization", pp. 47-57 (3) Section 4.1 "Reasonable Potential (RP) WET Analysis," pp. 13-14 (4) Section 2 "Evaluation of Dischargers for Reasonable Potential", pp. 7-10 (5) 40 CFR § 122.44(d)(1)(ii) – procedures accounting for controls on point and nonpoint; variability in effluent; sensitivity of species; and dilution. (6) 40 CFR § 122.44 (d)(1)(i) – WQS meet Section 303 of CWA including state narrative criteria for water quality. (7) 40 CFR § 122.44(d)(1)(iv) – must contain an effluent limit for WET if excursion of numeric WET criterion.

Topic	Subtopic	Final Regulations and Issued Guidance on Topic	Section/Page Number of Referenced Document
			(8) 40 CFR § 122.44(d)(1)(v) – must contain an effluent limit for WET if excursion of narrative WET criterion unless chemical specific limit controls toxicity. (9) 40 CFR § 122.2 – WET definition (10) 40 CFR § 122.47(a) – permit may specify a schedule of compliance (11) 40 CFR § 122.47(a)(1) – compliance as soon as possible (12) 40 CFR § 122.21(j)(5)(iv)(A) – minimum of four quarterly tests for a year, from the year preceding the permit application
	RPA when there is WET Data	(1) OWM NPDES WET Training Course (2) USEPA 1991a (3) USEPA 2010b (4) USEPA 1994 (5) 40 CFR Part 132 (6) 40 CFR § 122.41 (7) 40 CFR § 122.41 (8) 40 CFR § 122.21 (9) 40 CFR § 122.21 (10) 40 CFR § 122.44	(1) Module 4: Determining WET Reasonable Potential for NPDES Permits. (2) Section 3 "Effluent Characterization", pp. 47-57 (3) Section 4.1 "Reasonable Potential (RP) WET Analysis," pp. 13-14 (4) Section 2 "Evaluation of Dischargers for Reasonable Potential", pp. 7-10 (5) Appendix F – Great Lakes Water Quality Initiative Implementation Procedures (6) 40 CFR § 122.41(j)(1) – representative of monitored activity (7) 40 CFR § 122.41(l)(4)(ii) – all monitoring data must be included in DMR (8) 40 CFR § 122.21(g)(11) – all biological tests in last 3 years must be identified (9) 40 CFR § 122.21(j)(5) – effluent monitoring for WET (10) 40 CFR § 122.44(d)(1)(v) – discharge causes, has the reasonable potential to cause, or contributes to an in-stream excursion of an applicable state WQS
	Previous WET Test Result(s) that indicated Effluent Toxicity causing an Excursion of WET WQS	(1) OWM NPDES WET Training Course (2) USEPA 1991a (3) USEPA 2010b	(1) Module 4: Determining WET Reasonable Potential for NPDES Permits. (2) Section 3 "Effluent Characterization", pp. 47-57 (3) Section 4.1 "Reasonable Potential (RP) WET Analysis," pp. 13-14
NPDES WET Permit Limits	Establishing NPDES WET Limits	(1) 40 CFR § 125.3 (2) USEPA 1991a (3) 40 CFR § 122.45	(1) 40 CFR § 125.3(c)(4) – Limitations may be expressed, where appropriate, in terms of toxicity (2) Section 5.4.1 – general guidance on WQBELs for toxicity (3) 40 CFR § 122.45(d) – continuous discharges permits include MDL and AWL
	USEPA 1991 Technical Support Document for Water Quality-Based Toxics Control Approach	(1) CWA (2) OWM NPDES WET Training Course (3) USEPA 1991a	(1) Section 303(c) (2) Module 6: USEPA NPDES WET Permit Development (3) Section 5 "Permit Requirements", pp. 93-113
	Alternative Approaches	(1) 40 CFR § 122.41 (2) 40 CFR § 122.44 (3) 40 CFR § 122.44 (4) 40 CFR § 122.44	(1) 40 CFR § 122.41(l)(4)(iii) – utilize arithmetic mean for calculating limits (2) 40 CFR § 122.44(d)(1)(i) – Limits must control all pollutants or pollutant parameters.

Topic	Subtopic	Final Regulations and Issued Guidance on Topic	Section/Page Number of Referenced Document
		(5) 40 CFR § 122.44	(3) 40 CFR § 122.44(d)(1)(ii) – procedures must account for variability in pollutant, species sensitivity, and dilution (4) 40 CFR § 122.44(d)(1) – achieve WQS under section 303 of the CWA including state narrative criteria for water quality 40 CFR § 122.44(d)(1)(iv) – discharge cause, has the reasonable potential to cause, or contributes to an in-stream excursion for WET, the permit must contain an NPDES limit for WET
	TRE/TIE in Permits and NPDES WET Permit Limits	(1) USEPA 1991a	(1) Section 5.8 "Toxicity Reduction Evaluations," pp. 114-121
NPDES Permit Compliance Monitoring Conditions	Dilution Water Selection for Toxicity Tests	(1) USEPA 2002a (2) USEPA 2002b (3) USEPA 2002c (4) USEPA 1995b	(1) Section 7 "Dilution Water", pp. 31-36 (2) Section 7 "Dilution Water", pp. 26-29 (3) Section 7 "Dilution Water", pp. 26-30 (4) Section 7 "Dilution Water", pp. 36-42
	Toxicity Test (e.g., WET Testing) Monitoring Frequency	(1) USEPA 1991a (2) OWM WET Training Course (3) USEPA 2010a	(1) Section 3.3.3 "Effluent Characterization for Whole Effluent Toxicity," pp. 53-59 (2) Module 6: USEPA NPDES WET Permit Development (3) Section 8.1.3 "Monitoring Frequency" pp. 8-5—8-6
	Type of Toxicity Test required in NPDES Permit	(1) USEPA 2010a (2) USEPA 1995b	(1) Section 6.5.1 "Types of WET Tests," pp. 6-36 – 6-37 (2) Chronic toxicity test methods utilizing West Coast marine and estuarine species
	Accelerated Toxicity Testing and Possible Use of a TRE when Effluent Sample is Toxic	(1) USEPA 1991a	(1) Section 5.8.3, "Circumstances Warranting a TRE," pp. 117-118
	Quality Assurance/Quality Control (QA/QC)	(1) OWM WET Training Course	(1) Module 3: USEPA NPDES Reviewing WET Tests and WET QA/QC
	Statistical Test Endpoints	(1) USEPA 2002a (2) USEPA 2002b (3) USEPA 2002c (4) USEPA 1995b (5) USEPA 2017 (6) USEPA 2010c	(1) Chapter 11 "Acute Toxicity Data Analysis", pp. 71-98 (2) Chapter 9 "Chronic Toxicity Test Endpoints and Data Analysis", pp. 37-45 (3) Chapter 9 "Chronic Toxicity Test Endpoints and Data Analysis", pp. 40-47 (4) Chapter 9 "Chronic Toxicity Test Endpoints and Data Analysis", pp. 55-67 (5) Chapter 8, Section C "Analysis of WET Data", pp. 159-161 (6) Section 1.3 "Test of Significant Toxicity," pp. 4-5
	Toxicity Identification Evaluation/Toxicity Reduction Evaluation (TIE/TRE)	(1) USEPA 2017 (2) USEPA 1991a (3) USEPA 1989a (4) USEPA 1991c (5) USEPA 1992 (6) USEPA 1993a (7) USEPA 1993b	(1) Chapter 8, Subsection D "Toxicity Reduction Evaluations and Toxicity Identification Evaluations (TREs/TIEs), pp. 168-171 (2) Section 5.8 "Toxicity Reduction Evaluations," pp. 114-118 (3) "Generalized Methodology for Conducting Industrial Toxicity Reduction Evaluations (TREs)," pp. 1-107

Topic	Subtopic	Final Regulations and Issued Guidance on Topic	Section/Page Number of Referenced Document
			(4) "Methods for Aquatic Toxicity Identification Evaluations." pp. 1-87 (5) "Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase 1", pp. 1-59 (6) "Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity," pp. 1-71 (7) "Methods for Aquatic Toxicity Identification Evaluations: Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity," pp. 1-32
NPDES Permit Language for Reporting WET Monitoring Results	Reporting, Frequency; Recommended Content	(1) USEPA 2010a	(1) Section 8.1.3 "Monitoring Frequency," pp.8-5 to 8-6
	Discharge Monitoring Reports for WET Data and EPA's Integrated Compliance Information System (ICIS)	(1) USEPA 2010a (2) USEPA 2017 (3) 40 CFR § 122.22(c) (4) USEPA 1991a	(1) Section 11.5.1 "Compliance Monitoring," pp. 11-22 to 11-26 (2) Section G "Inspection Report," pp. 55-56 (3) 40 CFR § 122.22(c) – changes to authorization (4) Section 6.3.2 "Discharge Monitoring Reports/Quality Assurance," p. 124
	NPDES Electronic Rule (eRule)	(1) 40 CFR Part 127	(1) 40 CFR Part 127 – NPDES Electronic Reporting Rule (NPDES eRule)
Reviewing NPDES WET Compliance Reports	WET Data Evaluation Checklist	(1) USEPA 2010a	(1) Section 11.5.1.1 "Compliance Review," pp. 11-22 to 11-23
	Determining NPDES Effluent Representativeness of WET Data	(1) 40 CFR § 122.41 (2) USEPA 1994 (3) USEPA 1997 (4) 40 CFR § 122.44	(1) 40 CFR § 122.41(j)(1) - Effluent data should be representative of the monitored activity. (2) Summary statement No. 7 "Whole Effluent Toxicity Controls and the Pollutants Ammonia and Chlorine", p. 3 (3) Item No. 1 "pH and Ammonia Control" p. 2 (4) 40 CFR § 122.44(d)(1)(v) - TRE may be able to determine chemical limit instead of NPDES WET limit
	Accounting for Effluent Variability	(1) 40 CFR § 122.21 (2) 40 CFR § 122.41 (3) USEPA 2000b (4) USEPA 2002a (5) USEPA 2002b (6) USEPA 2002c (7) USEPA 1995b (8) 40 CFR § 122.41 (9) 40 CFR § 122.21 (10) USEPA 2000a	(1) 40 CFR § 122.21(j)(5)(iv)(A) – minimum of four quarterly WET tests (2) 40 CFR § 122.41(l)(4)(ii) – all monitoring data must be included in DMR (3) Section 5.5 "Conducting the Statistical Analysis to Determine the Effect Concentration," pp. 5-10 – 5-11 (4) Section 11 "Acute Toxicity Data Analysis", pp. 71-108 (5) Section 9 "Chronic Toxicity Test Endpoints and Data Analysis", pp. 37-46; Section 10.2 "Test Review" pp. 49-52 (6) Section 9 "Chronic Toxicity Test Endpoints and Data Analysis", pp. 40-48; Section 10.2 "Test Review" pp. 51-54 (7) Section 9 "Chronic Toxicity Test Endpoints and Data Analysis", pp. 55-67 (8) 40 CFR § 122.41(j) – Monitoring and records. (9) 40 CFR § 122.21(j)(5) – Effluent monitoring for WET. (10) Section 2 "Nominal Error Rate Adjustments", pp. 2-1—2-13

Topic	Subtopic	Final Regulations and Issued Guidance on Topic	Section/Page Number of Referenced Document
	Compliance Monitoring	(1) 40 CFR § 122.22 (2) USEPA 1991a (3) USEPA 2017	(1) 40 CFR § 122.22(e) – electronic reporting (2) Section 6.3 "Compliance Monitoring," pp. 123-124 (3) Section 8c "Analysis of WET Data", pp. 159-168
EPA NPDES WET Enforcement Procedures	EPA NPDES WET Basic Permitting Principles and Enforcement Strategy	(1) USEPA 1991a (2) USEPA 2010a (3) USEPA 1989b (4) USEPA 1989c	(1) Section 6 "Compliance Monitoring and Enforcement", pp. 123-127 (2) Section 11.5 "Permit Compliance and Enforcement," p. 11-21 (3) Section IV "Scope and Implementation," Subsection A "Compliance and Tracking Review," pp. 4-5 (4) Table 1 – Violation Review Action Criteria
	EPA NPDES Enforcement Management System	(1) USEPA 1991a (2) USEPA 1995c (3) USEPA 1989c	(1) Section 6.5 "Enforcement" and Section 6.6 pp. 125 -126 (2) Single NPDES WET Permit Limit Exceedance – p. 1; Inconclusive TREs – p. 2 (3) Table 1 – Violation Review Action Criteria
	Types of NPDES Enforcement Actions	(1) USEPA 2010a (2) USEPA 1989b	(1) Section 11.5.3 "Enforcement," pp. 11-25 to 11-26 (2) Section IV "Scope and Implementation of Strategy," Section D "Enforcing Toxic Control Permit Conditions," pp. 7-12
	Other Factors to Consider When Deciding an Appropriate Response	(1) CWA	(1) Section 309(a)(3) Compliance Order", C. p.1
	Invalid NPDES WET Test Results	(1) USEPA 2000b (2) USEPA 2017	(1) Section 12.2.3 "Test Acceptability Criteria" and Section 12.2.4.2 "Test Conditions," p.111 (2) Chapter 8 Toxicity; Subsection C Analysis of WET Data; Review of Test Conditions, p. 161
	Noncompliance with Other Narrative NPDES WET Permit Conditions	(1) USEPA 1989b	(1) Section IV, Subsection D "Enforcing Toxic Control Permit Conditions" Subsection c "Reporting/Other Violations, pp. 7-10

APPENDIX B

Glossary of Terms

Glossary of Terms

Absolute toxicity is the toxicity of the effluent without considering dilution.

Acute in aquatic toxicity tests means a stimulus severe enough to rapidly induce an effect. An effect observed in 96 hours or less typically is considered acute. When referring to aquatic toxicology or human health, an *acute* effect is not always measured in terms of lethality.

Acute toxicity test is a test to determine the concentration of a sample (e.g., reference toxicant, effluent, or receiving water) that causes an acute adverse effect (usually death) on a group of test organisms during a short-term exposure (e.g., 24, 48, or 96 hours). Acute toxicity is measured using statistical approaches (e.g., point estimate techniques or a hypothesis test).

Acute-to-chronic ratio (ACR) is the ratio of the acute toxicity of an effluent or toxicant to its chronic toxicity. It is used as a factor for estimating chronic toxicity based on acute toxicity data or for estimating acute toxicity based on chronic toxicity data.

Ambient toxicity is measured by a toxicity test on a sample collected from a water body.

Anti-degradation policies are developed and adopted as part of a state's, territory's, or authorized Tribe's water quality standards to ensure protection of existing uses and maintains the existing level of water quality at which that water quality exceeds levels necessary to protect fish and wildlife propagation and recreation on and in the water. These policies also include special protection of waters designated as Outstanding National Resource Waters.

Aquatic community is an association of interacting populations of aquatic organisms in a water body or habitat.

Average monthly limit (AML) is the highest allowable value for the average of daily discharges obtained over a calendar month.

Biological assessment is an evaluation of the biological condition of a water body using biological surveys and other direct measurements of resident biota in surface waters.

Biological criteria, also known as **biocriteria**, are narrative expressions or numeric values of the biological characteristics of aquatic communities based on appropriate reference conditions. They serve as an index of aquatic community health.

Biological integrity is the condition of the aquatic community inhabiting unimpaired water bodies of a specified habitat as measured by community structure and function.

Biological monitoring, also known as **biomonitoring**, describes the living organisms in water quality surveillance used to indicate compliance with water quality standards or effluent limits and to document water quality trends. Methods of *biological monitoring* may include, but are not limited to, toxicity testing such as ambient toxicity testing or whole effluent toxicity testing.

Biological survey, or **biosurvey,** is the collecting, processing, and analyzing of a representative portion of the resident aquatic community to determine its structural and/or functional characteristics.

Chronic in aquatic toxicity tests means a stimulus that results in an adverse effect over a long period of time, often one-tenth of the life span or more. *Chronic* should be considered a relative term

depending on the life span of an organism. The measurement of a chronic effect can be reduced growth, reduced reproduction, and so forth, in addition to lethality.

Chronic toxicity test is a short-term test, usually 96 hours or longer in duration, in which sublethal effects (e.g., significantly reduced growth or reproduction) are measured in addition to lethality.

Coefficient of variation (CV) is a standard statistical measure of the relative variation of a distribution or set of data defined as the standard deviation divided by the mean.

Concentration-response relationship (CRR) is the relationship between sample concentration and magnitude of effects. For toxicity testing, the *CRRs* are displayed in terms of biological test endpoint (e.g., survival, growth, and reproduction) at certain sample concentrations. Ideally, for toxicity testing, a *CRR* would be a monotonically decreasing response, thus as the concentration of a sample increases, the measured biological test endpoint would decrease.

Continuous discharge occurs without interruption throughout the operating hours of the facility, except for infrequent shutdowns for maintenance, process changes, or other similar activities [40 CFR § 122.2].

Criterion continuous concentration (CCC) is the EPA national water quality criteria recommendation for the highest in-stream concentration of a toxicant or an effluent to which aquatic organisms can be exposed indefinitely without causing an unacceptable effect.

Criterion maximum concentration (CMC) is the EPA national water quality criteria recommendation for the highest in-stream concentration of a toxicant or an effluent to which aquatic organisms can be exposed for a brief period without causing an acute effect.

Designated uses are those uses specified in water quality standards for each water body or segment whether they are being attained or not [40 CFR § 131.3(f)].

Discharge monitoring report (DMR) is a report submitted by NPDES permittees that contains self-monitoring results for wastewater required by NPDES permits [40 CFR § 122.2].

Effect concentration (EC) is a toxicant concentration identified through point estimate statistical analysis that would cause an observable adverse effect (e.g., death, immobilization, or serious incapacitation) in a certain percentage of the toxicity test organisms.

Effluent is treated or untreated wastewater that flows out of a treatment plant, sewer, or industrial outfall. Generally, it refers to wastes discharged into surface waters.

Effluent flow (Q_e) is the flow (in cubic feet per second or million gallons per day) of a wastewater discharge from an NPDES-regulated facility expressed in standard NPDES formulas by a permit writer as "Q_e" to calculate water quality-based effluent limits. NPDES permit authority policy or procedures might specify which flow measurement to use as the critical effluent flow value(s) in various water quality-based permit calculations (e.g., the maximum daily flow reported on the permit application, the maximum of the monthly average flows from DMRs for the past three years, or the facility design flow).

Endpoint is a toxicity test result that describe a stimulus severe enough to induce a specific level of response. Biological test *endpoints* include survival, growth, biomass, reproduction, and so forth; while statistical *endpoints* include LC_{50}, IC_{25}, NOEC, LOEC, and so forth.

Frequency of a water quality criterion is how often the criterion can be exceeded without unacceptably affecting the community. The purpose of the average *frequency* of allowed excursions is to provide an appropriate average period of time during which the aquatic community can recover from the effect of an excursion and then function normally for a period of time before the next excursion. The average *frequency* is intended to ensure that the community is not constantly recovering from effects caused by excursions of aquatic life criteria.

Hypothesis testing is a statistical technique (e.g., Dunnett's test, Test of Significant Toxicity [TST]) for determining whether a tested concentration results in a statistically different response than that observed in the control. For multi-concentration toxicity tests, the reported values determined by hypothesis testing are the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC). For WET tests being evaluated with the TST (incorporating EPA's regulatory management decision), the reported results are pass and fail.

In-stream waste concentration (IWC) is the concentration of a toxicant or effluent in the receiving water after mixing. The *IWC* is the inverse of the dilution factor. It is sometimes referred to as the "receiving water concentration" or "critical dilution".

Inhibition concentration (IC) is a point estimate of the toxicant concentration that would cause a given percent reduction (e.g., IC_{25}) in a nonlethal biological measurement of test organisms, such as reproduction or growth.

Lethal concentration (LC) is the point estimate of the toxicant concentration that would be lethal to a given percentage of test organisms during a specific period.

Load allocations (LA) are the portion of a receiving water's total maximum daily load that is attributed either to one of its existing or future nonpoint sources of pollution or to natural background sources [40 CFR § 130.2].

Long-term average (LTA) represents expected performance from the permitted facility required to achieve the associated wasteload allocation (WLA). The *LTA* of pollutant concentration or effluent toxicity is calculated from a WLA, typically assuming that the WLA is a 99th percentile value (or another upper bound value) based on the lognormal distribution. One *LTA* is calculated for each WLA (typically an acute *LTA* and a chronic *LTA* for aquatic life protection).

Lowest observed effect concentration (LOEC) is the lowest tested concentration of an effluent or a toxicant at which adverse effects are observed on the aquatic test organisms at a specific time of observation. It is determined using hypothesis testing.

Magnitude of a water quality criterion is how much of a pollutant (or pollutant parameter such as toxicity) expressed as a concentration or toxic unit can be present without impacting water quality.

Maximum daily limit (MDL) is the highest allowable daily discharge of a pollutant measured during a calendar day or 24-hour period representing a calendar day.

Minimum significant difference (MSD) is a measure of test sensitivity that establishes the minimum difference required between a control and a test treatment for that difference to be considered statistically significant.

Mixing zone is an area in which an effluent undergoes initial dilution and is extended to cover the secondary mixing in the ambient water body. A *mixing zone* is an allocated impact zone where water quality criteria can be exceeded if acutely toxic conditions are prevented.

National Pollutant Discharge Elimination System (NPDES) is the national program for issuing, modifying, revoking and reissuing, terminating, monitoring, and enforcing permits and imposing and enforcing pretreatment requirements under CWA Sections 307, 318, 402, and 405 [40 CFR § 122.2]. *NPDES* permits regulate discharges of pollutants from point sources to waters of the United States.

No observed effect concentration (NOEC) is the highest tested concentration of a sample at which no adverse effects are observed on the aquatic test organisms at a specific time of observation. It is determined using hypothesis testing.

Percent minimum significant difference (PMSD) is the minimum significant difference divided by the control mean expressed as a percent (see **minimum significant difference [MSD]**).

Point estimate is a statistical inference that estimates the true value of a parameter by computing a single value of a statistic from a set of sample data.

Priority pollutants are those pollutants listed in Appendix A to 40 CFR Part 423 by the Administrator in support of CWA Section 307(a).

Probability is a number expressing the likelihood of occurrence of a specific event, such as the ratio of the number of outcomes that will produce a given event to the total number of possible outcomes.

Publicly owned treatment works (POTWs) are facilities owned by a state or municipality (as defined by CWA Section 502(4) that treat domestic wastewater or wastewater from indirect dischargers (e.g., industrial facilities). (See 40 CFR § 403.3.)

Quality assurance (QA) is a practice in toxicity testing that addresses all activities affecting the quality of the final effluent toxicity data. *QA* includes evaluation of effluent sampling and handling, source and condition of test organisms, equipment condition, test conditions, instrument calibration, replication, use of reference toxics, recordkeeping, data, and other aspects of the test and testing procedures.

Quality control (QC) is the set of focused, routine, day-to-day activities conducted as part of an overall quality assurance program.

Reasonable potential (RP) is the determination of an effluent that is projected or calculated to cause, have the potential to cause, or contribute to an in-stream excursion above any state's, territory's, or authorized Tribe's water quality standard based on several factors, including, as a minimum, the four factors listed in 40 CFR § 122.44(d)(1)(ii).

Reasonable potential analysis (RPA) is used to determine whether a discharge, alone or in combination with other sources of pollutants to a water body and under a set of conditions arrived at by making a series of reasonable assumptions, could lead to an excursion above a state's, territory's, or authorized Tribe's water quality standard.

Reasonable potential multiplying factor (RPMF) is a numerical value based on the variability of a data set and number of samples in that data set and used to multiply the maximum observed effluent value in an effluent data set to account for the variability in a determination of whether a discharge has reasonable potential.

Receiving water concentration (RWC) is the concentration of a toxicant or the parameter toxicity in the receiving water after mixing. It is sometimes referred to as the "in-stream waste concentration" (IWC).

Receiving water flow (Q_s), as used in steady-state modeling approaches for permit limit calculations and reasonable potential analyses, is the upstream flow of the river or stream receiving the discharge expressed in cubic feet per second or millions of gallons per day. State, territory, or Tribal policies may direct the permit writer to use a particular receiving water flow, such as the lowest consecutive 7-day average stream flow during any 10-year period, in development of permit requirements.

Reference toxicant test is a check of the sensitivity of the toxicity test organisms and the suitability of the test methodology in a toxicity test. Reference toxicant data are part of a routine quality assurance/quality control program to evaluate the performance of laboratory personnel and the robustness and sensitivity of the toxicity test organisms.

Significant difference is defined as a statistically significant difference (e.g., 95% confidence level) in the means of two distributions of WET sampling toxicity test results.

Standard deviation is a measure of the variability of a set of data, calculated as the square root of the variance.

Statistic is a computed or estimated quantity, such as the mean, standard deviation, or coefficient of variation.

Steady-state model is a fate and transport model that has been used in the NPDES program and uses constant values of input variables to predict constant values of receiving water quality concentrations.

Sublethal means a stimulus below the level that causes death.

Test acceptability criteria (TAC) are specific criteria for determining whether toxicity test results are acceptable pursuant to EPA's toxicity test methods in 40 CFR Part 136 (additional *TAC* may be established by an NPDES permit authority). The effluent and reference toxicant must meet specific criteria as defined in the toxicity test method (e.g., for the *Ceriodaphnia dubia* survival and reproduction test, the criteria are 80% or higher survival of all control organisms, an average of 15 or more young per surviving female in the control solution, and 60% of the surviving control females must produce three broods).

Total maximum daily load (TMDL) is *the calculation of the maximum amount of a pollutant loading a water body can receive without violating* water quality standards for that pollutant. The *TMDL* is the sum of the individual wasteload allocations, load allocations, and a margin of safety [40 CFR § 130.2(i)].

Toxic pollutant is a pollutant listed in 40 CFR § 401.15 by the Administrator under CWA Section 307(a).

Toxic unit (TU) is a measure of toxicity in a sample as determined by the acute toxicity units or chronic toxicity units measured.

Toxic unit-acute (TU_a) is the reciprocal of the sample concentration that causes 50% of the organisms to die by the end of the acute exposure period (i.e., $100/LC_{50}$).

Toxic unit-chronic (TU_c) is the reciprocal of the sample concentration that causes no observable effect on the test organisms by the end of the chronic exposure period (i.e., 100/NOEC) or an allowable 25% effect (i.e., $100/IC_{25}$), depending on the applicable NPDES permit authority implementation procedures or water quality standards.

Toxicity identification evaluation (TIE) is a set of site-specific procedures used to identify the specific chemical(s) causing toxicity.

Toxicity reduction evaluation (TRE) is a site-specific study conducted in a step-wise process to identify the causative agents of toxicity, isolate the source of toxicity, evaluate the effectiveness of toxicity control options, and then confirm the reduction in toxicity after the control measures are put in place.

Toxicity test is a procedure to determine the toxicity of a chemical or a sample using living organisms. A *toxicity test* measures the degree of effect on exposed test organisms of a specific chemical or sample.

Toxic is a pollutant that can have an adverse effect on living organisms. CWA Section 307(a) "priority" pollutants are a subset of this group of pollutants.

Variance is a measure of the dispersion in a set of values, defined as the sum of the squared deviations from the mean divided by the total number of values in the set.

Wasteload allocation (WLA) is the portion of a receiving water's assimilative capacity that is allocated to one of its existing or future point sources of pollution [40 CFR § 130.2(h)].

Water quality assessment is an evaluation of the condition of a water body using biological surveys, chemical-specific analyses of pollutants in water bodies, and toxicity tests.

Water quality criteria (WQC) are elements of a state's, territory's, or authorized Tribe's water quality standards, expressed as constituent concentrations, levels, or narrative statements, representing a quality of water that supports a particular designated use. When criteria are met, water quality will generally protect the designated use [40 CFR § 131.3(b)]. Numeric criteria are scientifically derived ambient concentrations developed by EPA or states, territories, or authorized Tribes for various pollutants of concern to protect human health and aquatic life. Narrative criteria are statements that describe the desired water quality goal.

Water Quality Portal (WQP) is a source of discrete water quality data in the United States. This cooperative service integrates publicly available water quality data from the U.S. Geological Survey, Environmental Protection Agency, and other federal, state, territorial, Tribal, and local agencies.

Water quality-based effluent limit (WQBEL) is an effluent limitation in an NPDES permit calculated to meet all applicable water quality standards (e.g., aquatic life, human health, wildlife, and translation of narrative criteria) for a specific point source to a specific receiving water.

Water quality standards (WQS) are provisions of a state's, territory's, authorized Tribe's, or federal law that include designated use or uses for the waters of the United States and water quality criteria for those waters based on their designated uses. *Water quality standards* are intended to protect the public health or welfare, enhance the quality of water, and serve the purposes of the CWA [40 CFR § 131.3(i))].

Whole effluent toxicity (WET) is the aggregate toxic effect of an effluent measured directly by a toxicity test in which the test results are represented by acute (lethal) and/or chronic (lethal and sublethal) endpoints [40 CFR § 122.2].

APPENDIX C

Summary of Toxicity Test Conditions and Test Acceptability Criteria for EPA Toxicity Tests

Table C-1. Summary of toxicity test conditions and test acceptability criteria for EPA acute toxicity tests (USEPA 2002a).

Toxicity Test Condition	***C. dubia***	***D. pulex*** ***D. magna***	***P. promelas***	***C. variegatus***	***M. beryllina*** ***M. menidia*** ***M. peninsulae***	***M. bahia***	***O. mykiss*** ***S. fontinalis***	***H. costata***[1]
Toxicity Test Method #	2002.0	2021.0	2000.0	2004.0	2006.0	2007.0	2019.0	NA
Test Type	Static non-renewal, static-renewal, or flow-through							Static non-renewal, static renewal
Test Duration	24, 48, or 96 h							24, 48, or 96 h
Average Temperature	20 °C±1 °C or 25 °C±1 °						12 °C±1°	15 °C±1° or 13 °C±1°[2]
Temperature Deviation	Test temperature must not deviate (i.e., maximum minus minimum temperature) by more than 3°C during the test.							NA
Light Quality	Ambient lab illumination							Ambient lab illumination
Light Intensity	10 - 20µE/m^2/s (50 – 100 ft-c)							10 - 20µE/m^2/s (50 – 100 ft-c)
Photoperiod	16 h light, 8 h darkness[3]							16 h light, 8 h darkness[3]
Test Chamber Size	30 mL		250 mL				5 L	1,000 mL
Test Solution Volume	15 mL	25 mL	200 mL				4 L	200 mL
Renewal of Test Solution	After 48 h							After 48 h
Test Organism Age	< 24-h		1 – 14 d		9 – 14 d	1 – 5 d	O. mykiss 15 – 30 d; S. fontinalis 30 – 60 d	3 – 4 d post hatch juveniles
# of Organism per Chamber	5		10					5
# Replicates per Test Concentration	4		2 for effluent 4 for receiving water					5
# of Organisms per Test Concentration	20		20 for effluent 40 for receiving water					25
Test Chamber Cleaning	Not Required							
Feeding Regime	Feed yeast, Cerophyll, and TetraMin® (YCT) and green algae prior to test; 0.1 mL each 2 h prior to renewal		Feed Artemia prior to test; 0.2 mL Artemia concentrate 2 h prior to test renewal			Artemia prior to test; 0.2 mL ≤24 h old Artemia concentrate daily (~100 nauplii per mysid)	Not Required	Artemia prior to test; 0.2 mL ≤24 h old Artemia concentrate daily (40 nauplii per mysid)

Toxicity Test Condition	***C. dubia***	***D. pulex*** ***D. magna***	***P. promelas***	***C. variegatus***	***M. beryllina*** ***M. menidia*** ***M. peninsulae***	***M. bahia***	***O. mykiss*** ***S. fontinalis***	***H. costata***[1]
Test Chamber Aeration	None		None, unless DO below 4 mg/L; 100 bubbles/min				None, unless DO below 6 mg/L; 100 bubbles/min	None, unless DO below 4 mg/L; 100 bubbles/min
Dilution Water	Moderately hard[4]			5 – 32 ppt[5]		5 – 30 ppt[5]	Moderately hard[4]	34 ± 2ppt[5]
Test Concentrations – Effluent	5 test concentrations and control							5 and control
Test Concentrations – Receiving Water	100% and a control							100% and a control
Dilution Factor	Effluent: ≥0.5 Receiving water: none or ≥0.5							Effluent: ≥0.5 Receiving water: none or ≥0.5
Test Endpoint	Effluent: Mortality Receiving Water: Mortality							Effluent: Mortality Receiving Water: Mortality
Sample Type	Grab or Composite							Grab or Composite
Effluent Holding Requirements	Onsite 24 h							Onsite 24 h
	Offsite 36 h							Offsite 36 h
Receiving Water Holding Requirements	First use within 36 h							First use within 36 h
Sample Volume Required	1 L		2 L	1 L for effluents 2 L for receiving water			20 L for effluents 40 L for receiving water	1 L for effluents 2 L for receiving water
Test Acceptability Criteria	≥ 90% survival in controls							90% or greater survival in controls

Available options
Recommended or recommended minimum
Required or required minimum

Notes:

1. This method is specific to Pacific Coast waters and is not listed at 40 CFR Part 136 for nationwide use. This method has been proposed but not yet approved at 40 CFR Part 136.
2. 15 °C±1° for organisms collected South of Pt. Conception; 13 °C±1° for organisms collected North of Pt. Conception.
3. For the *O. mykiss* and *S. fontinalis* test, light intensity should be raised/lowered gradually over a 15-min period at the beginning/end of the photoperiod using a dimmer or other suitable device.
4. Water prepared using MILLIPORE MILLI-Q® or equivalent deionized water and reagent grade chemicals or 20% dilute mineral water; receiving water, ground water, or synthetic water, modified to reflect receiving water hardness.
5. Uncontaminated source of seawater, deionized water mixed with HSB or artificial sea salts prepared with MILLI-Q® or equivalent deionized water.

Table C-2. Summary of toxicity test conditions and test acceptability criteria for EPA freshwater chronic toxicity tests (USEPA 2002b).

Toxicity Test Condition	*C. dubia* Survival and Reproduction	*P. promelas* Larval Survival and Growth	*P. promelas* Embryo-Larval Survival and Teratogenicity	*S. capricornutum* Growth Toxicity
Toxicity Test Method #	1002.0	1000.0	1001.0	1003.0
Test Type	Static renewal			Static non-renewal
Test Duration	Until 60% or more of surviving control females have three broods (max 8 d)	7 d		96 h
Average Temperature	25 ± 1 °C			
Temperature Deviation	Test temperature must not deviate (i.e., maximum minus minimum temperature) by more than 3° C during the test.			
Light Quality	Ambient lab illumination			"Cool white" fluorescent lighting
Light Intensity	10 - 20µE/m^2/s (50 – 100 ft-c)			86 ± 8.6µE/m^2/s (400 ± 40 ft-c)
Photoperiod	16 h light, 8 h darkness			Continuous illumination
Test Chamber Size	30 mL	500 mL	150 mL	125 – 250 mL
Test Solution Volume	15 mL	250 mL	70 mL	50 or 100 mL[1]
Renewal of Test Solution	Daily			None
Test Organism Age	< 24; all released in an 8 h period	< 24 h (Max 48 h if shipped)	< 36 h (Max 48 h if shipped)	4 – 7 days
# of Organism per Chamber	1 assigned by using blocking by known parentage	10	15 10	10,000 cells/mL initial cell density
# Replicates per Test Concentration	10	4	4 3	4
# of Organisms per Test Concentration	10	40	60 30	NA
Shaking Rate	NA			100 rpm continuous, or twice daily by hand
Test Chamber Cleaning	Clean beakers or new cups daily	Siphon Daily	NA	NA
Source of Food	NA	< 24 h old *Artemia* nauplii	NA	NA
Feeding Regime	Feed 0.1 mL each of YCT and algal suspension per test chamber daily	Feed 0.1 g < 24 h old brine shrimp nauplii three times daily on days 0 – 6 (Minimum feed 0.15 g twice daily at 6 h intervals)	Not required.	NA
Test Chamber Aeration	None	None, unless DO below 4 mg/L (100 bubbles/ minute)		None
Dilution Water	Uncontaminated source of receiving or other natural water, synthetic water prepared using MILLIPORE MILLI-Q® or equivalent deionized water and reagent grade chemicals or dilute mineral water. Hardness ≥ 25 mg/L ($CaCO_3$) to ensure hatchability for Method 1001.			Algal stock culture medium[b]

Toxicity Test Condition	*C. dubia* Survival and Reproduction	*P. promelas* Larval Survival and Growth	*P. promelas* Embryo-Larval Survival and Teratogenicity	*S. capricornutum* Growth Toxicity
Test Concentrations – Effluent	5 test concentrations and a control			
Test Concentrations – Receiving Water	100% and a control			
Dilution Factor	Effluent: ≥0.5 Receiving water: none or ≥0.5			
Test Endpoint	Survival and Reproduction	Survival and growth (weight)	Combined mortality (dead and deformed organisms)	Growth (cell counts, chlorophyll fluorescence, absorbance, or biomass)
Sample Type	Composite			
Effluent Holding Requirements	Onsite 24 h Offsite 36 h			
# of Samples	Onsite – 7 Offsite – 3 (Days 1, 3, and 5)			1
Sample Volume Required	1 L/day	2.5 L/day	1.5 – 2.5 L/day	1 – 2 L
Test Acceptability Criteria	≥80% survival of all control organisms; average of 15 or more neonates per surviving control female; 60% of surviving control produce three broods	≥80% survival in controls; average dry weight per surviving organism in control ≥ 0.25 mg	≥ 80% survival in controls	Mean cell density of at least 1 x 10^6 cells/mL in the controls; and variability (CV%) among control replicates ≤ 20%

Available options
Recommended or recommended minimum
Required or required minimum

Notes:

a. For tests not continuously shaken, use 25 mL in 125 mL flasks and 50 mL in 250 mL flasks.

b. Enriched uncontaminated source of receiving or other natural water, synthetic water prepared using MILLIPORE MILLI-Q® or equivalent deionized water and reagent grade chemicals or dilute mineral water.

Table C-3. Summary of toxicity test conditions and test acceptability criteria for EPA East Coast marine chronic toxicity tests (USEPA 2002c).

Toxicity Test Condition	***C. variegatus* Larval Survival and Growth**	***M. beryllina* Larval Survival and Growth**	***M. bahia* Survival, Growth, and Fecundity**	***C. variegatus* Embryo-Larval Survival and Teratogenicity**	***A. punctulata* Fertilization**	***C. parvula* sexual reproduction[a]**
Toxicity Test Method #	1004.0	1006.0	1007.0	1005.0	1008.0	NA
Test Type	Static-renewal				Static	Static non-renewal
Salinity	20–30 ppt (±2 ppt of selected test salinity)	5–32 ppt (±2 ppt of selected test salinity)	20–30 ppt (±2 ppt of selected test salinity)	5–32 ppt (±2 ppt of selected test salinity)	30 ppt (±2 ppt of selected test salinity)	30 ppt (±2 ppt of selected test salinity)
Test Duration	7 d			9 d	1 h and 20 m	2 days in effluent; 5 – 7 days in control
Average Temperature	25 ± 1 °C		26 ± 1 °C	25 ± 1 °C	20 ± 1 °C	23 ± 1 °C
Temperature Deviation	Test temperature must not deviate (i.e., maximum minus minimum temperature) by more than 3° C during the test.					NA
Light Quality	Ambient lab illumination					Cool-white fluorescent
Light Intensity	10 - 20µE/m^2/s (50 – 100 ft-c)					75 µE/m^2/s (500 ft-c)
Photoperiod	16 h light, 8 h darkness				NA	16 h light, 8 h darkness
Test Chamber Size	600 mL – 1 L		400 mL	400 – 500 mL	20 mL[b]	200 – 250 mL
Test Solution Volume	500 – 750 mL		150 mL	250 – 400 mL	5 mL	100 mL
Renewal of Test Solution	Daily	Daily	Daily	Daily	NA	
Test Organism Age	< 24 h (≤ 24 h range in age)	7 – 11 d post hatch (≤ 24 h range in age)	7 d	< 24 h	Pooled sperm from 4 males; pooled eggs from 4 females	NA
# of Organism per Chamber	10		5	15	~2000 eggs; ~5,000,000 sperm per vial	5 female branch tips and 1 male plant
				10		
# Replicates per **Test Concentration**	4		8	4	4	4
				3		
# of **O**rganisms per **Test Concentration**	40			60	~8000 eggs; ~20,000,000 sperm	24
				30		
Test **Chamber Cleaning**	Siphon Daily		Pipette daily	NA	NA	NA
Source of **F**ood	< 24 h old *Artemia* nauplii	< 24 h old *Artemia* nauplii	< 24 h old *Artemia* nauplii	NA	NA	
Feeding **R**egime	Feed 0.1 g wet weight < 24 h old *Artemia* nauplii on days 0 -2; feed 0.15 g wet weight *Artemia* nauplii per replicate on days 3 - 6		Feed 150 24 h old *Artemia* nauplii per mysid daily, half after test solution renewal and half after 8 – 12 h	Not Required.	NA	NA

Toxicity Test Condition	***C. variegatus* Larval Survival and Growth**	***M. beryllina* Larval Survival and Growth**	***M. bahia* Survival, Growth, and Fecundity**	***C. variegatus* Embryo-Larval Survival and Teratogenicity**	***A. punctulata* Fertilization**	***C. parvula* sexual reproduction[a]**
Test Chamber Aeration	None, unless DO below 4 mg/L (100 bubbles/ minute)				NA	NA
Dilution Water	Uncontaminated source of natural seawater; deionized water mixed with HSB or artificial sea salts (HW MARINEMIX®, FORTY FATHOMS®, GP2 or equivalent).					
Test Concentrations	Effluent - 5 and a control					5 and a control
	Receiving Water - 100% and a control					100% and a control
Dilution Factor	Effluent: ≥0.5 Receiving water: none or ≥0.5	Effluent: ≥0.5 Receiving water: none or ≥0.5	Effluent: ≥0.5 Receiving water: none or ≥0.5	Effluent: ≥0.5 Receiving water: none or ≥0.5	Effluent: ≥0.5 Receiving water: none or ≥0.5	Effluent: ≥0.5 Receiving water: none or ≥0.5
Test Endpoint	Survival and Growth (weight)		Survival and Growth Egg Development	Percent hatch; percent larvae dead or with debilitating morphological and/or behavior abnormalities	Fertilization of sea urchin eggs	Reduction in cystocarp production compared to controls
Sample Type	Composite					Composite
Effluent Holding Requirements	Onsite 24 h					Onsite 24 h
	Offsite 36 h					Offsite 36 h
# of Samples	Onsite – 7 and Offsite – 3 (Days 1, 3, and 5)				1	1
Sample Volume Required	6 L/day		3 L/day	5 L/day	1 L	2 L
Test Acceptability Criteria	≥80% survival in controls; average dry weight per surviving organism in control ≥ 0.6 mg if unpreserved, or ≥ 0.5 mg after no more than 7 days in 4% formalin or 70% ethanol	≥80% survival in controls; average dry weight per surviving organism in control ≥ 0.5 mg when test starts with 7 d old larvae and unpreserved, or ≥ 0.43 mg after no more than 7 days in 4% formalin or 70% ethanol	≥80% survival in controls; average dry weight per surviving organism in control ≥ 0.2 mg; Fecundity may be used if 50% or more of females in control produce eggs	≥80% survival in controls	70 – 90% egg fertilization in controls	≥ 80% survival and an average of 10 cystocarps per plant in controls

Available options
Recommended or recommended minimum
Required or required minimum

Notes:

a. This method is not listed in 40 CFR Part 136 for nationwide use.
b. Liquid scintillation vials presoaked in control water.

Table C-4. Summary of toxicity test conditions and test acceptability criteria for EPA West Coast marine chronic toxicity tests (USEPA 1995b).

Toxicity Test Condition	Topsmelt, *A. affinis* Larval Growth and Survival	Mysid, *H. costata*, Survival and Growth	Pacific Oyster, *C. gigas* and Mussel, *Mytilus* sp. Larval Development	Red Abalone, *H. rufescens* Larval Development	Purple Urchin, *S. purpuratus* and Sand Dollar, *D. excentricus* Larval Development	Purple Urchin, *S. purpuratus* and Sand Dollar, *D. excentricus* Fertilization	Giant Kelp, *M. pyrifera* Germination and Germ-Tube Growth
Toxicity Test Type	Static-renewal		Static non-renewal				
Salinity	5 – 34‰ (± 2‰ of the selected test salinity)	34 ± 2‰	30 ± 2‰	34 ± 2‰	34 ± 2‰	34 ± 2‰	34 ± 2‰
Test Duration	7 d		48 h (or until complete development up to 54 h)	48 h	72 ± 2h	40 min (20 min plus 20 min)	48 h
Temperature	20 ± 1 °C	13 ± 1° C (mysids collected north of Pt. Conception) 15 ± 1 °C (mysid collected south of Pt. Conception)	20 ± 1 °C (oysters) 15 or 18± 1 °C (mussels)	15 ± 1 °C	15 ± 1 °C	12 ± 1 °C	15 ± 1 °C
Light Quality	Ambient lab illumination						
Light Intensity	10 - 20µE/m^2/s						50 ± 10µE/m^2/s
Photoperiod	16 h light, 8 h darkness					NA	16 h light, 8 h darkness
Test Chamber Size	600 mL	1000 mL	30 mL	600 mL	30 mL	16 x 100 or 16 x 125 mm	600 mL
Test Solution Volume	200 mL		10 mL	200 mL	10 mL	5 mL	200 mL
Renewal of Test Solution	Daily	75% renewal at 48 and 96 hours	NA				
Test Organism Age	9 – 15 days post-hatch	3 – 4 d post hatch juveniles	NA	NA	NA	NA	NA
# of Organism per Chamber	5		150 - 300	5 – 10 per mL	NA	About 1,120 eggs and not more than 3,360,000 sperm per test tube	7500 spores/mL of test solution
# Replicates per Test Concentration	5		4	5	4	4	5
# of Organisms per Test Concentration	25		600 - 1200	1000 - 2000	NA	About 4,480 eggs and not more than 13,440,000 sperm	7,500,00 spores
Test Chamber Cleaning	Siphon Daily	Siphon at renewal	NA				
Source of Food	< 24 h old *Artemia* nauplii	< 24 h old *Artemia* nauplii	NA				

Condition	Topsmelt, *A. affinis* Larval Growth and Survival	Mysid, *H. costata*, Survival and Growth	Pacific Oyster, *C. gigas* and Mussel, *Mytilus* sp. Larval Development	Red Abalone, *H. rufescens* Larval Development	Purple Urchin, *S. purpuratus* and Sand Dollar, *D. excentricus* Larval Development	Purple Urchin, *S. purpuratus* and Sand Dollar, *D. excentricus* Fertilization	Giant Kelp, *M. pyrifera* Germination and Germ-Tube Growth
Feeding Regime	Feed 40 nauplii per larvae twice daily (morning and night)		NA				
Test Chamber Aeration	None, unless DO below 4 mg/L (100 bubbles/ minute)		NA				
Dilution Water	Uncontaminated 1-µm-filtered natural seawater or HSB prepared from natural seawater.						
Test Concentrations	Effluent - 5 and a control						
	Receiving Water - 100% and a control						
Dilution Factor	Effluent: ≥0.5						
	Receiving water: none or ≥0.5						
Endpoint	Survival and Growth (weight)		Survival and Normal Larval Development	Normal Larval Development	Normal Larval Development; mortality can be included	Fertilization of eggs	Germination and germ-tube length
Sample Type	Composite						
Effluent Holding Requirements	Onsite 24 h						
	Offsite 36 h						
# of Samples	Onsite – 7 and Offsite – 3 (Days 1, 3, and 5)	1					
Sample Volume Required	2 L/day	2 L/renewal	1 L	2 L	1 L	1 L	2 L
Test Acceptability Criteria	≥80% survival in controls, 0.85 mg average weight of control larvae (9 day old), LC50 with copper must be ≤205 µg/L, <25% MSD for survival and <50% MSD for growth	≥75% survival in controls; average dry weight per surviving organism in control ≥ 0.4 µg; survival MSD <40%, growth MSD <50 µg, and both survival and growth NOECs must be less than 100 µg/L with zinc	Control survival must be ≥70% for oyster embryos and ≥50% for mussel embryos in control vials; ≥90% normal shell development in surviving controls; and must achieve a %MSD of <25%	≥80% normal shell development in the controls; must have statistically significant effect at 56 µg/L zinc; must achieve a %MSD of <20%	≥80% normal shell development in the controls; must achieve a %MSD of <25%	≥ 70% egg fertilization in controls; %MSD of <25%; and appropriate sperm counts	≥ 70% germination in the controls; ≥ 10 µm germ-tube length in the controls and the NOEC must be below 35 µg/L in the reference toxicant test; must achieve a %MSD of <20% for both germination and germ-tube length in the reference toxicant

APPENDIX D

Percentiles for Mean, Standard Deviation, and Coefficient of Variation for Toxicity Test Methods

APPENDIX D – Percentiles For Mean, Standard Deviation, and Coefficient of Variation for Toxicity Test Methods

This appendix provides information on the national percentiles for mean control reproduction, control coefficient of variation (CV) and standard deviation (SD), which can be used for training and evaluating laboratory staff to ensure they can consistently achieve adequate within-test precision.

EPA has quantified the within-test variability (expressed as both SD and CV) and mean control responses for many EPA toxicity test methods on a national basis (USEPA 2010c). For each of the nine EPA toxicity test methods examined, the control CV was calculated on the basis of WET test data compiled as described in Section 2.2 of USEPA 2010c. Cumulative frequency plots were used to identify various percentiles of observed toxicity test method-specific CVs (e.g., 25th, 50th, 75th percentiles). These measures were calculated to characterize typical achievable toxicity test performance in terms of control variability. A similar analysis was performed for the control endpoint responses for each of the nine toxicity test methods (e.g., mean offspring per female in the chronic *Ceriodaphnia dubia* test method) to characterize typical achievable toxicity test performance in terms of control response.

Freshwater Chronic Endpoints

Table D-1. Summary of mean control reproduction and control coefficient (CV) and standard deviation (SD) derived from analyses of 792 chronic *Ceriodaphnia dubia* (invertebrate, freshwater water flea) toxicity tests (USEPA 2010c).

Percentile	Mean Control Reproduction	Control CV	Control SD
10th	17.7	0.08	2.07
25th	21.2	0.10	2.64
50th	25.5	0.15	3.79
70th	28.4	0.22	5.27
75th	29.4	0.24	5.82
85th	31.6	0.31	7.24
90th	33.3	0.35	8.41
95th	35.6	0.40	10.25

Table D-2. Summary of mean control growth and control CV and SD derived from analyses of 472 chronic *Pimephales promelas* (vertebrate, freshwater fathead minnow) toxicity tests (USEPA 2010c).

Percentile	Mean Control Growth	Control CV	Control SD
10th	0.34	0.04	0.02
25th	0.43	0.06	0.03
50th	0.62	0.09	0.05
70th	0.76	0.12	0.07
75th	0.79	0.13	0.08
85th	0.86	0.16	0.10
90th	0.89	0.17	0.11
95th	0.94	0.21	0.13

Table D-3. Summary of mean control growth, CV, and SD derived from the analyses of all chronic *Raphidocelis subcapitata* (freshwater green alga) (formerly *Selenastrum capricornutum*) toxicity test data and compared with the analysis of only the chronic *Raphidocelis subcapitata* (formerly *Selenastrum capricornutum*) toxicity test in which it was assumed that the chelating agent ethylenediaminetetraacetic acid (EDTA) was added to the control (USEPA 2010c).

All Tests (*n* = 223)				Only Tests with EDTA Addition (*n* = 173)			
Percentile	Mean Cell Density	Control CV	Control SD	Percentile	Mean Cell Density	Control CV	Control SD
10th	1233050.0	0.02	44928.62	10th	1554500.0	0.02	43664.06
25th	2245833.5	0.04	108449.85	25th	2502500.0	0.03	135154.20
50th	3331250.0	0.06	277653.90	50th	3430000.0	0.06	309232.90
70th	4869000.0	0.10	407505.12	70th	5581650.0	0.10	417361.66
75th	6179667.0	0.11	444887.25	75th	8220000.0	0.11	447446.50
85th	9265500.0	0.13	545764.05	85th	9785000.0	0.14	543717.8
90th	9888000.0	0.16	599644.32	90th	10048000.0	0.16	583299.40
95th	10149500.0	0.18	751884.62	95th	10279000.0	0.18	669780.04

Marine Chronic Endpoints

Table D-4. Summary of mean control growth and control CV and SD derived from analyses of 210 chronic *Americamysis bahia* (invertebrate, marine/estuarine shrimp) (formerly *Mysidopsis bahia*) toxicity tests (USEPA 2010c).

Percentile	Mean Control Growth	Control CV	Control SD
10th	0.22	0.08	0.02
25th	0.25	0.10	0.03
50th	0.30	0.14	0.04
70th	0.36	0.17	0.06
75th	0.38	0.18	0.06
85th	0.41	0.22	0.07
90th	0.43	0.27	0.08
95th	0.47	0.35	0.11

Table D-5. Summary of mean control larval development and control CV and SD derived from analyses of 136 chronic *Halio is rufescens* (invertebrate, marine/estuarine, red abalone) toxicity tests (USEPA 2010c).

Percentile	Mean Control Larval Development	Control CV	Control SD
10th	0.839	0.02	0.01
25th	0.900	0.02	0.02
50th	0.938	0.03	0.03
70th	0.961	0.04	0.04
75th	0.968	0.05	0.04
85th	0.977	0.06	0.05
90th	0.982	0.06	0.06
95th	0.988	0.07	0.07

Table D-6. Summary of mean control germination and control CV and SD derived from analyses of 135 chronic *Macrocystis pyrifera* (plant, marine/estuarine, giant kelp) toxicity tests (USEPA 2010c).

Percentile	Mean Control Germination	Control CV	Control SD
10th	0.783	0.02	0.02
25th	0.859	0.03	0.02
50th	0.908	0.04	0.03
70th	0.936	0.05	0.04
75th	0.940	0.05	0.05
85th	0.958	0.07	0.06
90th	0.965	0.07	0.06
95th	0.973	0.10	0.09

Table D-7. Summary of mean control germ-tube length and control CV and SD derived from analyses of 135 chronic *Macrocystis pyrifera* toxicity tests (USEPA 2010c).

Percentile	Mean Control Germ-Tube Length	Control CV	Control SD
10th	11.965	0.03	0.46
25th	12.704	0.05	0.71
50th	14.014	0.07	1.04
70th	15.210	0.09	1.22
75th	15.554	0.09	1.29
85th	16.848	0.11	1.54
90th	17.568	0.12	1.74
95th	18.694	0.14	1.89

Table D-8. Summary of mean control fertilization and control CV and SD derived from analyses of 177 chronic *Dendraster excentricus* (invertebrate, marine/estuarine, sand dollar) and *Strongylocentrotus purpuratus* (invertebrate, marine/estuarine, purple sea urchin) toxicity tests (USEPA 2010c).

Percentile	Mean Control Fertilization	Control CV	Control SD
10th	0.826	0.01	0.00
25th	0.875	0.01	0.01
50th	0.953	0.03	0.02
70th	0.975	0.05	0.04
75th	0.978	0.07	0.06
85th	0.990	0.09	0.07
90th	0.993	0.11	0.09
95th	0.996	0.14	0.11

Table D-9. Summary of mean control growth, CV, and standard deviation derived from analyses of 83 chronic *Atherinops affinis* (vertebrate, marine/estuarine, topsmelt) toxicity tests (USEPA 2010c).

Percentile	Mean Growth (mg)	Control CV	Control SD
10th	1.122	0.05	0.07
25th	1.259	0.08	0.10
50th	1.455	0.10	0.15
70th	1.651	0.12	0.20
75th	1.725	0.12	0.22
85th	2.026	0.15	0.26
90th	2.187	0.17	0.27
95th	2.357	0.18	0.29

Table D-10. Summary of mean control growth and control CV and SD for saltwater fish chronic toxicity tests (e.g., *Menidia beryllina* [vertebrate, marine/estuarine, inland silverside]*, Cyprinodon variegatus* [vertebrate, marine/estuarine, sheepshead minnow) that use the same test design as the fathead minnow chronic test (USEPA 2010c).

Percentile	Mean Control Growth	Control CV	Control SD
10th	0.34	0.04	0.02
25th	0.43	0.06	0.03
50th	0.62	0.09	0.05
70th	0.76	0.12	0.07
75th	0.79	0.13	0.08
85th	0.86	0.16	0.10
90th	0.89	0.17	0.11
95th	0.94	0.21	0.13

Freshwater Acute Endpoints

Table D-11. Summary of mean control survival and control CV and SD derived from analyses of 347 acute *Pimephales promelas* toxicity tests (USEPA 2010c).

Percentile	Mean Control Survival	Control CV	Control SD
10th	0.95	0.00	0.00
25th	1.00	0.00	0.00
50th	1.00	0.00	0.00
70th	1.00	0.00	0.00
75th	1.00	0.00	0.00
85th	1.00	0.09	0.15
90th	1.00	0.12	0.18
95th	1.00	0.19	0.23

Table D-12. Summary of mean control growth, CV and SD derived from analyses of 239 acute *Ceriodaphnia dubia* toxicity tests (USEPA 2010c).

Percentile	Mean Survival (%)	Control CV	Control SD
10th	0.95	0.00	0.00
25th	1.00	0.00	0.00
50th	1.00	0.00	0.00
70th	1.00	0.00	0.00
75th	1.00	0.00	0.00
85th	1.00	0.00	0.00
90th	1.00	0.11	0.10
95th	1.00	0.11	0.10

APPENDIX E

EXAMPLE: NPDES WET Permit Limit Derivation Procedures

APPENDIX E – NPDES WET Permit Limit Derivation Procedures

This appendix presents an example of how to derive a permit limit for WET.

To calculate WET permit limits, all WET data collected under the previous permit cycle (up to five years) should be obtained and organized by test species (e.g., *Ceriodaphnia dubia -water flea, Pimephales promelas* - fathead minnow, etc.) and by toxicity test type (e.g., acute or chronic). The point estimates or hypothesis testing statistical endpoints should be converted to TUs by calculating the reciprocal (e.g., $100/IC_{25}$ or 100/NOEC). The mean and standard deviation of TUs are calculated for each pollutant using historical effluent data. Where historical data regarding effluent variability are insufficient (e.g., the number of samples (abbreviated as "n") < 10), the default CV should be 0.6 (see the TSD, Appendix E, p. E-3). In that case only, the variance of TU is calculated from the CV using formulas in Box 5-2 of the TSD (page 100). Statistical derivation procedures for the AML for WET should assume that at least four samples (*n*) will be taken per month.

The WLA required to protect against both acute and chronic effects under critical conditions may be calculated using either steady-state or dynamic models. However, for derivation of the WLA, the equation is rearranged to solve for the effluent concentration (C_d), or WLA, necessary to achieve the appropriate applicable criterion. For compliance purposes, the water quality criterion for aquatic life (toxicity criterion) is set equal to C_r, where C_r is the applicable criterion:

$$WLA = C_d = [C_r(Q_d + Q_s)] - [(C_s)(Q_s)]/Q_d$$

where:

Q_d = waste discharge flow in cubic feet per second (cfs) or mgd

C_d = waste discharge pollutant concentration in TUs for WET (TU_a or TU_c)

Q_s = stream flow in cfs or mgd above point of discharge

C_s = background in-stream pollutant concentration in TUs for WET (TU_a or TU_c)

Q_r = resultant in-stream flow after discharge in cfs or mgd: $Q_s + Q_d$

C_r = applicable toxicity criterion = resultant in-stream pollutant concentration in TUs for WET (TU_a or TU_c), in the stream (after complete mixing)

In most cases, a steady-state model can be used to calculate the WLA (i.e., allowable effluent concentration) that will meet acute and chronic WQC for the protection of aquatic life at the critical stream flow conditions, for example, lowest one-day stream flow during any 10-year period and 7Q10, respectively (see TSD, p. 68). Ambient flow data from the U.S. Geological Survey are available on the Water Quality Portal (WQP) (https://www.waterqualitydata.us/).

When calculating the WLA, it should be noted that, if the applicable state's, territory's, or authorized Tribe's WQS and plans do not explicitly allow the application of mixing zones, the appropriate applicable criterion must be met at the end-of-pipe (i.e., applicable criterion = $C_r = C_d$ = WLA). Where mixing zones are allowed, the appropriate state's, territory's, or authorized Tribe's procedures should be applied.

If adequate receiving water flow and effluent concentration data are available to estimate frequency distributions, dynamic modeling techniques can be used to calculate allowable effluent loadings that will more precisely maintain the state's, territory's, or authorized Tribe's WQS (see TSD, p. 97). The steady-

state mass balance equation, however, when coupled with the recommended conservative assumptions, is often adequate for protection of WQS.

WLAs determined using the state's, territory's, or authorized Tribe's WQC for WET may be converted to MDLs and AMLs. The following recommended methodology is designed to derive permit limits for specific pollutants and WET to achieve calculated WLAs at the 99% confidence level for MDLs and the 95% confidence level for AMLs (see TSD, Box 5-2, p. 100; Figure 5-4, p. 101; and Tables 5-1, 5-2, and 53, pp. 102–103, 106).

1. Using the mass-balanced equation to solve for the allowable effluent concentration (C_d), or WLA, for WET:

   a. Set C_r equal to acute, chronic criteria.

   b. Background receiving water (Q_s), discharge (Q_d) flows, and background pollutant concentration (C_s) should represent critical conditions.

   c. Solve for acute (WLA_a) and chronic (WLA_c) wasteload allocations.

2. Convert the acute WLA to chronic toxic units ($WLA_{a,c}$), using the acute-to-chronic ratio (ACR). (See the TSD, Section 1.3.4, p.17.)

$$WLA_{a,c}\,(\text{in } TU_c) = WLA_a\,(\text{in } TU_a) \times ACR$$

3. To calculate the standard deviation or CV:

   a. Use the effluent data set of "n" observations (n is > 10) to calculate the mean (μ) and standard deviation (σ) of log(TU) or log(chemical concentration) (see TSD, Appendix E).

   b. Where the effluent data set is small (n < 10), the conservative value of 0.6 is recommended (see TSD, Appendix E, page E-3) to estimate the CV, from which the variance is then calculated using formulas in Box 5-2 of the TSD (page 100). Numerical values for the case when CV = 0.6 are provided in the TSD (Tables 5-1 and 5-2, pp. 102 – 103).

4. To determine LTA discharge conditions:

   Use the following equations to calculate acute and chronic LTA discharge conditions and long-term average chronic ($LTA_{a,c}$ and LTA_c) that will satisfy the acute and chronic wasteload allocation ($WLA_{a,c}$ and WLA_c). The CV calculated above is used to estimate both acute and chronic WLA multipliers (see TSD, Table 5-1, p. 102).

$$LTA_{a,c} = WLA_{a,c} \times e^{[0.5\sigma^2 - \sigma]}$$

$$LTA_c = WLA_c \times e^{[0.5\sigma_4^{\,2} - \sigma_4]}$$

where:

$e^{[0.5\sigma^2 - \sigma]}$ = acute WLA multiplier

$e^{[0.5\sigma_4^2 - \sigma_4]}$ = chronic WLA multiplier

z = 2.326 for the 99th percentile occurrence probability for the LTA is recommended

5. Determine the lower (more limiting) LTA discharge condition.

$$\text{LTA} = \text{minimum } (\text{LTA}_{a,c} \text{ or } \text{LTA}_c)$$

6. Calculate the MDL and AML using the lower (more limiting LTA) discharge condition.

   Use the following equations to calculate the MDL and AML. The CV calculated above is used to estimate both acute and chronic LTA multipliers (see TSD, Table 5-2, p. 103).]

$$\text{MDL} = \text{LTA} \times e^{[z\sigma - 0.5\sigma^2]}$$

$e^{[z\sigma - 0.5\sigma^2]}$ = MDL LTA multiplier

z = 2.326 for the 99th percentile occurrence probability for the MDL is recommended

$$\text{AML} = \text{LTA} \times e^{[z\sigma_n - 0.5\sigma_n^2]}$$

where:

$e^{[z\sigma_n - 0.5\sigma_n^2]}$ = AML LTA multiplier

z = 1.645 for the 95th percentile occurrence probability for the AML is recommended

n = number of samples/month

Following these procedures, the MDL and AML may then be incorporated into the permit as justifiable WQBELs.

APPENDIX F

EXAMPLE: Deriving NPDES WET Permit Limits for Low-flow Dilution Situations

APPENDIX F – Deriving NPDES WET Permit Limits for Low-flow Dilution Situations

Many facilities across the country discharge to streams where the facility flow comprises a high percentage of the available stream flow during critical conditions or for large parts of the year so an alternative approach for deriving permit limits is needed. Receiving waters where little dilution is available still often have a full aquatic life use designation and are afforded all protections based on a state's, territory's, or authorized Tribe's narrative and/or numeric WET WQS. Due to the low margin of safety in such waters, effluent toxicity may cause ambient impacts and lead to exceedance of numeric or narrative a state's, territory's, or authorized Tribe's narrative and numeric WET WQS. Where such discharges cause or have the reasonable potential to cause or contribute to excursions of such criteria, WET limits are required by 40 CFR § 122.44(d)(1)(iv) and (v).

Due to the inherent small margin of safety, only limited regulatory flexibility from chronic WET limit requirements is available for discharges to waters where limited or no dilution is available.

Following is an example of a POTW discharging to a receiving water for which no assimilative capacity is available (i.e., no dilution). The example shows the steps that a NPDES permit authority should take to establish a WQBEL for WET.

General site description and information. This facility discharges up to 5.8 mgd. Based on the available information, the ACR is 10:1. The CV, based on available data, is 0.6; the water quality criterion for chronic toxicity is 1.0 TU_c and the acute criterion for acute toxicity is 0.3 TU_a. The state's, territory's, or authorized Tribe's WQS allow an assumption of complete mixing.

Determine waste load allocation (WLA). The WLA is used to determine the level of effluent concentration that will comply with the state's, territory's, or authorized Tribe's WQS in receiving waters. Using the information available for dilution, WLAs were calculated for WET using the complete mix equation:

$$WLA\ (C_d) = ([C_r(Q_d + Q_s)] - [(C_s)(Q_s)])/Q_d$$

Because this is an effluent-dominated situation, and background concentration C_s is set to zero, the equation simplifies to:

$$WLA = C_r[(Q_d + Q_s)/Q_d]$$

$$WLA_a = 0.3 \times 1 = 0.3\ TU_a$$

$$WLA_{a,c} - WLA_a \times ACR = 0.3 \times 10 = 3.0\ TU_{a,c}$$

Calculate LTAs. The process for calculating LTAs for toxicity is the same as that for chemical-specific pollutants, except for the additional step of needing to express the WLA for acute toxicity in equivalent chronic TUs.

$$\mathrm{LTA_{a,c}} = \mathrm{WLA_{a,c}}\ \mathrm{x}\ \mathrm{e}^{[0.05\sigma^2 - \mathrm{z}\sigma]}$$

$$LTA_{a,c} = 3 \times 0.321$$

where:

0.321 is the acute WLA multiplier for CV = 0.6 at the 99th percentile (from Table 5-1, p. 102 of the TSD)

$LTA_{a,c} = 0.963\ TU_c$

$$\mathrm{LTA_c} = \mathrm{WLA_c}\ \mathrm{x}\ \mathrm{e}^{[0.5\sigma_4^2 - \mathrm{z}\sigma_4]}$$

$LTA_c = 1 \times 0.527$

where:

0.527 is the chronic WLA multiplier at the 99th percentile (from Table 5-1, p. 102 of TSD)

$LTA_c = 0.527\ TU_c$.

Select the minimum LTA. The LTA based on the chronic WLA is more limiting and will be used to develop permit limits.

Calculate the maximum daily limit (MDL). The MDL is calculated using the following formula:

$$\mathrm{MDL} = \mathrm{LTA}\ \mathrm{x}\ e^{[z\sigma - 0.5\sigma^2]}$$

where:

$e^{[z\sigma - 0.5\sigma^2]}$ = MDL LTA multiplier

z = 2.326 for the 99th percentile occurrence probability for the MDL is recommended

MDL = 0.527 × 3.11 (from the LTA multiplier in Table 5-2, on p. 102 of the TSD)

$MDL = 1.6\ TU_c$.

Calculate the AML. Using the 95th percentile and monthly sampling, the AML is calculated using the following formula:

$$\mathrm{AML} = \mathrm{LTA}\ \mathrm{x}\ e^{[z\sigma_n - 0.5\sigma_n^2]}$$

where:

$e^{[z\sigma_n - 0.5\sigma_n^2]}$ = AML LTA multiplier

z = 1.645 for the 95th percentile occurrence probability for the AML is recommended

n = number of samples/month (the TSD recommends that a minimum n of 4 be used, even if monitoring is less frequent)

AML = 0.527 × 1.55

where:

1.55 is the LTA multiplier from Table 5-2 on p. 103 of the TSD.

$AML = 0.82\ TU_c$.