



EPA Regions 8, 9 and 10 Toxicity Training Tool



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EXECUTIVE SUMMARY

The U.S. Environmental Protection Agency's Pacific Southwest Region and Pacific Northwest Region (EPA Regions 9 and 10) have developed a Whole Effluent Toxicity (WET) Technical Training Tool for implementing WET in National Pollutant Discharge Elimination System (NPDES) permitting programs and Clean Water Act surface water quality monitoring programs. This training tool is designed for use with the EPA regional guidance document, *Regions 9 and 10 Guidance for Implementing Whole Effluent Toxicity* (Denton and Narvaez 1996), and EPA's national guidance document, *Technical Support Document for Water Quality-based Toxics Control* (USEPA 1991a). Since 1996, EPA has issued several important national guidance documents addressing WET implementation, and promulgated updated biological methods for acute and chronic toxicity at 40 CFR 136. These publications are described in the documentation for this training tool in order to provide a concise summary of current EPA program documents and regulations for WET. As such, this tool provides the basis for technical training on the topic of WET for EPA Regions 9 and 10. It is being made available for use by other EPA Regions and States (including Tribes and Territories) seeking basic training on the topic of WET for NPDES permitting and ambient water quality monitoring. This training tool is divided into seven topics with training slides and accompanying documentation (chapters and appendices). The training slides are provided in a Microsoft PowerPoint format. The topics covered are:

- Introduction to WET
- Developing WET Permit Conditions
- Chronic and Acute Toxicity Testing
- Test Review and Evaluation of Test Results
- Toxicity Reduction Evaluations
- Ambient Toxicity Testing and Watershed Assessment
- Enforcement Procedures for WET

This training tool is designed to assist EPA Regions and States implementing existing national policy on WET. It does not substitute for the Clean Water Act, or EPA or State regulations applicable to NPDES permits or WET testing; nor is this document a regulation, itself. This training tool does not impose legally binding requirements on EPA, States, or NPDES permittees, and may not apply in site-specific situations based upon the circumstances. EPA Regions 9 and 10 will change this training tool in the future, as appropriate. Those seeking more information on WET are referred to the documents referenced in this training tool, EPA's webpage at <http://www.epa.gov/> (Search: whole effluent toxicity, NPDES, etc.), and EPA's national and regional WET Program staffs.

References

Denton DL, Narvaez M. 1996. EPA Regions 9 and 10 guidance for implementing whole effluent toxicity testing programs. USEPA Regions 9 and 10.

USEPA. 1991a. Technical support document for water quality-based toxics control. Office of Water. Washington, DC. EPA/505/2-90-001.

LIST OF ACRONYMS AND ABBREVIATIONS

| | |
|----------|---|
| ‰ | parts per thousand |
| α | alpha error |
| β | beta error |
| AA | atomic absorption |
| ACR | acute-to-chronic ratio |
| AML | average monthly limit |
| ANOVA | analysis of variance |
| APC | areas of probable concern |
| APO | administrative penalty order |
| AO | administrative order |
| ASTM | American Society for Testing and Materials |
| AVS | acid volatile sulfide |
| AWL | average weekly limit |
| BMP | best management practices |
| BSAB | Biomonitoring Science Advisory Board |
| CABW | California aquatic bioassessment workshop |
| CAMLnet | California Aquatic Macroinvertebrate Laboratory Network |
| CCC | criteria continuous concentration |
| CDFG | California Department of Fish and Game |
| CFR | Code of Federal Regulations |
| CFS | cubic feet per second |
| CMC | criteria maximum concentration |
| CSBP | California stream bioassessment protocol |
| CV | coefficient of variation |
| CWA | Clean Water Act |
| DMR | discharge monitoring report |
| DO | dissolved oxygen |
| DOC | dissolved organic carbon |
| DQO | data quality objective |

| | |
|----------------|---|
| EC | effect concentration, e.g., EC ₂₅ , EC ₅₀ |
| EDTA | ethylenediamine tetraacetic acid |
| EMS | Enforcement Management System |
| EPA | U.S. Environmental Protection Agency (also, the Agency) |
| FAQ | frequently asked questions |
| FIFRA | Federal Insecticide, Fungicide, Rodenticide Act |
| FR | Federal Register |
| GC | gas chromatography |
| H ₀ | null hypothesis |
| H _a | alternative hypothesis |
| HPLC | high performance liquid chromatography |
| IC | inhibition concentration, e.g., IC ₂₅ , IC ₅₀ |
| ICP | ion-coupled plasma |
| IWC | instream waste concentration (sometimes referred to as receiving water concentration) |
| IWS | industrial waste surveys |
| LC | lethal concentration |
| LOEC | lowest observed effect concentration |
| LSTE | list of standard taxonomic effort |
| LOV | letter of violation |
| LTA | long-term average (LTAA = acute LTA; LTAc = chronic LTA; LTAA,c = acute-to-chronic LTA) |
| MGD | million gallons per day |
| MDL | maximum daily limit |
| MML | median monthly limit |
| MQO | measurement quality objective |
| MS | mass spectrometry |
| MSD | minimum significant difference |
| MSE | mean square error |
| NOAEC | no observed adverse effect concentration |
| NOEC | no observed effect concentration |

| | |
|-------|---|
| NOV | notice of violation |
| NPDES | National Pollutant Discharge Elimination System |
| OWM | Office of Wastewater Management |
| OST | Office of Science and Technology |
| PBO | piperonyl butoxide |
| PMSD | percent minimum significant difference |
| POTW | Publicly owned treatment works |
| QA | quality assurance |
| QAPP | quality assurance project plan |
| QC | quality control |
| RBP | rapid bioassessment protocol |
| RP | reasonable potential |
| RWC | receiving water concentration (sometimes referred to as instream waste concentration) |
| RWQCB | Regional Water Quality Control Board |
| SEM | simultaneously extracted metals |
| SETAC | Society of Environmental Toxicology and Chemistry |
| SFEI | San Francisco Estuary Institute |
| SI | stressor identification |
| SMWP | State Mussel Watch Program |
| SOP | standard operating procedure |
| SWAMP | Surface Water Ambient Monitoring Program |
| SWRCB | State Water Resources Control Board |
| TAC | test acceptability criteria |
| TIE | toxicity identification evaluation |
| TDS | total dissolved solids |
| TMDL | total maximum daily load |
| TOC | total organic carbon |
| TRE | toxicity reduction evaluation |
| TSD | EPA's Technical Support Document for Water Quality-based Toxics Control |

LIST OF ACRONYMS AND ABBREVIATIONS

| | |
|-------|---|
| TU | toxic unit (TU _a = acute toxicity; TU _c = chronic toxicity) |
| USEPA | United States Environmental Protection Agency |
| USGS | United States Geological Survey |
| WET | whole effluent toxicity |
| WLA | waste load allocation |
| WQBEL | water quality based effluent limit |
| WQC | water quality criteria |
| WQS | water quality standards |

Note: These acronyms and abbreviations may have other meanings in other EPA programs or documents.

DEFINITIONS

Acute-to-Chronic Ratio (ACR) is the ratio of the acute toxicity of an effluent or a toxic to its chronic toxicity. It is used as a factor for estimating chronic toxicity on the basis of acute toxicity data, or for estimating acute toxicity on the basis of chronic toxicity data.

Acute Toxicity Test is a test to determine the concentration of effluent or ambient waters that causes an 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 procedures (e.g., point estimate techniques or a hypothesis test).

Ambient Toxicity is measured by a toxicity test performed using solely receiving water.

Average Monthly Limit (AML) is the highest allowable average of “daily discharges” over a calendar month, calculated as the sum of all “daily discharges” measured during a calendar month divided by the number of “daily discharges” measured during that month.

Chronic Toxicity Test is a short-term test, usually 96 hours or longer in duration, in which sublethal effects (e.g., significantly reduced growth, reproduction) are usually measured in addition to lethality. Chronic toxicity is defined as $TUc = 100/NOEC$ or $TUc = 100/ECp$ or ICp .

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. It is also called the relative standard deviation (RSD). The CV can be used as a measure of precision within (within-laboratory) and between (between-laboratory) laboratories, or among replicates for each treatment concentration.

Confidence Interval is the numerical interval constructed around a point estimate of a population parameter.

Criterion Continuous Concentration (CCC) is the highest in-stream concentration of a toxic or an effluent to which organisms can be exposed indefinitely without causing unacceptable effects such as the exceedance of a chronic water quality criterion.

Criterion Maximum Concentration (CMC) is the highest in-stream concentration of a toxic or an effluent to which organisms can be exposed for a brief period of time without causing an acute effect such as the exceedance of an acute water quality criterion.

Daily Discharge is the discharge of a pollutant measured during a calendar day or any 24-hour period that reasonably represents the calendar day for purposes of sampling.

Discharge Monitoring Report (DMR) is EPA’s standardized reporting form for the reporting of self-monitoring results by permittees. DMRs must be used by “NPDES-approved States,” as well as by EPA. States with NPDES programs may modify the EPA standardized forms to substitute the State agency’s name, address, logo, and other similar information, as appropriate, in place of EPA’s.

Effect Concentration (EC) is a point estimate of the toxicant concentration that would cause an observable adverse effect (e.g., death, immobilization, or serious incapacitation) in a given percent of the test organisms, calculated from a continuous model (e.g., Probit Model). EC_{25} is a point estimate of the toxicant concentration that would cause an observable adverse effect in 25 percent of the test organisms.

Effluent Flow (Q_e) is the flow (in cubic feet per second or million gallons per day) of a wastewater discharge from a facility expressed in standard NPDES formulas used by permit writers as “ Q_e ” to calculate water quality based effluent limits.

Endpoint is a biological measurement used to quantify the results obtained from analytical methods such as whole effluent toxicity testing [e.g., lethal concentration (LC_{50}); inhibition concentration (IC_{25}); and no observed effect concentration (NOEC)]. Such endpoints are quantitative measurements of the responses of test organisms (e.g., survival, growth, mobility, reproduction, and weight gain or loss) in response to exposure to a serial dilution of effluent.

Hypothesis Testing is a statistical technique (e.g., Dunnett’s test) for determining whether a tested concentration results in a statistically different response from that observed in the control. For the multi-concentration tests, the reported values determined by hypothesis testing are the “no observed effect concentration (NOEC)” and “lowest observed effect concentration (LOEC).” The two hypotheses commonly tested in WET are:

Null hypothesis (H_0): The effluent is not toxic.

Alternative hypothesis (H_a): The effluent is toxic.

Inhibition Concentration (IC) is a point estimate of the toxicant concentration that would cause a given percent reduction in a non-lethal biological measurement (e.g., reproduction or growth), calculated from a continuous model (i.e., Interpolation Method). IC_{25} is a point estimate of the toxic concentration that would cause a 25-percent reduction in a non-lethal biological measurement.

Instream Waste Concentration (IWC) is the concentration of a toxicant in the receiving water after mixing. It is also referred to as the receiving water concentration (RWC).

Lethal Concentration, 50 Percent (LC_{50}) is the toxic or effluent concentration that would cause death in 50 percent of the test organisms over a specified period of time.

Long-term Average (LTA) of pollutant concentration or effluent toxicity is calculated from a wasteload allocation (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). The LTA represents expected long-term average performance from the permitted facility required to achieve the associated WLA.

Lowest Observed Effect Concentration (LOEC) is the lowest concentration of an effluent or test sample with an effect different from the control effect according to the statistical test used for analysis of toxicity that results in adverse effects on the test organisms (i.e., where the values for the observed endpoints statistically differ from the control).

Maximum Daily Limit (MDL) is the highest allowable discharge measured during a calendar day or 24-hour period representing a calendar day.

Median is the value of the middle score in the distribution.

Median Monthly Limit (MML) is the highest allowable median of “daily discharges” over a calendar month, calculated as the middle value of all “daily dischargers” measured during a calendar month.

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

Mixing Zone is an area where an effluent discharge undergoes initial dilution with water from upstream and is extended to cover the secondary mixing in the ambient waterbody; an allocated impact zone in which water quality criteria can be exceeded provided that acutely toxic conditions are prevented. States determine whether mixing zones are allowed.

National Pollutant Discharge Elimination System (NPDES) is the EPA program that regulates discharges to the nation’s waters. Discharge permits issued under the NPDES program are required by EPA regulation to contain, where necessary, effluent limits based on water quality criteria for the protection of aquatic life and human health.

No Observed Effect Concentration (NOEC) is the highest tested concentration of an effluent or toxicant that causes no observable adverse effect on the test organisms (i.e., the highest concentration of toxicant at which the values for the observed responses are not statistically different from the controls).

Percent Minimum Significant Difference (PMSD) is the minimum significant difference divided by the control mean, expressed as a percent (see minimum significant difference).

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.

Power is the probability of correctly detecting an actual toxic effect (i.e., declaring an effluent toxic when, in fact, it is toxic).

Precision is a measure of reproducibility within a data set. Precision can be measured both within a laboratory (within-laboratory) and between laboratories (between-laboratory) using the same test method and toxicant.

Publicly Owned Treatment Works (POTWs) are facilities, operated by local communities or States or their contractors, that treat domestic wastewater or wastewater from indirect dischargers (e.g., industrial facilities).

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, record keeping, data, and other aspects of the test and testing procedures.

Quality Control (QC) is the set of focused, routine, day-to-day activities carried out as part of an overall QA program.

Reasonable Potential (RP) is the likelihood that an effluent will cause or contribute to an excursion above a water quality standard based on a number of factors, including the use of data (e.g., whole effluent toxicity test data). In the context of this document, references to RP and WET limits include both lethal and sublethal effects.

Reasonable Potential Multiplier Factor (RPMF) is a numerical value that multiplies the maximum observed effluent value in an effluent data set.

Receiving Water Concentration (RWC) is the concentration of a toxic in the receiving water after mixing, sometimes referred to as the in-stream waste concentration (IWC).

Receiving Water Flow (Q_s) is the flow of the water receiving the discharge expressed in cubic feet per second or millions gallon per day.

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

Significant Difference is defined as a statistically significant difference (e.g., 95 percent confidence level) in the means of two distributions of sampling 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.

Test Acceptability Criteria (TAC) are specific criteria for determining whether toxicity test results are acceptable, pursuant to EPA's WET test methods in 40 CFR 136 (additional TAC may be established by a State Permitting Authority). The effluent and reference toxicant must meet specific criteria as defined in the test method (e.g., for the *Ceriodaphnia dubia* survival and reproduction test, the criteria are: 80% or greater survival of all control organisms and an

average of 15 or more young per surviving female in the control solution. Of the surviving control females, 60% must produce three broods.)

Total Maximum Daily Load (TMDL) is the allocation of the pollutant load to each source, which is calculated by estimating the maximum amount of a pollutant that a waterbody can receive and still meet water quality standards.

t-Test (formally Student's t-test) is a statistical analysis comparing two sets of replicate observations, in the case of WET, only two test concentrations (e.g., a control and 100% effluent). The purpose of this test is to determine if the means of the two sets of observations are different [e.g., if the 100% effluent concentration differs from the control (i.e., the test pass or fails)].

Toxicity Test is a procedure using living organisms to determine whether a chemical or an effluent is toxic. A toxicity test measures the degree of the effect of a specific chemical or effluent on exposed test organisms.

Toxic Unit (TU) is a measure of toxicity in an effluent as determined by the acute toxicity units (TU_a) or chronic toxicity units (TU_c) measured. The larger the TU, the greater the toxicity.

Toxic Unit - Acute (TU_a) is 100 times the reciprocal of the effluent concentration that causes 50 percent of the organisms to die in an acute toxicity test ($TU_a = 100/LC_{50}$) (see LC_{50}).

Toxic Unit - Chronic (TU_c) is 100 times the reciprocal of the effluent concentration that causes no observable effect on the test organisms in a chronic toxicity test ($TU_c = 100/NOEC$ or $100/EC_{25}$) (see NOEC).

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

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

Type I Error (alpha) is the rejection of the null hypothesis (H_0) when it is, in fact true (i.e., determining that the effluent is toxic when the effluent is not toxic).

Type II Error (beta) is the acceptance of the null hypothesis (H_0) when it is not true (i.e., determining that the effluent is not toxic when the effluent is toxic). Beta is related to the power of the test.

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 TMDL that is allocated to one of its existing or future point sources of pollution.

Water Quality Criteria are numeric scientifically derived ambient concentrations developed by EPA or States for various pollutants of concern to protect human health and aquatic life. Narrative criteria typically are statements that describe the desired water quality goal.

Water Quality-based Effluent Limit (WQBEL) is a NPDES permit limit established by either an EPA or a State permit writer that is developed to assure protection of aquatic life or human health consistent with applicable State or Tribal water quality standards, including the designated uses for a particular waterbody, the established criteria, and measured analytical data (e.g., chemical, WET or biosurvey), in accordance with the recommendations provided in EPA's 1991 Technical Support Document (TSD).

Water Quality Standard (WQS) Water quality standards are provisions of State or Federal law which consist of a designated use or uses for the waters of the United States and water quality criteria for such waters based upon such uses. Water quality standards are to protect the public health or welfare, enhance the quality of water, and serve the purposes of the Act. States and authorized Tribes are required to develop and adopt a statewide antidegradation policy and identify the methods for implementing the policy.

Whole Effluent Toxicity (WET) is the total toxic effect of an effluent measured directly with a toxicity test.

WET Permit Limit is the water quality-based effluent limit for WET, established by either an EPA or State permit writer, that is used to trigger accelerated WET monitoring and TRES.

WET Permit Trigger is a threshold level for WET in an NPDES permit, established by either an EPA or State permit writer, this is used to trigger accelerated WET monitoring and TRES when there is no reasonable potential for WET and no WET permit limit.

CHAPTER 1. INTRODUCTION

1.1 Overview

This chapter briefly describes the background and history of the whole effluent toxicity (WET) testing program and use of the integrated strategy to achieve and maintain water quality standards required by the U.S. Environmental Protection Agency (EPA).

1.2 Background

The Federal Water Pollution Control Act, commonly known as the Clean Water Act (CWA), was enacted in 1972 with the objective to “*restore and maintain the chemical, physical, and biological integrity of the Nation’s waters.*” In order to achieve this objective, goals and policies were established in the Act, including:

- Eliminating the discharge of pollutants into navigable waters by 1985;
- Wherever attainable, achieving an interim goal of water quality which provides for the protection and propagation of fish, shellfish, and wildlife, and provides for recreation in and on the water by July 1, 1983; and
- Prohibiting the discharge of toxic pollutants in toxic amounts.

In the 35 years since the CWA was enacted, the EPA and States authorized to administer EPA’s National Pollutant Discharge Elimination System (NPDES) permitting program have made significant progress toward achieving these goals and policies. Under the EPA’s integrated water quality-based “standards to permits” approach for toxics control, NPDES permits are designed to achieve and maintain water quality standards. A point source that discharges pollutants to surface waters of the United States must do so under the limitations and conditions of an NPDES permit. In setting these limitations and conditions, the EPA and States protect aquatic life using three control approaches discussed in the Technical Support Document for Water Quality-based Toxics Control (USEPA 1991a, referred to as the TSD):

- Chemical-specific control approach,
- WET control approach, and
- Biological criteria/bioassessment and biosurvey approach.

A detailed discussion of the capabilities and limitations of these three approaches is provided in Section 1.5 of the TSD (USEPA 1991a). Since each approach has unique as well as overlapping attributes, sensitivities, and program applications, no single approach for detecting impact should be considered uniformly superior to any other approach. An integrated approach to water quality-based toxics control is essential for a strong toxics control program.

The WET control approach to water quality protection is the primary subject of this document.

EPA defines whole effluent toxicity as “*the aggregate toxic effect of an effluent measured directly by an aquatic toxicity test*” [54 Federal Register (FR) 23868 at 23895, June 2, 1989]. Aquatic toxicity tests are laboratory experiments that measure the biological effect (e.g., survival, growth, and reproduction) of effluents or receiving waters on aquatic organisms. In aquatic toxicity tests, groups of organisms of a particular species are held in test chambers and exposed to different concentrations of an aqueous test sample (e.g., reference toxicant, effluent, or receiving water). Observations are made at predetermined exposure periods. At the end of the test, the responses of test organisms are used to estimate the effects of the aqueous sample.

Beginning in the 1980s, EPA published methods (USEPA 1985a, 1988, 1989a) for estimating the acute and chronic toxicity of effluents and receiving waters to freshwater and marine organisms. WET data gathered in the 1980s indicated that approximately 40 percent of NPDES facilities nationwide discharged effluent with sufficient toxicity to cause water quality problems.

Further reductions in the toxicity of NPDES effluents were needed to comply with State narrative “free from toxics in toxic amounts” water quality criteria. Responding to these findings, EPA implemented a national policy for assessing and controlling the discharge of toxic substances to ensure protection of water quality. 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 and recommend both chemical-specific analyses and biological techniques to assess effluent discharges and express permit limitations. To support this policy, EPA developed new regulations governing water quality-based permitting in the NPDES program (54 FR 23868, June 2, 1989) and the TSD (USEPA 1991a). Originally published in 1985 and updated in 1991, the TSD provides national guidance to Permitting Authorities implementing WET testing in NPDES permits.

On October 16, 1995, EPA promulgated WET test methods (USEPA 1993a, 1994a, 1994b, 1999a) and added them to the list of EPA methods approved under Section 304(h) of the CWA (40 CFR 136) for use in the NPDES program. These methods were subsequently challenged and under a settlement agreement, EPA conducted a round-robin study which evaluated 12 of the test methods (USEPA 2001a, 2001b). EPA also prepared a WET test methods guidance document (USEPA 2000a) and a WET test method variability guidance document (USEPA 2000b). On November 19, 2002, EPA promulgated revised WET test methods (USEPA 2002a, 2002b, 2002c) [67 FR 69952, November 19, 2002]. These methods were also challenged and ultimately, the U.S. Court of Appeals upheld the validity of the WET test methods against a variety of constitutional, statutory, and administrative law challenges. In *Edison Electric Institute et al. v. EPA*, 391 F.3d 1267 (D.C. Cir. 2004), the Court found that:

- EPA reasonably validated the standardized testing procedures, including their precision and bias, as well as their high rates of successful test completion.
- The methods did not produce unacceptably variable results.
- The method procedures (i.e., replication and comparison to controls) adequately compensated for the inability to determine a method detection limit, and
- The results produced with methods were representative of receiving water toxicity, including receiving waters of the arid West.

It is the position of EPA Regions 9 and 10 that WET test methods yield reproducible and precise results. WET testing plays a vital role in water pollution control programs by regulating complex mixtures of chemicals and helping to identify toxicity in wastewater effluents, stormwater, and ambient waters. We have summarized frequently asked questions (FAQs) to assist Permitting Authorities implementing WET programs (see Appendix A).

1.3 EPA's Integrated Strategy

Based on the stated goals of the CWA, the EPA and individual States implement three approaches to protect water quality. These approaches include chemical-specific control, toxicity testing control, and biological criteria/bioassessments (USEPA 1991a). This document only addresses the protection of aquatic life, not human health. Each of the three control approaches has advantages and limitations.

The chemical-specific approach involves the development of water quality criteria (WQC) for chemicals as expressed in terms of the acute criterion and the chronic criterion. These criteria are developed following EPA water quality guidelines (USEPA 1985b). EPA has developed water quality criteria for the 126 priority pollutants as required under CWA Section 308. These WQC are based on minimum data requirements that include both acute and chronic toxicity tests with the specified numbers and types of aquatic species. WQC are intended to protect most of the tested species, most of the time. The chemical-specific approach can allow prediction of ecological impacts before they occur. It also considers bioaccumulation and human health impacts. A limitation of the chemical-specific approach is that not all toxicants in wastewaters or aqueous samples may be known, and therefore, control requirements can only be established for those that are known. For mixtures of chemicals with unknown interactions or for chemicals having no chemical-specific criteria, sole use of chemical-specific criteria to safeguard aquatic resources would not be protective. Toxicity testing is needed because the chemical-specific approach only addresses individual chemicals and does not address chemical interactions or chemicals that are not known to be in the effluent. In addition, criteria have been developed for only a limited universe of chemicals. This is why the toxicity testing and bioassessment approaches for protecting aquatic life are also critical components for protection of aquatic resources.

The primary advantage of using the toxicity testing approach is that this tool can be used to assess toxic effects (acute and chronic) of all the chemicals in aqueous samples of effluent, receiving water, or stormwater. This allows the effect of the aqueous mixture to be evaluated, rather than the toxic responses to individual chemicals. Some advantages of WET testing include the toxicity of effluent or ambient water is measured directly for the species tested; the aggregate toxicity of all constituents in a complex effluent is measured; and ecological impacts can be predicted before they occur. Toxicity tests can be used to assess ambient waterbodies (i.e., receiving water) making these tools effective in the assessment of small and large watersheds (de Vlaming et al. 2000). This has been demonstrated by the State of California which has successfully used an ambient toxicity testing approach to identify and regulate frequently occurring toxic chemicals. This approach includes pinpointing critical sampling locations for collecting the ambient waters to be assessed using acute and chronic toxicity tests. If toxicity is detected, then additional samples are collected to determine the spatial and temporal

toxicity patterns. Subsequently, EPA's Toxicity Identification Evaluation (TIE) procedures are used to identify the causative toxicant(s). The goal of the TIE is to identify the chemical(s) causing toxicity in an aqueous sample. This ambient toxicity testing approach has led to the 303(d) listing of chemicals beyond the 126 priority pollutants commonly tested; one such listing is the pesticide diazinon, which is not a priority pollutant (SWRCB 2003). In addition, the approach of toxicity testing in conjunction with TIE analysis may be used to determine chemical interactions. These interactions can be additive, synergistic, or antagonistic. Lydy et al. (2004) provides a synthesis review of challenges in regulating pesticide mixtures and pesticide toxicity to aquatic organisms. Limitations of WET are that it directly measures only the immediate bioavailability of a toxicant(s) in the aqueous sample, and the long-term cumulative toxicity of a compound is not measured.

The bioassessment approach can directly assess the status of a waterbody, since biological communities reflect overall ecological integrity; it provides a holistic measure of the aggregate impact of pollutant stressors and can measure historical trends and fluctuating environmental conditions. The primary advantage of the bioassessment approach is that it integrates both the physical and biological stressor effects on aquatic biota. Biological assessments are based on the premise that the structure and function of an aquatic biological community can provide critical information about the quality of the surface water. The waterbodies being evaluated are assessed and compared to predetermined criteria for impairment and non-attainment of a designated use. The stressor identification (SI) process is a method for identifying biological and physical stressors of the impaired waterbody (USEPA 2000c). The bioassessment approach is limited in that bioassessments conducted at critical low flow conditions can be difficult to accomplish; data may not be sufficient to detect impacts without appropriate reference conditions or suitable biocriteria; the methods detect problems after they have occurred; and causes of impairment may not be assigned readily to any one permittee or other source.

Based on the individual strengths of each of the three approaches (chemical-specific, toxicity testing, and biological criteria/bioassessment) protection of aquatic life will be most thorough if all three approaches are used. If a waterbody is impaired, as measured by any of these three approaches (i.e., WQC are not attained) the CWA requires that impaired waterbodies be listed on the State's 303(d) list and that a total maximum daily load (TMDL) be developed to address the pollutant(s) causing the impairment. The TMDL provides the basis for actions to be taken to restore the water to its designated use.

It is EPA's position that the concept of "independent application" be applied to water quality-based situations (USEPA 1991b). One aspect of the policy expresses that water quality standards are to be independently applied. This means that any single assessment method (chemical criteria, toxicity testing, or biocriteria) can provide conclusive evidence that water quality standards are not attained. Since each method has unique, as well as overlapping attributes, sensitivities, and program applications, no single approach for detecting impact should be considered superior to any other approach. The most protective results from each assessment conducted should be used in the effluent characterization process. EPA regulations at 40 CFR 122.44(d)(1), in effect, require independent application of chemical-specific and whole effluent data and criteria when characterizing effluents and making water quality assessments.

1.4 SETAC Technical Workshop

In September 1995, the Office of Wastewater Management (OWM) and Office of Science and Technology (OST) helped fund a Society of Environmental Toxicology and Chemistry (SETAC) technical workshop on WET. The workshop explored the science involved in WET testing and published a peer-reviewed SETAC book, titled “Whole Effluent Toxicity Testing: An Evaluation of Methods and Prediction of Receiving System Impacts” edited by Grothe, Dickson, and Reed-Judkins (1996). The conclusions are highlighted in Attachment 1-1.

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Attachment 1-1. Pellston Workshop

1. WET exposure methods are technically sound and require no immediate modifications.
 2. WET testing is an effective tool for predicting impact in lotic receiving systems. Additional laboratory to field validation is not essential for the continued use of WET testing.
 3. The guidance provided in the U.S. EPA's *Technical Support Document for Water Quality Based Toxics Control* must be followed closely to meet the objectives of the WET testing program.
 4. A number of problems with WET tests are caused by misapplication of the tests, misinterpretation of data, quality of the WET test laboratory, and the lack of training and experience of laboratory personnel, regulators and permittees.
 5. Current WET permit limits have sufficient margins of safety so that episodic exceedances should not cause receiving water impacts. The significance of an exceedance of WET limits depends on receiving water conditions, especially dilution at the time of the exceedance, and the duration of the toxic event.
 6. Variability in the use of both WET test methods and bioassessment techniques influences test interpretation and acceptability and the extrapolation of WET test results to field impacts.
 7. The largest sources of variability in WET testing are the level of analyst expertise and judgment and test organism condition/ health. Deviation from established methods can be controlled by an effective QA/QC program.
 8. Currently used statistical methods are widely used and accepted. However, improvements are available that should be considered.
 9. Biological assessment approaches, when properly designed, can accurately assess environmental impact to aquatic biota.
 10. Bioassessments are needed to compensate for the limitations of WET tests to predict phytotoxicity, sediment toxicity, bioaccumulation, genotoxicity, indirect biotic effects, and effects of persistent chemicals.
 11. In addition to WET testing, results from *in situ* testing, ambient toxicity testing, and bioassessments are useful to evaluate WET limits and margins of safety.
 12. The relationship between WET tests and receiving water impacts is based largely on animal effects in streams. Minimal data exist describing the effect of effluent toxicity exposure in wetlands, estuaries, and large rivers.
 13. Careful thought must be given to selecting appropriate reference conditions for field assessments. Regional reference conditions strengthen assessments of receiving water impacts and facilitate characterization of natural variation.
 14. Effluent toxicity is one of several factors that can adversely impact biological communities and is not always the major cause of observed community impacts.
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CHAPTER 2. DEVELOPING WET PERMIT CONDITIONS

2.1 Overview

Chapter 2 discusses the development of WET permit conditions. The subjects covered in this chapter include: (1) mixing zones; (2) water quality criteria for WET; (3) reasonable potential determinations with and without facility-specific effluent data; (4) derivation and expression of WET permit limits; and (5) derivation and expression of WET permit triggers (WET permit triggers) used in conjunction with accelerated monitoring and TRES when there is no reasonable potential for WET and no WET permit limits.

When determining reasonable potential and deriving and expressing water quality-based effluent limits (WQBELs) and permit conditions for WET, the Permitting Authority needs to examine the State's water quality criteria for WET, mixing zone policy, and NPDES implementation procedures for determining reasonable potential and calculating WET permit limits or WET permit triggers for accelerated monitoring and TRES. We note that current State practices may differ from EPA's recommended approach for WET implementation outlined in the TSD (USEPA 1991a) and described in this chapter. In all cases, State practices must meet requirements of the Clean Water Act (CWA) and federal NPDES regulations.

Permitting Authorities determining reasonable potential and establishing WQBELs for WET must follow 40 CFR 122.44(d)(1) and should consider EPA guidance for water quality-based permitting in Chapters 3 and 5 of the TSD. For these types of calculations, EPA recommends that WET data be expressed using toxic units (TUs). Section 1.3.1 of the TSD defines TUs as 100 divided by the measured effect concentration expressed as a percentage of whole effluent. Thus, $TU_a = 100/LC_{50}$ and $TU_c = 100/NOEC$ or $100/EC_{25}$. When statistically estimating effluent variability for determining WET reasonable potential procedures following TSD procedures, TU_c data for an effluent should be based on point estimate results (e.g., EC_{25}) rather than hypothesis testing results (e.g., NOEC), in order to obtain a better estimate of the effluent coefficient of variations (CV) used for WET permitting (USEPA 2000b). However, WET permit limits or WET permit triggers should continue to be expressed in accordance with State water quality standards and NPDES implementation procedures using either NOEC or EC_{25} .

2.2 Mixing Zones

When deriving WET permit limits, or WET permit triggers for accelerated monitoring and TRES, mixing zones may be considered for an NPDES discharge based on available dilution and assimilative capacity, if authorized and allowed by State water quality standards (WQS). Section 4.3 of the TSD provides background information on mixing zones and discusses EPA's mixing zone policy and how this policy affects the allowable toxic load that can be discharged from a point source. Section 4.4 of the TSD discusses mixing zone analyses for situations in which the discharge does not mix completely with the receiving water within a short distance of the discharge. If complete mixing does not occur near the discharge point and the effluent plume is discernible downstream, then modeling techniques that simulate and predict mixing conditions are more appropriate. Section 4.5 of the TSD discusses the steady-state models most used by States and EPA Regions to calculate wasteload allocations (WLAs) for contaminants. An

example of a steady-state model is the mass balance equation, i.e., the continuity equation, described later in this chapter. Steady-state models assume that the effluent is completely mixed with the receiving water near the discharge point, such as in effluent-dominated streams. Steady-state models require single constant inputs for effluent flow, effluent concentration, receiving water flow, and background receiving water concentration. The critical conditions for receiving stream design flows used in steady-state modeling should reflect water quality criteria durations and frequencies and the hydrologically- and biologically-based design flows generally specified in State WQS. EPA's recommended receiving stream design flows for water quality criteria and their calculation are summarized in Appendix D of the TSD. Critical design flows recommended for use with EPA's acute criterion for WET are the 1Q10 and 1B3. Critical design flows recommended for use with EPA's chronic criterion for WET are the 7Q10 and 4B3. If mixing zones for acute or chronic WET are not authorized for an NPDES discharge, or not allowed by State WQS, then water quality criteria for WET must be applied at the end of the discharge pipe.

2.3 Water Quality Criteria for WET

Water quality standards are provisions of State (or federal) law or regulation which define the water quality goals of a waterbody, or portion thereof, by designating the uses of the waterbody and setting the water quality criteria necessary to protect those uses. States adopt WQS to protect public health or welfare, enhance the quality of water, and serve the purposes of the CWA. Such standards serve the dual purposes of establishing the water quality goals for a specific waterbody and serve as the regulatory basis for the establishment of water quality-based controls and strategies beyond the technology-based levels of treatment required by sections 301(b) and 306 of the CWA. (40 CFR 131.2)

Water quality criteria are elements of State WQS, expressed as constituent concentrations, levels, or narrative statements representing a quality of water that supports a particular use. When these criteria are met, water quality will generally protect the designated use. (40 CFR 131.3) While States have adopted a variety of criteria expressed as constituent concentration levels (or numeric criteria) for various pollutants, all States have adopted criteria expressed as narrative statements (or narrative criteria). These narrative criteria, often referred to as "free-from" criteria (in the case of WET, "no toxics in toxic amounts"), are an effective tool for controlling the discharge of pollutants where numeric criteria are not available. Numeric or narrative criteria for WET serve as the basis for establishing WET controls in NPDES permits. (40 CFR 122.44(d)(1))

EPA's national water quality criteria are developed under the requirements of CWA section 304(a) and are published by EPA in individual criteria documents. The water quality criteria for aquatic life consider a wide range of toxic endpoints, including acute and chronic impacts, and consist of two values—a criterion maximum concentration (CMC) to protect against acute (short-term effects) and a criterion continuous concentration (CCC) to protect against chronic (long-term) effects. At present, EPA has no national criteria developed under CWA section 304(a) for acute and chronic WET. In the absence of such criteria, EPA's recommended magnitudes for WET are as follows. For acute protection, the CMC should be set at 0.3 acute toxic units (TUa) to the most sensitive of at least two test species. For chronic protection, the CCC should be set at 1.0 chronic toxic units (TUc) to the most sensitive of at least three test

species. Also, State procedures for implementing narrative criteria for WET should specify the testing procedure, the duration of the tests (acute or chronic), the test species, and the frequency of testing required. (TSD Section 2.3.3)

2.4 Determining Reasonable Potential for WET

This section follows 40 CFR 122.44(d)(1) and discusses the possible outcomes of a reasonable potential (RP) determination for WET, as described in Chapter 3 of the TSD. Where there is either a numeric or narrative water quality criterion for WET, Permitting Authorities need to characterize WET in NPDES discharges and implement WQBELs for WET, as required by 40 CFR 122.44(d)(1). Following EPA's recommendations in Chapter 3 of the TSD, there are two ways to characterize an effluent to determine the need for WET permit limits. First, an assessment may be conducted using facility-specific effluent data for WET, following procedures outlined in Section 3.3 of the TSD or other State NPDES implementation procedures. Second, an assessment may also be conducted without generating facility-specific effluent data for WET, using the factors described in Section 3.2 of the TSD. Following 40 CFR 122.44(d)(1)(ii), in all situations when determining the need for a WET permit limit, the Permitting Authority is required to consider, at minimum, existing controls on point and nonpoint sources of pollution, the variability of the pollutant or pollutant parameter in the effluent, the sensitivity of the species to toxicity testing and, where appropriate, the dilution of the effluent in the receiving water.

Section 3.3.3 of the TSD describes four possible outcomes of a reasonable potential determination for WET. These are:

- *Outcome 1.* The discharge causes or contributes to an excursion above a numeric or narrative water quality criterion for WET and a WQBEL for WET is required;
- *Outcome 2.* The discharge has the reasonable potential to cause or contribute to an excursion above a numeric or narrative water quality criterion for WET and a WQBEL for WET is required;
- *Outcome 3.* The discharge does not [have the reasonable potential to] cause or contribute to an excursion above a numeric or narrative water quality criterion for WET and a WQBEL for WET is not required; however, WET permit triggers used in conjunction with accelerated monitoring and TREs are recommended by EPA; or
- *Outcome 4.* There is inadequate information to determine whether or not the discharge causes, has the reasonable potential to cause, or contributes to an excursion above a numeric or narrative water quality criterion for WET and a WQBEL for WET is not required; however, WET permit triggers used in conjunction with accelerated monitoring and TREs are recommended by EPA.

When determining the need for WQBELs for WET, Permitting Authorities should use all available effluent data, together with information like that discussed in the following sections, as a basis for this decision. The Permitting Authority may already have facility-specific WET data from NPDES self-monitoring reports, or may decide to require the discharger to generate WET

data prior to permit issuance or as a condition of the permit. NPDES application requirements at 40 CFR 122.21 specify effluent monitoring requirements for WET, based on several factors, including the type of discharge. EPA recommends that WET data be generated prior to permit issuance for the following reasons: (1) the presence or absence of toxicity can be more clearly established or refuted, and (2) where toxicity is shown, effluent variability can be more clearly defined and addressed. (TSD Section 3.3.1)

2.4.1 Determining the Need for Permit Limits with Facility WET Data

As described in Section 3.3.2 of the TSD, for facilities with WET data, EPA recommends finding that a discharger has the “reasonable potential” to exceed a water quality criterion for WET if it is demonstrated with a high level of confidence that the upper bound of the lognormal distribution of effluent values for WET are above water quality criteria for WET, at specified critical flow conditions. EPA’s recommended statistical approach for determining reasonable potential is a sequential, tiered process that is shown in Box 3-2 of the TSD. First, for each test method and species, effluent data for WET are reviewed to determine the total number of sample observations (n) and identify the maximum observed effluent value. Second, if there is enough sample observations ($n \geq 10$), these data are used to calculate statistics—a mean, standard deviation and coefficient of variation (CV)—which characterize the variability of WET in the effluent. However, if fewer than ten sample observations are available ($n < 10$), then Section 5.5.2 of the TSD recommends using the default CV of 0.6 to characterize the variability of WET in the effluent. Third, following the instructions in Section 3.3.2 of the TSD, the values for “ n ” and “CV” are used to calculate a reasonable potential multiplier factor. Fourth, the identified maximum observed effluent value for WET is multiplied by the reasonable potential multiplier factor to obtain a probability-based estimated maximum effluent value. Generally for WET, both the identified maximum observed effluent value and the probability-based maximum effluent value are used in the steady-state mass balance equation to project in-stream maximum values for WET, at specified critical flow conditions. Fifth, these projected in-stream maximum values are calculated and compared to the water quality criterion for WET (acute or chronic). If both projected in-stream maximum values are less than, or equal to, the water quality criterion for WET, then the Permitting Authority should exercise judgment as to whether reasonable potential exists. If either of these projected in-stream maximum values is greater than the water quality criterion for WET, then reasonable potential is established for the discharge and the permit must contain WQBELs for WET. Appendix B of this document provides an example of how the steady-state mass balance equation is used by EPA to calculate dilution and establish reasonable potential for acute and chronic WET.

2.4.2 Determining the Need for Permit Limits without Facility WET Data

As described in Section 3.2 of the TSD, the Permitting Authority may choose to develop and require WQBELs to control WET without facility-specify monitoring data, or prior to the generation of effluent data. In doing so, the Permitting Authority needs to follow the requirements in 40 CFR 122.44(d)(1) and clearly document these decisions in the record for the permit. When determining whether or not a discharge causes, has the reasonable potential to cause, or contributes to an excursion above a narrative or numeric water quality criterion for WET, the Permitting Authority can use a variety of factors and information where facility-

specific effluent monitoring data are not available. Also, these factors should be considered when effluent monitoring data are available. Some of the factors described in the TSD include:

- *Dilution.* Toxic impact is directly related to available dilution for the effluent. Dilution is related to the receiving water stream flow, the size of the discharge, whether or not there is an outfall diffuser, etc. The lower the available dilution, the higher the potential is for toxic effects. For example, as discussed in Section 3.3.3 of the TSD, if an effluent's dilution (i.e., in-stream waste concentration; IWC) at the edge of a mixing zone authorized by the Permitting Authority is expected to reach one percent or higher during critical or worst-case design periods, then the effluent may require a WET limit.
- *Type of industry.* Although NPDES discharges should be individually characterized because toxicity problems are site-specific, the "primary" industrial categories are of principal concern. Factors to consider can include the type and efficiency of treatment applied, general materials handling practices, and the functional target of the compound(s) produced.
- *Type of POTW.* POTWs with loadings from indirect dischargers (particularly primary industries) may be candidates for WET limits. However, the absence of industrial input does not guarantee an absence of toxicity problems. Down-the-drain disposal of pesticides, detergents, and other toxicants can result in toxic concentrations in POTW effluents. The types of industrial users, their product lines, raw materials, potential and actual discharges, and control equipment should be evaluated. POTW effluents should be evaluated for potential toxicity due to ammonia and chlorine.
- *Existing data on toxic pollutants.* Discharge monitoring reports (DMRs) and data from NPDES permit application forms may provide some indication of the presence of toxicants. The presence or absence of the 126 priority toxic pollutants (CWA section 307(a) and 40 CFR 131.3(d)) may or may not be an indication of the presence or absence of WET. There are thousands of toxicants not on the list of 126 priority toxic pollutants which are by definition "nonconventional" pollutants that may cause toxicity. Also, combinations of toxicants can produce toxicity where individual toxicants would not. NPDES regulations at 40 CFR 122.21(j)(5) specify that POTWs with design flows equal to or greater than 1 mgd and POTWs required to operate pretreatment programs, must perform specified WET testing and submit these results with their permit applications. Also, for certain types of dischargers, 40 CFR 122.21 allows Permitting Authorities to request additional data, including WET data, at the time of permit application. Also, data may be obtained using CWA section 308, or similar State authority.
- *History of compliance problems and toxic impact.* Permitting Authorities may consider particular dischargers that have had difficulty complying with limits on toxicants or that have a history of known toxicity impacts, as probable candidates for WET limits.
- *Type of receiving water and designated use.* Data on water quality can include reports of fish kills, State lists of priority waterbodies, and State lists of waters that do not meet water quality standards. Sources of this information are the lists of waters generated under CWA section 304(l) and 40 CFR 130.10(d)(6).

The presence of a factor (or combination of factors) described above, such as low available dilution, high quality receiving waters, poor compliance record, and clustered industrial and municipal discharges, could constitute a high priority for WQBELs for WET. If the Permitting Authority chooses to require a WET limit without facility-specific effluent monitoring data, then adequate justification for the limit needs to be provided in the fact sheet or statement of basis for the permit. EPA recommends that the more information the Permitting Authority can acquire to support WET limits, the better a position the authority will be in to defend the limit, if necessary.

2.4.3 Other State Regulations for Determining Reasonable Potential

The Permitting Authorities needs to follow applicable State regulations and policies which govern how reasonable potential for WET is determined. These State requirements must be consistent with the Clean Water Act and EPA's regulations for implementing WET in the NPDES permitting program (e.g., 40 CFR 136, 40 CFR 122.41(j), 40 CFR 122.44(d), 40 CFR 122.21(j)). In the absence of detailed State regulations and policies, EPA recommends that Permitting Authorities follow the approaches and statistical procedure for determining reasonable potential recommended in Chapter 3 of the TSD.

In the *California Ocean Plan* (SWRCB 2005), the California State Water Resources Control Board has adopted general reasonable potential language and specified statistical reasonable potential analysis procedures for both parametric effluent data sets and non-parametric effluent data sets. If there are three or more detected observations for the effluent and these observations are censored by 80% or less, then the parametric reasonable potential analysis procedure is used. This procedure assumes that effluent data are lognormally distributed and calculates an upper confidence bound (i.e., the one-sided, upper 95 percent confidence bound for the 95th percentile of the effluent distribution after complete mixing) for comparison with the water quality criterion. If the upper confidence bound is greater than the water quality criterion, then the California ocean discharge has reasonable potential to cause an excursion above the water quality criterion and a WQBEL is needed. Instructions for conducting a nonparametric reasonable potential analysis or a reasonable potential analysis based on best professional judgment are also given. To support these procedures, State Water Board staff have developed a stand-alone, Windows-based computer program called "RPcalc", the California Ocean Plan Reasonable Potential Analysis Calculator (SWRCB 2005), to assist Permitting Authorities conducting reasonable potential analyses for discharges regulated under the California Ocean Plan. This approach is found at <http://www.swrcb.ca.gov/> and an example is provided in Appendix B of this document.

2.4.4 Reasonable Potential Determination Outcomes for WET

Based on Outcomes 1 and 2, described in Section 2.4 of this document, if WET in an NPDES discharge is at levels that cause, have the reasonable potential to cause, or contribute to an excursion above State water quality standards, then the permit must contain WQBELs for WET. This conclusion can be based on one effluent sample observation for WET. Based on Outcome 3, described in Section 2.4 of this document, if WET in an NPDES discharge is below levels that cause, have the reasonable potential to cause, or contribute to an excursion above State water quality standards, then the permit need not contain WQBELs for WET. Based on Outcome 4,

described in Section 2.4 of this document, if there is inadequate information to determine whether WET in an NPDES discharge is below levels that cause, have the reasonable potential to cause, or contribute to an excursion above State water quality standards, then the permit need not contain WQBELs for WET. Although the Permitting Authority does not need to establish WQBELs for WET, there still may be a basis for concern under Outcomes 3 and 4.

Consequently, under each of these four outcomes, EPA recommends that WET monitoring in permits be conducted at frequency sufficient to ascertain discharge compliance with WQBELs for WET, WET permit conditions and, ultimately, State water quality standards. Whether or not WET limits are included in a permit, WET monitoring conditions need to specify: (1) an accelerated monitoring schedule following the exceedance of either a WET permit limit or WET permit trigger; and (2) the number of WET test failures during this schedule that will automatically initiate a TRE. Also, permits should contain a WET reopener condition which allows the Permitting Authority to “reopen” the permit and establish additional WET permit conditions or effluent limits based on monitoring results or other factors indicating that the effluent causes, has the reasonable potential to cause, or contributes to an excursion above water quality standards.

2.5 Deriving Permit Limits for WET

When a Permitting Authority determines, using reasonable potential procedures, that a discharge causes, has the reasonable potential to cause, or contributes to an in-stream excursion above State numeric water quality criteria for WET, the permit must contain WQBELs for WET. (40 CFR 122.44(d)(1)(iv)) If State WQS contain only narrative water quality criteria for WET and it is documented in the record for the permit (i.e., fact sheet or statement of basis) that chemical-specific WQBELs are sufficient to attain and maintain the narrative water quality criteria, then WQBELs for WET are not necessary. This is only authorized when the causative toxicant(s) in the effluent have been identified and confirmed. (40 CFR 122.44(d)(1)(v))

As explained in Section 5.1.1 of the TSD, once the decision has been made to develop WQBELs, there is an element of judgment inherent in the specific permit limit derivation procedures used for an individual NPDES discharger. Case-specific considerations will usually dictate the most appropriate conditions in individual situations (e.g., chronic or acute toxicity test, freshwater or marine test organisms, monitoring frequency, etc.); however, the general assumptions used when developing WQBELs should be consistent with the assumptions and principles inherent in effluent characterization and exposure assessment steps preceding the development of WQBELs. The WQBEL derivation procedure used by Permitting Authorities should be fully enforceable and should adequately account for effluent variability, consider available receiving water dilution when appropriate, protect against acute and chronic impacts, account for compliance monitoring sampling frequency, and protect pollutant wasteload allocations (WLAs) and ultimately the WQS.

Chapter 5 of the TSD explains the strengths and weaknesses of different approaches often used by Permitting Authorities to develop WQBELs, including permit limits for WET. This section discusses:

- The development of WLAs for WET using either steady-state models or dynamic models (TSD Chapter 4);
- The “statistical approach” where WQBELs are statistically calculated from the more stringent acute or chronic WLA for WET (TSD Section 5.4.1);
- The “direct application approach” where an acute or chronic WLA for WET is directly applied as a WQBEL (TSD Section 5.4.2); and
- Other approaches used to develop WQBELs for WET based on State WQS and NPDES implementation procedures.

As described in Section 5.2.2 of the TSD, WQBELs for NPDES discharges are established based on the need to maintain effluent quality for a pollutant at a level that will comply with WQS even during critical conditions in the receiving water. This level is determined by the WLA for the pollutant. The WLA, in turn, dictates the necessary level of treatment plant performance for the pollutant—or target long-term average (LTA)—discussed later in this chapter.

2.5.1 Developing Wasteload Allocations for WET

How are wasteload allocations (WLAs) developed for a pollutant in an effluent? There are two major types of water quality models used to develop WLAs for NPDES discharges: dynamic and steady-state. Dynamic models use estimates of effluent variability and the variability of receiving water assimilation factors to develop effluent requirements expressed in terms of the concentration of the pollutant and variability. As a result, the outputs of dynamic models can be used to base WQBELs on probability estimates of receiving water concentrations rather than worst-case assumptions. EPA only recommends using dynamic models to develop WLAs if adequate pollutant data for an effluent and receiving water flow are available to estimate frequency distributions. Traditional steady-state WLA models calculate WLAs at critical conditions, using worst-case assumptions for effluent and receiving water flows and pollutant levels. WQBELs derived from steady-state WLA models are designed to be protective of WQS during critical environmental conditions and all environmental conditions less than critical. Although steady-state WLA models tend to be more conservative than dynamic models because they rely on worst case assumptions, EPA recommends that steady-state WLA models generally be used by Permitting Authorities in most cases and especially where few or no WET data are available, or where daily receiving water flow records are not available. (TSD Section 5.3.2)

When using steady-state models, WLA calculations are always made using critical conditions. To calculate acute and chronic WLAs for WET using a steady-state model, the Permitting Authority needs to choose values for:

- Chronic criterion (CCC) for WET
- Fraction of 7Q10 (or 4B3) receiving water flow available for dilution, as authorized by State mixing zone policy
- Acute criterion for WET (CMC)

- Fraction of 1Q10 (or 1B3) receiving water flow available for dilution, as authorized by State mixing zone policy
- Maximum background level for WET in the receiving water
- Maximum effluent flow

Where receiving water data for WET are not available, EPA recommends assuming a default maximum background value of 0 (zero) TUs when calculating WLAs for acute and chronic WET.

Shown below, the mass balance equation, i.e., the continuity equation, is a simplified steady-state model for calculating WLAs that is generally recommended by EPA for calculating WLAs for acute and chronic WET.

Steady-State Model: Mass Balance Equation

$$C_r Q_r = C_e Q_e + C_s Q_s, \text{ where}$$

| | |
|---------|--|
| $C_r =$ | Water quality criterion for WET, in TUs |
| $C_e =$ | Wasteload allocation for WET, in TUs |
| $Q_e =$ | Effluent flow, in million gallons per day (MGD) or cubic feet per second (cfs) |
| $C_s =$ | Background in-stream value for WET above the discharge point, in TUs |
| $Q_s =$ | Percent (%) in-stream critical flow allowed for dilution, in MGD or cfs |

Use of this steady-state mass balance equation for calculating dilution and WLAs for acute and chronic WET assumes that the NPDES discharge achieves complete mixing across the width of the stream near the point of discharge and the effluent plume is not discernible downstream. If this is not the case, then modeling techniques that can simulate and predict mixing conditions are more appropriate for defining the mixing zone and dilution for the discharge. If a mixing zone is allowed and actual background in-stream pollutant levels are considered, then the mass balance equation is: $WLA = C_e = (C_r) [(Q_e + Q_s) / Q_e] - [C_s \times (Q_s / Q_e)]$, and water quality criteria are applied at the edge of the mixing zone. For WET, if a mixing zone is allowed and background in-stream toxicity is set equal to 0 TU, then the mass balance equation reduces to: $WLA = C_e = (C_r) [(Q_e + Q_s) / Q_e]$, and WET criteria are applied at the edge of the mixing zone. If a dilution model is used, e.g., UM3 from Visual Plumes (USEPA 2003a), and it is not necessary to consider the actual, nonzero, ambient concentration of a pollutant in the effluent, then the flux-averaged volumetric dilution factor (S_a) can be used in the mass balance equation: $WLA = C_e = C_r \times S_a$. If a mixing zone is not allowed, then the mass balance equation reduces to: $WLA = C_e = C_r$, and WET criteria are applied at the end of the pipe. Once both acute and chronic WLAs for

WET have been developed for an NPDES discharge, then QBELs need to be calculated. Appendix C of this document provide detailed examples of how the steady-state mass balance equation is used by EPA to calculate dilution and WLAs for acute and chronic WET, and QBELs for acute and chronic WET.

[Note: Where the volumetric dilution factor, $S_a = [(V_e + V_a) / V_e] = C_e / C_p$, in *Dilution Models for Effluent Discharges* (USEPA 1994c). Thus, if $S_a = 30$ (which means one volume of effluent is diluted with 29 volumes of ambient water), then the concentration of any volumetric tracer or conservative pollutant in the effluent is one thirtieth the concentration in the effluent, only if the ambient concentration is zero. In this definition of S_a , the volumetric dilution factor is very nearly 1 in the region outside the discharge orifice. Following the mass-balance equation, i.e., the continuity equation in Visual Plumes, because the dilution ratio, $D = Q_s / Q_e$, then $S_a = [(Q_e + Q_s) / Q_e] = 1 + D$. In other State documents, e.g., the California Ocean Plan (SWRCB 2005), the volumetric dilution factor, S_a , is considered the dilution ratio, D . In the California Ocean Plan definition, the volumetric dilution factor approaches zero near the discharge orifice. Page 9 of the Visual Plumes manual (USEPA 1994c) notes that above a dilution value of 30, the difference between the two definitions is progressively less than 3%, an inconsequential amount for most regulatory purposes.]

2.5.2 Statistical Approach for Developing WET Permit Limits

The statistical approach for developing QBELs from WLAs is described in Section 5.4.1 of the TSD. Because effluent quality varies over time, EPA recommends that the Permitting Authority establish QBELs using this statistical derivation procedure, in conjunction with WLAs, to adequately account for the variability observed in pollutant levels in NPDES discharges. Using this statistical approach, a WLA value is first set at the 99th percentile of necessary treatment plant performance and then translated into the average treatment performance level—long-term average (LTA) and coefficient of variation (CV)—that will ensure the WLA is met under critical conditions over the long-term. When two-value, or three-value steady-state WLAs have been developed for a pollutant (e.g., acute, chronic, and human health), the most stringent LTA is then translated into upper bound percentile values for effluent quality (i.e., 99th percentile and 95th percentile) and expressed as a maximum daily limit (MDL) and an average monthly limit (AML). (TSD Section 5.5.4) In making these translations for WET, the Permitting Authority needs to obtain values for:

- Acute-to-chronic ratio (TSD Section 5.4.1)
- Effluent variability expressed as CV (TSD Section 5.5.2)
- Number of compliance monitoring samples required per month (TSD Section 5.5.3)

Appendix C of this document provides detailed examples of EPA's recommended statistical approach for calculating WET permit limits.

2.5.3 Direct Application Approach for Developing WET Permit Limits

Several direct application approaches are described in Section 5.4.2 of the TSD. One type of direct application is when the Permitting Authority applies the WLA directly as a permit limit, generally a MDL. When a chronic WLA is set as a MDL, the MDL (1-day) should ensure protection of both acute (1-day) and chronic (4-day) water quality criteria. In the absence of additional information, permit writers may sometimes divide the MDL by 1.5 or 2.0 to derive an AML, depending on the expected range of effluent variability. Because this AML is derived without information about the variability of the effluent, this step may not ensure that the AML is protective of water quality criteria. Another type of direct application is when the acute WLA is applied as a MDL and the chronic WLA is applied as the AML; EPA discourages this approach since effluent variability has not been specifically addressed and compliance with the AML (30-day) during critical conditions could exceed the chronic (4-day) water quality criterion.

2.5.4 Other State Regulations

A State may have technology-based permit requirements for WET or use modified versions of the approaches described above to set WQBELs for WET. Permitting Authorities need to follow applicable State regulations and policies which govern how WET is implemented in NPDES permits. State requirements must be consistent with EPA's regulations for implementing WET in the NPDES permitting program (e.g., 40 CFR 136, 40 CFR 122.41(j), 40 CFR 122.44(d), 40 CFR 122.21(j)).

2.6 Permit Limit Expression

NPDES regulations at 40 CFR 122.45(d) require that all permit limits be expressed, unless impracticable, as both a MDL and an AML for all dischargers other than POTWs, and as an average weekly limit (AWL) and AML for POTWs. Following Section 5.2.3 of the TSD, the use of an AWL is not appropriate for WET. In lieu of an AWL for POTWs, EPA recommends establishing an MDL for toxic pollutants and pollutants in water quality permitting, including WET. This is appropriate for two reasons. The basis for the average weekly requirement for POTWs derives from secondary treatment regulations and is not related to the requirement to assure achievement of WQS. Moreover, an average weekly requirement comprising up to seven daily samples could average out daily peak toxic concentrations for WET and therefore, the discharge's potential for causing acute and chronic effects would be missed.

The MDL is the highest allowable value for the discharge measured during a calendar day or 24-hour period representing a calendar day. The permit should contain a condition indicating that the MDL is interpreted as the maximum acute or chronic WET result for that calendar month unless otherwise specified by State requirements. The AML is the highest allowable value for the average of daily discharges obtained over a calendar month. For WET, this is the average of individual WET test results for that calendar month, unless otherwise specified by State requirements.

In cases where an acute mixing zone is either not authorized, or authorized such that a critical instream waste concentration (IWC) for acute WET is set at a percent effluent value greater than 100% effluent, EPA Regions 9 and 10 continue to recommend that the acute WET permit limit should be expressed as a Pass/Fail limit, as described below. In cases where a chronic mixing zone is not authorized, EPA Regions 9 and 10 continue to recommend that the AML for chronic WET should be expressed as a median monthly limit (MML), as described below.

2.6.1 Acute WET Permit Limits for Low-Flow Situations

The following procedure is recommended for monitoring and limiting acute WET in NPDES discharge situations when an acute mixing zone is either not authorized, or authorized such that a critical IWC is set at a percent effluent value greater than 100% effluent. In these situations, where the critical IWC is set at a percent effluent value greater than 100% effluent, calculated WLAs and WQBELs for acute WET—based on EPA’s recommended water quality criterion for acute toxicity (CMC) of 0.3 TU_a = 100/LC₅₀, and the steady-state mass balance equation—can range from 0.999 TU_a down to 0.3 TU_a. (TSD Section 5.4.1). For these discharge situations, EPA Regions 9 and 10 continue to recommend hypothesis testing (Denton and Narvaez 1996). This is because the point estimate techniques used to evaluate compliance with EPA’s recommended acute toxicity criterion of 0.3 TU_a, i.e., “no acute toxicity”, cannot be used until the discharge-specific critical percent effluent concentration (LC₅₀) is able to be set at (or below) 100% effluent.

For these discharge situations, the acute WET permit limit should be “Pass” for any one test result. The determination of Pass or Fail from a single-effluent-concentration (paired) acute toxicity test is determined using a one-tailed hypothesis test called a t-test. The objective of a Pass or Fail test is to determine if survival in the single treatment (100% effluent) is significantly different from survival in the control (0% effluent). Following Section 11.3 in the acute test method manuals (USEPA 2002a), the t statistic for the single-effluent-concentration acute toxicity test is calculated and compared with the critical t set at the 5% level of significance. If the calculated t does not exceed the critical t, then the mean responses for the single treatment and control are declared “not statistically different” and the permittee reports “Pass” on the DMR form. If the calculated t does exceed the critical t, then the mean responses for the single treatment and control are declared “statistically different” and the permittee reports “Fail” on the DMR form. The permit should require additional toxicity testing and, ultimately, a TRE, if an acute WET permit limit or trigger is reported as “Fail”.

2.6.2 Chronic WET Permit Limits for Low-Flow Situations

When no mixing zone or dilution allowance is authorized, or an NPDES discharge is to a zero flow stream, EPA Regions 9 and 10 continue to recommend that Permitting Authorities establish a monthly median limit (MML) of 1.0 TU_c for chronic WET (Denton and Narvaez 1996). Under these discharge situations, chronic WET test results showing no chronic toxicity in 100 percent effluent are reported as censored values at the most hazardous effluent concentration possible to test (i.e., RWC = <1.0 TU_c in 100 percent effluent). Such results present unique issues for Permitting Authorities evaluating compliance with average monthly limits for chronic WET that are statistically calculated following EPA’s recommendations in Section 5.4.1 of the TSD, as

these calculated values are lower than 1.0 TUc (e.g., 0.8 TUc). While EPA Regions 9 and 10 continue to recommend the use of statistically-calculated maximum daily limits for chronic WET using TSD procedures, discharges without a mixing zone or dilution allowance—where the governing magnitude for the monthly limit is set at 1.0 TUc in 100 percent effluent—differ from discharge situations where the governing magnitudes for WET are set at other effluent dilutions. This is because the 100 percent effluent dilution represents both the censoring level for the toxicity test and the most hazardous effluent concentration possible to test.

Consequently, EPA Regions 9 and 10 continue to recommend direct application of 1.0 TUc as the monthly compliance level for NPDES discharges without a mixing zone or dilution allowance. In conjunction and limited to this discharge situation, because: (1) there are no values below 1.0 TUc and (2) an arithmetic average is sensitive to extremely large and small values, the median is favored as the better measure of central tendency for the monthly compliance level. EPA Regions 9 and 10 continue to believe that setting a median monthly limit at 1.0 TUc, rather than an average monthly limit at either 1.0 TUc or a statistically-calculated value lower than 1.0 TUc, allows Permitting Authorities to: (1) make the best use of all monthly WET test results—including those reported as censored values at the 100 percent effluent concentration—when evaluating compliance with monthly permit limits; and (2) continue to protect against short-term excursions above the 4-day average chronic criterion for WET of 1.0 TUc by establishing the traditional, statistically-calculated maximum daily limit for chronic WET recommended in the TSD.

In summary, use of the MML of 1.0 TUc for chronic WET is recommended only in conjunction with the following permit conditions:

- A statistically calculated MDL for chronic WET (TSD Section 5.4.1); and
- Routine WET monitoring using the most sensitive test species identified through screening using species representing three different phyla (TSD Section 1.3.4).

Appendix C of this document provides an example of EPA Region 9 and 10's recommended approach for calculating chronic WET permit limits for low-flow situations.

While continuing to affirm these recommendations for NPDES discharges when a mixing zone or dilution allowance is not authorized, EPA Regions 9 and 10 recognize that some Permitting Authorities may choose to establish only a maximum daily limit of 1.0 TUc for chronic WET, but no monthly limit. This alternative will protect against short-term excursions above the 4-day average chronic criterion for WET of 1.0 TUc and meet WQS, if used in lieu of the statistical procedure described in this document and in Section 5.4.1 of the TSD.

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CHAPTER 3. CHRONIC AND ACUTE TOXICITY TESTING

3.1 Overview

Regardless of whether the permit requirement is a WET permit limit or monitoring trigger (MT), the permit writer will need to develop appropriate testing conditions such as test method/species, testing frequency, and steps to address toxicity (which we have termed “stepwise approach” to addressing toxicity). In the 1996 document (Denton and Narvaez 1996), the EPA Regions 9 and 10 recommended, and continue to recommend the stepwise approach of accelerated testing; if continued toxicity is demonstrated then the permittee needs to conduct a toxicity reduction evaluation (TRE).

The first decision for a permit writer to make in selecting the appropriate toxicity tests is whether to conduct acute and/or chronic tests to address both the acute and chronic criteria. The next question to answer is whether to test with freshwater or marine species. Once these decisions have been made, the following parameters need to be considered when selecting the appropriate test species: taxonomic diversity; type of facility and toxicants; and seasonal and temporal effects. See Appendix D for an example of WET permit language.

3.2 Toxicity Test Methods

3.2.1 Acute Tests

Acute toxicity tests are used to determine the concentration of effluent or ambient water that results in mortality within a group of test organisms during a 24-, 48- or 96-hour exposure. In an acute toxicity test, an effluent sample is collected, diluted, and placed in test chambers with the chosen test species. After 24, 48 or 96 hours, the number of live organisms remaining in each test concentration and in a control is recorded. The acute test methods are listed in Attachment 3-1.

3.2.2 Chronic Tests

A chronic toxicity test is defined as a short-term test in which sublethal effects, such as fertilization, growth or reproduction, are measured in addition to lethality (in some tests). Traditionally, chronic tests are full life-cycle tests or shortened tests (approximately 30 days) known as early life stage tests. Measuring the chronic toxicity of effluents is difficult because of the potential for effluent toxicity to change over time. Thus, even a shortened chronic early life stage test conducted in one month would have to be repeated at intervals to ensure that process or receiving water changes were not altering toxicity in ambient waters. In addition, toxicity spikes occurring during any one portion of a 30-day test could produce a different level of toxic response than an identical spike occurring during a different time of the test. The duration of chronic toxicity tests precludes the use of a single effluent sample due to probable reduction in toxicity with storage and requires extensive logistical arrangements for sampling and handling of effluent. Chronic toxicity test methods of 7 days duration require a minimum of three samples.

As a result of such considerations EPA has developed a suite of shorter toxicity tests (short-term chronic tests) that aim to detect toxicity at chemical concentrations near those that produce chronic toxicity in longer term tests. The short-term chronic tests were developed and selected based on characteristics such as sensitive species, sensitive life-stages and endpoints, taxonomic and ecological diversity, short duration, availability of organisms for testing, and low volume requirements for test solutions. These resulting tests have typical durations of 40 minutes to 7 days, enabling tests to be run with effluent or receiving water samples at lower costs and increased test frequency. The chronic test methods are listed in Attachment 3-2 and 3-3.

EPA standardized the test procedures for conducting the approved acute and chronic WET test methods in the following three method manuals (USEPA 2002a, 2002b, 2002c), which were incorporated by reference into the WET final rule (67 Federal Register 69953). See Attachment 3-4 for a summary of WET method changes. In addition, since first promulgating acute and chronic WET methods in 1995, EPA has continued to recommend that NPDES permitting authorities implement chronic WET in permits for West Coast facilities based on *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms* (USEPA 1995b; West Coast manual) and other alternative guidance, as directed by State Permitting Authorities. This practice corresponds with the 2002 Final WET Rule (67 Federal Register 69952, 69955). In the preamble to this rulemaking, EPA states: “Because test procedures for measuring toxicity to estuarine and marine organisms of the Pacific Ocean are not listed at 40 CFR part 136, permit writers may include (under 40 CFR 122.41(j)(4) and 122.44[d](1)(iv)) requirements for the use of test procedures that are not approved at part 136, such as the *Holmesimysis costata* Acute Test and other West Coast WET methods (USEPA 1995b) on a permit-by-permit basis.” Indeed, regulations for POTWs at 40 CFR 122.21(j)(5)(viii) clarify that West Coast NPDES permit applicants, including those in Hawaii, are “exempted” from 40 CFR 136 chronic methods and must use alternative guidance as directed by the Permitting Authority.

3.3 Selection of Freshwater or Estuarine/Marine Test Methods

The decision of whether to use freshwater or estuarine/marine test methods is based on the salinity of the effluent and that of the receiving water. EPA provides technical discussion regarding the selection of test species (see TSD, Section 3.3.6). A summary paper by Goodfellow et al. (2000) provides information on the role of ion imbalance (either excess or deficiency) in aquatic toxicity testing and provides various recommendations that could be considered in addressing these issues. The Goodfellow et al. (2000) paper discusses procedures that use weight-of-evidence approaches to identify ion imbalance toxicity, including direct measurement, predictive toxicity models for freshwater, exchange resins, mock effluents and ion imbalance toxicity with tolerant/susceptible test species. Toxicity associated with ion imbalance of the effluent occurs when the ion concentrations and molar ratios of the effluent exceed or do not meet the physiological tolerance range of the selected test organism. States may have prescribed approaches which require toxicant characterization of the effluent to ascertain whether the toxicity is strictly due to ion imbalance and/or other toxicants within the effluent. If the toxicity is strictly due to ion imbalance and has been demonstrated in the weight-of-evidence approach then, on a site-specific basis, an alternate test species may be substituted.

3.3.1 Freshwater organisms

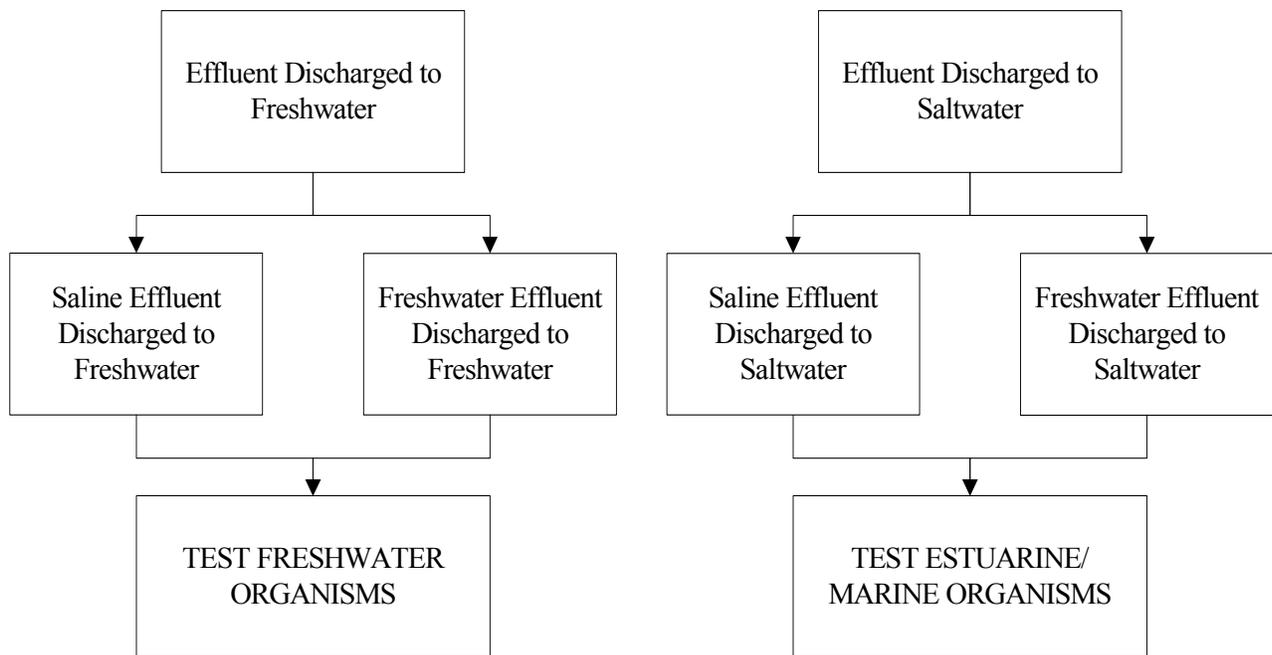
Freshwater organisms are used when the receiving water salinity is <1,000 mg/L (1‰). Species selection for freshwater is straightforward since there are methods for only three species: a fish (fathead minnows), an invertebrate (water flea), and a plant (green algae).

3.3.2 Estuarine/marine organisms

Estuarine/marine test organisms are used when the receiving water salinity is ≥1,000 mg/L (1‰). The EPA has test methods for test species resident to the East Coast and West Coast. There are two fish species (one East Coast and one West Coast), six invertebrate species (one East Coast and five West Coast), and one plant species (West Coast).

The selection of test organism is based on effluent and receiving water characteristics insert, as shown in the decision tree below (Figure 3-1).

Figure 3-1. Selection of Test Species Based on Effluent and Receiving Water Salinity



Saline Effluent Discharged to Estuarine/Marine Waters

The dissolved salts in the effluent are possible toxicants because the type and/or proportion of dissolved salts in the effluent may be different from that of the dissolved salts in the receiving water. The toxicity test should determine if these salts contribute to receiving water toxicity. For this reason, **estuarine/marine organisms are the preferred test species.**

Saline Effluent Discharged to Freshwater

The dissolved salts in the effluent are possible toxicants that are not present in the receiving water. The toxicity test should determine whether the dissolved salts are contributing to receiving water toxicity. For this reason, **freshwater organisms are the preferred test species.**

3.3.3 Freshwater Effluent Discharged to Estuarine/Marine Waters

The lack of dissolved salts in the effluent can affect marine toxicity test organisms. In contrast to the scenarios presented above, the toxicity test does not need to measure this effect since lack of salts is not considered a toxic effect. The estuarine/marine toxicity test methods account for this by requiring the salinity of the effluent be adjusted to the protocol salinity using either dry salts or hypersaline brine. For this reason, **estuarine/marine organisms are the preferred test species.**

3.3.4 Other Considerations

Factors that may be considered in selecting a marine invertebrate are the types of organisms found at the discharge location, types of toxicants discharged by the facility, and the relative sensitivity of the test organisms to known toxicants in the discharge. If the discharge is located near the intertidal zone, then an intertidal test species may be important (e.g., red abalone or bivalves). If the pollutants will be discharged near a kelp forest, where mysids are commonly located, the mysid test method may be more appropriate.

Sometimes, marine test species such as invertebrates and plants may not be amenable for testing at high effluent concentrations such as 100% effluent. For example, if the effluent salinity is 0‰ and hypersaline brine salinity is 100‰, then 66% effluent is the highest concentration that can be attained for tests with a salinity requirement of 34‰ when using only hypersaline brine (USEPA 1995a). **Therefore, a freshwater organism or a marine/estuarine organism that does not require hypersaline brine in the dilution water must be used if the permit limit or trigger is greater than the highest effluent concentration that can be tested.** However, the marine fish test methods, *Menidia* and *Atherinops* can be tested up to 100% effluent. Thus, these fish species can be used for freshwater discharges to saltwater with 100% effluent because dry sea salts (artificial) can be used to attain the method-required salinity (5-36‰).

3.4 Factors to Consider When Selecting Test Species

The Permitting Authority should select the appropriate species to be tested based on taxonomic diversity, type of facility, types of potential toxicants, and effluent seasonal and temporal effects.

In addition, the Permitting Authority should evaluate any existing toxicity data provided by the permittee.

3.4.1 Taxonomic Diversity

In the selection of test species, **EPA recommends the use of species from ecologically diverse taxa** (see TSD, Section 1.3.4). The recommendation is to screen an effluent with at least three species (a fish, an invertebrate, and a plant) for chronic testing and two species (a fish and an

invertebrate) for acute testing. This recommendation is based upon the fact that there are species sensitivity differences among different groups of organisms to different toxicants. The initial multiple species screening should be conducted at least three times before selecting the most sensitive species. There are no acute test methods with plant species.

After this screening period, monitoring should be conducted on the most sensitive test species (e.g., the species demonstrating the lowest NOEC or IC₂₅ value). It is also recommended in the permit that the permittee shall also re-screen once every year with three species (or two species for acute testing). If the same test species is the most sensitive, then the permittee shall continue to monitor with this test species. It is important to consider re-screening at a different time each year to evaluate effects of potentially different toxicants at different times of the year. For example, POTWs may have pesticide usage from homeowners in the spring and not in the winter months. Other factors to consider are the type of facility and seasonal and temporal effects from a facility.

3.4.2 Type of Facility

It is important to consider the type of toxicants that may be discharged from a facility and which species would be appropriate for such toxicants. For example, if a facility is discharging effluent that primarily consists of herbicides, a plant test method may be more appropriate. Certain species have been found to be sensitive to certain toxicants. Invertebrates are more sensitive to organophosphate pesticides (e.g., diazinon) than fish. Fish are more sensitive to ammonia than invertebrates. In situations where multiple species screening is not practical (such as ambient toxicity testing programs) it may be appropriate to test with the species with known sensitivity to the toxicants of concern.

3.4.3 Seasonal and Temporal Effects

It may be necessary to consider potential seasonal or temporal changes in the effluent when selecting the appropriate testing species. For example, pesticides may be of concern after spring runoff or first fall flush, and typically invertebrates such as water fleas or mysids are typically more sensitive.

Note: The *Selenastrum capricornutum* growth test (USEPA 2002b) now requires the addition of ethylenediamine tetraacetic acid (EDTA) to nutrient stock solutions when conducting this test under NPDES permits; Permitting Authorities are cautioned to consider this possibility when selecting test methods for monitoring effluents that are suspected to contain metals, as EDTA may interfere (i.e., mask) with the potential to ascertain the toxicity of metals.

Note: For controlling pathogen interference in the fathead minnow larval survival and growth test (USEPA 2002b), EPA recommends pathogen control techniques that do not modify the sample, such as the modified test-design technique. Upon approval by the Permitting Authority, however, analysts also may use various sample sterilization techniques that modify the sample to control pathogen interference, provided that parallel testing of unaltered samples further confines the presence of pathogen interference and demonstrates successful pathogen control (See chronic freshwater toxicity test methods manual, Sections 11.3.4.6.1 – 11.3.4.6.4).

3.5 Monitoring Frequency Recommendations

Once the need for a WET limit or monitoring requirement has been determined, the frequency of WET testing needs to be determined. The frequency for monitoring pollutants or pollutant parameters such as WET should be determined on a case-by-case basis, and decisions for setting the monitoring frequency should be set forth in the permit fact sheet. Some states have their own recommended sampling guidelines that can help a permit writer determine an appropriate monitoring frequency. The intent is to establish a frequency of monitoring that will detect most events of noncompliance without requiring needless or burdensome monitoring (Table 3-1).

Table 3-1 Likelihood of Detecting at Least One Toxic Event

| <i>Number of Tests (N)</i> | <i>True Probability of Occurrence^A</i> | | |
|--------------------------------|---|------------|------------|
| | <i>10%</i> | <i>20%</i> | <i>30%</i> |
| 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 |

a Assumes negligible serial correlation among observations, and true rate of occurrence over time. Probability of occurrence is stated as a percentage of the possible independent sampling events.

3.5.1 Example of Probability of Detecting Toxicity

For example, suppose the (unknown) probability is 0.20 (e.g., probability of occurrence is 20%) that the NOEC for a chronic *Ceriodaphnia* test will occur at or above the permitted TU value. Then, if testing is performed once per quarter (n=4), the probability that, in the course of one year, at least one of the four tests will demonstrate a toxicity at or above the permitted TU value is 0.59. The same would apply to monitoring once per year for four years (n=4). As another example using the same true probability of occurrence (20%), quarterly monitoring for three years (n=12) would be expected to exhibit at least one result exceeding the permitted TU value with high probability (0.93).

EPA recommends that the permit contain a monitoring schedule that increases or decreases in frequency depending on the results of WET testing after at least 20 tests have been completed under consistent treatment operations. **EPA Regions 9 and 10 recommend a minimum of monthly WET testing for majors (>1 MGD) and quarterly for minors (< 1 MGD).** The rationale for this is that majors, given such factors as type, size and variability of the discharge, as well as receiving waters, are generally expected to cause more receiving water impacts than minors. However, a group of minors clustered together could have the same effect as a major. When establishing monitoring frequency for a given facility, the permit writer should consider all available information, and not rely only upon the “major” or “minor” classification.

3.6 Sample Collection

3.6.1 Effluent Sampling

Effluents are usually collected as flow-proportional or time-weighted composite samples, except in instances where the residence time in the treatment plant is very short and the purpose of the sampling is to detect peaks (spikes) in toxicity. **The sampling site should be after the last treatment process (including disinfection and dechlorination) and at a location in the discharge stream as close to the actual discharge point as feasible.** There may be no removal of chlorine or any other constituent by chemical or physical means prior to testing without specific approval from the Permitting Authority. See EPA test method manuals (USEPA 2002a, 2002b, 2002c), Section 8, for discussion on selection of sample types and discussion of sample techniques and equipment. Composite samples should be chilled to the specified temperature in the test method manuals as grab sample is being collected. Grab samples should be chilled immediately following collection.

As recommended in the test method manuals, EPA has not modified the default maximum 36 hour sample holding time (up to 72 hours with Permitting Authority approval), which must be met for the first use of the sample. However, EPA has provided additional clarification and additional flexibility for use of samples for test renewals when the samples meet the initial sample holding times for first use. Sample holding times apply to “first use of the sample,” and samples may be used for renewal at 24, 48, and/or 72 hours after first use. The test method manuals also now provide additional flexibility when shipment of renewal samples is delayed during an ongoing test. If shipping problems (e.g. the unsuccessful Saturday delivery) are encountered with renewal samples after a test has been initiated, the Permitting Authority may allow the continued use of the most recently used sample for test renewal. EPA also clarified that sample collection on days 1, 3, and 5 is the recommended (not required) sample collection scheme. A minimum of three samples are required for seven-day chronic tests, but variations in the sampling scheme (i.e., the days on which new samples are collected) are also allowed.

3.6.2 Sample Collection

Grab samples should be collected beneath the surface in chemically clean, pre-labeled plastic or glass sample containers depending on the physical-chemical properties of the suspected target contaminants. For example, polar constituents and metals can be collected in plastic containers, while non-polar (hydrophilic) constituents such as pesticides must be collected in glass

containers. The container must be filled without head space to avoid loss of volatile constituents.

Composite samples are typically collected using refrigerated programmable electronic samplers that deliver a selected volume of sample to a collection container at predetermined times. Steps must be taken to assure that all collection system components are clean and free from contamination prior to use.

3.6.3 Sample Transport and Storage

Samples should be immediately placed in ice chests and covered with wet ice to assure that samples arrive at the test lab at the recommended range of 0–6 °C. The single allowable exception is when a grab sample is delivered to the test laboratory within 4 hours of collection. Samples must be stored in the dark at 0-6 °C until tested within 36 hours. Note that the composite sample holding time begins when the last volume in the 24-hour sample is collected.

3.7 Dilution Water

3.7.1 Selection of Dilution Water

The use of dilution water is an important part of toxicity testing. Dilution water may be either standard laboratory water and/or receiving water. The type of dilution water used in effluent toxicity tests will depend largely on the objectives of the test.

- If the objective of the test is to estimate the absolute acute or chronic toxicity of the effluent, which is the primary objective of NPDES permit-related toxicity testing, standard laboratory dilution water as defined in each test method is used.
- If the objective of the test is to estimate the toxicity of the effluent in uncontaminated receiving water, the test may be conducted using dilution water consisting of a single grab sample of receiving water (if non-toxic), collected either upstream and outside the influence of the outfall, or with other uncontaminated natural water (ground or surface) or standard dilution water having approximately the same characteristics (hardness and/or salinity) as the receiving water.
- If the objective of the test is to determine the additive or mitigating effects of the discharge on already contaminated receiving water, the test is performed using dilution water consisting of receiving water collected immediately upstream or outside the influence of the outfall.

Note: If the test organisms have been cultured in water which is different from the test dilution water, a second control, using culture water, should be included in the test.

3.7.2 Criteria for Acceptable Dilution Water

Acceptable dilution water for WET testing has the following properties:

- appropriate for the objectives of the test;
- supports adequate performance of the test organisms with respect to survival, growth, reproduction, or other responses that may be measured in the test (i.e., consistently achieves test acceptability criteria for control responses);
- consistent in quality; and
- does not contain contaminants that could produce toxicity.

In the test method manuals (USEPA 1995a, 2002a, 2002b, 2002c), Section 7 describes the types of dilution water that may be used for WET testing depending upon the objectives of the test.

3.7.3 Selection of Dilution Series

The selection of a dilution series (number and spacing of test concentrations) for WET tests is important in producing reliable and precise results. This is most obvious for effect concentrations such as NOEC and lowest-observable-effect-concentration (LOEC) values generated by hypothesis testing. These values are by definition limited to one of the effluent concentrations selected for the test. The precision of these values also is determined by the distance from the NOEC or LOEC to the next highest or lowest effluent concentration.

The test method manuals (USEPA 1995a, 2002a, 2002b, 2002c) suggest, but do not require, a dilution series of 6.25%, 12.5%, 25%, 50%, and 100% effluent for most effluents. This dilution series should be used as a default when little information is known about the effluent being tested and when initial range finding indicates that the effect concentration of interest is within the 6.25% to 100% effluent range. In many situations, a more appropriate dilution series can be selected based on experience from repeated testing of a given effluent. The WET test method manuals do recommend a dilution factor of 0.5 for preparing test concentrations. This recommendation does not fix the dilution factor, but is provided to establish a lower limit on the dilution factor. The use of dilution factors greater than 0.5 is encouraged when historical testing indicates that an effluent is relatively consistent and effect concentrations generally fall within a given range.

For effluent dominated waters, using a standard dilution series of 6.25%, 12.5%, 25%, 50%, and 100% effluent, a measured NOEC value of 50% indicates that the transition from no observable effects to observable effects occurs somewhere between 50% and 100% effluent concentration (the NOEC-LOEC interval). **Therefore, the following dilution series is recommended for effluent dominated (i.e., low dilution) waters: 12.5%, 25%, 50%, 62.5%, and 100%.**

References

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USEPA. 2002a. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fifth Edition. Office of Water, Washington, DC. EPA/821/R-02/012.

USEPA. 2002b. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Fourth Edition. Office of Water, Washington, DC. EPA/821/R-02/013.

USEPA. 2002c. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. Third Edition. Office of Water, Washington, DC. EPA/821/R-02/014.

Attachment 3-1. Acute Test Methods

| <i>Species Category</i> | <i>Receiving Water Type</i> | <i>Species</i> | <i>Typical Toxicants</i> | <i>Salinity Range of Effluent Dilutions</i> |
|-------------------------|-----------------------------|---|--------------------------|---|
| Fish | Freshwater | Fathead minnow, <i>Pimephales promelas</i> | ammonia, chlorine | 1-6‰ |
| | | Rainbow trout, <i>Oncorhynchus mykiss</i> | ammonia, chlorine | 1-2‰ |
| | Marine | Silverside, <i>Menidia beryllina</i> | ammonia, chlorine | 1-36‰ Note: Can be used for end of pipe testing, if the effluent is \geq 5‰ |
| | | Topsmelt, <i>Atherinops affinis</i> | ammonia, chlorine | 5-36‰ Note: Can be used for end of pipe testing, if the effluent is \geq 5‰ |
| Invertebrate | Freshwater | Water flea, <i>Ceriodaphnia dubia</i> | pesticides | 1-3‰ |
| | | Water flea, <i>Daphnia pulex</i> and <i>Daphnia magna</i> | pesticides | 1-6‰ |
| | Marine | Atlantic mysid, <i>Mysidopsis bahia</i> | metals | 15- 36‰ |
| | | Pacific mysid, <i>Holmesimysis costata</i> | metals, insecticides | 32-36‰ |

Source: USEPA. 2002a. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fifth Edition. Office of Water, Washington, DC. EPA/821/R-02/012.

Attachment 3-2. Chronic Freshwater Test Methods

| <i>Species Category</i> | <i>Species</i> | <i>Test Type</i> | <i>Endpoints</i> | <i>Type Toxicants</i> |
|-------------------------|---|-------------------------|---------------------------|----------------------------|
| Fish | Fathead minnow, <i>Pimephales promelas</i> | 7-day renewal test | survival, growth | surfactants, ammonia |
| Invertebrate | Water flea, <i>Ceriodaphnia dubia</i> | 7-day renewal test | Reproduction, survival | pesticides, surfactants |
| Plant | Green alga, <i>Selenastrum capricornutum</i> | 96-hour non- renewal | growth | metals, herbicides |

Source: USEPA. 2002b. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Fourth Edition. Office of Water, Washington, DC. EPA/ 821/R-02/013.

Attachment 3-3. Chronic East Coast and West Coast Estuarine/Marine Test Methods

| <i>Category (Method)</i> | <i>Species</i> | <i>Test Type</i> | <i>Test Endpoint</i> | <i>Type of Toxicants</i> | <i>Salinity Range of Effluent Dilutions</i> |
|--|---|-------------------|--|--------------------------------|---|
| Fish (West Coast) ^a | Topsmelt, <i>Atherinops affinis</i> | 7-day renewal | survival, growth | ammonia, chlorine, surfactants | 10-36‰ |
| Fish (East Coast) ^b | Inland silverside, <i>Menidia beryllina</i> | 7-day renewal | survival, growth | surfactants, chlorine, ammonia | 5-36‰ |
| Invertebrate (West Coast) ^a | Pacific mysid, <i>Holmesimysis costata</i> | 7-day renewal | survival, growth, fecundity | metals, insecticides | 32-36‰ |
| | Red abalone, <i>Haliotis rufescens</i> | 48-hr non-renewal | shell development | metals, surfactants | 32-36‰ |
| | Mussels, <i>Mytilus sp.</i> , Oyster, <i>Crassostrea gigas</i> | 48-hr non-renewal | larval development | metals, chlorine | 28-32‰ |
| | Purple urchin, <i>S. purpuratus</i> , Sand dollar, <i>Dendraster excentricus</i> | 48-hr non-renewal | larval development; fertilization | metals, chlorine | 32-36‰ |
| Invertebrate (East Coast) ^b | Atlantic Mysid, <i>Mysidopsis bahia</i> | 7-day renewal | survival, growth, fecundity | metals, ammonia, insecticides | 15-36‰ |
| Plant (West Coast) ^a | Giant kelp, <i>Macrocystis pyrifera</i> | 48-hr non-renewal | germination, germ-tube length (growth) | metals | 32-36‰ |

a USEPA. 1995a Short-term methods for estimating the chronic toxicity of effluents and receiving waters to West Coast marine and estuarine organisms. Office of Research and Development. Cincinnati, OH. EPA/600/R-95/136.

b USEPA. 2002c. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. Third Edition. Office of Water, Washington, DC. EPA/821/R-02/014.

Attachment 3-4. Wet Test Method Rule

The EPA administrator signed the WET methods rule on November 19, 2002, and promulgated in the FR Notice (67 Federal Register 69952 et seq., November 19, 2002) test methods in 40 CFR Part 136, which are further detailed in USEPA 2002a, 2002b, and 2002c. This FR notice provides the statutory authority, background of WET, summary of final rule, changes from proposed rule, response to major comments, statutory and executive order reviews, and references, all of which are useful to permitting authorities implementing WET. The following is a list of those method changes. This document does not include the WET rule details that are specific to conducting a specific test method (e.g., blocking by known parentage) as that is necessary for the testing laboratory to understand and conduct properly.

Summary of Final Rule

WET Method Changes

- Minor corrections and clarifications,
- Incorporation of updated method precision data,
- Requirement for “blocking” by known parentage in the *Ceriodaphnia dubia* Survival and Reproduction test,
- Specification of procedures to control pH drift that may occur during testing,
- Review procedures for the evaluation of concentrations-response relationships,
- Clarification of limitations in the generation of confidence intervals,
- Guidance on dilution series selection,
- Clarification of requirements regarding acceptable dilution waters,
- Procedures for determining and minimizing the adverse impact of pathogens in the Fathead Minnow Survival and Growth Test,
- Requirement for the use of ethylenediaminetetraacetic acid (EDTA) in the *Selenastrum capricornutum* Growth Test.

Additional Revisions to WET Test Methods

- Requirement to meet specific variability criteria when NPDES permits require sublethal WET testing endpoints expressed using hypothesis testing,
- Increases in the required minimum number of replicates for several tests,
- Clarification of required and recommended test conditions for the purposes of reviewing WET test data submitted under NPDES permits,
- Additional clarification of sample holding times,

- Clarification of requirements for reference toxicant testing and additional guidance on evaluating reference toxicant test results,
- Clarification of allowable sample holding temperatures,
- Clarification of biomass as the measured endpoint in survival and growth tests,
- Clarification of requirements for measuring total residual chlorine in WET samples,
- Modification of the test terminations criteria for the *Ceriodaphnia dubia* Survival and Reproduction Test to exclude the counting of fourth brood neonates,
- Additional minor corrections identified by commenters.

References

USEPA. 2002a. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fifth Edition. Office of Water, Washington, DC. EPA/821/R-02/012.

USEPA. 2002b. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Fourth Edition. Office of Water, Washington, DC. EPA/821/R-02/013.

USEPA. 2002c. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. Third Edition. Office of Water, Washington, DC. EPA/821/R-02/014.

CHAPTER 4. TEST REVIEW AND EVALUATION OF TEST RESULTS

4.1 Overview

This chapter is designed to provide the permit writer a background for evaluating and reviewing WET test results. The statistics used to analyze WET test results are discussed, as well as the quality assurance procedures necessary to implement a successful WET testing program. Test review is an important part of an overall quality assurance program (see Section 4 of test method manuals) and is necessary for ensuring that all test results are reported accurately. **Test review should be conducted on each test by both the testing laboratory and the Permitting Authority.**

This chapter will describe the two statistical approaches typically used to generate the toxicity test effect concentrations. Effect concentrations are concentrations of a test material (i.e., effluent, reference toxicant, receiving or stormwater) associated with the observed biological endpoints (e.g., mortality, growth) followed by data for which is analyzed using either hypothesis testing procedures or point estimate techniques. This chapter will also discuss the test review process for Permitting Authorities which includes:

- examination of the sample handling and collection,
- review of test conditions,
- review of test acceptability criteria (TAC),
- review of concentration-responses and
- evaluation of percent minimum significant differences (PMSDs; test variability)

4.2 Terms and Definitions

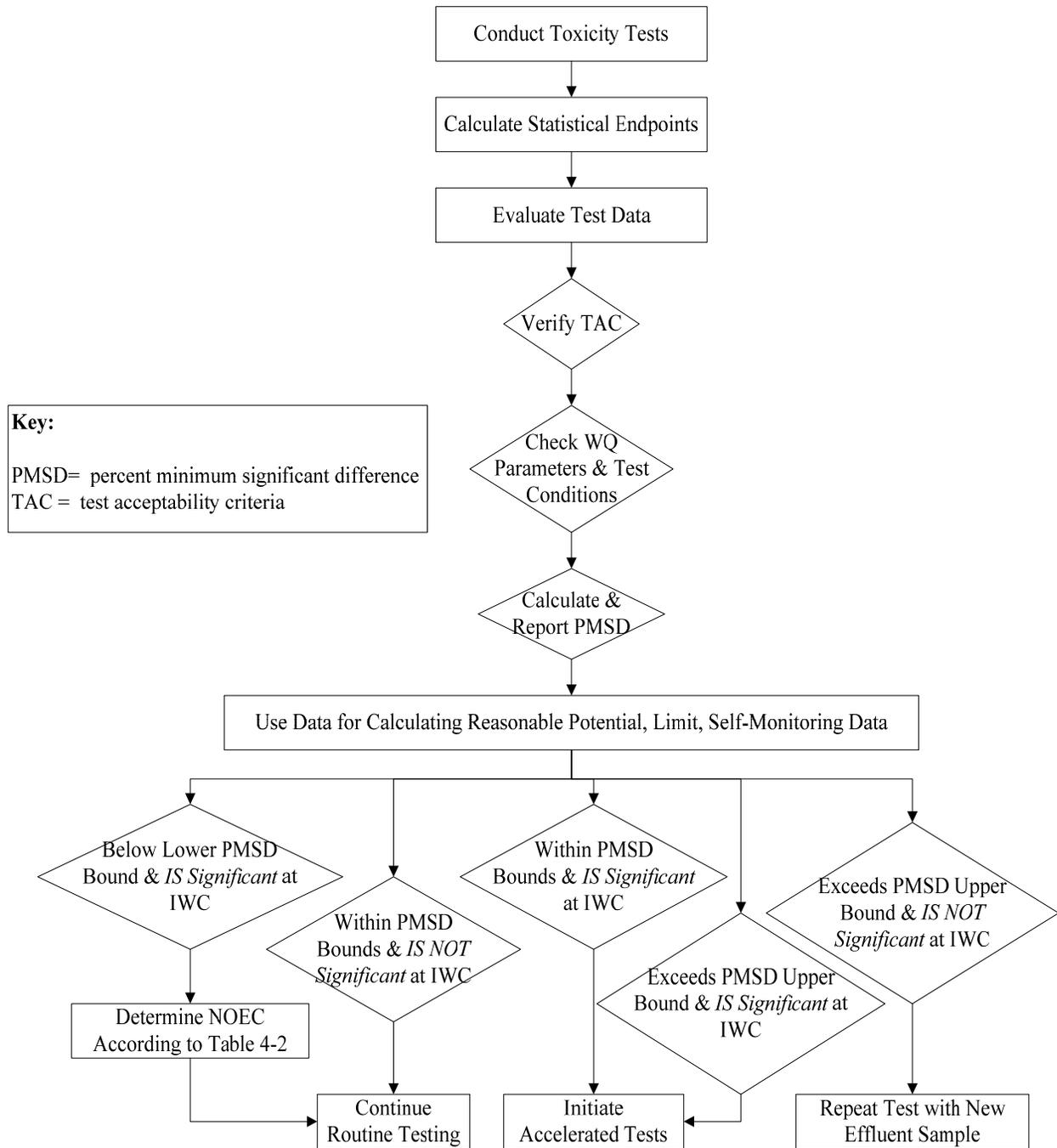
Effect concentrations are concentrations of the test material (e.g., effluent) that produce a specified degree of toxic response or effect (e.g., the LC_{50} is the concentration that produces 50% mortality). Effect concentrations are derived from the observed biological endpoints using either hypothesis testing procedures or point estimate techniques.

Hypothesis testing is a statistical procedure (e.g., Dunnett's test) for determining whether a test concentration is statistically different from the control. Endpoints determined from hypothesis testing in aquatic toxicity methods are NOECs and LOECs.

Point estimate procedures are used to determine the toxic concentration that would cause an observable adverse effect (e.g., reduced growth, expressed as EC_{25}) in a given percent of test organisms, calculated from a continuous model (e.g., Probit model). Endpoints determined from point estimates include LC_{50} for acute and EC_{25} for chronic methods. EC_{25} is a point estimate of the toxicant concentration that would cause an observable adverse effect in 25 percent of the test organisms.

A flow chart of the test review and evaluation process is shown in Figure 4-1 below.

Figure 4-1. Test Review and Evaluation Process Flow Chart



4.3 Statistical Approaches to Evaluate Multiple-Concentration Test Designs

This section will highlight some of the statistical discussions covered in the EPA acute (USEPA 2002a) and chronic test methods (USEPA 1995a, 2002b, 2002c). The objective of a toxicity test

is to estimate the highest "safe" or "no-effect concentration" of an effluent, stormwater or ambient water. When a single WET test is conducted, the observed toxicological measured biological endpoints (e.g., survival, reproduction, growth) are recorded. At the end of a test, the data are subjected to an array of statistical analyses to quantify the effects observed during the test. EPA test methods currently recommend two statistical approaches to estimate effect concentrations either hypothesis testing approaches and point estimate techniques both of which are applicable for acute and chronic testing. A good review and discussion of pros and cons of these two statistical approaches is highlighted in Fox and Denton (2002).

The statistical methods used for analyzing test data should be reviewed to verify that the recommended flowcharts for statistical analysis were followed. Any deviation from the recommended flowcharts for selection of statistical methods should be noted in the data report. **In all cases (flowchart recommended statistical approaches or flowchart deviations), the data reviewers should verify that necessary assumptions are met for the statistical approach used.**

4.3.1 Hypothesis testing procedures

Hypothesis testing procedures, such as the Dunnett's test, are used to determine the NOEC. The NOEC is the highest concentration of toxicant to which organisms are exposed in a toxicity test that causes no observable adverse effects on the test organisms (i.e., the highest concentration of toxicant in which the values for the observed responses are not statistically significantly different from the controls). Determining the NOEC does not mean, though, that there was "no toxic effect", only that no statistically significant effect was observed.

The procedures consist of an analysis of variance (ANOVA) to determine the error term, which is then used in a multiple comparison procedure for comparing each of the treatment means with the control mean, in a series of paired tests. The assumptions when using ANOVA are that the data are distributed normally when tested by Shapiro-Wilk's Test and that the group variances are homogenous when tested by Bartlett's Test. In cases where the number of replicates for each concentration is not equal, a test may be performed with Bonferroni's adjustment for multiple comparisons, instead of using Dunnett's procedure. If either of the two statistical assumptions (normality or homogeneity of variance) fails, then one of the two non parametric tests should be used. The Steel's Many-One Rank Test should be used if there are four replicates per test concentration. If the number of replicates is not equal, then Wilcoxon Rank Sum Test with Bonferroni's adjustment should be used. (See EPA test method manuals, Chapter on Data Analysis, USEPA 1995a, 2002a, 2002b, 2002c).

Hypothesis tests provide comparisons between one or more effluent concentrations and an appropriate dilution water control. The benefits of hypothesis testing include the following:

- the results can provide statistical information regarding test variability (e.g., minimum significant difference (MSD))
- the results inform the regulator of the NOEC

- the researcher can use the same statistical methods for many different test methods and endpoints;
- the researcher can test just the instream waste concentration (IWC) vs. the control (by using a standard t-test); and,
- the researcher can use routine statistical analyses (USEPA 1995a, 2002a, 2002b).

An important criticism of hypothesis tests is that they might have either poor or excessive statistical power since the majority of analyses do not constrain statistical beta error (see Attachment 4-1 for a discussion on defining false positives and false negatives). In one case, a large effect size (e.g., significant biological effect) might not be statistically significant, but in another case a small effect size (e.g., small biological effect) might be statistically significant. Another criticism of hypothesis testing is that no true dose-response relationship can be derived using the hypothesis test, since the NOEC is dependent upon the selection of the dilution series. The true effect level might lie somewhere in between the NOEC and the LOEC. For example, with an NOEC of 25% and an LOEC of 50%, the actual NOEC might lie somewhere between these values. The inability to generate precision estimates with NOECs is also a criticism.

To alleviate some of these concerns, the spacing of the dilution series should be carefully selected. Ideally the concentrations should bracket the IWC and include the IWC as one of the test concentrations. In addition, the within-test variability of individual tests should be reviewed (see discussion on PMSDs). When NPDES permits require sublethal hypothesis testing endpoints, the within-test variability must be reviewed and variability criteria must be applied (see Attachment 4-2 on defining test precision).

4.3.2 Point estimate techniques

Point estimation technique is used to determine the toxicant concentration that would cause an observable adverse effect in a given percent "p" of the organisms. For point estimates, typically the results are reported as the effective concentration (EC) or the inhibition concentration (IC). EC_p is generally used with quantal endpoints (e.g., survival or fertilization). When survival is the quantal endpoint, the EC_p is typically expressed as the LC_p (lethal concentration). The inhibition concentration, IC_p, is generally used for tests where a nonquantal continuous endpoints (e.g., length, weight, or reproduction) are measured.

Most point estimate endpoints, such as the LC, EC, or IC are derived from a mathematical model that assumes a continuous concentration-response relationship. By definition, any LC, EC, or IC value is an estimate of some amount of adverse effect. Thus the assessment of a "safe" concentration must be made from a biological standpoint rather than with a statistical test. The biologist must determine some amount of adverse effect that is deemed to be "safe," in the sense that from a practical biological viewpoint it will not affect the normal propagation of fish and other aquatic life in receiving waters.

The statistical models are highlighted in the EPA test method manuals flowchart. Probit analysis is used to estimate LC or EC values from 1 to 50 percent effect of the test organisms measuring quantal endpoints (e.g., survival, fertilization, germination, larval development). The analysis

consists of adjusting the data for mortality in the control, and then using a maximum likelihood technique to estimate the parameters of the underlying log tolerance distribution, which is assumed to have a particular shape.

Probit analysis is contingent on the assumption of a normal distribution of log tolerances. If the normality assumption is not met, and at least two partial mortalities are not obtained, Probit analysis should not be used. It is important to check the results of the Probit analysis to determine if use of this analysis is appropriate. The chi-square test for heterogeneity provides a good test of appropriateness of the analysis. The computer program checks the chi-square statistic calculated for the data set against the tabular value, and provides an error message if the calculated value exceeds the tabular value.

If an acute toxicity data does not fit the Probit model, then LC_{50} may be estimated by Spearman-Kärber method or the trimmed Spearman-Kärber for acute toxicity only. If a test results in 100% survival and 100% mortality in adjacent treatments (all or nothing effect), the LC_{50} may be estimated using the Graphical method. **If chronic toxicity endpoints, the Linear Interpolation method should be used when Probit analysis is not appropriate, since the effect concentration needed to be observed is less than a 25% effect.**

The Linear Interpolation method is a procedure to calculate a point estimate of the effluent or other toxicant concentration that causes a given percent reduction of the test organisms (e.g., $\leq 25\%$ effect) in continuous endpoints (e.g., reproduction or growth). Use of the Linear Interpolation method is based on the assumptions:

- the responses are monotonically non-increasing (the mean response for each higher concentration is less than or equal to the mean response for the previous concentration)
- the responses follow a piece-wise linear response function, and
- the responses are from a random, independent, and representative sample of test data.

4.3.3 Point estimate confidence intervals

EPA acknowledges that some point estimation techniques do not generate confidence intervals, but this does not preclude the use of point estimates in compliance determinations. Confidence intervals are not currently reported in the Permit Compliance System (the national database tracking compliance with NPDES permits) nor are they used in compliance determinations. Compliance with permit requirements is based on the point estimate itself and not confidence intervals surrounding the estimate. This approach is no different in WET testing than in chemical testing, where compliance is also based on the analytical result itself.

4.4 Statistical Approaches to Evaluate 2 Sample-Concentration Test Designs

Often in ambient and stormwater toxicity testing design, a laboratory control and a single concentration (e.g., 100% stormwater or ambient water) is tested. In these pass/fail tests, the objective is to determine if the survival in the single treatment (e.g., effluent, ambient, stormwater) is significantly different from the control survival. In this testing design the determination of pass or fail from a single aqueous concentration is ascertained with a standard t-

test (USEPA 2002a, see figure 12 of the acute toxicity “Data analysis section” or in the chronic test method manuals, the appendix on “Single-concentration toxicity test - comparison of control with 100% effluent or receiving water”). First, after the data have been transformed, a test of the assumption of normality is conducted with the Shapiro Wilk's test. The F test for equality of variances is used to test the homogeneity of variance assumption. To perform the t-test, obtain values for the means and variances and use the one-tailed test at the 0.05 level of significance. If the calculated t is greater than the critical t, the conclusion is that the survival in the 100% ambient or stormwater test concentration is significantly less than the survival in the control (i.e., the sample is toxic). **EPA Regions 9 and 10 recommend that the statistical significance (i.e., pass/fail) of a two-sample test design be determined with either a modified t-test (if homogeneity of variance is not achieved) or a standard t-test (if homogeneity of variance is achieved).**

4.5 Test Review Considerations

Test review is an important part of an overall quality assurance program (see QA/QC chapter in the test methods manual). It is necessary to ensure that all test results are reported accurately. Test review should be conducted on each test by both the testing laboratory and the Permitting Authority. The components of test review include:

- review of sample handling and collection,
- review of test acceptability criteria,
- review of test conditions,
- review of concentration-response relationships,
- review of reference toxicant tests, and
- review of test variability (i.e., examination of PMSD values).

4.6 Review of Sampling and Handling

The collection and handling of samples are reviewed to verify that the sampling and handling procedures (see Section 8 of the test method manuals) were followed. Chain-of-custody forms are reviewed to verify that samples were tested within allowable sample holding times. Any deviations from the procedures given in Section 8 of the test method manuals should be documented and described in the data report.

4.7 Review of Test Acceptability Criteria

Test acceptability criteria (TAC) set minimum requirements for performing toxicity tests. These minimum requirements are clearly identified in the test method manuals. Both effluent and reference toxicant tests must meet these TAC. As should be stated in the NPDES permit, if a test fails either the effluent or reference toxicant TAC, then the permittee must repeat the test as soon as possible. For example, the control for both the effluent test and the reference toxicant test must achieve 80% or greater survival and produce an average of 15 young per female for the chronic water flea survival and reproduction test method. These requirements are stated in the

summary of test conditions and test acceptability criteria table in each chapter for the test method manuals. Note, for each test method there is a table in the manuals titled, “Summary of test conditions and test acceptability criteria” for each test species. The Permitting Authority should be familiar with these summary test conditions and TAC.

Test data are reviewed to verify that TAC requirements for a valid test have been met. **Any test not meeting the minimum TAC is considered invalid. All invalid tests must be repeated with a newly collected sample, as soon as possible, but no later than 14 days.**

4.8 Review of Test Conditions

Test conditions are reviewed and compared to the specifications listed in the summary of test condition tables provided for each method. Physical and chemical measurements taken during the test (e.g., temperature, pH, and dissolved oxygen) also are reviewed and compared to specified ranges. Any deviations from specifications should be documented and described in the data report.

The summary of test condition tables presented for each method identifies test conditions as required or recommended. For WET test data submitted under NPDES permits, all “required” test conditions must be met or the test is considered invalid and must be repeated with a newly collected sample. Deviations from “recommended” test conditions must be evaluated on a case-by-case basis to determine the validity of test results. Deviations from recommended test conditions may or may not invalidate a test result depending on the degree of the departure and the objective of the test. The reviewer should consider the degree of the deviation and the potential or observed impact of the deviation on the test result before rejecting or accepting a test result as valid. For example, if dissolved oxygen is measured below 4.0 mg/L in one test chamber, the reviewer should consider whether any observed mortality in that test chamber corresponded with the drop in dissolved oxygen.

Also, an individual test may be conditionally acceptable if temperature, dissolved oxygen and other specified conditions fall outside specifications, depending on the degree of the departure and the objectives of the tests (see test conditions and test acceptability criteria specified for each test method). The acceptability of the test will depend on the experience and professional judgment of the laboratory investigator and the Permitting Authority (see section on data evaluation in the test method manuals). Whereas slight deviations in test conditions may not invalidate an individual test result, test condition deviations that continue to occur frequently in a given laboratory may indicate the need for improved quality control in that laboratory.

4.9 Review of Reference Toxicants

The purpose of generating reference toxicant data is (1) to assess the health and sensitivity of test organisms over time, and 2) to document and demonstrate initially and ongoing acceptable laboratory performance. Satisfactory laboratory performance is demonstrated by performing at least one acceptable test per month with a reference toxicant for each toxicity test method conducted in the laboratory during a month. **For a given test method, successive tests must be performed with the same reference toxicant, at the same concentrations in the same dilution water, using the same data analysis methods.** Regardless of the source of test

organisms (in-house cultures or purchased from external suppliers), the testing laboratory must perform at least one acceptable reference toxicant test per month for each type of toxicity test method conducted in that month. If a test method is conducted only monthly, or less frequently, a reference toxicant test must be performed concurrently with each effluent toxicity test. This requirement will document ongoing laboratory performance and assess organism sensitivity and consistency when organisms are cultured in-house. When organisms are obtained from external suppliers, concurrent reference toxicant test must be performed with each effluent sample, unless the test organism supplier provides control chart data from at least the last five months of reference toxicant testing. This requirement assesses organism sensitivity and health when organisms are obtained from external vendors.

The test review of a given effluent or receiving water should include review of the associated reference toxicant test and current control chart. The test reviewer should verify that a quality control reference toxicant test was conducted according to the specified frequency required by the Permitting Authority or recommended by the method. The TAC, test conditions, concentration-response relationship, and test sensitivity of the reference toxicant tests are reviewed to verify that the reference toxicant tests conducted were valid. The results of the reference toxicant tests are then plotted on a control chart and compared to the current control chart limits. Reference toxicant tests that fall outside of the recommended control chart limits are evaluated to determine the validity of associated effluent and receiving water tests (see chapter on quality assurance of test method manuals). Reference toxicant tests should not be used as a *de facto* criterion for rejection of individual effluent or receiving water tests. An out of control reference toxicant test does not necessarily invalidate the associated test results. The reviewer should consider the degree to which the reference toxicant test fell outside of the control chart limits, the width of the limits, the direction of the deviation (toward increasing test organism sensitivity or toward decreasing test organism sensitivity), the test conditions of both the effluent and the reference toxicant tests, and the objective of the test. More frequent and/or concurrent reference toxicant testing may be advantageous if recent problems (e.g. invalid tests, reference toxicant test results outside of control chart limits, reduced health of organism cultures, or increased within-test variability) have been identified in testing.

4.10 Review of Concentration-Response Relationships

In toxicology, it is conventional to plot the data in the form of a curve relating the dose of the chemical to cumulative percentage of test organism demonstrating a response such as death or reduced growth. Typically, as the toxicant increases in concentration a greater biological response is measured (e.g., increase in lethality, or decrease in growth or reproduction).

The concept of a concentration-response or a dose-response relationship is “the most fundamental and pervasive one in toxicology” (Casarett and Doull 1975). Note, a concentration-response relationship is analogous to the dose-response relationship employed in mammalian toxicity testing. This concept assumes that there is a causal relationship between the dose of a toxicant (or concentration for toxicants in solution) and a measured response. A response may be any measurable biochemical or biological parameter that is correlated with exposure to the toxicant. The classical concentration-response relationship is depicted as a sigmoidal-shaped curve, however, the particular shape of the concentration-response curve may differ for each

coupled toxicant and response pair. In general, more severe responses (such as acute effects) occur at higher concentrations of the toxicant, and less severe responses (such as chronic effects) occur at lower concentrations. A single toxicant also may produce multiple responses, each characterized by a concentration-response relationship. A corollary of the concentration-response concept is that every toxicant should exhibit a concentration-response relationship, given that the appropriate response is measured and given that the concentration range evaluated is appropriate. Use of this concept can be helpful in determining whether an effluent is toxic and in identifying anomalous test results.

The concentration-response relationship generated for each multi-concentration test must be reviewed to ensure that calculated test results are interpreted appropriately. EPA's document (USEPA 2000a) provides guidance on evaluating concentration-response relationships to assist in determining the validity of WET test results. **All WET test results (from multi-concentration tests) reported under the NPDES program should be reviewed and reported according to EPA guidance on the evaluation of concentration-response relationships (USEPA 2000a).** The EPA guidance (2000a) provides review steps for 10 different concentration-response patterns that may be encountered in WET test data. Based on the review, the guidance provides one of three determinations:

- that calculated effect concentrations are reliable and should be reported,
- that calculated effect concentrations are anomalous and should be explained, or
- that the test was inconclusive and should be repeated with a newly collected sample.

It should be noted that the determination of a valid concentration-response relationship is not always clear cut. Data from some tests may suggest consultation with professional toxicologists and/or regulatory officials. Tests that exhibit unexpected concentration-response relationships also may indicate a need for further investigation and possible retesting.

4.11 Review of Test Variability

When NPDES permits require sublethal hypothesis testing endpoints for the chronic test methods USEPA 2002b, 2002c (e.g., growth or reproduction NOECs and LOECs), the within-test variability must be reviewed and variability criteria must be applied as described in this section. When the methods are used for non-regulatory purposes, the variability criteria are recommended but are not required, and their use (or the use of alternative variability criteria) may depend upon the intended uses of the test results and the requirements of any applicable data quality objectives and quality assurance plan. Good test precision or low within-test variability is a general measure of test quality (see Attachment 4-2). Note: the Permitting Authority may always be more stringent than specified as above.

To measure test variability, calculate the PMSD achieved in the test. The PMSD is the smallest percentage decrease in growth or reproduction from the control that could be determined as statistically significant in the test. The PMSD is calculated as 100 times the MSD divided by the control mean. The MSD equation is shown in Attachment 4-1. PMSD may be calculated

legitimately as a descriptive statistic for within-test variability, even when the hypothesis test is conducted using a non-parametric method. The PMSD bounds were based on a representative set of tests, including tests for which a non-parametric method was required for determining the NOEC or LOEC. The hypothesis testing procedures to determine test results should follow the statistical flow charts provided for each method. That is, when test data fail to meet assumptions of normality or heterogeneity of variance, a nonparametric method (determined following the statistical flowchart for the method) should be used to calculate test results, but the PMSD may be calculated as described above (using parametric methods) to provide a measure of test variability.

Compare the PMSD measured in the test with the upper PMSD bound variability criterion listed in Table 4-1. When the test PMSD exceeds the upper bound, the variability among replicates is unusually large for the test method. Such a test should be considered insufficiently sensitive to detect toxic effects on growth or reproduction of substantial magnitude. A finding of toxicity at a particular concentration may be regarded as trustworthy, but a finding of "no toxicity" or "no statistically significant toxicity" at a particular concentration should not be regarded as a reliable indication that there is no substantial toxic effect on growth or reproduction at that concentration.

If the PMSD measured for the test is less than or equal to the upper PMSD bound variability criterion in Table 4-1, then the test's variability measure lies within normal bounds and the effect concentration estimate (e.g., NOEC or LOEC) would normally be accepted unless other test review steps raise serious doubts about its validity. If the PMSD measured for the test exceeds the upper PMSD bound variability criterion in Table 4-1, then one of the following two cases applies.

If toxicity is found at the permitted receiving water concentration (RWC) based upon the value of the effect concentration estimate (NOEC or LOEC), then the test shall be accepted and the effect concentration estimate may be reported, unless other test review steps raise serious doubts about its validity.

If toxicity is not found at the permitted RWC based upon the value of the effect concentration estimate (NOEC or LOEC) and the PMSD measured for the test exceeds the upper PMSD bound, then the test shall not be accepted, and a new test must be conducted promptly on a newly collected sample (preferably within 14 days).

To avoid penalizing laboratories that achieve unusually high precision, lower PMSD bounds shall also be applied when a hypothesis test result (e.g., NOEC or LOEC) is reported. Lower PMSD bounds, which are based on the 10th percentiles of national PMSD data, are presented in Table 4-1. The 10th percentile PMSD represents a practical limit to the sensitivity of the test method because few laboratories are able to achieve such precision on a regular basis and most do not achieve it even occasionally. In determining hypothesis test results (e.g., NOEC or LOEC), a test concentration shall not be considered toxic (i.e., significantly different from the control) if the relative difference from the control is less than the lower PMSD bounds in Table 4-1.

If the permit specifies that self-monitoring data are to be generated using hypothesis testing approaches, then the analyst should report the NOEC as follows. Find the smallest concentration for which (a) the treatment mean differs significantly from the control mean and (b) the relative difference (see example below) is not smaller than the 10th percentile in Table 4-2. Therefore, the NOEC is the next smaller test concentration. In other words, concentrations having a very small relative difference from the control (smaller than the lower PMSD bound) would be treated as if they do not differ significantly from control (even if they do so), for the purpose of determining the NOEC.

Table 4-1 Variability Criteria (Upper and Lower PMSD Bounds) for Sublethal Hypothesis Testing Endpoints Submitted Under NPDES Permits^a

| <i>Test Method</i> | <i>Endpoint</i> | <i>Lower PMSD Bound</i> | <i>Upper PMSD Bound</i> |
|---|-----------------|-------------------------|-------------------------|
| Method 1000.0 Fathead Minnow Larval Survival and Growth Test ^b | Growth | 12 | 30 |
| Method 1002.0 <i>Ceriodaphnia dubia</i> Survival and Reproduction Test ^b | Reproduction | 13 | 47 |
| Method 1003.0 <i>Selenastrum capricornutum</i> Growth Test ^b | Growth | 9.1 | 29 |
| Method 1006.0, Inland Silverside Larval Survival and Growth Test ^c | Growth | 11 | 28 |
| Method 1007.0, <i>Mysidopsis bahia</i> Survival, Growth and Fecundity Test ^c | Growth | 11 | 37 |

a Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA 2001a; USEPA 2001b)

b USEPA 2002b

c USEPA 2002c

Table 4-2 illustrates the application of the lower PMSD bound for the reproduction endpoint of a *Ceriodaphnia* chronic test. In this example, the effluent test's PMSD was 9.9, smaller than the 10th percentile value of 13 (USEPA 2002b). The IWC concentration differed significantly from the control. The test falls under outcome number 5, a significant but very small difference at the IWC. The first step is to calculate the relative differences from control (Table 4-1) as [(control mean - treatment mean) divided by (control mean)] × 100. The next step is to determine which relative differences exceed the PMSD lower bound, 13 in this case (see the 3rd column of Table 4-1). Finally, the NOEC is determined as described above. The NOEC is 12.5 percent effluent for this example.

Table 4-2 Example of Applying the Lower PMSD Bound for the Chronic *Ceriodaphnia dubia* Test with the Reproduction Endpoint

| <i>Concentration</i> | <i>Reproduction</i> | <i>Relative Difference</i> | <i>Does Relative Difference</i> |
|----------------------|---------------------|----------------------------|---------------------------------|
|----------------------|---------------------|----------------------------|---------------------------------|

| <i>(Percent Effluent)</i> | <i>(Mean Of Ten Replicates)</i> | <i>From Control</i> | <i>Exceed 13?</i> |
|---------------------------|---------------------------------|---------------------|-------------------|
| 100% | 5.08 * | 82 | Yes |
| 50% | 12.4 * | 56 | Yes |
| 25% | 23.4 * | 17 | Yes |
| IWC = 12.5% | 25.3 * | 10 | No |
| 6.25% | 26.1 | 7.4 | No |
| Control | 28.2 | 0 | No |

* Differs statistically from the control as determined by $MSD = 2.8$ neonates. Thus, treatment means that are less than $28.2 - 2.8 = 25.4$ would be statistically significant. These correspond to relative differences greater than 100 $(2.8 / 28.2) = 9.9$ percent.

NOTE: The lower PMSD bound for this method and endpoint is 13. In this example, the statistically determined NOEC is 6.25 percent effluent using the test's (very small) PMSD of 9.9. Therefore, the reported NOEC should be 12.5 percent effluent after applying the lower PMSD bound.

To assist in reviewing within-test variability, EPA recommends maintaining control charts of PMSDs calculated for successive effluent tests (USEPA 2000b). A control chart of PMSD values characterizes the range of variability observed within a given laboratory, and allows comparison of individual test PMSDs with the laboratory's typical range of variability. Control charts of other variability and test performance measures, such as the MSD, standard deviation or CV of control responses, or average control response, also may be useful for reviewing tests and minimizing variability.

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Attachment 4-1. Background Statistics: Hypothesis Testing

One objective of a toxicity test is to determine if the toxicological measurement endpoint in one treatment (an effluent dilution) differs from the endpoint in another treatment (a control). The null hypothesis (H_0) is that there is no difference between the two treatments (i.e., the effluent or ambient water is not toxic). The alternative hypothesis (H_a) is that there is a statistical difference between the control and the treatment (i.e., the effluent or ambient water is toxic). The table below presents the possible outcomes and decisions that can be reached in hypothesis testing.

Comparison of Type I and Type II Statistical Decision Errors^a

| <i>Decision</i> | <i>True Condition Treatment = Control</i> | <i>True Condition Treatment > Control</i> |
|---------------------|---|--|
| Treatment = Control | Correct Decision (1 - α) | False Negative Type II error (β) |
| Treatment > Control | False Positive Type I error (α) | Correct Decision (1 - β) (power) |

a The alpha, α , represents the probability of a type I statistical error (i.e., false positive) and beta, β , is the probability of making a type II statistical error (i.e., false negative).

Hypothesis tests can be designed to control (minimize) the chances of making incorrect decisions. A Type I error (alpha, α) results in the false conclusion that an effluent is toxic when the effluent is not toxic. A Type II error (beta, β) results in the false conclusion that the effluent is not toxic, when the effluent is actually toxic. Traditionally, acceptable values for α have ranged from 1 to 10% with 5% used most commonly. This choice should depend upon the consequences of making a Type I error. Historically, having chosen α , environmental researchers have ignored β and the associated power of the test (1 - β). Power is the probability of correctly detecting a true toxic effect (i.e., declaring an effluent toxic when in fact it is toxic).

α and β are dependent on each other (as α increases, β decreases), assuming that sample size (number of treatments, number of replicates), the amount of difference to detect and the variability are held fixed. Increasing α level of a statistical test increases the power of the test, if all other factors are held constant. Selection of the appropriate α level of a test is a function of the costs associated with making Type I errors. For a given α , β decreases (power increases) as the sample size increases and the variance decreases. The desired power of the statistical analysis should be considered in the study plan development.

The use of the statistical tests can protect regulators from concluding the effluent is toxic when it is not. The statistical tests can control the risk of a Type I error, which is important when the results are shown to be toxic. Without a power analysis, the assurance that a sample is not toxic is questioned, and the possibility exists that a false negative has occurred.

Although the EPA test method manuals (USEPA 1995a, 2002a, 2002b, 2002c) require an α of 0.05 (5%), a level of β is not specified. If β is not specified, then we might not detect toxicity when, in fact, an effluent is toxic. Without specifying the level of β , there is little incentive for a

testing laboratory to produce precise test results (i.e., limit test variability). Therefore, EPA requires the review of percent minimum significant difference (PMSDs) by testing laboratories and Permitting Authorities. Note, the EPA (2000b) discussed using an α level of 0.01 under specific conditions. **However, in the final WET methods rule, EPA recommended that only an α rate of 0.05 is to be used.**

Test sensitivity and minimum significant difference

The MSD is a measure of the within-test variability and represents the amount of difference from the control that can be detected statistically.

The following formula is used to calculate MSD (described by USEPA 1995a, 2002a, 2002b, 2002c):

$$MSD = d s_w \sqrt{(1/n_1) + (1/n)}$$

Where

d = critical value for the Dunnett's procedure.

s_w = the square root of the within mean square error (MSE).

n_1 = number of experimental units in the control treatment.

n = the number of experimental units per treatment, assuming an equal number at all other treatment.

The MSD is often expressed as a percentage of the toxicological endpoint in the control response (%MSD = 100 x MSD/control mean). The MSD, though, incorporates alpha (type I error) and experimental design (number replicates, treatments), in addition to an estimate of test variability (i.e., MSE). Distributions of the MSD values of multiple tests for a specific reference toxicant and test method can be used to determine the level of sensitivity that can be achieved by a certain percentage of the tests. The MSD should increase as the MSE increases when the number of replicates and treatments and alpha are constant.

To summarize, the sensitivity of the toxicity test will depend in part on the number of replicates of experimental units per treatment, the alpha and beta (provided beta is used to determine the effect size desired), and the variability (e.g., MSE). The power to detect differences increases (i.e., MSD decreases) as the variability decreases and the effect size increases. These discussions demonstrate the importance of measuring test sensitivity and setting the power for toxicity test methods. The issue of false positive and false negative errors needs to be evaluated along with test power and sensitivity to decide the appropriate testing frequency for compliance purposes.

References

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Attachment 4-2. Importance of Quality Control Procedures and Defining Test Precision

Quality Control Procedures

This quality assurance (QA) section will only highlight the general discussions from the test method manuals, such as the use of reference toxicants, and defining test precision with reference toxicants. Development and maintenance of a toxicity test laboratory QA program requires an ongoing commitment by laboratory management. As stated in the toxicity test method manuals each toxicity test laboratory should:

- Appoint a QA officer with the responsibility and authority to develop and maintain a QA program;
- Prepare a quality assurance plan with stated data quality objectives;
- Prepare written descriptions of laboratory standard operating procedures for culturing, toxicity testing, instrument calibration, sample chain-of-custody procedures, laboratory sample tracking system, glassware cleaning, etc.; and
- Provide an adequate, qualified technical staff for culturing and testing organisms, and suitable space and equipment to assure reliable data.

The EPA acute and chronic toxicity test method manuals each contain a chapter on QA procedures. Topics covered in the chapter include handling of effluents and receiving waters, quality of test organisms, food quality, calibration and standardization, reference toxicant testing, and record keeping. Of particular importance is the requirement to conduct satisfactory reference toxicant tests in conjunction with effluent or ambient water tests. Reference toxicant tests confirm the sensitivity of the test organisms and demonstrate a laboratory's ability to obtain consistent results with WET test methods. Appropriate laboratory practices are essential in obtaining quality test data. QA practices for toxicity tests include all aspects of the test that affect the quality of the data such as:

- Effluent/ambient water sampling and handling
- source and condition of the test organisms
- condition of equipment
- test conditions
- instrument calibration
- replication
- use of reference toxicants
- record keeping
- data evaluation

Test Precision

Precision is a measure of test consistency or repeatability both within a laboratory (intralaboratory) and among several laboratories (interlaboratory). Precision is quantified by a variety of measures including the CV of point estimates (e.g., LC₅₀ for acute endpoints and EC/IC₂₅ for chronic endpoints) from multiple tests conducted with the same test method and reference toxicant. EPA (2000b) analysis demonstrated and concluded that comparisons of WET method precision with method precision for analytes commonly limited in NPDES permits clearly demonstrate that the variability of the WET methods is within the range of variability experienced in other types of analyses. In addition, several researchers (Grothe et al. 1996, Burton et al. 1996, DeGraeve et al. 1998) noted that method performance improves when prescribed methods are followed closely by experienced analysts.

Test results will depend upon the species tested, source of the test organisms, water quality parameters (e.g., use of temperature as specified in the test method manuals) and food and dilution water quality. The repeatability or precision of toxicity tests is also a function of the number of test organisms used in each test concentration.

Factors which can affect test success and precision include:

- the experience and skill of the laboratory analyst
- test organism condition and sensitivity;
- dilution water quality;
- chemical and physical water quality parameters (e.g., temperature, DO); and
- quality and quantity of food provided.

The EPA TSD (USEPA 1991a) contains the summarized intra- and interlaboratory precision data for the freshwater and east coast marine test methods. Grothe and Kimerle (1985), Rue et al. (1988), Morrison et al. (1989), Grothe et al. (1990) discussed the precision of select toxicity test methodologies and found them to be comparable to commonly accepted chemical analytical methodologies. Grothe and Kimerle (1985) concluded that the reproducibility of the *D. magna* toxicity test was as good as, if not better than, commonly accepted analytical methods. They postulated that one of the main reasons those low CVs were obtained in their study was because the method was clearly defined and uniformly followed by all laboratories. Anderson (1991) and the Biomonitoring Science Advisory Board (BSAB 1994) have examined the precision of test methods used on the west coast and generally found the tests had very good precision. Denton et al. (1992) also found the overall interlaboratory CVs for four west coast marine species ranged from 11.5% for *Haliotis rufescens*, the red abalone larval development test to 38.7% for *Strongylocentrotus purpuratus*, the purple urchin fertilization test. The BSAB report (1994) also concluded that toxicity tests should not be gauged by variability alone. The report also concluded that other factors at least as important as precision included sensitivity, accuracy and ecological relevance. WET testing can be improved most usefully by decreasing intra-test variability.

Specific factors that affect variability in WET analyses have been described in several papers (Burton et al. 1996; Ausley 1996; Erickson et al. 1998; Davis et al. 1998). The most important initial consideration in developing precise data is a laboratory's experience and success in performing a specific analysis. Most critical reviews of WET data precision emphasize this initial consideration. Experienced professionals most likely will be able to develop the most consistent and reliable information and can interpret anomalous conditions in the testing or results.

An additional factor in considering WET test method variability is whether the prescribed methods (e.g., see chapter on test review of the EPA test method manuals) are being followed appropriately. Both the Permitting Authority and permittee should strive to ensure that such practices are in place for any program developing WET data, whether by national laboratory accreditation, State regulatory certification, direct permittee oversight, or specific contractual agreement with the laboratory.

When the variability of WET analyses is viewed in the context of the NPDES program, these techniques produce data that are as precise as those from chemical analyses (USEPA 2000b). As with any other analytical system, lack of experience in performing the analyses, lack of adherence to prescribed QA practices or failure to follow good laboratory practices will reduce the precision of the results. Studies of these factors by independent researchers from both the regulatory and regulated communities support these conclusions. While examples of poor-quality, highly variable results from chemical analyses have also been publicized, these results are frequently influenced by the shortcomings mentioned above. **Permittees who must generate and use WET data should become well-educated in data quality interpretation, and permittees should require that QC practices be followed by laboratories generating the data.** See "Conclusions and Guidance to Laboratories, Permittees, and Regulatory Authorities" chapter of EPA (2000b) for more detailed discussion and approaches to address to minimize test method variability.

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Attachment 4-3 Evaluation of Toxicity Data

Permit Review

1. Examine the test results to verify that the laboratory is using the test method and dilution series as required in the NPDES permit. The dilution series being tested should always include the receiving water concentration (RWC). Note: This may need to be performed only after a permit has been first issued.
2. Evaluate the test results against the permit requirements for WET to assess whether the limit or numeric monitoring trigger is being achieved. For example, where a 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 a WET limit or numeric monitoring trigger is expressed in terms of “% effluent at the RWC,” the value is expressed as a value that the % effluent must be at or above.

Test Review

Test review is an important part of the overall quality assurance program and is necessary for ensuring that all test results are reported accurately. Test review should be conducted on each test by both the testing laboratory and the Permitting Authority. Note, see the chapter on Test Review of the specified toxicity test methods manual.

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, does the sample meet the test initiation and renewal holding time requirements?
2. Evaluate the test results for the effluent to verify that the laboratory met the TAC as specified in the test method. See the individual “Summary of Test Conditions and TAC” section for each test method (USEPA 1995a, 2002a, 2002b, 2002c). All invalid tests must be repeated with a newly collected sample, as specified in permit.
3. Examine the “Summary of Test Conditions and TAC” section for the specific method to determine whether the required and recommended test conditions were met. Below is a single example for a required test condition and a recommended test condition that would be specific to the particular toxicity test method listed in the permit.
 - a. Did the laboratory conduct the test using the required test conditions? Some of the test conditions listed which are specified as “required” and therefore the condition must be met. For example, did the test use the required minimum number of replicates, number of test organisms, test type, etc.? All required test conditions must be met or the test is considered invalid and must be repeated with a newly collected sample.
 - b. Did the laboratory conduct the test using the recommended test conditions? Some of the listed test conditions are specified as “recommended” and therefore the

range should be obtained. For example, when the test method specifies number of test organisms per test chamber, the test condition will provide a recommended number of test organisms (e.g., 10 larvae) per test chamber. A testing laboratory can use more than the recommended number of test organisms per chamber as long as the loading capacity is maintained.

4. Examine the statistical results to verify the recommended flowcharts for statistical analysis were followed. Any deviation from the recommended flowcharts for selection of statistical methods should be noted in the data report.
5. Examine the concentration-response relationships as these must be reviewed to ensure that calculated test results are interpreted appropriately. All WET test results (from multi-concentration tests) reported under the NPDES program should be reviewed and reported according to EPA guidance on the evaluation of concentration-response relationship (USEPA 2000a).
6. Test review of a given effluent or receiving water test should include review of the associated reference toxicant test and current control chart. Were out-of-control reference toxicant test results evaluated to determine appropriate corrective action?
7. The within-test variability of individual tests should be reviewed. When NPDES permits require sublethal hypothesis testing endpoints from Methods 1000.0, 1002.0, 1003.0, 1006.0, and 1007.0 (e.g., growth or reproduction NOECs and LOECs), within-test variability must be reviewed and variability criteria must be applied as described in the Method Manuals Section on Test Review. See “Conclusions and Guidance to Laboratories, Permittees, and Regulatory Authorities” chapter of EPA (2000b) for more detailed discussion and approaches to address to minimize test method variability.

References

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CHAPTER 5. TOXICITY REDUCTION EVALUATIONS

5.1 Overview

When WET testing demonstrates that effluent toxicity exceeds the NPDES permit limit or monitoring trigger, the principal mechanism for bringing a permittee into compliance is a Toxicity Reduction Evaluation (TRE). The TRE is a methodical, stepwise and iterative process that uses information generated in each step to identify the causative toxicant(s) of WET and either remove them at the source or implement in-plant treatment to reduce their concentration(s) below toxic levels, and then confirm the reduction in effluent toxicity through WET monitoring.

Ultimately, the goal of the TRE is to achieve compliance with permit WET requirements. TREs can vary widely in complexity and expense, ranging from simply improving housekeeping procedures to conducting intensive Toxicity Identification Evaluations (TIEs). EPA and others have published extensive TRE/TIE technical guidance that is referenced at the end of this chapter. In addition, numerous TIE papers and case studies have been published, which demonstrate the efficacy of the TIE process in identifying the cause(s) of WET.

5.2 Approaches for Reducing Toxicity

Toxicity may be reduced by implementing one of two approaches within the TRE: (1) a TIE, or (2) treatability studies. The decision to pursue either the TIE or treatability approach depends on a number of site-specific and cost considerations. Generally, the TIE approach is favored because it results in control of toxics at the source rather than modifying plant operations to treat or degrade the toxicity with subsequent discharge to the environment. In practice, the TIE approach is usually implemented first, with the treatability option applied if the TIE approach is unsuccessful in identifying and controlling toxicity. Identification of the toxicant(s) and reduction at the source would likely lead to chemical-specific limits, whereas toxicity reduction using a treatability approach would generally result in a WET limit. Regardless of which approach is used, toxicity must be reduced to levels that ensure compliance with permit requirements and attainment water quality standards as demonstrated by continued WET monitoring.

5.3 TRE/TIE Work Plan

EPA Regions 9 and 10 recommend that an initial TRE/TIE Work Plan be developed by the permittee within 60-90 days of the effective date of the permit. The TRE Plan developed by the permittee is intended to be a written description of activities that will take place in the event of a WET exceedence. The TRE Work Plan, at a minimum, has the following characteristics (Norberg-King et al. 2005, Chapter 3):

- Identify the roles and responsibilities of the TRE team
- Describe a complete list of data types to be reviewed
- Provide an overview of proposed steps to address and resolve toxicity. The plan should be detailed yet allow flexibility for inclusion of other approaches as additional TRE information is obtained.

- Include a schedule for conducting the TRE and reporting progress to the Permitting Authority.

Because most TRE/TIE work plans are developed before any permit violation or monitoring trigger occurs, they initially must be fairly generic in nature. **However, the work plan should be updated with an implementation time schedule, as the TRE progresses, to incorporate site-specific information and altered TIE approaches suggested by results of the initial TIE activities.** Any alterations to the approaches or implementation schedule should be thoroughly justified and a rationale for the proposed course of action must be presented. Reasonable time should be allowed for each aspect of the study. The time it takes to conduct a TRE can vary considerably depending on the facility type, and complexity and characteristics of the effluent toxicity. For example, an industrial facility with limited processes and waste streams should be easier to characterize than a large POTW with multiple influents from industrial and urban sources. Other factors, such as multiple toxicants, qualitative and quantitative changes in toxicity, intermittent and/or non-persistent toxicity all tend to increase the time it takes to complete a TRE. EPA indicates that most TREs are resolved within 28 months (Norberg-King et al. 2005). Ausley et al. (2005) has suggested the following time frames for the various TRE components.

Time Frame for Conducting a TRE

| <i>Task</i> | <i>Time Frame (Months)</i> |
|---------------------------------------|----------------------------|
| Data, process and housekeeping review | 1 – 3 |
| Phase I TIE | 1 – 6 |
| Phase II TIE | 1 – 6 |
| Phase III TIE | 1 – 3 |
| Toxicity Source Tracking | 1 – 3 |
| Toxicity Treatability | 1 – 3 |
| Total | 6 – 30 months |

Source: Adapted from Ausley et al. 2005

Permittees should seek technical review and comment from their Permitting Authority when developing TRE plans that outline investigative and problem resolution techniques, including reasonable timelines and milestones, in order to avoid delays and maximize consideration of relevant factors that may affect toxicity. The Permitting Authority should then approve the TRE schedule and completion date. The Permitting Authority should either concur with the technical merit of the plan or recommend modifications that would improve its technical merit. A close cooperative relationship should be established among the permittee (and, if applicable, the permittee's technical consultant) and the Permitting Authority early in the TRE process. This relationship should be maintained until the TRE is successfully completed and any controls necessary to prevent unacceptable levels of toxicity are fully implemented. This process allows

all parties to understand the requirements and expectations, and encourages evolution of the plan toward the most effective resolution. Collaboration among the parties throughout the TRE process will add to its effectiveness. EPA describes a 7-step TRE process, which is shown in Figure 5-2 and briefly described below.

Step 1: Accelerated WET Monitoring

The Permitting Authority should establish in the permit conditions under which the permittee must initiate accelerated monitoring and the TRE. Generally, this will be when WET testing results obtained during routine WET monitoring indicate toxicity above either WET permit limit or monitoring trigger. **This document recommends that accelerated monitoring consist of six WET tests conducted at approximately 2-week intervals over a 12-week period.** During this accelerated monitoring phase, if more than one sample demonstrates an unacceptable level of toxicity, the permittee must initiate the TRE work plan. When intermittent toxicity is found (i.e., when toxicity is not detected in every test event with each subsequent sampling event) the permittee should alter sampling procedures to obtain and store adequate sample volume such that WET testing and subsequent TIE procedures can be conducted on the same sample (if the WET testing indicates toxicity).

Step 2: Information and Data Acquisition

The first step in the TRE is the collection of information and analytical data pertaining to effluent toxicity. This information includes data on the operation and performance of the treatment plant, including:

- Industrial waste surveys (IWS)
- Permit applications
- Industrial user compliance reports
- Plant design criteria

The importance of this initial information gathering phase cannot be overstated in terms of optimizing a successful outcome of the TRE. By carefully reviewing the information collected and comparing trends in flow patterns, treatment efficiency, wastewater loading and effluent constituents with toxicity patterns over time, permittees may be able to narrow the scope of further investigations and possibly even identify problem constituents.

Step 3: Facility Performance Evaluation

POTW treatment deficiencies that cause poor pollutant removal can have an adverse effect in toxicity reduction. As an initial step, effluent toxicity, operations and performance data should be carefully examined to identify treatment deficiencies or in-plant sources of toxicity. In addition, the POTW pretreatment program data should be reviewed to indicate possible sources of toxicity. The municipal TRE manual (USEPA 1999b) provides in-depth discussion of parameters to be evaluated in this part of the TRE.

If a treatment deficiency is identified, studies should be conducted to evaluate treatment modifications before proceeding with the TRE. If plant performance is not a cause of toxicity, or treatment modifications do not reduce toxicity, the permittee should proceed with the TIE.

Step 4: Toxicity Identification Evaluation

EPA has published TIE procedures to determine the causes of acute and chronic effluent toxicity to freshwater species (USEPA 1989b, 1989c, 1991c, 1992, 1993b, 1993c) and to estuarine/marine organisms (USEPA 1996a). The generic TIE protocols are performed in three phases: toxicity characterization (Phase I), toxicant identification (Phase II), and toxicant confirmation (Phase III). A flow diagram of the TIE process is shown in Figure 5-2.

The Phase I TIE manipulations are designed to sequester or remove toxicity caused by specific classes of chemicals, as shown in Table 5-1.

Table 5-1. Purpose of Phase I TIE Treatments

| <i>TIE Treatment</i> | <i>Treatment Identifies</i> |
|---|---|
| Initial toxicity (unaltered effluent) | Initial toxicity test demonstrating toxicity of sample |
| Baseline toxicity (unaltered effluent tested simultaneously with other TIE manipulations) | Results compared to TIE manipulations to assess effectiveness of TIE manipulations |
| pH adjustment/filtration (pH 3 and pH 11) | Particulate-bound toxicants |
| pH adjustment/aeration (pH 3 and pH 11) | Ammonia and volatile, oxidizable toxicant |
| C18 (or C8) solid-phase extraction (SPE) at pH 3, pH 9, and pH 11 | Polar and non-polar organic chemicals |
| Sodium thiosulfate addition | Oxidants and some cationic metals ^a |
| Ethylene diaminetetraacetic acid (EDTA) addition | Cationic metals |
| Graduated pH adjustments | Ammonia and pH-sensitive toxicants |
| Piperonyl butoxide (PBO) | Organophosphate insecticides (decreases toxicity) Pyrethroid insecticides (increases toxicity) |

a Copper, silver and selenium

Each of these TIE treatments are applied to the test sample and comparison of the level of baseline toxicity with the TIE treatments identify the physical/chemical characteristics of the

toxicants. It is essential that proper controls and blanks be used with each TIE treatment, and that a high level of QA/QC is maintained throughout the TIE process. EPA cautions that the investigator should approach the TIE without a preconceived notion as to the cause of toxicity and therefore all treatments should be conducted. On the other hand, if one wants to know the role of a single chemical or class of chemicals, ammonia for example, the TIE can be designed to accomplish that goal. If the standard suite of Phase I treatments are ineffective in identifying cause(s) of toxicity, other techniques can be used, including anion and cation ion exchange resins and activated charcoal molecular sieves (Burgess et al. 1997).

Application of the TIE process over the years has demonstrated its applicability to virtually every test species used in WET, including marine species (Burgess et al. 1995), although the use of marine species require that test samples be adjusted (after the TIE treatment). Note, the samples need adjustment of the salinity before the TIE manipulations to insure salinity consistency between treatments and because some of the manipulations must be performed with salinity adjusted samples; for example, the graduated pH manipulation) to the appropriate salinity using dry sea salts or hypersaline brine (Ho et al. 1995; USEPA 1996a). One other caveat in marine TIEs is that due to the strong carbonate buffering capacity of sea water, the only effective means of controlling pH is to use controlled atmospheric chambers.

The EPA Phase I TIE manuals (USEPA 1991c, 1992, 1996a) describe a process where the sample is split into aliquots, each of which is subjected to a single TIE manipulation concurrently with the other treatments (“parallel” treatment approach). However, EPA points out that the Phase I TIE characterization procedures are relatively broad and can indicate more than one class of toxicant. Additional tests or an altered approach may be needed to delineate/confirm the role of a particular chemical class in the effluent toxicity, especially when multiple toxicants are present (USEPA 1993b, 1993c). For example, when the primary toxicant is present in high concentrations, it may mask the other potential toxicant(s) in the sample – ammonia is a common example. In these cases, sequential treatments (“stacked” treatment approach) can be used to evaluate the role of secondary toxicants; for example, removal of ammonia by zeolite followed by removal of non-polar organics by SPE treatment in cases where multiple toxicants are present at toxic concentrations.

Results of Phase I can be compared with pretreatment program data and chemical-specific effluent data to identify potential toxicants. However, chemical analysis conducted in the absence of Phase I TIE information (i.e., chemical class of toxicant(s), to guide the type of analysis) are usually wasted expenditures. For this reason, EPA cautions that chemical-specific tracking should be conducted after the toxicant(s) are identified and confirmed in Phase II and Phase III TIEs, respectively (USEPA 1993b, 1993c).

The Phase I TIE process should be repeated with multiple samples until a clear pattern of toxicity emerges.

5.4 Interpretation of Phase I TIE Results

Phase I characterization provides information on the chemical class(es) responsible for the effluent toxicity, and is applicable to both acute and chronic endpoints. The following guidance

is given by EPA for interpreting Phase I TIE results for various classes of toxicants. Note that the toxic response is assessed by comparing the results for each of the TIE treatments, against the toxicity measured in the baseline (pre-treatment) test.

Ammonia

- Ammonia toxicity can be assessed by zeolite removal or the graduated pH test.
- Toxicity increases in the graduated pH test at higher pH
- Toxicity decreases after zeolite treatment. If the zeolite removal procedure is used, an ammonia add-back step (spiking the zeolite-treated sample with ammonia at the original concentration in the sample) is essential to ensure that the zeolite has not removed other constituents.
- The effluent is more toxic to *P. promelas* than to *C. dubia*.

Note: If the concentration of total ammonia (as nitrogen) is 5 mg/L or more and chronic toxicity is a concern, the potential for ammonia toxicity should be evaluated.

Drawing conclusions about ammonia toxicity based solely on observed concentrations can be misleading. Ammonia is an example of a toxicant that acts independently of other toxicants in effluents. Even though ammonia concentrations may appear to be sufficient to cause all of the effluent toxicity, other toxicants may be present and may contribute to toxicity when ammonia is removed. Thus, it is important to ascertain if ammonia is masking other potential toxicant(s) in the test sample using the sequential TIE approach previously described.

Oxidants

- Toxicity is reduced or removed in the sodium thiosulfate addition test.
- Toxicity is reduced or removed in the aeration test.
- The sample is less toxic over time when held at 4 °C (and the type of container does not affect toxicity).
- *C. dubia* are more sensitive to the effluent than *P. promelas*.

Non-polar organic toxicants

- Toxicity in the post C18 SPE column test is absent or reduced
- Toxicity was recovered in the methanol eluate test ('stronger' solvents may be required to elute highly lipophilic chemicals from SPE columns)
- Toxicity was dramatically changed by piperonyl butoxide (PBO) addition (PBO decreases toxicity of organophosphate insecticides and increases toxicity of pyrethroid insecticides)

Cationic metals

- Toxicity is removed or reduced in the EDTA addition test

- Toxicity is removed or reduced in the post-C18 SPE column test
- Toxicity is removed or reduced in the filtration test, especially when pH adjustments are coupled with filtration
- Toxicity is removed or reduced in the sodium thiosulfate addition test
- Erratic dose response curves are observed

None of these characteristics is definitive, with the possible exception of EDTA. In addition, toxicity may be pH sensitive in the range at which the graduated pH test is performed, but may become more or less toxic at lower or higher pH depending on the particular metal involved.

Surfactants

- Toxicity is removed or reduced in the filtration test.
- Toxicity is removed or reduced by the aeration test. In some cases, toxicity may be recovered from the walls of the aeration vessel using a dilution water or methanol rinse.
- Toxicity is removed or reduced in the post-C18 SPE column test. The toxicity may or may not be recovered in the methanol eluate test. If a series of methanol concentrations (e.g., 25, 50, 75, 80, 85, 90, 95 and 100% in water) is used to elute the column, toxicity may be observed in multiple fractions.
- Toxicity is removed or reduced in the post-C18 SPE column test using unfiltered effluent. Toxicity reduction/removal is similar to that observed in the filtration test and toxicity may or may not be recovered in the methanol eluate test or by extraction from the glass fiber filter used in the filtration test.
- Toxicity degrades over time as the effluent sample is held in cold storage (4 °C). Degradation is slower when the effluent sample is stored in glass containers instead of plastic containers.

Total Dissolved Solids (TDS)

- pH adjustments do not remove or reduce toxicity and a precipitate is not visible in the pH adjustment test, pH adjustment and filtration test, or pH adjustment and aeration test.
- There is no loss of toxicity in the post-C18 SPE column tests, or a partial loss of toxicity, but no change in conductivity measurement.
- There is no change in toxicity with the EDTA addition test, sodium thiosulfate addition test, or the graduated pH test.
- There is a greater sensitivity by *C. dubia* and *D. pulex* compared to *D. magna*, together with high conductivity readings.
- A mock effluent prepared with the same ions as the effluent exhibits similar toxicity as the effluent (Goodfellow et al. 2000).
- Toxicity is removed or reduced by ion exchange resin.

- Toxicity is not removed or reduced by passing the effluent over activated carbon.

A list of toxicants identified in the TIE process is provided in Table 5-2 at the end of this chapter.

5.5 Phase II TIE Procedures

The Phase II guidance manual (USEPA 1993a) describes procedures for use in identification of specific classes of toxicants, including:

- Ammonia
- Cationic metals
- Polar and non-polar organic chemicals
- Chlorine
- Filterable toxicants

Phase II treatment techniques are similar to Phase I and are applicable to acute and chronic test methods with most WET test species. Phase II incorporates chemical-specific analytical procedures, including gas-chromatography (GC), GC/mass spectrometry (GC/MS), high-performance liquid chromatography (HPLC)/MS, atomic absorption (AA), and/or ion-coupled plasma (ICP)/MS to identify toxicants. The reader is referred to the EPA Phase II manual (USEPA 1993b), Municipal TIE Guidance (USEPA 1999b) and the SETAC TRE/TIE book (Norberg-King et al. 2005) for a detailed description of Phase II TIE procedures and examples of TIE case studies.

5.6 Phase III TIE Procedures

A thorough confirmation of the cause(s) of toxicity is a key part of the TIE process, although it is often the most laborious and difficult aspect. This confirmation must be performed over a considerable period to be certain that the cause(s) of toxicity is not changing over time. This is particularly true for POTWs, where control over influent is not complete. USEPA (1993c) emphasizes that sample integrity is particularly important in Phase III. Field replicates, system blanks and controls should be used as appropriate to prevent interferences and toxicity artifacts.

Suspect toxicant(s) identified in Phases I and Phase II are confirmed through application of one or more Phase III steps, including:

- Correlation approach
- Symptom approach
- Species sensitivity approach
- Spiking approach

- Mass balance approach

These approaches are not discussed in detail here, but are fully explained along with specific examples in EPA TIE manuals (USEPA 1991c, 1992, 1993b, 1993c). The reader is encouraged to review this material before reviewing TIE reports.

Step 5: Toxicity Source Evaluation

Once the TIE has identified and confirmed the chemical(s) responsible for the toxicity, efforts are initiated to identify the source(s) of the chemical(s). This process entails sampling of influent trunk lines from residential and industrial dischargers. Two types of source identification studies may be performed: chemical tracking or toxicity-based tracking. In some circumstances, both approaches have been conducted concurrently.

Chemical-specific tracking is recommended when the POTW effluent toxicant(s) have been identified and confirmed in the TIE, and can be readily traced to the responsible sewer dischargers. Toxicity tracking is used when TIE data indicate the type of effluent toxicant, but the specific toxicant(s) is not identified. Toxicity tracking involves treating the sewer samples in a bench-scale treatment simulation prior to toxicity measurements to account for the toxicity removal that is provided by the POTW.

The sampling strategy for toxicity source evaluations involves two tiers. Tier I focuses on sampling and analysis of the main sewer lines in the collection system. Tier II involves testing sewer lines and indirect dischargers upstream of the main lines identified as being toxic in Tier I. This tiered approach can be used to identify the contributors of toxicity and/or toxicants by eliminating segments of the collection system that do not contribute toxicity/toxicants.

Step 6: Toxicity Control Evaluation

Using the results of each of the above TRE elements, alternatives for effluent toxicity reduction are evaluated and the most feasible option(s) is selected for implementation. Effluent toxicity may be controlled either through pretreatment regulations or in-plant treatment modifications or additions. In some cases, several control methods may be required to achieve the desired toxicity reduction. Selection of control options is usually based on technical and cost criteria.

If the toxicity source evaluation is successful in locating the sources that are contributing to the POTW effluent toxicants, local limits can be developed and implemented. If in-plant control appears to be a feasible approach, treatability testing may be used to evaluate methods for optimizing existing treatment processes and to assess options for additional treatment. A description of treatability approaches can be found in the municipal TRE manual (USEPA 1999b).

Step 7: Toxicity Control Implementation and Follow-Up Monitoring

The toxicity control method or technology is implemented and follow-up WET testing is conducted at increased frequency to ensure that the control method achieves the TRE objectives and meets permit limits.

5.7 Inconclusive TRE/TIEs

TIEs that fail to characterize toxicants effectively frequently do so for one of two reasons. The first is the inability of inexperienced individuals to properly conduct and/or interpret results of TIE steps and obtain unambiguous identification of the toxicant(s). The second is difficulty in applying TIE techniques to samples with intermittent toxicity, toxicity caused by changing toxicants, and/or unstable effluent toxicity (Ausley et al. 1998; USEPA 2001c; SETAC 2005). Effluents with one or more of these characteristics pose significant challenges for even experienced analysts. EPA (USEPA 2001c) states that under conditions where the permittee has implemented an exhaustive TRE plan and all other permit requirements, but is still unable to attain or maintain compliance with toxicity-based limits, special technical evaluation may be warranted and civil relief granted. Solutions to these cases are pursued jointly with TIE experts at Regional or State offices or the EPA Duluth laboratory.

5.8 Conclusions

- The TRE/TIE process has been well described and updated in numerous documents published by EPA and others.
- A key aspect to successful TRE/TIEs is the development of a detailed TRE study plan early in the process.
- Application of these methods has generally resulted in toxicant(s) identification and mitigation of effluent toxicity problems, allowing the permittee to return to compliance.
- Enforcement decisions should be guided on a case- and site-specific basis considering existing and historical toxicity, including magnitude, frequency and duration of toxicity and importantly, the permittee's diligence in resolving and preventing WET non-compliance.
- Finally, permittees and regulatory authorities should establish early in the TRE process a cooperative and communicative relationship that would be maintained until the TRE is successfully completed and controls fully implemented. Good communication and a well-conceived TRE plan will assure that all parties understand the requirements and expectations that will result in a more effective and faster resolution of the effluent toxicity.

Figure 5-1. Summary of the TRE Process

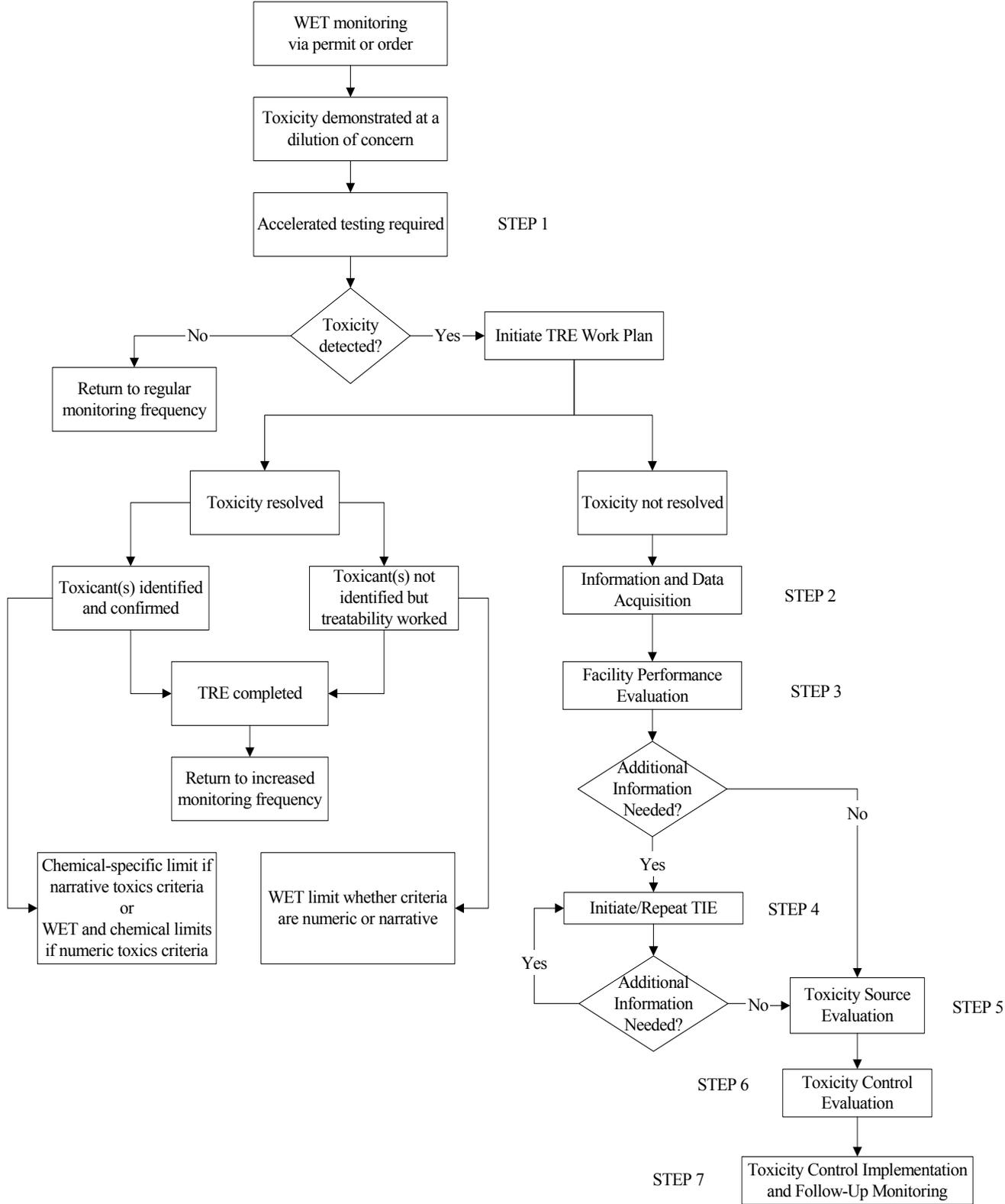


Figure 5-2. Flow Diagram of a Toxicity Identification Evaluation

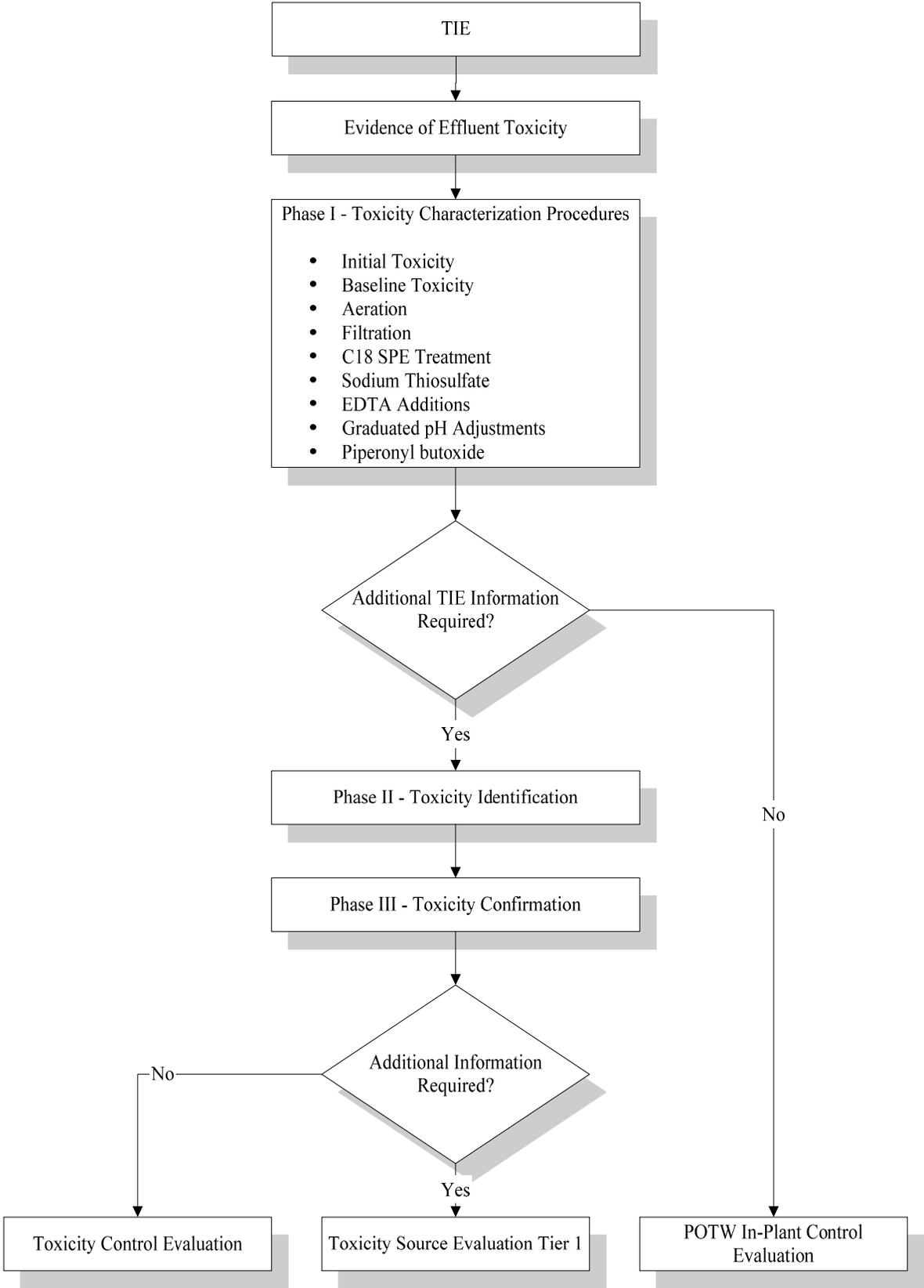


Table 5-2 Toxicants Identified in POTW Effluents

| <i>Toxicant Type</i> | <i>Level of Concern</i> | <i>Potential Source</i> | <i>Information Need</i> | <i>References and Case Studies</i> |
|---|---|---|---|---|
| Chlorine | 0.5-1 mg/L | POTW disinfection | Temp, pH during test; Cl ⁻ conc. Phase I TIE oxidant test | |
| Ammonia | 5 mg/L as NH ₃ -N | Domestic and industrial sources; POTW sludges; dewatering streams | NH ₃ -N conc, pH, temp, salinity, cond, at receipt and during the test. Phase I TIE graduated pH and zeolite treatment | USEPA 1999, Appendix G SETAC Case Study 6.5 |
| Non-Polar Organics; OP insecticides (e.g., diazinon and chlorpyrifos) | Effluent concentrations \geq EC ₂₅ ; Diazinon 0.12-0.58 μ g/L; Chlorpyrifos 0.03 μ g/L | Lawn pest control, pet care, veterinary, food vendors | High resolution analysis (GC/MS). Phase I TIE SPE test and SPE eluate add-back | USEPA 1999, Appendices A,B,F,G and H SETAC Case Studies 6.10, 6.11, 6.13, 6.14, 6.16, 6.17, 6.25, 6.29, 6.35, 6.36, 6.37 |
| Metals: cadmium, copper, lead, nickel, zinc | Various depending on water quality parameters and test species | POTW treatment additives; industrial users | Dissolved metals, hardness, alkalinity, and pH. Phase I TIE EDTA test | USEPA 1999, Appendix G SETAC Case Studies 6.6 and 6.22 |
| Treatment additives; dechlorination chemicals; polymers, biocides | Varies | Disinfection, dechlorination, sludge processing solid clarification | Information on toxicity of products; Use rates Phase I and II TIE results | SETAC Case studies 6.15, 6.18 |
| Surfactants | Varies | Industrial users | Methylene blue active substances (MBAS) Phase I and II TIE results | SETAC Case Study 6.19 |
| Ions and Total Dissolved Solids (TDS) | 1,000-6,000 μ mhos depending on test species, endpoint and TDS constituents | Industrial users; sludge processors; reverse-osmosis dischargers | TDS, ion analysis, anion, cation balance Phase I and II TIE results | SETAC Case studies 6.4, 6.5, 6.7, 6.8, 6.24, 6.28 |

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CHAPTER 6. AMBIENT TOXICITY TESTING AND WATERSHED ASSESSMENT

6.1 Overview

This chapter provides guidance to permit writers who are including stormwater or ambient conditions in permits. Although, WET tests are used as the primary tool for stormwater and ambient monitoring, the conditions under which they are used are generally different from monitoring continuous effluent discharges. Procedures which should be considered include:

- Experimental design – sample collection location, single vs. multiple concentrations
- Sampling – frequency, volume, container material, holding time
- Toxicity test method – organism selection, renewal frequency

Additionally, this chapter provides a broad overview of tools to be considered for stormwater and ambient monitoring, and provide examples of programs that have utilized tools including sediment toxicity testing, bioassessments, and in situ testing.

6.2 Introduction

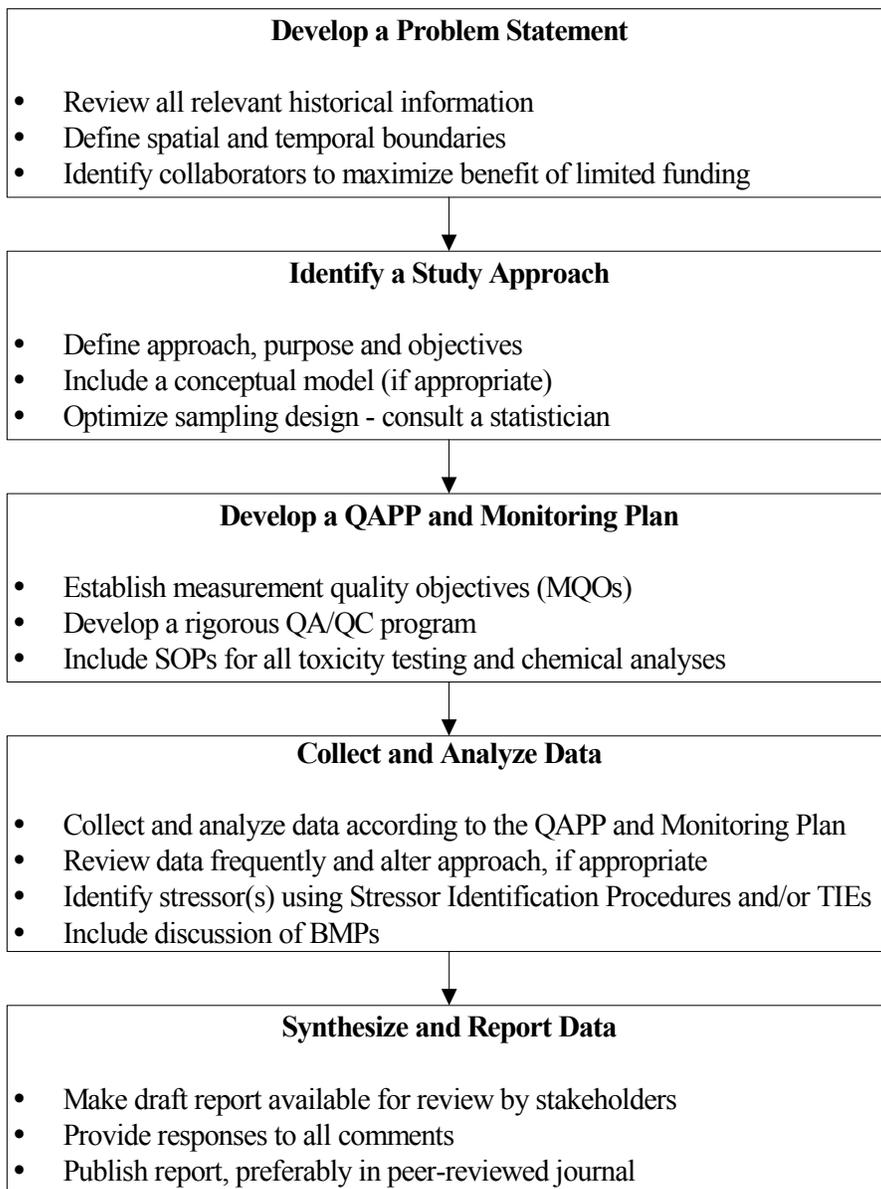
Permitting authorities are, by the very nature of what they do, stewards of the nation's water resources. As such, their ultimate goal is to maintain those resources in a condition that, "meets the needs of the present without compromising the ability of future generations to meet their needs" (Bruntland 1987). The Clean Water Act (CWA) states, "The objective of this act is to restore, and maintain the chemical, physical, and biological integrity of the nation's waters." It is no longer sufficient to think about aquatic ecosystems from a single perspective like point sources, non-point sources, sediment, stormwater, or the air/water interface. A holistic approach, using the watershed as the integrating unit, has clearly been recognized by EPA as the focal point for measuring how well the objectives of the CWA are being met.

According to the Watershed Information Network (www.epa.gov/owow/watershed/), a watershed is an area of land that drains to a common place, such as a stream, lake, estuary, wetland, aquifer, or the ocean. Since the goal of permitting authorities is to maintain healthy water resources, they are increasingly not only required to monitor effluent discharges, but potential watershed pollution in the form of stormwater discharges and non-point source toxicity to receiving waters, or ambient waters. Much like effluent outfalls are monitored with toxicity and chemistry, stormwater outfalls and receiving waters can be monitored with similar tools, but with specific considerations for their use.

Once, the Permitting Authority identifies the questions to be addressed, the development of the Quality Assurance Project Plan (QAPP) integrates all technical and data quality aspects of a project including planning, implementation, and assessment. EPA requires that all environmental data used in decision-making be supported by an approved QAPP. EPA requirements for QAPPs can be found at <http://www.epa.gov/quality/qs-docs/rsfinal.pdf>. Ambient water quality monitoring conducted in California using state funds must be compatible with the State's Surface Water Ambient Monitoring Program (SWAMP). The objective of

SWAMP is to provide high quality data that is comparable and accessible. The current requirements necessary to be considered SWAMP-compatible are detailed in the links found at www.swrcb.ca.gov/swamp. Before any study is undertaken, there are certain common steps, regardless of the study, that should be performed. These steps are outlined in Figure 6-1.

Figure 6-1. Recommended Steps in Development and Implementation of Environmental Monitoring Studies



6.3 Use of WET Testing in Stormwater and Ambient Monitoring

Toxicity testing procedures that are typically used in WET testing compliance, coupled with other biological assessments, have become increasingly important tools for identification of waterbodies which fail to meet goals of the CWA. In general the same organisms, testing protocols and sampling methods used in WET testing can be used in stormwater and ambient water monitoring. However, stormwater and ambient water study designs may need to incorporate different test organisms and sampling strategies to meet the goals of the study.

Monitoring in freshwater ecosystems typically employs EPA three-species toxicity tests with freshwater algae (*Selenastrum capricornutum*), the cladoceran (*Ceriodaphnia dubia*), and the fathead minnow (*Pimephales promelas*) (USEPA 2002a, 2002b). There are numerous advantages in using established WET test species for ambient monitoring including well understood life history and husbandry of the test organism, and established test protocols with a robust statistical basis for endpoint interpretation. Depending on site-specific water quality conditions, it may be appropriate to utilize other species. For example, standard WET species may not tolerate high TDS waters characteristic of some ambient and storm waters. In cases where water quality characteristics are not compatible with standard test species, the permitting authority should use best scientific judgment within local and state agencies and EPA to select alternate species and/or testing approaches.

For testing of estuarine environments, EPA has published short-term chronic toxicity test methods for several West Coast species which could be used for environmental monitoring in estuarine and marine environments (USEPA 1995a). The estuarine species include topsmelt (*Atherinops affinis*) and mysid (*Holmesimysis costata*). For testing marine waters, protocols for Pacific oyster (*Crassostrea gigas*), mussel (*Mytilus* sp.), red abalone (*Haliotis rufescens*), giant kelp (*Macrocystis pyrifera*), sea urchin (*Strongylocentrotus purpuratus*), and sand dollar (*Dendraster excentricus*) are available. Monitoring programs may be conducted in areas that contain species of special concern. EPA has provided guidance on selection of standard test organisms that would predict responses of species that are threatened or endangered (USEPA 2003b).

6.4 Stormwater Monitoring

Stormwater monitoring for toxicity is really a special case of effluent monitoring, the main difference being that stormwater is episodic. There are special conditions associated with stormwater monitoring in cities and towns where collected stormwater is conveyed through separate storm sewer systems or through combined sewers to a treatment plant prior to discharge. In most cases, stormwater is directly discharged to the receiving system without treatment. Ultimately, a successful stormwater program minimizes the level of contaminants in the stormwater. The most severe receiving water problems due to wet weather flows are likely associated with chronic exposures to contaminated sediment and to habitat destruction.

Since 1990, EPA has developed Phase I of the NPDES Stormwater Program (http://cfpub.epa.gov/npdes/home.cfm?program_id=6). Most stormwater discharges are considered point sources and require coverage by an NPDES permit. The Phase I program

addressed sources of stormwater runoff that had the greatest potential to negatively impact water quality. Under Phase I, EPA required NPDES permit coverage for stormwater discharges from medium and large separate stormwater systems, eleven categories of industrial activity, and construction activity that disturb five or more acres of land. Phase II of the program requires NPDES coverage for stormwater from certain regulated small municipal separate storm sewer systems and construction activity disturbing between 1 and 5 acres of land.

The “quality” of the wet weather flow is dependent in large part on the use designations of the land it flows over. There are differences between constituents in wet weather flows originating in high mountain forested areas and those originating in fully developed urban areas. According to Pitt (2003) urban receiving waters may have many beneficial goals, including:

- stormwater conveyance (flood protection),
- biological uses (warm water fishery, biological integrity, etc.),
- noncontact recreation (linear parks, aesthetics, boating, etc.),
- contact recreation (swimming), and
- water supply.

However, with full-scale development and lack of stormwater controls, severely degraded streams will be commonplace in highly urbanized areas. Some studies have shown significant aquatic life impacts even in watersheds that are less than 10% urbanized (Pitt 2003; Booth and Jackson 1997). In the Pacific Northwest, Horner et al. (1997) found that when imperviousness reached about 8% in the watershed, there was a rapid decline in the biological conditions in the receiving water. Severe problems were found when imperviousness reached 30%. Claytor (1996) found that when only conventional water quality measures are used to evaluate the status of non-tidal streams, 87% supported their designated biological uses. However, when biological assessments were included, only 13% of the streams supported their designated biological uses. According to the EPA Stormwater website designed to provide guidance for reducing contaminant input into receiving waters, the primary method to control stormwater discharges is through the use of Best Management Practices (BMPs). EPA maintains a web site <http://www.bmpdatabase.org/index.htm> that contains a database of roughly 200 BMPs.

6.5 Ambient Monitoring

The receiving waters of either an effluent or stormwater discharge are monitored to achieve a greater understanding of the potential effects of the discharge. Standard effluent monitoring tools, such as toxicity testing and water chemistry are used gather data on receiving water impacts, but other tools include in situ toxicity tests, bioassessments, and sediment toxicity testing. The experimental design of the ambient monitoring study will be based on the study questions and the tools that are chosen. Water column toxicity tests will pick up more ephemeral toxicity, and therefore should be used in fewer places, but perhaps more often. In situ water column toxicity tests can integrate toxicity over time, and could probably be used more sparingly, at least temporally. Sediment acts as a sink for many chemicals, particularly hydrophobic contaminants, and sediment toxicity testing tends to monitor the potential for longer

term effects. Sediment toxicity tests could be used less often temporally, but over a wider spatial range. Bioassessment also monitors long term trends, and is not generally considered a diagnostic tool, but could be used to assess long term impacts.

Several studies in California have successfully used ambient toxicity testing to identify and regulate frequently occurring toxic chemicals (Foe and Sheipline 1993; Kuivila and Foe 1995; de Vlaming et al. 2000). In these studies integral sampling locations were selected and ambient waters were collected to be assessed acute and chronic toxicity. If toxicity was detected, additional samples were collected for testing to determine spatial and temporal patterns, as well as for conducting toxicity identification evaluations (TIEs) to identify the causative agents. This approach has led to the listing of chemicals broader than the 126 priority pollutants commonly tested. For example, diazinon was identified as causing water quality impairments and lead to 303(d) listings in several watersheds in California.

6.6 Special Considerations

Unlike effluents, where the constituents in the discharge remain fairly consistent, the constituents in stormwater and ambient samples can be ephemeral. Storm events are episodic, and depending on land use, a variety of contaminants can be present in the runoff. Receiving waters are similarly dynamic depending on inputs from point and non-point sources. Because of their inherent differences from effluents, toxicity testing of stormwater and receiving water have some specific method considerations. Areas which need to be considered differently for stormwater or ambient testing than the effluent testing program include: (1) sampling location and sample type, (2) sample containers, (3) sample initiation test, (4) sample renewals, and (5) experimental test design (single vs. multiple concentration testing).

6.6.1 Sampling Location

Selection of appropriate sample sites and sampling regimes are critical to the success of environmental monitoring studies. Sampling design in environmental monitoring programs is inevitably a compromise between cost/effort and accurately reproducing the regimen to which the organisms are actually exposed in the environment. Many sampling scenarios involve the use of integrator sites where multiple discharges and/or tributary flows combine. The United States Geological Survey's (USGS) "Seamless Data Distribution System" (<http://seamless.usgs.gov>) enables a user to view and download many geospatial layers, such as the National Evaluation Data set, National Land Cover Data set, and High Resolution Orthoimagery. If toxicity is detected at the integrator site, each of the contributing sources is tested to determine the source of the toxicity. Although this seems intuitive, care must be taken to assure that the samples are taken in such a way that takes into account the hydrology of the system being studied. USGS maintains a web site (<http://water.usgs.gov/waterwatch>) that reports in real time flows in mainstem rivers and major tributaries. In addition, real-time stream flows for California are posted the California Department of Water Resources website (<http://cdec.water.ca.gov>), which is useful in developing sampling plans. Land use information is critical for designing monitoring studies when it is important to know the contribution of flows from agricultural and urban areas. In addition, for agricultural areas, knowledge of crop type (<http://gis.ca.gov>) and pesticide use in

specific areas (<http://calpip.cdpr.ca.gov>) can be useful in tracing sources of toxicity from agricultural chemicals.

6.6.2 Timing of Sample Collection

Monitoring stormwater for toxicity requires a special understanding of what needs to be monitored (Herricks and Milone 1998) although the methods used to test stormwater may not be any different from those used to test ambient/receiving waters. The challenge associated with stormwater testing is in developing sampling strategies that incorporate realistic exposure scenarios. Routine stormwater monitoring can differ from a “first flush” event that is generally more toxic because of contaminant buildup on impervious surfaces during the dry season. Similarly, first flush events from agricultural settings can occur after winter dormant spraying and pesticide applications in the spring. The greater the period between rainfall events, the greater is the potential for build-up of contaminants.

Timing of sampling of stormwater discharge depend on the intensity of the storm as well as preexisting conditions surrounding the site such as amount of impervious surfaces, characteristics of the collection system and soil saturation. The effect of these factors on discharge volume can be monitored using a hydrograph plot (flow vs. time). Contaminants will usually move into the receiving water as the storm hydrograph increases (Burton and Pitt 2001). Depending on the purpose of the study, multiple samples can be collected and tested throughout the runoff event to assess short-term effects and contaminant loading.

If a study objective was to monitor the toxicity associated with a particular storm event in a particular watershed at a particular site, or multiple sites, then samples collected over the period of the storm, based on the watershed characteristics and hydrograph would provide the most realistic time-scale for exposure. Herricks and Milone (1998) discuss a variety of approaches for determining the appropriate time scale of exposure for a given watershed. Miller et al. 2005 present results of flow-through toxicity studies for studying stormwater in an urban creek using *C. dubia*.

At the other extreme of exposure would be water column organisms that are picked up and carried for an extended, but unknown, period of time with the first flush of water that enters the receiving system. In this case, samples of the first flush of water can be used to expose organisms in the laboratory using WET test methods with or without renewals, depending on what the investigator is attempting to mimic.

For ambient sampling, knowledge of land use, pesticide application patterns and timing, and system hydrology is required to select sample site locations and timing. For both stormwater and ambient samples, sites that demonstrate adverse effects, timely collection of additional site samples is essential to establish the frequency, magnitude, and duration of the toxicity at the site.

6.6.3 Sample Collection

Effluent monitoring generally utilizes composite sampling to collect water during a discreet period of discharge. Depending on the objectives of the study, composite sampling can also be

used for stormwater and ambient monitoring, but grab samples are used most often. The use of grab samples, the episodic nature of storm events, and the level of effort involved in the collection of receiving water samples can often lead to difficulties in adhering to a 36-hour sample holding time and the ability to collect multiple samples for renewals in an individual test.

All tests should be conducted as soon as possible following sample collection. EPA has allowed exceptions to the 36-hour holding time, for example, when effluents are shipped overseas for testing (Denton and Narvaez 1996). The primary reason for an extension of the holding time would be the consideration of the sampling and laboratory technicians safety (Burton and Pitt 2001; see page 255), and logistics of coordinating collection and transport of multiple samples within a short period. Since, storm events are not pre-determined and typically are occurring rapidly throughout a watershed; therefore, many site samples must be coordinated with short notification. The 36-hour holding time for test initiation should be targeted, but no more than 72 hours should elapse before initial use of a sample. Typically, environmental monitoring programs use a single sample for all toxicity test renewals. For acute studies (typically 96 hours), a single test sample is usually collected and used to renew test solutions daily or at 48 hrs. EPA specifies the use of a minimum of 3 samples for chronic toxicity studies with fish and invertebrates (USEPA 1995a, 2002b, 2002c), but depending on the study question, sampling for storm events, might occur only once, or several times throughout the hydrograph. Another solution is to renew the test solutions with a mixture of ambient waters and stormwaters, if such waters could be collected following test initiation while meeting WET test holding time specifications (Katznelson and Mumley 1997).

During sample collection, it is critical to confirm and record the site location using GPS coordinates, note site characteristics, measure basic water chemistry (temperature, dissolved oxygen, conductivity), and estimate flow velocity and volume. The latter information may be challenging to obtain but is critical for estimating toxicant loading. Generally, glass sample containers are recommended for ambient and stormwater samples. Samples must be immediately placed on wet ice and transported to the testing lab, where testing should be initiated as soon as possible. Even assuming that all conditions of sample holding (36 hrs maximum at $\leq 6^{\circ}\text{C}$) are met, significant quantities of some chemical classes of constituents (e.g., organophosphates, pyrethroid insecticides and surfactants) may sorb to sample containers during the holding period. Vigorous shaking of sample containers prior to distributing to test containers to re-dissolve sorbed constituents is recommended (Wheelock et al. 2005).

6.6.4 Data Analysis

Initially, samples are tested at without dilution such as 100% concentration. The test endpoint data is analyzed using a standard t-test approach as described in the test methods manual (see USEPA 2002a, page 86). Many sampling plans specify that if toxicity is detected, the site shall be re-sampled and retested using a dilution series to determine the duration, frequency and magnitude of the toxicity. Toxic samples should immediately be subjected to TIE procedures to attempt to identify the toxic chemical(s).

6.6.5 Stormwater *In Situ* vs. First Flush

There are potentially two entirely different kinds of exposure from stormwater events. For sessile organisms (e.g., organisms which do not move with the discharge flow), the exposure is the culmination of all the water and constituents that pass over them during an event. In this case, *in situ* monitoring, using methods that can withstand the changes in the flow regime, can characterize that exposure. The effects of that exposure may be more difficult to predict, as they may not occur until some time after the exposure. One way to address this is to remove the *in situ* systems after the storm event and monitor their responses in clean water. Herricks and Milone (1998) studied time-varying exposures in the laboratory using the cladoceran *C. dubia*, the fish *P. promelas*, and the amphipod *H. azteca*. Their work showed the need for appropriate time-scales of exposure. Organisms that reside in the water column would move with the stormwater flows. Therefore, exposing *C. dubia* to the first flush sample in a storm event would probably not represent the exposure most of these sorts of organisms would receive.

6.7 Additional Monitoring Tools

There are additional tools that can be utilized for monitoring of stormwater and ambient water. Three of these tools are discussed below: *in situ* toxicity testing, sediment toxicity testing, and bioassessments. The use of these tools, and others, can either lead to the identification of an impairment, or monitor a currently impaired waterbody. Once impairment has been identified, identification of the primary stressors is pursued through the EPA stressor identification process (USEPA 2000c). This process was developed to identify any type of stressor or combination of stressors that cause biological impairment. The Stressor Identification (SI) process entails critically reviewing the available environmental information, analyzing potential exposure scenarios, and developing monitoring programs to fill in data gaps. The reader is encouraged to review the SI document prior to developing or reviewing environmental monitoring programs. Some types of monitoring approaches and their applications are shown in Table 6-1.

Table 6-1. Types of Monitoring Approaches and Their Applications

| <i>Type</i> | <i>Approach</i> | <i>Applications</i> |
|----------------------|----------------------------|---|
| Chemical Condition | Water quality sampling | Screen for impairment; identify specific pollutants of concern; identify water quality trends; determine support of designated contact recreation uses; identify potential pollution sources. |
| Physical Condition | Watershed survey | Determine land use patterns; determine presence of current and historical pollution sources; identify gross pollution problems; identify water uses, users, diversions, and stream obstructions |
| | Habitat assessment | Determine and isolate impacts of pollution sources, particularly land use activities; interpret biological data; screen for impairments |
| Biological Condition | Macroinvertebrate sampling | Screen for impairment; identify impacts of pollution and pollution control activities; determine the severity of the pollution problem and rank stream sites; identify water quality trends; determine support of designated aquatic life uses. |

Source: USEPA 1997a.

6.7.1 *In-Situ* Testing

Toxicity tests using standard WET organisms and performed on ambient water samples are considered surrogate exposures for environmental realism. Exposing these organisms *in situ* can increase the environmental relevance. The test organisms used for *in situ* biomonitoring range from the same organisms used in WET toxicity testing to a wide array of other organisms. The list of references that follow are only a small number of articles on *in situ* toxicity testing: WET test organisms (Anderson 2002; Dickson et al. 1996; Hemming et al. 2001) amphipods (Maltby et al. 2003; Rainbow and Kwan 1995; Gerhardt et al. 1998); algae (Twist et al. 1997); real-time biomonitors (Allen et al. 1996; Waller et al. 1995; Kuster et al. 2004; Gerhardt et al. 1998; Kieu et al. 2001; Charoy et al. 1995).

Organisms can also be exposed *in situ* for bioaccumulation studies. Freshwater and marine mussels bioaccumulate both metals and organics and have been used extensively to evaluate sources of environmental pollution. Mussels can be placed in the field for varying periods and have the additional endpoints of growth and survival. Strategically located mussels can identify chemical inputs.

Several large monitoring programs have used mussels to monitor contaminants and determine contaminant bioavailability in the water column. The San Francisco Estuary Institute (SFEI) has a long history of using bivalves (resident clams and transplanted oysters and mussels) as sentinel species. Davis and Taberski (2002) reported on the use of mussels as part of a regional monitoring program of water quality in San Francisco Bay. California's Department of Fish and Game State Mussel Watch Program (SMWP) has been in effect since 1976. The Mussel Watch program is part of a worldwide monitoring effort designed to detect the presence and concentration of toxic pollutants in estuarine and marine waters (Martin and Severeid 1984). California has also employed mussels in the freshwater toxic substances monitoring program (SWRCB 1990).

6.7.2 Sediments

Because sediments can be sinks for many contaminants, they are potentially impacted by discharges to a receiving waters. The Contaminated Sediment Management Plan (USEPA 1998) has as its goal, "to reduce fragmentation, duplication, and increase more holistic approaches to pollution prevention." For example, NPDES permitted facilities may be meeting all their chemical-specific, parameter-specific, and WET requirements and yet sediment contamination could result from releases from these facilities. There are more than ten Federal statutes that provide authority to EPA program offices to address the problem of contaminated sediment. The EPA (1998) studied data from 1,372 of 2,111 watersheds in the continental United States and, based on the approach discussed below, identified 96 watersheds that contain "areas of probable concern" (APC). Four goals have been established to address the problem of contaminated sediment (USEPA 1998). These goals are:

- prevent the volume of sediment from increasing,
- reduce the volume of existing contaminated sediment,

- ensure that sediment dredging and dredged material disposal are managed in an environmentally sound manner, and
- develop scientifically sound sediment management tools for use in pollution prevention, source control, and dredged material management.

It is important to note that these 96 watersheds have been identified from existing databases and do not represent all the watersheds or portions of watersheds that might meet the criteria for APCs. A complete inventory of contaminated sediments in the United States has not as yet been established (USEPA 1997b). It is also important to note that the time span covered by the database from which these 96 APC watersheds were developed was 1980 to 1993. An updated report that is in draft form (USEPA 2001d) will provide new estimates for data up to and including 1999 as to the number and distribution of APCs.

Through the Federal Insecticide, Fungicide, Rodenticide Act (FIFRA), EPA has the authority to ban or restrict the use of pesticides that have the potential to contaminate sediments if the risk is judged to be unreasonable. However, sediment toxicity has not been a part of routine test procedures and risk assessments for pesticide registration, re-registration or special review, even though prevention is clearly a better strategy than remediation.

Sediment has been functionally defined as all of the detrital, organic and inorganic particles that settle to the bottom of a body of water. In many sediment types (depositional sediments), water is found between the particles in the sediment and is termed interstitial or porewater. This water becomes very important in consideration of toxicity of contaminated sediment. Power and Chapman et al. (1992) divide sediment into four main compartments: interstitial water, organic, inorganic, and anthropogenically derived materials, including contaminants and eroded topsoil. According to their classification scheme, the largest volume is occupied by interstitial water that may occupy over 50% by volume of surface sediments. The inorganic phase includes the rock and shell fragments and mineral grains that originate from natural erosion of terrestrial materials. Organic matter is a variable, but small, fraction that occupies a low volume but is an important component because it can regulate the sorption and bioavailability of many contaminants.

Sediment toxicity tests are utilized much like WET tests, but their focus is on evaluating ambient sediment conditions. Freshwater and marine sediment testing protocols are described fully by the EPA (USEPA 1994d, 1994e, 2000d). The objective of sediment toxicity testing is to determine if chemicals in the sediment are harmful to, or accumulated by, benthic organisms. Sediment toxicity tests can be used to (1) determine the relationship between toxic effects and bioavailability, (2) investigate interactions among chemicals, (3) compare the sensitivities of different organisms, (4) determine spatial and temporal distribution of contamination, (5) evaluate dredge material, (6) measure toxicity as part of product licensing or safety testing or chemical approval, (7) rank areas for cleanup, and (8) set cleanup goals and estimate the effectiveness of remediation or management practices (USEPA 2000d). In addition to the methods in EPA 2000d, standard methods for assessing the toxicity of contaminants associated with sediments have been developed using amphipods, midges, polychaetes, oligochaetes, mayflies, and cladocerans (ASTM 1999a, 1999b, ASTM 1999c; USEPA 1994d, 1994e; Environment Canada 1997a, 1997b).

The sediment quality triad is an integrative approach for evaluating sediments (Chapman et al. 1992). This process is defined as any three-component integrative assessment that includes sediment toxicity, sediment chemistry and some measure of *in situ* bioeffects (often benthic infaunal community structure). The sediment quality triad is based on a *weight of evidence* approach for determining impact. For example, if chemistry indicates a potential impact, and toxicity tests show adverse effects, then the weight of evidence is strong that contaminants are impacting the sediment. Multiple toxicity tests on a variety of species do not substitute for other part of the triad, but do increase the strength of the toxicity leg. Detection of resident community alterations through bioassessments also reinforces the possibility of an impact.

Often, when information is gathered for assessing impacts, a tiered approach is used. By starting with the least complex and least expensive testing methodologies, a weight-of-evidence can be built over multiple metrics. If the metrics of the triad provide mixed results, then additional information may be needed to resolve the conflicts. However, some conclusions from mixed effect results can guide additional studies (Table 6-2). As with most assessments of environmental quality, the more quality information that is available, the greater is the likelihood that the assessment will be accurate.

Table 6-2. Information Provided by Differential Triad Response

| <i>Contamination</i> | <i>Toxicity</i> | <i>Alteration</i> | <i>Possible Conclusions</i> |
|----------------------|-----------------|-------------------|--|
| + | + | + | Strong evidence for pollution-induced degradation |
| - | - | - | Strong evidence that there is no pollution-induced degradation |
| + | - | - | Contaminants are not bioavailable |
| - | + | - | Unmeasured chemicals or conditions exist with the potential to cause degradation |
| - | - | + | Alteration is not due to toxic chemicals |
| + | + | - | Toxic chemicals are stressing the system |
| - | + | + | Unmeasured toxic chemicals are causing degradation |
| + | - | + | Chemicals are not bioavailable or alteration is not due to toxic chemicals |

Source: Chapman et al. 1992

6.7.3 Sediment Collection

Procedures for collecting, storing, and manipulating sediments for chemical and toxicological analyses are well documented (USEPA 2001d; ASTM 2000). The EPA test methods manual represents a compilation of information from governmental documents to peer-reviewed literature and is an important source of information regarding the sediment phase of the aqueous

environment. ASTM has also published a guide for the collection, storage, characterization and manipulation of sediments for toxicological testing (ASTM 2000).

The goal of any sediment sampling program should be to collect sediment in a manner that produces minimally disturbed sediment. The methods used to sample, transport, handle, and store and manipulate sediments and interstitial waters can influence the physicochemical properties and the results of chemical, toxicity and bioaccumulation analyses (USEPA 2001d). Many of the areas covered in EPA's technical manual are subjects of active research programs and, while the intent of the manual is to provide methodologies that minimize sampling impact, the authors recognize that methods are likely to evolve and that new additions of the technical guidance will reflect those advances. To keep pace with the changes visit www.epa.gov and search on sediment sampling and sediment testing.

There are many devices that have been used to collect whole sediments. The choice of sampling method is dependent to a large degree on what the sample is to be used for. The EPA sediment technical manual (USEPA 2001d) has a good discussion of the various collection methods and their strengths and weaknesses. Sampling sediments to determine the average concentration of chemical contaminants can be problematic. For monitoring and assessment studies, the upper 10-15 cm of sediment is normally collected because this is the area where most of the epibenthic and benthic organisms and the most recently deposited sediments are found. These samples can be used for physical and chemical analyses, benthic community analysis, and toxicity tests. In many instances, sub-samples of equal size from sediment samples can eliminate or reduce the influence of unequal sized grab samples.

Interstitial water, or pore water, is the liquid contained within every sediment sample. This water may occupy up to 50% by volume in silt and depositional sediments (Sarda and Burton 1995; USEPA 2001d). Because interstitial water is in intimate contact with the sediment, it is assumed to be in thermodynamic equilibrium with contaminants in the sediment, and is generally to be considered the route of exposure for many sediment contaminants. In addition, contaminants in interstitial water can be transported to overlying waters through diffusion, bioturbation and re-suspension (Sarda and Burton 1995).

Interstitial water can be used to evaluate sediment toxicity with organisms that are normally used in aquatic toxicity tests (Carr and Nipper 2003). To evaluate interstitial water it must be separated from the sediment matrix. It should be noted that extraction of interstitial water can alter the chemistry of the sample (Sarda and Burton 1995). There are several methods used to isolate interstitial water from sediment including centrifugation, pressurization, or suction. *In situ* sampling devices for interstitial water have also been used. The most commonly used methods are "peepers" and suction devices. Peepers are samplers that have a rigid body with openings covered with permeable membranes. Prior to deployment, the openings are filled with a medium consistent with sample objectives. The peeper is then placed in the sediment and the medium in the openings is allowed to come into equilibrium with the surrounding interstitial water. The equilibration time varies, but multiple-week exposures are not unusual (USEPA 2001d; Sarda and Burton 1995). These methods generally produce smaller volumes of water (<500 mL) compared to centrifugation and pressurization and are often limited to shallower

water depths. A variety of peeper designs along with diffusion samplers, vapor diffusion samplers, and semi-permeable membrane devices are discussed on the EPA website (<http://clu-in.org/programs/21m2/sediment/>). Regardless of the method of collection porewater samples should be processed as soon as possible after collection.

6.7.4 Freshwater Sediment Test Organisms

The EPA sediment test methods manual (USEPA 2000d) describes five methods for three organisms to measure the toxicity and bioaccumulation of contaminants from freshwater sediments. Two of the methods, one for the amphipod *Hyalella azteca* and one for the insect *Chironomus tentans*, measure survival and growth over a 10-day exposure period. One of the methods measures survival, growth and reproduction of *H. azteca* over a 42-day test, and one measures effects on *C. tentans* over the life-cycle of the insect. A bioaccumulation test with *Lumbriculus variegatus* is also presented.

Recently, sediment toxicity has been documented in urban waterways (Amweg et al. 2006) and agriculturally dominated waterways (Weston et al. 2004). The reader is encouraged to consult these published studies prior to designing or reviewing sediment toxicity. Phillips et al. (2006) and Anderson et al. (2006) describe TIE procedures for identification of the causes of toxicity in sediments from agriculturally dominated watersheds in California.

6.7.5 Bioassessments

Benthic infauna surveys can be accurate indicators of ecosystem health, and benthic surveys are frequently used as biocriteria to assess ecological integrity (Gibson et al. 2000; Borja 2005). Benthic data can be evaluated against historical data, reference conditions, models and indices, and with consensus professional judgment. Although standard benthic evaluation tools exist, the interpretation of benthic data is often subjective and based on best professional judgment (SCCWRP 2006). Moreover, because the presence of resident biota is region-specific, interpretation of bioassessment data must be based on the ecoregion.

Rapid Bioassessment Protocols (RBPs) were developed for freshwater environments as inexpensive screening tools for determining if a stream was supporting its designated aquatic life use (Plafkin et al. 1989). EPA guidance for marine bioassessments is provided in Gibson et al. (2000), but there are also a number of published marine bioassessment studies (e.g. Thompson and Lowe 2004; Weisberg et al. 1997; Smith et al. 2001). As these protocols were applied and modified, the areas in which the protocols provided useful information expanded to include:

- Characterizing the existence and severity of impairment to the water resource
- Helping to identify sources and causes of impairment
- Evaluating the effectiveness of control actions and restoration activities
- Supporting use attainability studies and cumulative impact assessments
- Characterizing regional biotic attributes of reference conditions.

The revised RBPs have been adopted and modified by various states to meet their monitoring needs (Barbour et al. 1999). Once adapted to the characteristics of a state, consistent reproducible procedures can be used to evaluate the status of a wadeable river or stream. One of the goals of the application of RBPs is to develop *biocriteria* that can be tailored to reflect the kind of biological system that should be found in waters that have a particular designated use (public water supply, for protection of fish, shellfish, and wildlife, and for recreational, agricultural, industrial, and navigational purposes). Once biocriteria are developed, a biosurvey of a receiving system with a particular designated use can be performed to determine if that system meets the requirements for that designated use. There are only a few places in the country that have developed biocriteria.

Implementing biocriteria in California is the responsibility of the State Water Resources Control Board and the Regional Water Quality Control Boards. In California, there is not one single entity responsible for developing statewide bioassessment protocols. As a consequence, five candidate programs exist in California that could provide the framework for the implementation of statewide bioassessment methods (SWRCB 2003). Bioassessments have been conducted at over 3000 sites in California by a variety of agencies. The California Department of Fish and Game (CDFG) bioassessment methodology has been used the most, with over 2500 sites sampled (SWRCB 2003). The more recent organization of California's Surface Water Ambient Monitoring Program (SWAMP) should provide the impetus to implement a better organized and standardized biological and assessment program (SWRCB 2003).

The California DFG is a leader in establishing taxonomic standards for statewide bioassessment efforts, an immense undertaking, given the size and diversity of ecoregions in California. The CABW was established as a forum for researchers, agency personnel and private consultants working in the field of freshwater biology. In 1995 the California Aquatic Macroinvertebrate Laboratory Network (CAMLnet <http://www.dfg.ca.gov/cabw/camlnetste.pdf>) workgroup was started to develop consistent, sound methodological approaches to aquatic bioassessment, to provide mentoring and support, and to facilitate communication by promoting discussion of findings and bioassessment programs.

In 1999, CAMLnet produced the first edition of the CAMLnet List of Standard Taxonomic Effort (LSTE). This document defines the basic level of taxonomic resolution to be used by all CSBP data analyses. To conform to the CSBP standard effort levels, taxa may be identified to more, but not less precise, levels than those listed in the LSTE. The latest version (2003) of the list can be found at www.dfg.ca.gov/cabw/camlnetste.pdf. These protocols fit the essentials of the wadeable protocol to these specialized habitats.

An important and difficult step that is being pursued is the establishment of reference conditions for each of the types of waterbodies. The reference sites are, in theory, pristine sites for that waterbody type. Once the bioassessments of the reference conditions are in place, all streams of the same physical attributes (e.g., wadeable streams in a particular hydrologic unit) should have conditions equal to the reference site's conditions. In practice, it is difficult to find pristine sites for any given waterbody type, so the use of "least impacted" sites are often used instead. Regardless of the final choice of bioassessment protocols chosen for use, they will become an important tool in the arsenal of tools water quality managers have at their disposal.

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CHAPTER 7. ENFORCEMENT PROCEDURES FOR WET

7.1 Overview

The following discussion provides guidance on determining appropriate enforcement responses to violations of WET limits and conditions. This guidance incorporates the two main goals of EPA's NPDES compliance and enforcement program which are (1) to compel or require the permittee to expeditiously achieve and maintain compliance, and (2) to serve as a deterrent.

7.2 Background

CWA Section 309(a) states that any violation of a permit condition or limitation is subject to enforcement. Through EPA's 1989 national NPDES enforcement guidance, Enforcement Management System (EMS) guidance, the EPA Regional or State enforcement authority is encouraged to initiate an appropriate enforcement response to all permit violations. EPA's overall approach to enforcement applies to all parameters, including WET. Once a facility has been identified as having an apparent permit violation(s), the Permitting Authority reviews all available data on the seriousness of the violation, the compliance history of the facility, and other relevant facts to determine whether to initiate an enforcement action and the type of action that is appropriate. **The EMS recommends an escalating response to continuing violations of any parameter. Regions 9 and 10's enforcement follows the EMS.**

In a joint memorandum issued by EPA Headquarters Office of Regulatory Enforcement and Office of Wastewater Management (USEPA 1995b) EPA clarified National policy with regard to the two most common issues raised by the regulated community involving the enforcement of WET requirements in NPDES permits: 1) single exceedance of WET limits, and 2) inconclusive toxicity reduction evaluations (TREs).

EPA does not recommend that the initial response to a single exceedance of a WET limit, causing no known harm, be a formal enforcement action with a civil penalty. The regulated community has expressed concern about the potential for third party lawsuits for single exceedance of WET. Citizens cannot sue a permittee on the basis of a single violation of a permit limit. Under section 505(a) of the CWA, citizens are allowed to take a civil action against anyone who is alleged to "be in violation" of any standard or limit under the CWA. In Gwaltney of Smithfield, Ltd., v. Chesapeake Bay Foundation, Inc., 484 U.S. 49, 1008 S.Ct. 376, 98 L.Ed.2d 306 (1987), the Supreme Court held that the most natural reading of "to be in violation" is "a requirement that citizen-plaintiffs allege a state of either continuous or intermittent violation--that is, a likelihood that a past polluter will continue to pollute in the future." A State may have its own enforcement policy which may be more stringent.

In the case of inconclusive TREs, EPA recommends that solutions in these cases be pursued jointly with expertise from EPA and/or the States as well as the permittee. Solutions may involve special technical evaluation, as well as relief of civil penalties. The primary corrective action required for violations of WET limits is completion of a TRE, including, if necessary, a TIE. This requirement is incorporated into the Regions' NPDES permits. The permit language addressed in this document contains provisions requiring the permittee to: implement the generic

TRE plan; increase the testing frequency following a violation or monitoring trigger if necessary; and, if also necessary, initiate a facility-specific TRE and a TIE following additional toxic sample(s) during the accelerated monitoring period. The permits require permittees to develop and submit a generic TRE workplan within 90 days of permit issuance.

Table 7-1 summarizes the Regions' WET enforcement responses. The following sections discuss the types of noncompliance and the appropriate enforcement responses in more detail. Appropriate federal or State laws, policy and enforcement personnel need to be consulted prior to a determination of noncompliance or initiation of enforcement actions.

Table 7-1. Enforcement Response Summary

| <i>Noncompliance</i> | <i>Initial Response</i> | <i>Elevated Response Following Repeated or Sustained Violations</i> |
|--|-------------------------|---|
| Limit Violations | Phone call, LOV or NOV | NOV/AO; AO/APO; judicial referral |
| Failure to Conduct TRE, TIE, or Accelerate Testing | NOV/AO | AO/APO; judicial referral |
| Failure to Test | NOV/AO | AO/APO |
| Invalid Results | | |
| - Good Faith Effort | Tech. Assist. | NOV/AO; APO |
| - Lack of Good Faith | NOV/AO | AO/APO |
| - Failure to Re-Test | NOV/AO | AO/APO |
| Failure to Comply with Narrative Conditions of | NOV/AO | AO/APO |

LOV = Letter of Violation NOV = Notice of Violation
 AO = Administrative Order APO = Administrative Penalty Order

7.3 Types of Noncompliance

Noncompliance with the NPDES permit and the CWA includes:

- (a) violation of WET permit limit(s),
- (b) failure to conduct WET tests,
- (c) failure to provide valid test results (i.e., meet all test acceptability criteria) or otherwise comply with the permit's test and quality assurance procedures, including failure to re-test within 14 days following the failure to meet test acceptability criteria,
- (d) failure to comply with any other WET NPDES permit conditions, including the conditions requiring:
 - (1) an increase in the testing frequency following a violation or monitoring trigger requirement,

- (2) an initiation of a TRE within 15 days of a violation as described in AO or permit
- (3) initiation of a TIE following a subsequent violation during the accelerated monitoring period,
- (4) a submittal of a generic TRE work plan within 90 days of permit issuance,
- (5) initial screening, or annual re-screening, for the most sensitive species,
- (e) failure to comply with the permit's reporting requirements and,
- (f) failure to comply with the terms and conditions of an Administrative Order (AO) or consent decree.

7.4 Types of Enforcement Actions

EPA or an appropriate State has discretion to determine that enforcement action is warranted and the type of action that is appropriate. EPA's EMS recommends an escalating response to continuing violations. There are three major categories of potential responses: no action, informal enforcement action and formal enforcement action. In ascending order of severity, the enforcement actions available to EPA include Notice of Violations (NOVs) and Administrative Orders (AOs), Administrative Penalty Orders (APOs), Civil judicial action, and criminal prosecution. EPA Region 9 generally issues an AO along with all NOVs (with the exception of NOVs issued to Federal Facilities). Other EPA Regions and States may issue NOVs without an accompanying AO. Similar State actions are available to each authorized State. Determination of the appropriate enforcement response for WET violations will be based on the same factors used to determine the appropriate response for chemical-specific violations, that is, the need to compel or expedite a permittee's return to compliance, and the deterrent value of a particular enforcement response. EPA/State should consider such factors as:

- (a) the duration of noncompliance or number of violations;
- (b) the severity or significance of the violations, and the resultant environmental harm;
- (c) the cause or source of the violations and a permittee's degree of control over the causative agent of toxicity;
- (d) a permittee's history of violations/recalcitrance; and,
- (e) the economic benefit gained from noncompliance.

7.4.1 Notice of Violation and Administrative Order for Compliance

An AO, or its equivalent, issued in conjunction with a NOV, should require the permittee to comply with WET limits and conditions by specified dates. Required compliance with most narrative permit conditions should be immediate. The AO should specify the required corrective actions, or require the permittee to develop, submit for approval, and implement a corrective action plan. Generally, EPA/State should issue an NOV/AO or the equivalent under the following scenarios:

- (a) a permittee failed to conduct the required WET tests on one or more occasions;
- (b) after a WET limit violation, a permittee failed to initiate a TRE and/or TIE, or failed to increase the testing frequency;
- (c) a permittee failed to comply with any narrative WET permit condition on one or more occasions including conditions addressing reporting requirements, species screening requirements, or submittal of a TRE workplan;
- (d) a permittee failed to provide valid test results, or otherwise failed to comply with permit conditions regarding test procedures or quality assurance, including the requirement to re-test within 14 days following the failure to meet test acceptability criteria;
- (e) a permittee's TRE efforts are inadequate, the corrective actions are inadequate, or the time frames for completing corrective actions are unacceptable;
- (f) a permittee may need some additional incentive to complete the necessary corrective actions (e.g., when corrective actions require long construction schedules, or are expensive, or a permittee has a history of recalcitrance);
- (g) WET violations resulted in documented environmental impacts;
- (h) the permittee has not eliminated or reduced the toxicity within a reasonable amount of time, and the violations are ongoing, whether continuously or sporadically.

7.4.2 Administrative Penalty Order (APO)

Issuance of an APO would be appropriate if the permittee has demonstrated recalcitrance; if violations have continued over an extended time period or have repeatedly reoccurred; if the violations are especially serious; or if the violations could have reasonably been avoided. APOs only penalize permittees for past violations. Therefore, if additional corrective action is necessary, an AO should also be issued, or a civil judicial referral should be considered. EPA/State should consider issuing an APO, or its equivalent, for the following situations:

- (a) a permittee failed to initiate a TRE and/or TIE, or failed to increase the testing frequency, on several occasions or after an extended period of noncompliance;
- (b) a permittee repeatedly failed to comply with any narrative WET condition or repeatedly failed to provide valid test results;
- (c) a permittee repeatedly failed to conduct WET tests;
- (d) the WET limit violation(s) was caused by negligence, poor operation and maintenance practices, a poor pretreatment program, or other circumstances within the control of the permittee which could have reasonably been avoided. [Note: Certain types of negligence may be dealt with more appropriately through criminal prosecution. These cases should be referred to EPA's criminal investigations division, or to the appropriate State agency.];
- (e) the WET violation(s) resulted in, or contributed to, significant adverse environmental impacts;
- (f) the permittee gained significant economic benefit from noncompliance;

- (g) the permittee demonstrated recalcitrance in initiating or completing corrective actions; and,
- (h) the penalty calculation, which is based on economic benefit and gravity, is less than \$157,000.

7.4.3 Civil Judicial Action

A civil judicial action is appropriate under circumstances similar to an AO with an APO, but where the severity of violations or degree of recalcitrance is greater; additional corrective actions are required; or the economic benefit derived from noncompliance is greater. EPA and the State should consider a civil referral in response to the following:

- (a) a permittee's repeated failure to conduct a TRE or increase the testing frequency during an extended period of noncompliance or recurring periods of noncompliance despite previous enforcement actions or other direction from EPA or the State;
- (b) a permittee's repeated failure to conduct a TRE in an aggressive or good faith manner, or to otherwise eliminate or reduce toxicity;
- (c) a permittee's failure to adequately comply with an AO;
- (d) situations where extensive corrective action is required, especially extensive construction, or where a permittee may need extra incentive to complete corrective actions due to time, cost or potential recalcitrance;
- (e) situations where corrective actions are costly and allowed the permittee to gain significant economic benefit from delayed compliance;
- (f) situations where the violations resulted in or contributed to significant environmental impacts; and
- (g) the penalty calculation, based on economic benefit and gravity, exceeds \$157,000.

7.4.4 Criminal Prosecution

For willful, knowing, or negligent violations of the NPDES permit or CWA, the permittee can be subject to criminal penalties. These cases should be referred to the Criminal Investigations Division of EPA, or the appropriate State office.

7.5 Other Factors to Consider When Deciding an Appropriate Response:

In comparison to chemical-specific effluent limit violations, it can be more difficult to identify the causative agents of WET violations and to isolate the sources of toxicity. In addition, once the toxic agents and sources are identified, it can be more difficult to control these sources, especially without costly technological solutions. This is especially true for municipal treatment facilities where the public, commercial establishments and industry can all contribute to toxicity. Although these factors should not deter EPA or the State from taking enforcement action, they

should be considered when assessing the appropriate enforcement response and determining reasonable compliance dates.

In general, the EPA Regions or the State may decide enforcement action is not necessary following a violation of WET limitations if the permittee adequately complies with its NPDES permit requirements for accelerating testing and conducting a TRE. Enforcement action would be appropriate if the permittee failed to aggressively conduct a TRE or was otherwise recalcitrant in addressing the toxicity.

Exceptions to this general guideline include situations where the WET violation(s) are of large magnitude, or contributed to significant environmental impacts (there may be violations of chemical-specific effluent limits as well); the permittee may need additional incentive to complete corrective actions identified by the TRE; the permittee failed to eliminate/reduce toxicity within a reasonable time frame; or, the WET violations were caused by circumstances within the control of the permittee and could have been reasonably avoided. In cases like these, EPA/State should consider enforcement action even if the permittee did initiate a timely TRE.

7.6 Invalid Test Results

When a permittee is experiencing difficulty in meeting test acceptability criteria, EPA/State's initial response should be technical assistance (provided the permittee is making a good faith effort). If this proves unsuccessful, or the permittee is not making a good faith effort, EPA/State should then consider enforcement action. The initial enforcement action will typically be a Notice of Violation and Administrative Order (NOV/AO), or its equivalent, which would require the permittee to take appropriate measures to ensure the tests are properly conducted, such as finding a contract lab that is able to conduct the tests. In addition, if the permittee fails to re-test within 14 days following one or more failures to meet test acceptability criteria, EPA/State should issue an enforcement order.

7.7 Noncompliance with Other Narrative WET Permit Conditions

A permittee's failure to comply with any other narrative WET permit condition, such as the requirement to develop a TRE workplan, screen for the most sensitive species, or comply with reporting requirements, should also result in enforcement action. Initially, EPA or the State should issue an NOV/AO (or its equivalent) which requires immediate compliance. An exception could be made for first time or infrequent offenders who generally appear to be acting in good faith. In these cases, EPA/State could resolve issues of noncompliance through a verbal notice of violation, or a simple written NOV without an AO.

References

USEPA. 1995b. National policy regarding whole effluent toxicity enforcement. July 6, 1995. Memorandum from Brian Maas. Office of Enforcement and Compliance Assurance. Washington, DC.

APPENDIX A

FREQUENTLY ASKED QUESTIONS

Permitting:

Q: Are WET tests reliable and accurate to be used in the NPDES permitting program?

A: While some permittees may still contend that WET tests are inherently unreliable and inaccurate, the U.S. Court of Appeals recently rejected arguments that the variability observed in WET test methods (i.e., method variability) is excessive, concluding “. . . EPA has demonstrated that it is not.” (See *Edison Electric Institute, et al., v. Environmental Protection Agency, et al.*, 391 F. 3d 1267, 1272 (D.C. Cir. 2004)). In this case, the Court determined that EPA had “gone far enough” to minimize the effect of organic idiosyncrasy (the use of living specimens) by taking experimental and statistical precautions in designing and refining the WET test methods, denying the petitioners’ complaint that EPA had not gone far enough to minimize the potential for variability between and within-tests.

Q: Can a State use either point estimate or hypothesis testing techniques for analyzing toxicity test data?

A: EPA allows State Permitting Authorities the choice of either hypothesis testing or point-estimation techniques for developing permit conditions and determining compliance. While several important drawbacks of the NOEC have been identified, hypothesis testing, per se, with safeguards is acceptable (Fox and Denton 2002). Such safeguards can include: testing a series of concentrations to verify and quantify a concentration-response relationship; increasing power (i.e., decreasing the type II error rate); closely bracketing the IWC by adjacent concentrations; applying an percent minimum significant difference (PMSD) as a test sensitivity criterion. Note, that for reasonable potential determination EPA has recommended using point estimate procedures in NPDES testing even when NPDES self-monitoring data are required to be determined using hypothesis testing techniques (USEPA 2000b). However, the permit limit can be still expressed and reported using hypothesis testing techniques, while also requiring reporting of specified point estimates for calculating facility-specific CVs for determining reasonable potential of toxicity.

Q: Should detection or quantitation limits be set if toxicity limits are established?

A: EPA has stated that method detection limit concepts are not applicable to WET test methods and have not been applied historically to toxicity testing methods developed by EPA or other scientific entities. EPA also believes that the test design employed in WET testing including controls, replication, and hypothesis testing or point estimation techniques, all provide an adequate protection from false positives. Detection limits are applicable only to tests that rely on instrumental measurements; the detection limits represent the sensitivity thresholds of the technology below which measurements become unreliable or impossible. Because WET testing is a biological and experimental method, rather than an instrumental method,

detection limit concepts are not applicable. In the Edison electric Institute et al v EPA (D.C. Cir. 2004, pg 10 - 11), it was decided that the described safeguards in EPA's WET methods addressed the petitioners' concerns and that EPA had offered a reasoned, thorough explanation of its decision on this subject without further requirement by law.

Q: Is the use of a numeric limit justifiable?

A: Yes, EPA emphasizes that the Clean Water Act (CWA), NPDES regulations, EPA's Technical Support Document for Water Quality-based Toxics Control (TSD, USEPA 1991a) all clearly envision that effluent limits should be expressed numerically. (See CWA 301(b)(1)(C) and 502(11); 40 CFR 122.44(d)(1)(iv) and (k) and 122.2).

- a. By definition, 40 CFR 122.2 describes an effluent limitation as a restriction imposed . . . on quantities, discharge rates, and concentrations of 'pollutants';
- b. According to 40 CFR 122.44(d)(v), limits on whole effluent toxicity are necessary when chemical-specific limits are not sufficient to attain and maintain applicable numeric or narrative water quality;
- c. See chapter 5 of the TSD (USEPA 1991a), which describes the methodology to be used for calculating a statistical numeric limit for pollutants, including chronic toxicity;
- d. Appendix B of the TSD, Basic Principles for Whole Effluent Toxicity, describes EPA's intent to have numeric limits for chronic toxicity, "Final whole effluent toxicity limits must be included in permits where necessary to ensure that State Water Quality standards are met. These limits must properly account for effluent variability, available dilution, and species sensitivity." This does not fit the description of a numeric effluent limit, because a narrative effluent limit cannot account for variability or available dilution. A numeric limit, on the other hand, can be calculated in such a way as to account for variability or available dilution; and,
- e. In this document and (Denton and Narvaez 1996), both describe establishing limitations for chronic toxicity in the form of a daily maximum and a monthly median.

Q: When writing a permit for a discharge which only occurs intermittently throughout the year. What type of WET requirements should be incorporated into the permit?

A: Permit conditions describing the appropriate limits and monitoring triggers, test methods and species should clearly be defined in any permit. However in the situations of intermittent discharges it would be identified in the permit that during those periods of no discharge, the monitoring and testing conditions would not apply.

Q: What does that mean when it says that “permittees must certify on DMR statements that these are accurate”?

A: EPA clarified in its March 3, 2000 memorandum to EPA Regional Water Management Division Directors and Enforcement Division Directors that the purpose and meaning of the DMR certification was to certify only that all WET test results had been submitted and not tampered with or inappropriately modified prior to reporting on the DMR. The memorandum sought to resolve the confusion over the term accuracy which is sometimes used as a term to describe performance characteristic of a measurement system. In the context of DMR certification, the term accuracy is a certification of information submission, namely that information provided is accurate as a layperson uses the term, rather than accurate as the term is used to describe quantifiable performance of a measurement system. Therefore, the DMR certification is not intended to certify the WET test results are accurate including whether or not the WET test results are valid from a toxicity test standpoint (e.g., quality assurance/quality control on the tests was done properly by the analytical laboratory). Rather when a person certifies that the submission of WET testing information is accurate to the best of his/her knowledge and belief, the person certifies that the results obtained using the WET test procedures are faithfully and truthfully transcribed on the information submission, and the results were in fact results obtained using the specified test procedures.

Q: Is WET appropriate for circumstances of effluent dominated streams, and storm and ambient water applications?

A: The Permitting Authority, at the time of permit issuance, makes a determination as to whether WET testing, permit limitations for WET, or other requirements are appropriate and necessary to protect the receiving stream from potential toxic impacts from the permit to discharges. This determination is made on a case-by-case basis after considering the existing controls on point and non-point sources of pollution, the variability of the pollutant or plume parameters in the effluent, the sensitivity of the species to toxicity testing, and the dilution of the effluent in the receiving water (40 CFR 122.44(d)(1)(ii)).

Q: What is EPA's guidance to States in regards to a single exceedance of a WET limit?

A: EPA points to the August 15, 1995 national policy memo regarding WET enforcement and it specifies that the initial enforcement response to a single exceedance of a WET limit, causing no known harm, should not be a formal enforcement action with a civil penalty, but that any violation of a WET limit is of concern and should receive an immediate, professional review by the Permitting Authority (USEPA 1995b). EPA’s recommended response to an isolated or infrequent violation of a WET limit, causing no known harm, is issuance of a letter of violation or Administrative Order which does not include a penalty. EPA policy suggests that additional testing is an appropriate initial response to a single WET limit violation and an escalated enforcement response to repeated violations.

Q: Are mixing zones applicable for WET permit limits?

A: This depends on the authorization of mixing zones for toxicity under a States' water quality standards. However, mixing zones even if allowable in a State's water standards plan may not be appropriate for a specific discharge location. This depends on the receiving water habitat. For example, if there are threatened and endangered species to be protected or sensitive spawning grounds, then a mixing zone may not be appropriate.

Q: Do Permitting Authorities have discretion to evaluate and, if necessary, reject unrepresentative or invalid WET data before use them in making a reasonable potential determination?

A: Yes, however this does not mean that Permitting Authorities have the right to determine that valid and representative WET data that demonstrate effluent toxicity are to be considered irrelevant and disregarded when determining whether a WET limit is needed in a NPDES permit.

Q: How should potential ionic imbalance toxicity to be evaluated?

A: Ion imbalances can cause toxicity in effluents. When toxicity effluent limits or monitoring triggers are exceeded, the permittee shall implement a TRE as described in their TRE work plan. Where TDS is a suspected toxicant, the permittee should utilize EPA's TIE procedures, in conjunction with recommendations prescribed by Goodfellow et al. (2000) to identify the specific ions contributing to TDS toxicity; regulatory or technical solutions may be possible if ions are identified as the only responsible effluent toxicant. In situations where ions and another toxicant are identified, the initial responsibility is to effectively address the other toxicant. After that toxicant is dealt with, then ion-specific toxicity in the discharge can be appropriately addressed and potential management and regulatory options considered by the Permitting Authority.

Testing Issues:

Q: Are toxicity test methods as precise as analytical test methods?

A: EPA found that WET test methods are as precise as chemical methods (USEPA 2000b). WET test method variability can be minimized by focusing on strict adherence to the EPA WET test method procedures; by using additional test acceptability criteria (TAC); by ensuring that laboratory personnel are properly trained to perform the tests correctly; and other preventive measures such as proper sample collection and storage.

Q: How is precision calculated for a test method?

A: Coefficient of variation (CV) is the descriptive statistics for quantifying test method precision. CV is the ratio of the standard derivation to mean. The precision of the effect concentrations is quantified by obtaining multiple test results under similar test conditions using the same test material. For example, the standard deviation and mean for EC₂₅

obtained for a specific test method from multiple monthly reference toxicant tests conducted at one laboratory would quantify “within-laboratory” precision for that laboratory.

Q: How are the types of test method variability defined?

A: There are several measures of variability related to WET tests included are within-test variability, within-laboratory variability, and between-laboratory variability. Within-test (intra-test) variability is the variability in test organism response within a concentration averaged across all concentrations of the test material in a single test. Within-laboratory (intra-laboratory) variability is the variability that is measured when reference toxicant tests are conducted using specific methods under reasonably constant conditions in the same laboratory. Within-laboratory variability includes within-test variability. Between-laboratory (inter-laboratory) variability is the variability between laboratories. It is measured by obtaining results from different laboratories using the same test method and the same test material (e.g., reference toxicant).

Q: Define the applicability of method detection limit to WET test methods?

A: EPA established the method detection limit concept specifically for chemical methods, where results generally consist of a single measurement of the pollutant of interest by an analytical instrument. The method detection limit concept uses information about the variability of the measurement system to determine a response level at which the measurement can be reliably distinguished from background noise, thus providing protection from false positive results. In WET testing, the final result is not based on a single measurement, but is the product of a series of replicated measurements on a range of effluent concentrations. The additional measurements, controls, replication, and statistical approaches included in WET test method measurement system ensure that measured responses can be reliably distinguished from background noise.

Q: Are laboratories routinely able to achieve the required test acceptability criteria on a routine basis?

A: EPA conducted a national interlaboratory study (USEPA 2001a, 2001b) of toxicity test methods; EPA confirmed that the methods are adaptable to a wide variety of laboratories and that the methods generate reproducible results in laboratories.

Q: Are the chronic tests for NPDES effluent testing required to use multi-concentrations rather than a single concentration vs control?

A: The decision to use a multi-concentration or single-concentration tests approach is typically defined in a State’s water quality standard control plan or policy. The November 2002 WET methods rule did not address or change any EPA policy concerning multi-concentration versus single concentration testing. With regards to the method manuals, they do not definitively say that multi-concentration testing is required for NPDES effluent tests. In fact, Section 8.10.1 says "the tests recommended for use in determining discharge permit compliance in the NPDES program are multi-concentration, or definitive, tests which

provide...". Often a State will utilize the single-concentration testing approach for assessing the toxicity of ambient or stormwaters. EPA recommends using multi-concentration testing for NPDES testing of effluents, but it is not required.

Q: Does EPA or its test method manuals require a specific dilution series to be used?

A: EPA has not required a specific dilution series or procedure for selecting dilution series. EPA recommends that test concentrations be selected independently for each test based on the objective of the study, the expected range of toxicity, the receiving water concentration, and any available historical testing information on the effluent.

Q: When there is no mixing zone allowed for toxicity, what dilution series should be used?

A: The following is suggested 100%, 62.5%, 50%, 25%, and 12.5%. This is following the basic suggestion of using the 0.5 dilution series. However, it is encouraged to include an additional concentration between 50 and 100% effluent especially since compliance would be based at the 100 percent effluent. The 62.5% effluent concentration is suggested especially if compliance is assessed at both 1.0 TU as a monthly median and 1.6 TU (e.g., 62.5% effluent) as a monthly average.

Q: What is the interpretation of the terms of "required" (using the term "must") and those that are "recommended" (using the term "should") when following the EPA test method manuals?

A: When EPA promulgated the 2002 WET methods (USEPA 2002a, 2002b, 2002c), these test method manuals clearly distinguish between required and recommended test conditions for the purposes of reviewing WET test data submitted under NPDES permits. EPA defined in the manual tables on summary of test conditions and test acceptability criteria for each method, such that each test condition is identified as required or recommended to be clear. In addition, EPA clarified the section on test review to each test method manuals. This section of the test methods manual provide technical guidance on the review of sampling and handling procedures, test acceptability criteria, test conditions, statistical methods, concentration-response relationships, reference toxicant testing, and within-test variability. This section clarifies that for WET test data submitted under NPDES permits, all required test conditions must be met or the test is considered invalid and must be repeated with a newly collected sample. Deviations from recommended test conditions must be evaluated on a case-by-case basis to determine the validity of test results. Deviations from recommended test conditions may or may not invalidate the test results depending on the degree of the departure and the objective of the test. The data reviewer should consider the degree of the deviation and the potential or observed impact of the deviation on the test result before rejecting or excepting a test results. For example, if dissolved oxygen is measured below 4.0 mg/L in one test chamber, the reviewer should consider whether any observed mortality in that test chamber corresponded with the drop in dissolved oxygen.

Q: What effluent pH should be used?

A: If the objective of the WET test is to determine the toxicity of the effluent and the receiving water, the pH should be maintained at the pH of the receiving water (measured at the edge of the regulatory mixing zone). If the objective of the WET tests is to determine the absolute toxicity of the effluent, the pH should be maintained at the pH of the sample on completion of collection (for freshwater testing) or after adjusting the sample salinity (for marine testing). The objective of testing is to determine the absolute toxicity of the effluent, so the effluent pH should be the target. The target pH should not be an average or median of the effluent pH. The target for each WET test should be the pH of the individual sample when sample collection is complete (e.g., if the sample is a 24 hr composite, use the measured pH of the composite after the compositing is completed). An average or median effluent pH should not be used, because WET test results are snapshots and should not try to be otherwise.

Reference Toxicant Testing:

Q: What is the purpose of reference toxicant testing?

A: The purpose of reference toxicant testing is: 1) to assess the health and sensitivity of test organisms over time, and 2) to document and demonstrate initially and ongoing acceptable laboratory performance. These purposes of reference toxicant testing are reflected in the reference toxicant testing requirements under quality of test organisms in the test method manuals. For a given test method, successive tests must be performed with the same reference toxicant, at the same test conditions, in the same dilution water type, using the same data analysis methods.

Q: At what frequency should reference toxicant tests be required?

A: The test method manuals specify that, regardless of the source of test organisms (in-house cultures or purchased from external suppliers), the testing laboratory must perform at least one acceptable reference toxicant test per month for each toxicity test method conducted in that month. If the test method is conducted only monthly, or less frequently, a reference toxicant test must be performed concurrently with each effluent toxicity test. Reference toxicant tests performed by organism suppliers cannot substitute for this requirement, because in addition to assessing the sensitivity of test organisms, reference toxicant testing is used to document ongoing laboratory performance. The manuals do allow reference toxicant control charts from organism suppliers as a substitute for concurrent reference toxicant testing with each effluent test. While the method manuals require the conduct of reference toxicant tests, it is the responsibility of the Permitting Authority to determine the requirements for reporting test results and associated data and a State can always be more stringent than minimum requirements of the test method manuals.

Q: How should a failed reference toxicant test result be evaluated and how should it impact effluent testing?

A: EPA test method manuals include the added a caution that “reference toxicant test results should not be used as a *de facto* criterion for rejection of individual effluent or receiving

water tests. Reference toxicant tests do provide information on trends in organism sensitivity and laboratory performance that can be useful in evaluating and interpreting effluent and receiving water tests results. For this reason, EPA has recommended evaluating the following elements of reference toxicant test results in the review of effluent and receiving water test data: the degree to which the reference toxicant tests result is outside of control chart limits, the width of the limits, the direction of a deviation (toward increased test organism sensitivity or toward decreased test organism sensitivity), the test conditions of both the effluent tests and the reference toxicant tests, and the objective of the test. .” In addition, EPA added recommendations to track the ongoing performance of individual QC measures such as PMSD, average control response, and CV of control response.

Sample Handling, Collection Issues:

Q: Should effluent samples only be collected as composite samples?

A: Composite and grab samples are allowed for WET testing depending on the objectives of the tests. Both sampling techniques have advantages and disadvantages that are described in the manuals. Permitting Authority should evaluate these advantages and disadvantages in light of the test objectives when selecting a sample type.

Q: Are the stipulations on filtration of effluents described in Section 9.1.2 of EPA/821/R-02/012 (USEPA 2002a) recommendations or requirements?

A: According to Section 9.1.2 of the acute test methods manual, filtering the sample through a 60 um mesh is only a requirement when the sample contains indigenous organisms that will interfere with the test. For example, some predatory invertebrates could eat the test organisms. If these interfering organisms are not present, the sample does not have to be filtered.

Q: What is the sample temperature range that is specified in the test methods manual?

A: According to the test method manuals the storage and shipping temperature of samples is in the range of 0-6 degree C. This modification provides greater consistency with national environmental laboratory accreditation conference (NELAC) standards.

Q: When should total residual chlorine be measured in the test methods?

A: If total residual chlorine is not detected in effluent or dilution water at test initiation, it is unnecessary to measure total residual chlorine at test solution renewal or at test termination. If total residual chlorine is detected at test initiation, then measurement of total residual chlorine at test solution renewal and test termination would continue to be required. It is not necessary to measure total residual chlorine in the laboratory prepared synthetic dilution water.

Q: Should treatment plant effluents be dechlorinated prior to toxicity testing?

A: The goal of the WET test is to determine the potential toxicity of the final effluent; therefore, if the final effluent has been treated with chlorine and dechlorinated, then that is what is to be tested. Dechlorination using anhydrous sodium thiosulfate to reduce chlorine would only be allowed at the discretion of the Permitting Authority. For example, if the effluent is toxic and the suspected toxicant is total residual chlorine (TRC), then the Permitting Authority could suggest having the permittee conduct a side by side test (minimum of three tests) of final effluent (w/o dechlorination) and with dechlorination to assist in determining whether TRC is causing toxicity. In addition, the permittee would need to conduct a definitive TIEs to determine that TRC is the sole toxicant. If so, then the Permitting Authority needs to address whether there is an appropriate TRC limit for the effluent and facility.

Note language in paragraph, 8.8.5 the chronic toxicity test method manuals, states, "At a minimum, pH, conductivity, and TRC are measured in the undiluted effluent or receiving water, and pH, and conductivity are measured in the dilution water. Therefore, an effluent sample at test initiation that has TRC concentrations above the toxic effect level (see TIE procedures to obtain toxic effect levels) would be causing toxicity to the test species, which is not allowed.

Q: What is the holding time requirement for first-use effluent sample?

A: The holding time requirements for first use of a sample have not changed. This requirement continues to state that the lapsed time from collection to first use of the sample must not exceed 36 hours. The allowance for Permitting Authorities to issue a variance for up to 72 hours also remains in the test method manuals. However, EPA clarified in the 2002 test method manuals, that samples can be used for test renewal at 24, 48, and/or 72 hours after first use. In the previous version of the freshwater chronic test methods manual, it stated that samples can be used for renewal at "24 and 48 hours after test initiation." This statement was modified to add "72 hours" based on comments that were received on the proposed rule. For example, when conducting the chronic *Ceriodaphnia* test over the duration of six to eight days (the maximum test duration is 8 days) with samples collected as recommended on days one, three, and five, the third sample must be used at 72 hours after first use in order to make the final renewal of the test. Otherwise, a fourth sample would need to be collected for renewal on the final day. This fourth sample would also need to be collected whether it was used or not because the decision to extend the test to an eighth day is not made until renewal on the seventh day, when it is generally too late to collect and ship another sample. For this reason, the holding time requirement was modified to allow use of samples at 72 hours after first use.

The second modification that was made (to both the freshwater chronic and marine chronic manuals) was to add an allowance for the use of existing samples for renewal when shipping problems are encountered. This allowance states: "If shipping problems (e.g., unsuccessful Saturday delivery) are encountered with renewal samples after a test has been initiated, the Permitting Authority may allow the continued use of the most recently used sample for test renewal." This modification was also added in response to comments on the proposed rule.

Based on these holding time requirements, a sample could be 108 hours old when last used (36 hours from collection to first use plus 72 hours from first use to last use). It should be noted, however, that these represent maximum allowable times and should not represent standard practice. EPA still recommends the collection of three samples on days 1, 3, and 5. Using this regime, sample holding times will be well below the maximums unless test durations are extended to 8 days or shipping problems are encountered.

Q: Do the test method manuals allow flexibility in determining the sample renewal collection schedule?

A: Yes, the test method manuals specify that sample collection on days 1, 3, and 5 is an example and not required sample collection scheme. For example, if shipping problems (e.g., unsuccessful Saturday delivery) are encountered with renewal samples after a test has been initiated, the Permitting Authority may allow the continued use of the most recently used sample for test renewal.” This means that if the shipment of a renewal effluent sample is not received on the precise day, this does not necessitate the termination of the test.

pH Adjustments:

Q: What is pH shock and is it real?

A: EPA believes that pH drift alone is not considered a test interference if pH stays within the organism’s tolerance range. The degree of pH drift typically observed in effluent samples should generally only interfere with test results if the sample contains a compound with toxicity that is pH dependent and at a concentration that is nearer the toxicity threshold. EPA does not have evidence to suggest that pH shock resulting from transferring organisms from culture water pH to test solution pH produces toxicity, provided the changes in pH or within the organism tolerance range (pH 6-9). Belanger and Cherry (1990) showed that *Ceriodaphnia dubia* survival and reproduction did not differ significantly in tests conducted at pH values ranging from 6 to 9, regardless of pH acclimation history. Acclimating organisms to test pH (for four weeks) only affected test performance when testing at pH 5.0 and 10.0 (beyond the normal organism tolerance range

Q: Are pH adjustments allowed for the chronic test methods?

A: The use of pH control is a modification to the tests and procedures that affects the measure toxicity of the sample, so Permitting Approval of this modification is required. The procedure is intended to control for pH drift that could produce artifactual toxicity, however, the procedure could be misused to artificially reduce sample toxicity when pH control is unwarranted. Approval of the procedure by the Permitting Authority will ensure that pH control is warranted in the test procedure. Permitting Authority approval in this instance is consistent with other method modifications, such as modification of sample holding times. The issue is not pH adjustment; it is control of pH drift during the test when the drift itself (not adjustments) is responsible for artifactual toxicity. There needs to be more side-by-side testing (minimum of 3 side-side tests) to confirm that pH drift is responsible for artifactual toxicity. This side-by-side testing does not mean testing the effluent at two different pHs. It

means testing a split sample, where pH is uncontrolled in one treatment and controlled (avoiding drift) in the other treatment. For example, if the collected sample is pH 6.5, then both of the side-by-side treatments start the test at 6.5. The uncontrolled treatment may drift to 7.5 during the test, but the controlled treatment is maintained at 6.5. Then, assess whether the tests differ in toxicity, and if so what is causing toxicity?

Q: Is pH adjustments allowed for the acute test methods?

A: EPA has not provided additional techniques that include modification of the sample to control pH drift in acute test methods because the current acute methods provide adequate remedies for pH drift without modifying the sample. pH drift in acute tests may be remedied by more frequent test renewals or the use of flow-through testing.

Q: What is the optional treatment for controlling pH drift?

A: EPA believes that the CO₂ controlled atmosphere technique provides the best pH control with least amount of sample modification. This technique uses the existing carbonate buffering system in the sample to control pH. While the method modification provides guidance on using this technique, a particular method for ministering the technique is not prescribed. The manual describes two methods for using the CO₂ controlled atmosphere technique: injecting a predetermined volume of CO₂ into closed test containers, and; flushing a chamber containing the test vessels with a mixture of CO₂ and air. Another technique for pH control is to eliminate airspace in the test vessel with a lid. This is effective when the partial pressure of CO₂ in the test solution is higher than that in the atmosphere, since it prevents CO₂ from escaping and allowing pH to rise. This technique would be allowable provided that is capable of adequately controlling pH.

Q: Is the use of pH buffers or addition of chemical acceptable?

A: EPA has not recommended the use of organic buffers for controlling test pH because this technique represents a greater modification of the sample than the CO₂ controlled atmosphere technique. The use of organic buffers means adding a foreign substance to the sample that could potentially produce unknown interactions that may modify sample toxicity. EPA agrees that the addition of any foreign chemical to the sample is not ideal; however, atmospheric CO₂ alone is not always sufficient to adjust and maintain pH. EPA adds in the manual the following caution: “the addition of acids and bases should be minimized to reduce the amount of additional ions (Na or Cl) added to the sample.”

Specific Method Issues:

Acute Test Methods:

Q: Are the rainbow and brook trout approved test methods in Part 136?

A: Yes, these methods are approved in Part 136, and included as summary of test conditions and TAC as Table 15 of EPA (2002a).

Q: When conducting a 96-hour acute test method are renewal(s) required?

A: At 48-hour a renewal is required minimum for the 96-hour acute toxicity test methods.

Q: Explain the change in the growth endpoints (dividing by the number of surviving organisms) to the biomass endpoint (dividing by the number of original organisms) used in the fish test methods.

A: In the 1995 WET final rule, EPA changed the test endpoint from the growth endpoint that was based on the number of surviving organisms, to the biomass endpoint that combines growth and survival and is based on the number of initial organisms. EPA made this change: 1) to provide consistency with other methods (e.g., *C. dubia* survival and reproduction tests) that incorporate survival along with sublethal effects, and; 2) because the combined survival and growth (or biomass) endpoint is a more sensitive measure than the growth endpoint alone. Data from Markle et al. (2000) support this conclusion by showing that point estimates calculated using the biomass endpoint were always lower (i.e., more biologically sensitive) than point estimates calculated using the growth endpoint. While the 1995 WET final rule changed the test endpoint to a combined survival and growth endpoint, test method manuals continue to refer to the endpoint as a "growth" endpoint. In fact, a combined survival and growth endpoint is more accurately termed biomass.

Q: What is blocking by known parentage?

A: It is a block randomization procedure that distributes offspring from a single parent evenly among the test treatments. For a given replicate, one neonate from the same parent is distributed to each test treatment. Process is repeated for each replicate using a new parent.

Q: Are 4th brood neonates for the chronic *Ceriodaphnia dubia* test method in the 4th edition manual?

A: In the *C. dubia* test, offspring from 4th or higher broods should not be counted and should not be included in the total number of neonates produced during the test.

Q: When using the *Mysidopsis bahia* chronic toxicity test method is the fecundity endpoint required (i.e., mandatory)?

A: The WET methods clearly state that achievement of the fecundity endpoint is not required for an acceptable *Mysidopsis bahia* chronic test. The tests acceptability criteria for this method state that “the minimum requirements for an acceptable test are 80% survival and an average weight of the lease 0.20 mg/mysid in the controls. If fecundity in the controls is adequate (egg production by 50% of the females), fecundity should be used as the criterion of effect in addition to survival and growth.” The fecundity endpoint therefore is an optional endpoint in this test method, and the failure to generate this endpoint does not affect the validity or acceptability of the test.

Stormwater and Ambient Testing Issues:

Q: Have the toxicity test methods been used to assess agricultural, urban, and industrial stormwater runoff toxicity? If so, what toxicant(s) have been identified?

A: Toxicity testing of stormwaters has been used as a monitoring tool for urban and agricultural stormwater assessments in California. For example, researchers have identified the pesticides diazinon and chlorpyrifos in urban stormwaters (Katznelson and Mumley 1997; Bailey et al. 2000; Fong et al. 2000; Larsen et al. 2000; Larsen and List 2002; SRWP 2000). Toxicity testing of stormwaters from agricultural settings has identified rice pesticides, diazinon, chlorpyrifos, carbofuran, and carbaryl as toxicants (SRWP 1998; Foe et al. 1998; Reyes et al. 2000; Werner et al. 2000).

Q: Are acute and/or chronic test method(s) used to assess storm and ambient waters?

A: Typically, acute tests (96 hours or less) are primarily being used to initially assess the toxicity of storm and ambient waters. This is for several reasons, including the short-term nature of most storm events, the fact that renewals may not be necessary (except for the 96-hour test with a renewal at 48-hours), and the need to target and prioritize survival impacts first.

Q: What testing factors may need to be considered differently for stormwater testing compared to testing effluent from a continuous discharge?

A: The main factors include (1) sample collection and sample initiation holding time, (2) sample renewals, and (3) test design - single vs. multiple concentration testing (see below).

Q: Can an exception to the 36-hour holding time for initiation of the test be allowed for storm and ambient water testing?

A: All tests should be conducted as soon as possible following sample collection. EPA has allowed exceptions to the 36-hour holding time, for example, when effluents are shipped overseas for testing (Denton and Narvaez 1996). The primary reason for an extension of the holding time would be the consideration of the sampling, laboratory technician safety (Burton and Pitt 2001; see page 255), and logistics of coordinating collection and transport of multiple stormwater samples within a short period of time. Storm events are not pre-determined events and typically occur rapidly throughout a watershed; therefore, many site

samples must be coordinated and processed with short notification to the toxicity testing laboratories. It is encouraged that the 36-hour holding time for test initiation be targeted; however, the Permitting Authorities may allow an exception beyond the 36-hours. However, no more than 72 hours should elapse before initial use of a sample.

Q: How is the standard test renewal practices specified in the test method manuals followed, given that storm events may be of short duration?

A: EPA 5th edition acute test methods specify that test solutions be renewed after 48 hours for a 96-hour test. However, for storm events in short duration, this is not always feasible. A more realistic option, in cases when a second stormwater sample may not be available, would be to renew the test solutions with a mixture of ambient waters and stormwaters if such waters could be collected following test initiation while meeting WET test holding time specifications (Katznelson and Mumley 1997). Another option would be to collect sufficient volume during the storm event to use for the start of the test and at the 48-hour renewal.

Q: Are single concentrations (100% storm or ambient water) compared to a control in WET stormwater tests or are multiple dilutions of the stormwater or ambient water being tested?

A: Either testing approach may be applied, depending on the purpose of the testing and the discharge setting. For example, if the receiving stream is small and stormwater-dominated during storm events, “screening” tests of undiluted stormwater (100% stormwater or ambient water) discharges may be appropriate. Multiple-dilution WET tests would be needed to determine the magnitude of effect and to generate LC_{50s} (acute) or NOECs (chronic).

Q: When would a multiple dilution test be performed if a single concentration test is initially conducted?

A: A single concentration is typically compared to a control to determine the effect in 100% stormwater and ambient water exposures as a first tier to assess stormwaters and ambient water with a standard t-test approach as described in the test methods manual (see USEPA 2002a, page 86). A multiple concentration test could be considered for the next sampling event if toxicity is of significant magnitude in the 100% stormwater (e.g., 100% mortality within 24 to 48 hours). The testing facility may consider testing the original sample (assuming sufficient volume collected) with a dilution series to more fully characterize the sample, for those samples which demonstrate high mortality within a short timeframe.

Q: What is meant by the term “first flush” when referring to collection of stormwater samples?

A: “First flush” refers to the first waters released from a discharge point as a result of a storm event or runoff associated with ice and snow melt. Typically, constituent concentrations are highest in this “first flush” sample. “First flush” is operationally defined by a time-period in some states (e.g., waters discharged within the first 15 or first 30 minutes of a discharge event). However, the “first flush” may not always contain the highest concentrations of pollutants as this depends on the rain intensity, type of pollutant, and size of the watershed. The first flush phenomenon is more prevalent for rains with relatively constant intensities

and small watershed size (Burton and Pitt 2001). Therefore, it is important to understand the watershed in order to determine if sampling of first flush in a storm event is critical. Another consideration is to capture the first seasonal flush (e.g., after an extended dry period) in arid areas.

Q: Is capturing the first flush important?

A: The precedent has been established for chemical-specific stormwater sampling to sample first-flush discharges suggests the potential for higher chemical-specific toxicity in first-flush samples. This “first flush” effect depends on the nature and form of the pollutant (Ward and Elliot 1995). The chemograph peak slightly precedes that of the hydrograph for sediments or sediment-bound pollutants (e.g., chlorpyrifos, phosphorus) entrained in the water column. However, for dissolved pollutants like diazinon, the chemograph peak follows that of the hydrograph.

Q: Is timing of sample collection to a flow measurement important?

A: A measurement of flow should coincide with the collection of stormwater samples for WET testing. This typically entails measuring flow discharge from the site, in addition to the amount of rainfall causing the discharge event. It is important to establish when sampling occurred relative to the streamflow hydrograph (and subsequent chemograph) (Ward and Elliot 1995). Scientists must consider the magnitude of a toxic response in relation to flow of receiving waters when making chemical or toxicity assessments of receiving or stormwaters in the regulatory arena (permitting and TMDL development) and when developing study designs. Therefore, if assessment and quantification of the mass loadings are of interest, then concurrent flow measurements from a US Geological Survey gauging station located near the point of interest and within the same watershed should be collected (USGS 1999, 2000). Measurement of flow concurrent with sample collection should be considered if a nearby and representative gauging station is not available.

Test Review and Data Analysis:

Q: What steps should the Permitting Authority take to review the test result in determining whether the test is reliable?

A: See Attachment 4-3, Evaluation of Toxicity Data of this document and the test method manuals chapter on “Report Preparation and Test Review”.

Q: Should concentration-response curves be evaluated?

A: Yes, the test method manuals (see chapter on test review) requires the laboratory and the Permitting Authority to review concentration-response curves. The EPA guidance (USEPA 2000a) assists the data reviewer through a stepwise process to determine the cause for non-ideal concentration-response relationships, and determine whether the test result is reliable, anomalous, and/or whether a new sample and toxicity test is required. This standardization

of concentration-response relationship review will decrease discrepancies in data interpretation amongst Permitting Authorities.

Q: How should Permitting Authorities address variability?

A: EPA is aware that there has been concern about the variability of the WET test method. EPA undertook an evaluation of an extensive toxicity dataset and published the document that examines the issue of test variability entitled “Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications under the National Pollutant Discharge Elimination System Program” (USEPA 2000b). This document outlines approaches for Permitting Authorities to consider in the context of within-test variability.

Q: What is percent minimum significant difference (PMSD)?

A: PMSD is the minimum significant difference (MSD) divided by the control mean, expressed as a percent. MSD is a measure of test sensitivity that establishes the minimum difference required between a control and a test treatment in order for that difference to be considered statistically significant.

Q: How should a test with a PMSD greater than the upper PMSD bound (according to the chapter on test review in the test methods manual) be evaluated?

A: According to the chapter on test review, which includes a discussion on test variability, “The within-test variability of individual test should be reviewed.” Excessive within-test variability may invalidate a test result and warrant further testing. For additional guidance on evaluating within-test variability as measured by PMSD, reviewer should consult EPA (2000b). If the PMSD measured for the test exceeds the upper PMSD bound variability criterion as defined in the test methods manual, then one of the two following cases applies: 1) if toxicity is found at the permitted receiving water concentration (RWC) based upon the value of the effect concentration estimate (NOEC), then the test should be accepted and the effect concentration estimate may be reported, unless other test review steps raise serious doubts about its validity, or 2) if toxicity is not found at the permitted RWC based upon the value of the effect concentration estimate (NOEC) and the PMSD measured for the test exceeds the upper PMSD bound, then the test shall not be accepted, and a new test must be conducted promptly on a newly collected sample.

Q: How should a test with a PMSD less than the upper PMSD bound (according to the chapter on test review of the test methods manual) to be evaluated?

A: Lower PMSD bounds shall also be applied when a hypothesis test result (NOEC) is reported. In determining hypothesis test results (NOEC), a test concentration shall not be considered toxic (i.e., significantly different from the control) if the relative difference from the control is less than the lower PMSD bound (see pertinent table for values in test methods manual, chapter on report preparation and test review). See EPA (2000b) for specific examples of implementing lower PMSD bounds and Table 4-2 of this document.

Q: What is the advantage to implementing a PMSD bound versus a control CV that should be achieved?

A: In the chronic test method manuals, EPA has required variability criteria (i.e., PMSD) when NPDES permits require sublethal endpoints expressed using hypothesis testing. EPA chose to implement variability criteria based on the PMSD, rather than control CV, because the PMSD is most directly applied to the determination of hypothesis testing results. The PMSD includes exactly the variability affecting the NOEC determination, while the CV for the control or any one treatment represents only a portion of the variability affecting the NOEC determination. Permitting Authorities are free to continue the use of variability control strategies adopted within their jurisdiction, but when NPDES permits require sublethal WET testing endpoints expressed using hypothesis testing, the variability criteria must be implemented as well.

Q: Should the laboratory maintain control charts for PMSDs?

A: EPA recommends that laboratories track PMSD values over time so that the testing laboratory may assess the normal operating ranges of this parameter in the laboratory and identify periods of decreased consistency. This information is useful in quickly identifying and correcting potential problems and sources of variability. The tracking of PMSD values also is useful for evaluating whether a laboratory needs to increase test replication to consistently achieve the variability criteria.

Q: Can chronic tests be used to determine acute toxicity without needing to conduct a separate acute toxicity test? For example, if permit requires acute testing (48 or 96 hour) for the water flea, and the fathead minnow and chronic testing for the water flea, fathead minnow, and green algae can the chronic tests, be assessed for both acute and chronic toxicity results?

A: Currently, the acute test methods manual does not include an acute toxicity test method for algae or plants. For example, the 7-day chronic fathead minnow survival and growth test method both acute and chronic toxicity can be assessed concurrently. At the end of the toxicity test, statistical values can be determined for 7-day survival and growth with either NOECs or EC/IC₂₅s. In addition, any of the following acute assessments can be determined: 24-hr, 48-hr, 72-hr, or 96-hr for either LC₅₀s or NOAEC (no observed adverse effect concentrations) values. For the 7-day chronic *C. dubia* survival and reproduction test method both acute and chronic toxicity can be assessed concurrently. At the end of the toxicity test, statistical values can be determined for the 7-day survival with NOEC only (i.e., no LC₅₀s can be assessed based on experimental design) and reproduction with either NOECs or IC₂₅s. In addition, any of the following acute assessments can be determined: 24-hr, 48-hr, 72-hr, or 96-hr NOAEC (NOEC) values. Since, the experimental design for the chronic *C. dubia* is 10 replicates of one water flea/replicate, no point estimates can be determined. So, if the acute toxicity standard is based on LC₅₀ determination, then a separate acute *C. dubia* test would need to be conducted.

Q: What is the measured rate of false positives for WET test methods?

A: EPA evaluated and assessed the false positive rate in their WET interlaboratory variability study and conclusively showed that measured false positive rates were below the theoretical rate of 5% estimated for the methods.

Q: What are alpha and beta errors?

A: A type I (alpha) error (i.e., false positive) results in the false conclusion that an effluent is toxic when it is not toxic. A type II (beta) error (i.e., false negative) results in the false conclusion that an effluent is not toxic when it actually is toxic. Power (1 - beta) is the probability of correctly detecting a true toxic effect (i.e., declaring an effluent toxic when it is in fact toxic). The EPA test method manuals recommend an alpha rate of 0.05 or 5 percent in the toxicity test method manuals. The risks of a high rate of type II errors is the risk to the environment that toxicity is occurring however it is not detected for various reasons, such as infrequent sampling, and/or lack of test sensitivity (high within-test variability). The risk of a test producing a false positive result is that the permittee may need to conduct additional tests (i.e., accelerated testing).

Q: How are alpha and beta related?

A: Alpha and beta are related (i.e., as alpha increases, beta decreases), assuming that the sample size (number of treatments, number of replicates), size of difference to be detected, and variance are held constant.

Q: What alpha rate should be used for data analysis?

A: The recommended alpha rate to be used according to the test method manuals is 0.05. Note, the WET Methods Guidance document (USEPA 2000a) does discuss using an alternate alpha rate of 0.10 under very specific conditions, however this was not recommended in EPA's final WET methods rule action. Therefore, the alpha rate to be used is the rate of 0.05.

Q: Can a sample be deemed a false positive?

A: No. If a test is properly conducted and correctly interpreted, identifying any particular outcome as a "false positive" is impossible. An effluent that is deemed toxic should require that the permittee conduct additional toxicity tests to determine if toxicity is reoccurring. Even if no toxicity is demonstrated in follow-up test result, this does not rule out that the original toxic event was a true toxic spike in the effluent.

Chapter 5 of the variability document (USEPA 2000b) specifically addresses "false positives." The hypothesis test procedures prescribed in the WET methods should provide adequate protection against incorrectly concluding that an effluent is toxic when it is not. EPA strongly recommends that WET testing laboratories carefully review the statistical procedures used to produce WET test results and other factors (i.e., biological and statistical quality assurance), and verify that test conditions and test acceptability criteria were achieved. If a test is properly conducted and correctly interpreted, identifying any particular outcome as a "false positive" should not happen.

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APPENDIX B

DETERMINING REASONABLE POTENTIAL FOR WET

Table B-1. Effluent-specific Coefficient of Variation (CV) Equations for Lognormal Distribution

| | | | |
|----------------|---|---|-------------------|
| x_i | = | daily pollutant measurement i (in effluent) | |
| y_i | = | $\ln (x_i)$ | |
| n | = | sample size of effluent data set | |
| μ_y^* | = | $\Sigma (y_i) / n$ | $1 \leq i \leq n$ |
| $\sigma_y^2^*$ | = | $\Sigma [(y_i - \mu_y)^2] / (n - 1)$ | $1 \leq i \leq n$ |
| σ_y^* | = | $\sqrt{\sigma_y^2^*}$ | $1 \leq i \leq n$ |
| $E(X)^*$ | = | $\exp (\mu_y + 0.5 \sigma_y^2)$ | |
| $V(X)^*$ | = | $[\exp (2 \mu_y + \sigma_y^2)] [\exp (\sigma_y^2) - 1]$ | |
| $CV(X)^*$ | = | $[\exp (\sigma_y^2) - 1]^{1/2}$ | |

Note: Formulas are based on the lognormal distribution. “*” means “estimator”.

Table B-2. Reasonable Potential Multiplier Factor Equations

| | | |
|--------------------------|---|--|
| n | = | sample size of effluent data set |
| P_n | = | $(1 - \text{confidence level})^{1/n}$ |
| | = | $(1 - 0.99)^{1/n}$ |
| $C_{95 \text{ (or 99)}}$ | = | $\exp [z_{95 \text{ (or 99)}} \sigma_y^* - 0.5 \sigma_y^{2*}]$ |
| C_{P_n} | = | $\exp (z_{P_n} \sigma_y^* - 0.5 \sigma_y^{2*})$ |
| | = | $\exp [(z_{95 \text{ (or 99)}} - z_{P_n}) \sigma_y^*]$ |
| | = | reasonable potential multiplier factor (RPMF), where $\sigma_y^{2*} = \ln (CV^2 + 1)$ |

Note: Formulas are based on the lognormal distribution. “*” means “estimator”.

| | | |
|-----------|---|--|
| z_{95} | = | 1.645 |
| z_{99} | = | 2.326 |
| z_{P_n} | = | $4.91 [P_n^{0.14} - (1 - P_n)^{0.14}]$ |

“ z_{P_n} ” can be obtained from this formula, for $3 < n < 50$, with relative error less than 0.5%. “ z_{P_n} ” can also be obtained from the table of the Standard Normal distribution by linear interpolation, and it can be obtained from any statistical program, spreadsheet, or calculator that reports quantiles for the Standard Normal distribution.

**Table B-3. Reasonable Potential Multiplier Factors:
0.95 “confidence level” and 95% percentile (rounded to one digit after the decimal)**

| n | CV | | | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | 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 | 22.4 | 26.4 | 30.8 | 35.6 | 40.7 | 46.3 | 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 | 16.5 | 18.0 | 19.6 | 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 | 10.1 | 10.8 | 11.6 | 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 | 3.5 | 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 | 4.4 | 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 | 3.8 | 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 | 3.3 | 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 | 3.5 |
| 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 | 2.6 | 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 | 2.4 | 2.5 | 2.6 | 2.7 | 2.8 | 2.8 |
| 14 | 1.1 | 1.2 | 1.3 | 1.3 | 1.4 | 1.5 | 1.6 | 1.7 | 1.8 | 1.9 | 2.0 | 2.1 | 2.2 | 2.2 | 2.3 | 2.4 | 2.5 | 2.5 | 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 | 2.1 | 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 | 1.5 | 1.6 | 1.7 | 1.8 | 1.9 | 1.9 | 2.0 | 2.1 | 2.1 | 2.2 | 2.2 | 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 | 1.5 | 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 | 1.7 | 1.8 | 1.8 | 1.9 | 1.9 | 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 | 1.6 | 1.7 | 1.7 | 1.8 | 1.8 | 1.9 | 1.9 | 2.0 | 2.0 | 2.0 |

**Table B-4. Reasonable Potential Multiplier Factors:
0.99 “confidence level” and 99% percentile (rounded to one digit after the decimal)**

| n | CV | | | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|------|------|------|------|------|------|------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 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 | 1.6 | 2.5 | 3.9 | 6.0 | 9.0 | 13.2 | 18.9 | 26.4 | 36.0 | 48.1 | 63.0 | 81.0 | 102.3 | 127.3 | 156.2 | 189.2 | 226.5 | 268.3 | 314.7 | 366.0 |
| 2 | 1.4 | 2.0 | 2.9 | 4.0 | 5.5 | 7.4 | 9.8 | 12.6 | 16.1 | 20.2 | 24.8 | 30.2 | 36.2 | 42.9 | 50.2 | 58.3 | 67.0 | 76.4 | 86.5 | 97.2 |
| 3 | 1.4 | 1.9 | 2.5 | 3.3 | 4.4 | 5.6 | 7.1 | 8.9 | 11.0 | 13.4 | 16.0 | 18.9 | 22.1 | 25.6 | 29.4 | 33.4 | 37.7 | 42.2 | 47.0 | 52.0 |
| 4 | 1.3 | 1.7 | 2.3 | 2.9 | 3.8 | 4.7 | 5.9 | 7.2 | 8.7 | 10.3 | 12.2 | 14.1 | 16.3 | 18.6 | 21.0 | 23.6 | 26.3 | 29.1 | 32.0 | 35.1 |
| 5 | 1.3 | 1.7 | 2.1 | 2.7 | 3.4 | 4.2 | 5.1 | 6.2 | 7.3 | 8.6 | 10.0 | 11.5 | 13.1 | 14.8 | 16.5 | 18.4 | 20.3 | 22.3 | 24.4 | 26.5 |
| 6 | 1.3 | 1.6 | 2.0 | 2.5 | 3.1 | 3.8 | 4.6 | 5.5 | 6.4 | 7.5 | 8.6 | 9.8 | 11.1 | 12.4 | 13.8 | 15.2 | 16.7 | 18.2 | 19.8 | 21.4 |
| 7 | 1.3 | 1.6 | 2.0 | 2.4 | 2.9 | 3.1 | 4.2 | 5.0 | 5.8 | 6.7 | 7.6 | 8.6 | 9.7 | 10.8 | 11.9 | 13.1 | 14.3 | 15.5 | 16.8 | 18.1 |
| 8 | 1.2 | 1.5 | 1.9 | 2.3 | 2.8 | 3.3 | 3.9 | 4.6 | 5.3 | 6.1 | 6.9 | 7.8 | 8.7 | 9.6 | 10.5 | 11.5 | 12.5 | 13.6 | 14.6 | 15.7 |
| 9 | 1.2 | 1.5 | 1.8 | 2.2 | 2.7 | 3.2 | 3.7 | 4.3 | 4.9 | 5.6 | 6.3 | 7.1 | 7.9 | 8.7 | 9.5 | 10.4 | 11.2 | 12.1 | 13.0 | 13.9 |
| 10 | 1.2 | 1.5 | 1.8 | 2.2 | 2.6 | 3.0 | 3.5 | 4.1 | 4.6 | 5.3 | 5.9 | 6.6 | 7.3 | 8.0 | 8.7 | 9.4 | 10.2 | 11.0 | 11.7 | 12.5 |
| 11 | 1.2 | 1.5 | 1.8 | 2.1 | 2.5 | 2.9 | 3.4 | 3.9 | 4.4 | 4.9 | 5.5 | 6.1 | 6.7 | 7.4 | 8.0 | 8.7 | 9.4 | 10.0 | 10.7 | 11.4 |
| 12 | 1.2 | 1.4 | 1.7 | 2.0 | 2.4 | 2.8 | 3.2 | 3.7 | 4.2 | 4.7 | 5.2 | 5.8 | 6.3 | 6.9 | 7.5 | 8.1 | 8.7 | 9.3 | 9.9 | 10.5 |
| 13 | 1.2 | 1.4 | 1.7 | 2.0 | 2.3 | 2.7 | 3.1 | 3.5 | 4.0 | 4.5 | 5.0 | 5.5 | 6.0 | 6.5 | 7.0 | 7.6 | 8.1 | 8.7 | 9.2 | 9.8 |
| 14 | 1.2 | 1.4 | 1.7 | 2.0 | 2.3 | 2.6 | 3.0 | 3.4 | 3.8 | 4.3 | 4.7 | 5.2 | 5.7 | 6.2 | 6.6 | 7.1 | 7.6 | 8.1 | 8.6 | 9.1 |
| 15 | 1.2 | 1.4 | 1.6 | 1.9 | 2.2 | 2.6 | 2.9 | 3.3 | 3.7 | 4.1 | 4.5 | 5.0 | 5.4 | 5.9 | 6.3 | 6.8 | 7.2 | 7.7 | 8.1 | 8.6 |
| 16 | 1.2 | 1.4 | 1.6 | 1.9 | 2.2 | 2.5 | 2.8 | 3.2 | 3.6 | 4.0 | 4.4 | 4.8 | 5.2 | 5.6 | 6.0 | 6.4 | 6.9 | 7.3 | 7.7 | 8.1 |
| 17 | 1.2 | 1.4 | 1.6 | 1.9 | 2.1 | 2.4 | 2.8 | 3.1 | 3.5 | 3.8 | 4.2 | 4.6 | 5.0 | 5.4 | 5.8 | 6.1 | 6.5 | 6.9 | 7.3 | 7.7 |
| 18 | 1.2 | 1.4 | 1.6 | 1.8 | 2.1 | 2.4 | 2.7 | 3.0 | 3.4 | 3.7 | 4.1 | 4.4 | 4.8 | 5.2 | 5.5 | 5.9 | 6.3 | 6.6 | 7.0 | 7.4 |
| 19 | 1.2 | 1.4 | 1.6 | 1.8 | 2.1 | 2.3 | 2.6 | 2.9 | 3.3 | 3.6 | 3.9 | 4.3 | 4.6 | 5.0 | 5.3 | 5.7 | 6.0 | 6.4 | 6.7 | 7.0 |
| 20 | 1.2 | 1.3 | 1.6 | 1.8 | 2.0 | 2.3 | 2.6 | 2.9 | 3.2 | 3.5 | 3.8 | 4.1 | 4.5 | 4.8 | 5.1 | 5.5 | 5.8 | 6.1 | 6.4 | 6.7 |

Table B-5. Example Numerical Calculation for Reasonable Potential Determination

The following equations are recommended in Chapter 3 and Appendix E of the TSD (USEPA 1991a) for determining reasonable potential in accordance with 40 CFR 122.44(d)(1). These equations are based on the lognormal distribution and are suitable for WET data expressed in units of $TU_c = 100 / NOEC$ (or IC_{25}), or units of $TU_a = 100 / LC_{50}$. Note that “*” means “estimator”. This example uses three significant figures and the final result is expressed using two significant figures. The example assumes that WET data (in TU_c) have, at most, two significant figures.

Step 1

In this example, chronic toxicity effluent data for compliance monitoring are reported in units of $TU_c = 100 / NOEC$. No acute toxicity effluent data are available. The chronic WET data are reviewed and both the observed maximum effluent value ($maxCe$) and total sample size of effluent data set (n) are identified.

$$\begin{aligned}
 TU_c &= \{ 8, 4, 4, 4, 2, >16, 8, 8, 16, >16, 8, 4, >16, 2, 16, 8, 4, 2, >16, 2 \} \text{ in units of } TU_c = 100 / NOEC \\
 maxCe &= \text{observed maximum effluent value} \\
 &= >16 TU_c \\
 n &= \text{total sample size of effluent data set} \\
 &= 20
 \end{aligned}$$

Following Section 5.5.2 of the TSD, if n is <10 , then effluent variability (CV) is estimated using 0.6; proceed to Step 4. If n is ≥ 10 , then proceed to Step 2 in order to estimate effluent-specific variability. In this example, n is ≥ 10 ; proceed to Step 2.

Step 2

In order to better estimate effluent variability, chronic toxicity effluent data are also reported in units of $TUc = 100 / IC_{25}$. These data are used to calculate estimates for sigma (σ_y^*) and CV.

$$\begin{aligned}
 TUc &= \{ 4.3, 1.9, 2.0, 1.9, 1.9, 33.2, 5.6, 5.2, 9.1, 29.4, 9.3, 2.0, \\
 &\quad 38.5, 1.7, 6.4, 6.1, 2, 1.5, 28.6, 2.4 \} \text{ in units of } TUc = 100 / \\
 &\quad IC_{25} \\
 x_i &= \{ 4.3, 1.9, 2.0, 1.9, 1.9, 33.2, 5.6, 5.2, 9.1, 29.4, 9.3, 2.0, \\
 &\quad 38.5, 1.7, 6.4, 6.1, 2, 1.5, 28.6, 2.4 \} \text{ in units of } TUc = 100 / \\
 &\quad IC_{25} \\
 y_i &= \text{log base } e \text{ of daily pollutant measurement } i \\
 &= \ln (x_i) \\
 &= \{ 1.45, 0.641, 0.693, 0.641, 0.641, 3.50, 1.72, 1.64, 2.20, \\
 &\quad 3.38, 2.23, 0.693, 3.65, 0.530, 1.85, 1.80, 0.693, 0.405, \\
 &\quad 3.35, 0.875 \} \\
 n &= \text{sample size of effluent data set} \\
 &= 20 \\
 \mu_y^* &= \text{mean of logarithms} \\
 &= \Sigma (y_i) / n \qquad 1 \leq i \leq n \\
 &= 32.5 / 20 \\
 &= 1.62 \\
 \sigma_y^{2*} &= \text{variance of logarithms} \\
 &= \Sigma [(y_i - \mu_y)^2] / (n - 1) \qquad 1 \leq i \leq n \\
 &= 23.2 / 19 \\
 &= 1.22 \\
 \sigma_y^* &= \text{standard deviation of logarithms} \qquad 1 \leq i \leq n \\
 &= \sqrt{ \sigma_y^{2*} } \\
 &= \sqrt{ 1.22 } \\
 &= 1.10 \\
 E(X)^* &= \exp (\mu_y + 0.5 \sigma_y^2) \\
 &= \exp [1.62 + (0.5) (1.22)] \\
 &= 9.29
 \end{aligned}$$

$$\begin{aligned}
 V(X)^* &= [\exp(2\mu_y + \sigma_y^2)] [\exp(\sigma_y^2) - 1] \\
 &= \{ \exp[(2)(1.62) + 1.22] \} [\exp(1.22) - 1] \\
 &= [86.4] [2.38] \\
 &= 205
 \end{aligned}$$

$$\begin{aligned}
 CV(X)^* &= [\exp(\sigma_y^2) - 1]^{1/2} \\
 &= [\exp(1.22) - 1]^{1/2} \\
 &= 1.54
 \end{aligned}$$

Proceed to Step 3.

Step 3

The reasonable potential multiplier factor is calculated using estimates for sigma (σ_y^*) and CV from Step 2.

$$\begin{aligned}
 P_n &= (1 - \text{confidence level})^{1/n} \\
 &= (1 - 0.99)^{1/20} \\
 &= 0.794
 \end{aligned}$$

$$\begin{aligned}
 Z_{P_n} &= 0.8205 \text{ “}Z_{P_n}\text{” is found from a table of the Standard Normal} \\
 &\text{distribution, by linear interpolation between} \\
 &\text{tabled values } z_{0.7939} = +0.82 \text{ and } z_{0.7967} = +0.83.
 \end{aligned}$$

$$\begin{aligned}
 \text{Reasonable potential multiplier factor (RPMF)} &= \frac{C_{95 \text{ (or 99)}}}{C_{P_n}} \\
 &= \frac{\exp [Z_{95 \text{ (or 99)}} \sigma_y^* - 0.5 \sigma_y^2]}{\exp (Z_{P_n} \sigma_y^* - 0.5 \sigma_y^2)} \\
 &= \frac{\exp [(Z_{95 \text{ (or 99)}} - Z_{P_n}) \sigma_y^*]}{\exp [(2.326 - 0.8205) (1.10)]} \\
 &= \frac{\exp (1.66)}{5.26} ,
 \end{aligned}$$

$$\begin{aligned}
 \text{where } \sigma_y^* &= \sqrt{\sigma_y^2} \\
 &= \sqrt{1.22} \\
 &= 1.10
 \end{aligned}$$

Note: Using Table B-4 for the reasonable potential multiplier factor, at $n = 20$ for a $CV = 1.5$, the reasonable potential multiplier factor is 5.1; at $n = 20$ for a $CV = 1.6$, the reasonable potential multiplier factor is 5.5. Using linear interpolation to the

CV = 1.54 gives a reasonable potential multiplier factor of 5.26. This sometimes differs from the exact reasonable potential multiplier factor calculated above because the numbers in Table B-4 have been rounded.

Proceed to Step 4.

Step 4

The statistically estimated maximum effluent value ($\max C_{eRP}$) is calculated using the reasonable potential multiplier factor (RPMF) from Step 3 and the observed maximum effluent value ($\max C_e$) from Step 1.

$$\begin{aligned} \max C_e &= \text{observed maximum effluent value} \\ &= \{ 8, 4, 4, 4, 2, >16, 8, 8, 16, >16, 8, 4, >16, 2, 16, 8, 4, 2, >16, 2 \} \text{ in units of } T_{Uc} = 100 / \text{NOEC} \\ &= >16 T_{Uc} \\ \\ \max C_{eRP} &= \text{statistically estimated maximum effluent value} \\ &= (\text{RPMF}) (\max C_e) \\ &= (5.26) (>16) \\ &= 84 T_{Uc} \end{aligned}$$

In addition, because no acute toxicity effluent data are available to evaluate the reasonable potential to exceed the water quality criterion for acute toxicity, a default acute-to-chronic ratio is recommended in Section 1.3.4 of the TSD in order to estimate effluent levels for acute toxicity.

$$\begin{aligned} \text{ACR} &= \text{acute-to-chronic ratio in TSD Section 1.3.4} \\ &= LC_{50} / \text{NOEC} \\ &= T_{Uc} / T_{Ua} \\ &= 10 \\ T_{Uc,a} &= T_{Uc} / 10 \quad , \text{ where chronic toxicity is expressed} \\ &\quad \text{in acute toxic units (} T_{Uc,a} \text{)} \\ \\ \max C_e &= \text{observed maximum effluent value in units of } T_{Uc,a} \\ &= (\max C_e \text{ in units of } T_{Uc}) / 10 \\ &= >16 / 10 \\ &= >1.6 T_{Uc,a} \\ \\ \max C_{eRP} &= \text{statistically estimated maximum effluent value in units of} \\ &\quad T_{Uc,a} \\ &= (\text{RPMF}) (\max C_e \text{ in units of } T_{Uc,a}) \\ &= (5.26) (>1.6) \\ &= 8.4 T_{Uc,a} \end{aligned}$$

Proceed to Step 5.

Step 5

The resultant magnitudes of chronic and acute toxicity in the receiving water after effluent discharge (C_r) are calculated using the mass balance equation, a steady-state model, and compared with water quality criteria for chronic and acute toxicity. If a resultant magnitude for toxicity (C_r) is greater than a water quality criterion for toxicity, then reasonable potential is established and a WQBEL is needed, in accordance with 40 CFR 122.44(d)(1).

$$C_r Q_r = C_e Q_e + C_s Q_s$$

where C = critical value for WET (in units of TU_c , TU_a)
 Q = critical value for flow (in units of cfs or MGD)
 r = effluent plus upstream after discharge
 e = effluent discharge
 s = upstream before discharge

S_a = critical dilution factor authorized by Permitting Authority
 = $(1 + Q_s / Q_e)$ or output from dilution model

C_r = resultant magnitude for toxicity in the receiving water after effluent discharge

$$= \frac{C_e + [C_s (Q_s / Q_e)]}{1 + (Q_s / Q_e)}$$

$$= \frac{C_e + [C_s (S_a - 1)]}{S_a}$$

In this example, the resultant magnitude of chronic WET in the receiving water after effluent discharge (C_r) to compare with water quality criterion for chronic toxicity (CCC).

CCC = criterion continuous concentration to protect against chronic effects
 = 1.0 TU_c

S_{a_c} = chronic critical dilution factor
 = $(1 + Q_{S7Q10} \text{ (or } 4B3) / Q_e)$
 = 8

C_s = critical value for WET upstream before discharge
 = 0 TU

$$\begin{aligned}
 \text{Ce} &= \text{most critical value for WET in the effluent discharge in units of TUc} \\
 &= \text{maximum (maxCe or maxCe}_{RP} \text{)} \\
 &= \text{maximum (>16 or 84)} \\
 &= 84 \text{ TUc} \\
 \\
 \text{Cr} &= \frac{\text{Ce} + [\text{Cs} (\text{Sa} - 1)]}{\text{Sa}} \\
 &= \frac{84 + [0 (8 - 1)]}{8} \\
 &= 11 \text{ TUc} \\
 \\
 \text{Reasonable Potential} &= \text{Cr} > \text{CCC} \\
 &= 11 \text{ TUc} > 1 \text{ TUc} \\
 &= \text{Yes, permit needs a WQBEL.}
 \end{aligned}$$

In this example, the resultant magnitude of acute WET in the receiving water after effluent discharge (Cr) to compare with water quality criterion for acute toxicity (CMC).

$$\begin{aligned}
 \text{CMC} &= \text{criterion maximum concentration to protect against acute effects} \\
 &= 0.3 \text{ TUa} \\
 \\
 \text{Sa}_a &= \text{acute critical dilution factor} \\
 &= (1 + \text{QS}_{1Q10 \text{ (or 1B3)}} / \text{Qe}) \\
 &= 1 \\
 \\
 \text{Cs} &= \text{critical value for WET upstream before discharge} \\
 &= 0 \text{ TU} \\
 \\
 \text{Ce} &= \text{most critical value for WET in the effluent discharge in units of TUc,a} \\
 &= \text{maximum (maxCe or maxCe}_{RP} \text{)} \\
 &= \text{maximum (>1.6 or 8.4)} \\
 &= 8.4 \text{ TUc,a}
 \end{aligned}$$

$$\begin{aligned}
 Cr &= \frac{Ce + [Cs (Sa - 1)]}{Sa} \\
 &= \frac{8.4 + [0 (1 - 1)]}{1} \\
 &= 8.4 \text{ TU}_{c,a} \\
 \text{Reasonable Potential} &= Cr > CMC \\
 &= 8.4 \text{ TU}_{c,a} > 0.3 \text{ TU}_a \\
 &= \text{Yes, permit needs a WQBEL.}
 \end{aligned}$$

References

USEPA. 1991a. Technical support document for water quality-based toxics control. Office of Water. Washington, DC. EPA/505/2-90/001.

APPENDIX C

DERIVING PERMIT LIMITS FOR WET

Table C-1. Example Calculations for Developing Permit Limits from Two-value, Steady-state Wasteload Allocations for WET

The following equations are recommended in Chapter 5 and Appendix E of the TSD (USEPA 1991a) for calculating water quality-based effluent limits (WQBELs) for WET. These equations are based on the lognormal distribution and are suitable for WET data expressed in units of $TU_c = 100 / \text{NOEC}$ (or IC_{25}), or units of $TU_a = 100 / \text{LC}_{50}$. Note that “*” means “estimator”. This example uses three significant figures and the final result is expressed using two significant figures. The example assumes that WET data (in TU_c) have, at most, two significant figures.

Generally, wasteload allocations for WET in effluent discharge are calculated using the mass balance equation, a steady-state model.

$$C_r Q_r = C_e Q_e + C_s Q_s$$

where C = critical value for WET (in units of TU_c or TU_a)
 Q = critical value for flow (in units of cfs or MGD)
 r = effluent plus upstream after discharge
 e = effluent discharge
 s = upstream before discharge

S_a = critical dilution factor authorized by Permitting Authority
 = $(1 + Q_s / Q_e)$ or output from dilution model

C_e = wasteload allocation (WLA) in units of TU_c , TU_a , or $TU_{a,c}$
 = $C_r + [(Q_s / Q_e) (C_r - C_s)]$
 = $C_r + [(S_a - 1) (C_r - C_s)]$

The wasteload allocation (WLA_c) for chronic toxicity in the effluent discharge is calculated using the mass-balance equation.

$$\begin{aligned}
 Cr &= \text{criterion continuous concentration (CCC) to protect against chronic effects} \\
 &= 1.0 \text{ TUc} \\
 Cs &= \text{critical value for WET upstream before discharge} \\
 &= 0 \text{ TU} \\
 Sa_c &= \text{chronic critical dilution factor} \\
 &= (1 + QS_{7Q10} \text{ (or } 4B3) / Q_e) \\
 &= 8 \\
 Ce &= \text{WLA in units of TUc} \\
 &= Cr + (Sa - 1)(Cr - Cs) \\
 &= 1 + (8 - 1)(1 - 0) \\
 &= 8 \text{ TUc}
 \end{aligned}$$

The wasteload allocation for acute toxicity in the effluent discharge is expressed in chronic toxic units (WLA_{a,c}) and calculated using the mass-balance equation and an acute-to-chronic ratio.

$$\begin{aligned}
 ACR &= \text{acute-to-chronic ratio in TSD Section 1.3.4} \\
 &= LC_{50} / \text{NOEC} \\
 &= \text{TUc} / \text{TUa} \\
 &= 10 \\
 \text{TU}_{a,c} &= 10 \times \text{TUa}, \text{ where acute toxicity is expressed in chronic toxic units (TU}_{a,c}\text{)} \\
 Cr &= \text{criterion maximum concentration (CMC) to protect against acute effects} \\
 &= 0.3 \text{ TUa} \\
 Cs &= \text{critical value for WET upstream before discharge} \\
 &= 0 \text{ TU} \\
 Sa_a &= \text{acute critical dilution factor} \\
 &= (1 + QS_{1Q10} \text{ (or } 1B3) / Q_e) \\
 &= 1 \\
 Ce &= \text{WLA in units of TU}_{a,c} \\
 &= [Cr + (Sa - 1)(Cr - Cs)] \times ACR \\
 &= [0.3 + (1 - 1)(1 - 0)] \times 10 \\
 &= 3 \text{ TU}_{a,c}
 \end{aligned}$$

After both acute and chronic wasteload allocations are determined, the critical treatment performance level (coefficient of variation, CV, and long term average, LTA) that will allow the effluent to meet the wasteload allocations is calculated. Following Section 5.5.2 of the TSD, if k is <10 , then effluent variability (CV) is estimated using 0.6. If k is ≥ 10 , then the following equations are used to estimate effluent-specific variability.

$$\begin{aligned}
 x_i &= \text{daily pollutant measurement } i \text{ (in effluent) in units of TUc} \\
 &= 100 / \text{IC25, or TUa} = 100 / \text{LC50} \\
 &= \{ 4.3, 1.9, 2.0, 1.9, 1.9, 33.2, 5.6, 5.2, 9.1, 29.4, 9.3, 2.0, \\
 &\quad 38.5, 1.7, 6.4, 6.1, 2, 1.5, 28.6, 2.4 \} \text{ in units of TUc} = 100 / \text{IC25} \\
 \\
 y_i &= \text{log base } e \text{ of daily pollutant measurement } i \\
 &= \ln (x_i) \\
 &= \{ 1.45, 0.641, 0.693, 0.641, 0.641, 3.50, 1.72, 1.64, 2.20, \\
 &\quad 3.38, 2.23, 0.693, 3.65, 0.530, 1.85, 1.80, 0.693, 0.405, \\
 &\quad 3.35, 0.875 \} \\
 \\
 k &= \text{sample size of effluent data set} \\
 &= 20 \\
 \\
 \mu_y^* &= \text{mean of logarithms} \\
 &= \Sigma (y_i) / k \qquad 1 \leq i \leq k \\
 &= 32.5 / 20 \\
 &= 1.62 \\
 \\
 \sigma_y^{2*} &= \text{variance of logarithms} \\
 &= \Sigma [(y_i - \mu_y)^2] / (k - 1) \qquad 1 \leq i \leq k \\
 &= 23.2 / 19 \\
 &= 1.22 \\
 \\
 \sigma_y^* &= \text{standard deviation of logarithms} \qquad 1 \leq i \leq k \\
 &= \sqrt{ \sigma_y^{2*} } \\
 &= \sqrt{ 1.22 } \\
 &= 1.10 \\
 \\
 E(X)^* &= \exp (\mu_y + 0.5 \sigma_y^2) \\
 &= \exp [1.62 + (0.5) (1.22)] \\
 &= 9.29 \\
 \\
 V(X)^* &= [\exp (2 \mu_y + \sigma_y^2)] [\exp (\sigma_y^2) - 1] \\
 &= \{ \exp [(2) (1.62) + 1.22] \} [\exp (1.22) - 1] \\
 &= [86.4] [2.38] \\
 &= 205 \\
 \\
 CV(X)^* &= [\exp (\sigma_y^2) - 1]^{1/2}
 \end{aligned}$$

$$= [\exp(1.22) - 1]^{1/2}$$

$$= 1.54$$

The long-term average for chronic toxicity (LTAc) and the long-term average for acute toxicity (LTAa,c) in the effluent discharge are calculated using the following equations. Knowing CV(X) *, these long-term average values may be determined using the pre-calculated “WLA multipliers” in TSD Table 5-1.

$$\begin{aligned} \text{LTAc} &= \text{chronic (4-day average) long term average in units of TUc} \\ &= \text{WLAc} \times \exp(0.5 \sigma_4^2 - z_{0.99} \sigma_4) \\ &= 8 \times \exp[(0.5)(0.466) - (2.326)(0.683)] \\ &= 8 \times 0.258 \\ &= 2.06, \text{ or} \end{aligned}$$

$$\begin{aligned} &= \text{WLAc} \times \text{chronic WLA multiplier from TSD Table 5-1 for} \\ &\quad \text{CV and 99}^{\text{th}} \text{ percentile} \\ &= 8 \times 0.258 \\ &= 2.06 \end{aligned}$$

$$\begin{aligned} \text{LTAa,c} &= \text{acute (1-day average) long term average in units of TUa,c} \\ &= \text{WLAa,c} \times \exp(0.5 \sigma^2 - z_{0.99} \sigma) \\ &= 3 \times \exp[(0.5)(1.22) - (2.326)(1.10)] \\ &= 3 \times 0.142 \\ &= 0.426, \text{ or} \end{aligned}$$

$$\begin{aligned} &= \text{WLAc} \times \text{acute WLA multiplier from TSD Table 5-1 for} \\ &\quad \text{CV and 99}^{\text{th}} \text{ percentile} \\ &= 3 \times 0.141 \\ &= 0.423 \end{aligned}$$

$$\begin{aligned} \text{where CV} &= \text{CV(X) *} \\ &= 1.54 \\ \sigma_4^2 &= \ln[(\text{CV}^2 / 4) + 1] \\ &= 0.466 \\ \sigma^2 &= \ln(\text{CV}^2 + 1) \\ &= 1.22 \\ z_{0.99} &= 2.326 \text{ is recommended for WLA in TSD Section 5.5.4} \end{aligned}$$

Permit limits are calculated using the lower (more limiting) LTA discharge condition.

$$\begin{aligned} \text{LTA} &= \text{minimum (LTAc or LTA,a,c)} \\ &= \text{minimum (2.06 or 0.426)} \\ &= 0.426 \end{aligned}$$

A maximum daily limit (MDL) and average monthly limit (AML) are calculated using the more limiting discharge condition—defined by the LTA and CV—using the following equations. Knowing CV(X) *, the maximum daily limit and average monthly limit may be determined using the pre-calculated “LTA multipliers” in TSD Table 5-2.

$$\begin{aligned}
 \text{MDL} &= \text{maximum daily limit} \\
 &= \text{LTA} \times \exp (z_{0.99} \sigma - 0.5 \sigma^2) \\
 &= 0.426 \times \exp [(2.326) (1.10) - (0.5) (1.22)] \\
 &= 0.426 \times 7.02 \\
 &= 3.0 \text{ TU}_{a,c} \text{ , or} \\
 &= \text{LTA} \times \text{maximum daily limit LTA multiplier from TSD} \\
 &\quad \text{Table 5-2 for CV and 99}^{\text{th}} \text{ percentile} \\
 &= 0.426 \times 7.07 \\
 &= 3.0 \text{ TU}_{a,c} \\
 \\
 \text{AML} &= \text{average monthly limit} \\
 &= \text{LTA} \times \exp (z_{0.95} \sigma_n - 0.5 \sigma_n^2) \\
 &= \text{LTA} \times \exp (z_{0.95} \sigma_4 - 0.5 \sigma_4^2) \\
 &= 0.426 \times \exp [(1.645) (0.683) - (0.5) (0.466)] \\
 &= 0.426 \times 2.44 \\
 &= 1.0 \text{ TU}_{a,c} \text{ , or} \\
 &= \text{LTA} \times \text{average monthly limit LTA multiplier from TSD} \\
 &\quad \text{Table 5-2 for CV, 95}^{\text{th}} \text{ percentile, and } n \geq 4 \\
 &= 0.426 \times 2.43 \\
 &= 1.0 \text{ TU}_{a,c} \\
 \\
 \text{where CV} &= \text{CV(X) *} \\
 &= 1.54 \\
 \sigma^2 &= \ln (\text{CV}^2 + 1) \\
 &= 1.22 \\
 z_{0.99} &= 2.326 \text{ is recommended for MDL in TSD Section 5.5.4} \\
 n &= \text{number of samples per month } \geq 4 \\
 &= 4 \\
 \sigma_n^2 &= \ln [(\text{CV}^2 / n) + 1] \\
 \sigma_4^2 &= \ln [(\text{CV}^2 / 4) + 1] \\
 &= 0.466 \\
 z_{0.95} &= 1.645 \text{ is recommended for AML in TSD Section 5.5.4} \\
 \\
 \text{MDL} &= 3.0 \text{ TU}_{a,c} \\
 \text{AML} &= 1.0 \text{ TU}_{a,c}
 \end{aligned}$$

Following Section 2.6.2 in Chapter 2 of this document, EPA Regions 9 and 10 continue recommend that Permitting Authorities establish a monthly median limit (MML) of 1.0 TU_c for chronic WET, when the statistically-calculated AML is at or less than 1.0 TU_c. As a result, in this example where the acute-to-chronic ratio is 10, the recommended permit limits for chronic WET are:

| | | |
|-----|---|---|
| MDL | = | maximum daily limit 3.0 TU _{a,c} |
| MML | = | median monthly limit 1.0 TU _{a,c} |

In addition, because these permit limits have been developed using a default acute-to-chronic ratio of 10, the permit should include: (1) side-by-side acute and chronic WET monitoring in order to develop an effluent-specific acute-to-chronic ratio, and (2) a permit reopener condition authorizing revisions to these WQBELs, if appropriate, based on this new information.

Table C-2. Example Calculations for Developing Permit Limits from Two-value, Steady-state Wasteload Allocations for WET under Low Flow Conditions

The following equations are recommended in Chapter 5 and Appendix E of the TSD (USEPA 1991a) for calculating water quality-based effluent limits (WQBELs) for WET. These equations are based on the lognormal distribution and are suitable for WET data expressed in units of $TU_c = 100 / \text{NOEC}$ (or IC_{25}), or units of $TU_a = 100 / \text{LC}_{50}$. Note that “*” means “estimator”. This example uses three significant figures and the final result is expressed using two significant figures. The example assumes that WET data (in TU_c) have, at most, two significant figures.

Generally, wasteload allocations for WET in effluent discharge are calculated using the mass balance equation, a steady-state model.

$$C_r Q_r = C_e Q_e + C_s Q_s$$

where C = critical value for WET (in units of TU_c or TU_a)
 Q = critical value for flow (in units of cfs or MGD)
 r = effluent plus upstream after discharge
 e = effluent discharge
 s = upstream before discharge

S_a = critical dilution factor authorized by Permitting Authority
 = $(1 + Q_s / Q_e)$ or output from dilution model

C_e = wasteload allocation (WLA) in units of TU_c , TU_a , or $TU_{a,c}$
 = $C_r + [(Q_s / Q_e) (C_r - C_s)]$
 = $C_r + [(S_a - 1) (C_r - C_s)]$

The wasteload allocation (WLA_c) for chronic toxicity in the effluent discharge is calculated using the mass-balance equation.

C_r = criterion continuous concentration (CCC) to protect against chronic effects
 = 1.0 TU_c

C_s = critical value for WET upstream before discharge
 = 0 TU

$S_{a,c}$ = chronic critical dilution factor
 = $(1 + Q_{S7Q10} \text{ (or } 4B3) / Q_e)$
 = 1

C_e = WLA in units of TU_c
 = $C_r + (S_a - 1) (C_r - C_s)$

$$= 1 + (1 - 1)(1 - 0)$$

$$= 1 \text{ TUc}$$

The wasteload allocation for acute toxicity in the effluent discharge is expressed in chronic toxic units (WLA_{a,c}) and calculated using the mass-balance equation and an acute-to-chronic ratio.

| | | |
|-------------------|---|---|
| ACR | = | acute-to-chronic ratio in TSD Section 1.3.4 |
| | = | LC50 / NOEC |
| | = | TU _c / TU _a |
| | = | 10 |
| TU _{a,c} | = | 10 × TU _a , where acute toxicity is expressed in chronic toxic units (TU _{a,c}) |
| Cr | = | criterion maximum concentration (CMC) to protect against acute effects |
| | = | 0.3 TU _a |
| Cs | = | critical value for WET upstream before discharge |
| | = | 0 TU |
| Sa _a | = | acute critical dilution factor |
| | = | (1 + QS _{1Q10 (or 1B3)} / Q _e) |
| | = | 1 |
| Ce | = | WLA in units of TU _{a,c} |
| | = | [Cr + (Sa - 1) (Cr - Cs)] × ACR |
| | = | [0.3 + (1 - 1) (1 - 0)] × 10 |
| | = | 3 TU _{a,c} |

After both acute and chronic wasteload allocations are determined, the critical treatment performance level (coefficient of variation, CV, and long term average, LTA) that will allow the effluent to meet the wasteload allocations is calculated. If k is ≥ 10 , then the equations in Table C-1 are used to estimate effluent-specific variability. In this example, following Section 5.5.2 of the TSD, k is < 10 and effluent variability (CV) is estimated using 0.6.

$$\begin{aligned}
 x_i &= \text{daily pollutant measurement } i \text{ (in effluent) in units of TUc} \\
 &= 100 / \text{IC25, or TUa} = 100 / \text{LC50} \\
 &= \{ 1, 1, 1, 1.8, <1 \} \text{ in units of TUc} = 100 / \text{IC25} \\
 \\
 k &= \text{sample size of effluent data set} \\
 &= 5 \\
 \\
 \text{CV(X) } * &= [\exp (\sigma_y^2) - 1]^{1/2} \\
 &= 0.6
 \end{aligned}$$

The long-term average for chronic toxicity (LTAc) and the long-term average for acute toxicity (LTAA,c) in the effluent discharge are calculated using the following equations. Knowing CV(X) *, these long-term average values may be determined using the pre-calculated “WLA multipliers” in TSD Table 5-1.

$$\begin{aligned}
 \text{LTAc} &= \text{chronic (4-day average) long term average in units of TUc} \\
 &= \text{WLAc} \times \exp (0.5 \sigma_4^2 - z_{0.99} \sigma_4) \\
 &= 1 \times \exp [(0.5) (0.0862) - (2.326) (0.294)] \\
 &= 1 \times 0.527 \\
 &= 0.527 \text{ , or} \\
 \\
 &= \text{WLAc} \times \text{chronic WLA multiplier from TSD Table 5-1 for} \\
 &\quad \text{CV and 99}^{\text{th}} \text{ percentile} \\
 &= 1 \times 0.527 \\
 &= 0.527 \\
 \\
 \text{LTAA,c} &= \text{acute (1-day average) long term average in units of TUa,c} \\
 &= \text{WLAa,c} \times \exp (0.5 \sigma^2 - z_{0.99} \sigma) \\
 &= 3 \times \exp [(0.5) (0.307) - (2.326) (0.554)] \\
 &= 3 \times 0.321 \\
 &= 0.963 \text{ , or} \\
 \\
 &= \text{WLAc} \times \text{acute WLA multiplier from TSD Table 5-1 for} \\
 &\quad \text{CV and 99}^{\text{th}} \text{ percentile} \\
 &= 3 \times 0.321 \\
 &= 0.963
 \end{aligned}$$

$$\begin{aligned}
 \text{where CV} &= \text{CV(X) *} \\
 &= 0.6 \\
 \sigma_4^2 &= \ln [(\text{CV}^2 / 4) + 1] \\
 &= 0.0862 \\
 \sigma^2 &= \ln (\text{CV}^2 + 1) \\
 &= 0.307 \\
 z_{0.99} &= 2.326 \text{ is recommended for WLA in TSD Section 5.5.4}
 \end{aligned}$$

Permit limits are calculated using the lower (more limiting) LTA discharge condition.

$$\begin{aligned}
 \text{LTA} &= \text{minimum (LTA}_c \text{ or LTA}_{a,c}) \\
 &= \text{minimum (0.527 or 0.963)} \\
 &= 0.527
 \end{aligned}$$

A maximum daily limit (MDL) and average monthly limit (AML) are calculated using the more limiting discharge condition—defined by the LTA and CV—using the following equations. Knowing CV(X) *, the maximum daily limit and average monthly limit may be determined using the pre-calculated “LTA multipliers” in TSD Table 5-2.

$$\begin{aligned}
 \text{MDL} &= \text{maximum daily limit} \\
 &= \text{LTA} \times \exp (z_{0.99} \sigma - 0.5 \sigma^2) \\
 &= 0.527 \times \exp [(2.326) (0.554) - (0.5) (0.307)] \\
 &= 0.527 \times 3.11 \\
 &= 1.6 \text{ TUc } , \text{ or} \\
 &= \text{LTA} \times \text{maximum daily limit LTA multiplier from TSD} \\
 &\quad \text{Table 5-2 for CV and 99}^{\text{th}} \text{ percentile} \\
 &= 0.527 \times 3.11 \\
 &= 1.6 \text{ TUc} \\
 \\
 \text{AML} &= \text{average monthly limit} \\
 &= \text{LTA} \times \exp (z_{0.95} \sigma_n - 0.5 \sigma_n^2) \\
 &= \text{LTA} \times \exp (z_{0.95} \sigma_4 - 0.5 \sigma_4^2) \\
 &= 0.527 \times \exp [(1.645) (0.294) - (0.5) (0.0862)] \\
 &= 0.527 \times 1.55 \\
 &= 0.82 \text{ TUc } , \text{ or} \\
 &= \text{LTA} \times \text{average monthly limit LTA multiplier from TSD} \\
 &\quad \text{Table 5-2 for CV, 95}^{\text{th}} \text{ percentile, and } n \geq 4 \\
 &= 0.527 \times 1.55 \\
 &= 0.82 \text{ TUc}
 \end{aligned}$$

$$\begin{aligned}
 \text{where } CV &= CV(X) * \\
 &= 0.6 \\
 \sigma^2 &= \ln (CV^2 + 1) \\
 &= 0.307 \\
 z_{0.99} &= 2.326 \text{ is recommended for MDL in TSD Section 5.5.4} \\
 n &= \text{number of samples per month } \geq 4 \\
 &= 4 \\
 \sigma_n^2 &= \ln [(CV^2 / n) + 1] \\
 \sigma_4^2 &= \ln [(CV^2 / 4) + 1] \\
 &= 0.0862 \\
 z_{0.95} &= 1.645 \text{ is recommended for AML in TSD Section 5.5.4}
 \end{aligned}$$

Following Section 2.6.2 in Chapter 2 of this document, EPA Regions 9 and 10 continue recommend that Permitting Authorities establish a monthly median limit (MML) of 1.0 TUc for chronic WET, when no mixing zone is authorized or an NPDES discharge is to a zero flow stream and the statistically-calculated AML is at or less than 1.0 TUc. As a result, in this example, the recommended permit limits for chronic WET are:

$$\begin{aligned}
 \text{MDL} &= \text{maximum daily limit} \\
 &1.6 \text{ TUc} \\
 \text{MML} &= \text{median monthly limit} \\
 &1.0 \text{ TUc}
 \end{aligned}$$

References:

USEPA. 1991a. Technical support document for water quality-based toxics control. Office of Water. Washington, DC. EPA/505/2-90/001.

APPENDIX D

ACUTE WET PERMIT LANGUAGE

xx. Acute Whole Effluent Toxicity Requirements

For routine monitoring frequency (i.e., monthly, quarterly, semi-annual or annual), and permit years for split sampling of WET and other monitored parameters (i.e., 1, 2, 3, 4 and 5), select proper paragraph 1, as described in Chapter 3 of this document.

1. Monitoring Frequency

The permittee shall conduct *monthly/quarterly/semi-annual* acute toxicity tests on 24-hour composite effluent samples. Once each calendar year, at a different time of year from the previous years, the permittee shall split a 24-hour composite effluent sample and concurrently conduct two toxicity tests using a fish and an invertebrate species; the permittee shall then continue to conduct routine *monthly/quarterly/semi-annual* toxicity testing using the single, most sensitive species.

Acute toxicity test samples shall be collected for each point of discharge at the designated NPDES sampling station for the effluent (i.e., downstream from the last treatment process and any in-plant return flows where a representative effluent sample can be obtained). During years **1, 2, 3, 4 and 5** of the permit, a split of each sample shall be analyzed for all other monitored parameters at the minimum frequency of analysis specified by the effluent monitoring program.

1. Monitoring Frequency

The permittee shall conduct annual acute toxicity tests on 24-hour composite effluent samples. Each calendar year, at a different time of year from the previous years, the permittee shall split a 24-hour composite effluent sample and concurrently conduct two toxicity tests using a fish and an invertebrate species; the permittee shall then continue to conduct routine annual toxicity testing using the single, most sensitive species.

Acute toxicity test samples shall be collected for each point of discharge at the designated NPDES sampling station for the effluent (i.e., downstream from the last treatment process and any in-plant return flows where a representative effluent sample can be obtained). During years **1, 2, 3, 4 and 5** of the permit, a split of each sample shall be analyzed for all other monitored parameters at the minimum frequency of analysis specified by the effluent monitoring program.

To monitor acute whole effluent toxicity with proper species and test methods select proper paragraph 2, as described in Chapter 3 of this document. Please note that freshwater discharges to marine or estuarine receiving water bodies are monitored using either freshwater species and test methods or saltwater species and test methods, based on the magnitude of the discharge specific mixing zone or dilution allowance authorized by the permitting authority. Choose one vertebrate species and one invertebrate species.

2. Freshwater Species and Test Methods

Species and short-term test methods for estimating the acute toxicity of NPDES effluents are found in the fifth edition of *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms* (EPA/821/R-02/012, 2002; Table IA, 40 CFR Part 136). The permittee shall conduct 96-hour static renewal toxicity tests with the following vertebrate species:

- The fathead minnow, *Pimephales promelas* (Acute Toxicity Test Method 2000.0);
- The rainbow trout, *Oncorhynchus mykiss*, or brook trout, *Salvelinus fontinalis* (Acute Toxicity Test Method 2019.0);

And the following invertebrate species:

- The daphnid, *Ceriodaphnia dubia* (Acute Toxicity Test Method 2002.0);
- The daphnid, *Daphnia pulex*, or daphnid, *Daphnia magna* (Acute Toxicity Test Method 2021.0).

2. Marine and Estuarine Species and Test Methods

Generally, species and short-term test methods for estimating the acute toxicity of NPDES effluents are found in the fifth edition of *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms* (EPA/821/R-02/012, 2002; Table IA, 40 CFR Part 136). The permittee shall conduct 96-hour static renewal toxicity tests with the following vertebrate species:

- The topsmelt, *Atherinops affinis* (Larval Survival and Growth Test Method 1006.0¹ in the first edition of *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms* (EPA/600/R-95/136, 1995) (specific to Pacific Coast waters);
- The Inland silverside, *Menidia beryllina*; Atlantic silverside, *Menidia menidia*; or Tidewater silverside, *Menidia peninsulae* (Acute Toxicity Test Method 2006.0);
- The sheepshead minnow, *Cyprinodon variegates* (Acute Toxicity Test Method 2004.0);

¹ Daily observations for mortality make it possible to calculate acute toxicity for desired exposure periods (i.e., 96-hour LC50, etc.).

And the following invertebrate species:

- The West Coast mysid, *Holmesimysis costata* (Table 19 in the acute test methods manual) (specific to Pacific Coast waters);
- The mysid, *Americamysis bahia* (Acute Toxicity Test Method 2007.0).

Select proper paragraph 3, as described in Chapter 2 of this document. Acute WET permit limits or triggers established by the permitting authority must follow applicable water quality standards and NPDES regulations and are discharge specific. 40 CFR Part 122.44(d)(1). Note that WET permit limits or triggers specified in paragraph 3 are based on EPA's recommendations in Technical Support Document for Water Quality-based Toxics Control (EPA/505/2-90-001, 1991; TSD) and EPA's Regions 9 and 10 Guidance for Implementing Whole Effluent Toxicity Testing Programs (Denton and Narvaez, 1996).

If a mixing zone or dilution allowance for an effluent discharge is either not authorized or authorized such that a critical IWC is set at a % effluent value greater than 100% effluent, then a Pass or Fail test is recommended.

When a mixing zone or dilution allowance for an effluent discharge is authorized such that a critical IWC is set at a % effluent value at or lower than 100% effluent, then EPA's recommended procedures for calculating acute WET permit limits or triggers are found in Box 5-2 and Tables 5-1 and 5-2 of the TSD.

3. Acute WET Permit Trigger

There is no acute toxicity effluent limit for this discharge. The acute WET permit trigger for this discharge is "Pass" for any one test result. For this permit, the determination of Pass or Fail from a single-effluent-concentration (paired) acute toxicity test is determined using a one-tailed hypothesis test called a t-test. The objective of a Pass or Fail test is to determine if survival in the single treatment (100% effluent) is significantly different from survival in the control (0% effluent). Following Section 11.3 in the acute test methods manual (EPA/821/R-02/012, 2002), the t statistic for the single-effluent-concentration acute toxicity test shall be calculated and compared with the critical t set at the 5% level of significance. If the calculated t does not exceed the critical t, then the mean responses for the single treatment and control are declared "not statistically different" and the permittee shall report "Pass" on the DMR form. If the calculated t does exceed the critical t, then the mean responses for the single treatment and control are declared "statistically different" and the permittee shall report "Fail" on the DMR form. This permit requires additional toxicity testing if the acute WET permit trigger is reported as "Fail".

3. Acute WET Permit Triggers

There are no acute toxicity effluent limits for this discharge. The acute WET permit triggers are any one test result greater than xxx TUa (during the monthly reporting period), or any one or more test results with a calculated average value greater than yyy

TUa (during the monthly reporting period). Results shall be reported in TUa, where $TUa = 100/LC50$. The Lethal Concentration, 50 Percent (LC50) is the toxic or effluent concentration that would cause death in 50 percent of the test organisms over a specified period of time. This permit requires additional toxicity testing if an acute WET permit trigger is exceeded.

3. Acute WET Permit Limit

There is an acute toxicity effluent limit for this discharge. The acute WET permit limit for this discharge is “Pass” for any one test result. For this permit, the determination of Pass or Fail from a single-effluent-concentration (paired) acute toxicity test is determined using a one-tailed hypothesis test called a t-test. The objective of a Pass or Fail test is to determine if survival in the single treatment (100% effluent) is significantly different from survival in the control (0% effluent). Following Section 11.3 in the acute test methods manual (EPA/821/R-02/012, 2002), the t statistic for the single-effluent-concentration acute toxicity test shall be calculated and compared with the critical t set at the 5% level of significance. If the calculated t does not exceed the critical t, then the mean responses for the single treatment and control are declared “not statistically different” and the permittee shall report “Pass” on the DMR form. If the calculated t does exceed the critical t, then the mean responses for the single treatment and control are declared “statistically different” and the permittee shall report “Fail” on the DMR form. This permit requires additional toxicity testing if the acute WET permit limit is reported as “Fail”.

3. Acute WET Permit Limits

There are acute toxicity effluent limits for this discharge. The acute WET permit limits are any one test result greater than **xxx** TUa (during the monthly reporting period), or any one or more test results with a calculated average value greater than **yyy** TUa (during the monthly reporting period). Results shall be reported in TUa, where $TUa = 100/LC50$. The Lethal Concentration, 50 Percent (LC50) is the toxic or effluent concentration that would cause death in 50 percent of the test organisms over a specified period of time. This permit requires additional toxicity testing if an acute WET permit limits is exceeded.

4. Quality Assurance

- a. Quality assurance measures, instructions, and other recommendations and requirements are found in the test methods manual previously referenced. Additional requirements are specified, below.

The acute instream waste concentrations and effluent dilution series specified by the permitting authority are discharge specific and are determined based on applicable water quality standards, NPDES regulations, and requirements and recommendations in the test methods manuals. Note that the instream waste concentrations and dilution series specified in paragraph 4.b are based on EPA's recommendations in Technical Support Document for Water Quality-based Toxics Control (EPA/505/2-90-001, 1991; TSD), EPA's Regions 9 and 10 Guidance for Implementing Whole Effluent Toxicity Testing Programs (Denton and Narvaez, 1996), test method manuals previously referenced, and on Chapters 2 and 3 of this document.

- b. This discharge is subject to a determination of Pass or Fail from a single-effluent-concentration (paired) acute toxicity test using a one-tailed hypothesis test called a t-test. The acute instream waste concentration (IWC) for this discharge is 100% effluent. The 100% effluent concentration and a control shall be tested.
- b. For this discharge, a mixing zone or dilution allowance is authorized such that critical IWCs are set at % effluent values at or lower than 100% effluent. The acute instream waste concentrations (IWCs) for this discharge are **XXX%** effluent and **YYY%** effluent. A series of at least five effluent dilutions and a control shall be tested. At minimum, the dilution series shall include and bracket the IWCs.

Select for dilution water based on test methods required in paragraph 2.

- c. Effluent dilution water and control water should be prepared and used as specified in the test methods manual *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms* (EPA/821/R-02/012, 2002); and/or, for *Atherinops affinis*, *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms* (EPA/600/R-95/136, 1995). If the dilution water is different from test organism culture water, then a second control using culture water shall also be used. If the use of artificial sea salts is considered provisional in the test method, then artificial sea salts shall not be used to increase the salinity of the effluent sample prior to toxicity testing without written approval by the permitting authority.
- d. If organisms are not cultured in-house, then concurrent testing with a reference toxicant shall be conducted. If organisms are cultured in-house, then monthly reference toxicant testing is sufficient. Reference toxicant tests and effluent toxicity tests shall be conducted using the same test conditions (e.g., same test duration, etc.).
- e. If either the reference toxicant or effluent toxicity tests do not meet all test acceptability criteria in the test methods manual, then the permittee must resample and retest within 14 days.
- f. Following Paragraph 12.2.6.2 of the test methods manual, all acute toxicity test results from the multi-concentration tests required by this permit must be reviewed and reported according to EPA guidance on the evaluation of concentration-response

relationships found in *Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing (40 CFR 136)* (EPA/821/B-00/004, 2000).

Select proper paragraph 4.g for review of with-in test variability based on test methods required in paragraph 2.

- g. Within-test variability of individual toxicity tests should be reviewed for acceptability and variability criteria (upper and lower PMSD bounds) should be applied, as directed under Section 12.2.8 - *Test Variability* of the test methods manual, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*. Under Section 12.2.8, the calculated percent minimum significant difference (PMSD) for both reference toxicant test and effluent toxicity test results must be compared with the upper and lower PMSD bounds variability criteria specified in Table 3-6 - *Range of Relative Variability for Endpoints of Promulgated WET Methods, Defined by the 10th and 90th Percentiles from the Data Set of Reference Toxicant Tests*, taken from *Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System Program* (EPA/833/R-00/003, 2000), following the review criteria in Paragraphs 12.2.8.2.1 and 12.2.8.2 of the test methods manual. Based on this review, only accepted effluent toxicity test results shall be reported on the DMR form. If excessive within-test variability invalidates a test result, then the permittee must resample and retest within 14 days.
- g. Because this permit provides for a 96-hour LC50 endpoint from Method 1006.0 in *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms* (EPA/600/R-95/136, 1995), with-in test variability must be reviewed for acceptability and a variability criterion (upper %MSD bound) must be applied, as directed under the test method. Based on this review, only accepted effluent toxicity test results shall be reported on the DMR form. If excessive within-test variability invalidates a test result, then the permittee must resample and retest within 14 days.
- h. If the discharged effluent is chlorinated, then chlorine shall not be removed from the effluent sample prior to toxicity testing without written approval by the permitting authority.
- i. Where total ammonia concentrations in the effluent are ≥ 5 mg/L, toxicity may be contributed by unionized ammonia. pH drift during the toxicity test may contribute to artifactual toxicity when ammonia or other pH-dependent toxicants (e.g., metals) are present. This problem is minimized by conducting toxicity tests in a static-renewal or flow-through mode, as outlined in Paragraph 9.5.9 of the test methods manual.

5. Initial Investigation TRE Workplan

Within 90 days of the permit effective date, the permittee shall prepare and submit a copy of their Initial Investigation Toxicity Reduction Evaluation (TRE) Workplan (1-2 pages) to the permitting authority for review. This plan shall include steps the permittee intends to follow if toxicity is measured above an acute WET permit limit or trigger and should include, at minimum:

- a. A description of the investigation and evaluation techniques that would be used to identify potential causes and sources of toxicity, effluent variability, and treatment system efficiency.
- b. A description of methods for maximizing in-house treatment system efficiency, good housekeeping practices, and a list of all chemicals used in operations at the facility.
- c. If a Toxicity Identification Evaluation (TIE) is necessary, an indication of who would conduct the TIEs (i.e., an in-house expert or outside contractor).

6. Accelerated Toxicity Testing and TRE/TIE Process

- a. If an acute WET permit limit or trigger is exceeded and the source of toxicity is known (e.g., a temporary plant upset), then the permittee shall conduct one additional toxicity test using the same species and test method. This test shall begin within 14 days of receipt of test results exceeding an acute WET permit limit or trigger. If the additional toxicity test does not exceed an acute WET permit limit or trigger, then the permittee may return to their regular testing frequency.
- b. If an acute WET permit limit or trigger is exceeded and the source of toxicity is not known, then the permittee shall conduct six additional toxicity tests using the same species and test method, approximately every two weeks, over a 12 week period. This testing shall begin within 14 days of receipt of test results exceeding an acute WET permit limit or trigger. If none of the additional toxicity tests exceed an acute WET permit limit or trigger, then the permittee may return to their regular testing frequency.
- c. If one of the additional toxicity tests (in paragraphs 6.a or 6.b) exceeds an acute WET permit limit or trigger, then, within 14 days of receipt of this test result, the permittee shall initiate a TRE using, based on the type of treatment facility, EPA manual *Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants* (EPA/833/B-99/002, 1999) or EPA manual *Generalized Methodology for Conducting Industrial Toxicity Reduction Evaluations* (EPA/600/2-88/070, 1989). In conjunction, the permittee shall develop and implement a Detailed TRE Workplan which shall include: further actions undertaken by the permittee to investigate, identify, and correct the causes of toxicity; actions the permittee will take to mitigate the impact of the discharge and prevent the recurrence of toxicity; and a schedule for these actions.

- d. The permittee may initiate a Toxicity Identification Evaluation (TIE) as part of a TRE to identify the causes of toxicity using the same species and test method and, as guidance, EPA test method manuals: *Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures* (EPA/600/6-91/003, 1991); *Methods for Aquatic Toxicity Identification Evaluations, Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity* (EPA/600/R-92/080, 1993); *Methods for Aquatic Toxicity Identification Evaluations, Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity* (EPA/600/R-92/081, 1993); and *Marine Toxicity Identification Evaluation (TIE): Phase I Guidance Document* (EPA/600/R-96-054, 1996).

7. Reporting of Acute Toxicity Monitoring Results

- a. A full laboratory report for all toxicity testing shall be submitted as an attachment to the DMR for the month in which the toxicity test was conducted and shall also include: the toxicity test results—for determination of Pass/Fail; LC50; TUa = 100/LC50; NOAEC; TUa = 100/NOAEC—reported according to the test methods manual chapter on report preparation and test review; the dates of sample collection and initiation of each toxicity test; all results for effluent parameters monitored concurrently with the toxicity test(s); and progress reports on TRE/TIE investigations.
- b. The permittee shall notify the permitting authority in writing within 14 days of exceedance of an acute WET permit limit or trigger. This notification shall describe actions the permittee has taken or will take to investigate, identify, and correct the causes of toxicity; the status of actions required by this permit; and schedule for actions not yet completed; or reason(s) that no action has been taken.

8. Permit Reopener for Acute Toxicity

In accordance with 40 CFR Parts 122 and 124, this permit may be modified to include effluent limitations or permit conditions to address acute toxicity in the effluent or receiving waterbody, as a result of the discharge; or to implement new, revised, or newly interpreted water quality standards applicable to acute toxicity.

APPENDIX D

CHRONIC WET PERMIT LANGUAGE

xx. Chronic Whole Effluent Toxicity Requirements

For routine monitoring frequency (i.e., monthly, quarterly, semi-annual or annual), yearly determination of test species sensitivity (i.e., fish, invertebrate, or alga), and permit years for split sampling of WET and other monitored parameters (i.e., 1, 2, 3, 4 and 5), select proper paragraph 1, as described in Chapter 3 of this document.

1. Monitoring Frequency

The permittee shall conduct ***monthly/quarterly/semi-annual*** chronic toxicity tests on 24-hour composite effluent samples. Once each calendar year, at a different time of year from the previous years, the permittee shall split a 24-hour composite effluent sample and concurrently conduct three toxicity tests using a fish, an invertebrate, and an alga species; the permittee shall then continue to conduct routine ***monthly/quarterly/semi-annual*** toxicity testing using the single, most sensitive species.

Chronic toxicity test samples shall be collected for each point of discharge at the designated NPDES sampling station for the effluent (i.e., downstream from the last treatment process and any in-plant return flows where a representative effluent sample can be obtained). During years ***1, 2, 3, 4 and 5*** of the permit, a split of each sample shall be analyzed for all other monitored parameters at the minimum frequency of analysis specified by the effluent monitoring program.

1. Monitoring Frequency

The permittee shall conduct annual chronic toxicity tests on 24-hour composite effluent samples. Each calendar year, at a different time of year from the previous years, the permittee shall split a 24-hour composite effluent sample and concurrently conduct three toxicity tests using a fish, an invertebrate, and an alga species.

Chronic toxicity test samples shall be collected for each point of discharge at the designated NPDES sampling station for the effluent (i.e., downstream from the last treatment process and any in-plant return flows where a representative effluent sample can be obtained). During years ***1, 2, 3, 4 and 5*** of the permit, a split of each sample shall be analyzed for all other monitored parameters at the minimum frequency of analysis specified by the effluent monitoring program.

To monitor chronic whole effluent toxicity with proper species and test methods select proper paragraph 2, as described in Chapter 3 of this document. Please note that freshwater discharges to marine or estuarine receiving water bodies are monitored using either freshwater species and test methods or saltwater species and test methods, based on the magnitude of the discharge specific mixing zone or dilution allowance authorized by the permitting authority.

3. Freshwater Species and Test Methods

Species and short-term test methods for estimating the chronic toxicity of NPDES effluents are found in the fourth edition of *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* (EPA/821/R-02/013, 2002; Table IA, 40 CFR Part 136). The permittee shall conduct static renewal toxicity tests with the fathead minnow, *Pimephales promelas* (Larval Survival and Growth Test Method 1000.0¹); the daphnid, *Ceriodaphnia dubia* (Survival and Reproduction Test Method 1002.0¹); and the green alga, *Selenastrum capricornutum* (also named *Raphidocelis subcapitata*) (Growth Test Method 1003.0).

3. Marine and Estuarine Species and Test Methods

Species and short-term test methods for estimating the chronic toxicity of NPDES effluents are found in the first edition of *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms* (EPA/600/R-95/136, 1995) and applicable water quality standards; also see 40 CFR Parts 122.41(j)(4) and 122.44(d)(1)(iv) and 40 CFR Part 122.21(j)(5)(viii) for POTWs. The permittee shall conduct a static renewal toxicity test with the topmelt, *Atherinops affinis* (Larval Survival and Growth Test Method 1006.0¹); a static non-renewal toxicity test with the giant kelp, *Macrocystis pyrifera* (Germination and Growth Test Method 1009.0); and a toxicity test with one of the following invertebrate species:

- Static renewal toxicity test with the mysid, *Holmesimysis costata* (Survival and Growth Test Method 1007.0¹);
- Static non-renewal toxicity test with the Pacific oyster, *Crassostrea gigas*, or the mussel, *Mytilus* spp., (Embryo-larval Shell Development Test Method 1005.0);
- Static non-renewal toxicity test with the red abalone, *Haliotis rufescens* (Larval Shell Development Test Method);
- Static non-renewal toxicity test with the purple sea urchin, *Strongylocentrotus purpuratus*, or the sand dollar, *Dendraster excentricus* (Embryo-larval Development Test Method); or
- Static non-renewal toxicity test with the purple sea urchin, *Strongylocentrotus purpuratus*, or the sand dollar, *Dendraster excentricus* (Fertilization Test Method 1008.0).

¹ Daily observations for mortality make it possible to calculate acute toxicity for desired exposure periods (i.e., 7-day LC50, 96-hour LC50, etc.).

If laboratory-held cultures of the topsmelt, *Atherinops affinis*, are not available for testing, then the permittee shall conduct a static renewal toxicity test with the inland silverside, *Menidia beryllina* (Larval Survival and Growth Test Method 1006.0¹), found in the third edition of *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms* (EPA/821/R-02/014, 2002; Table IA, 40 CFR Part 136).

Select proper paragraph 3, as described in Chapter 2 of this document. Chronic WET permit limits or triggers established by the permitting authority must follow applicable water quality standards and NPDES regulations and are discharge specific. 40 CFR Part 122.44(d)(1). Note that the median monthly chronic WET permit limit or trigger specified in paragraph 3 is based on EPA's recommendations in Technical Support Document for Water Quality-based Toxics Control (EPA/505/2-90-001, 1991; TSD) and EPA's Regions 9 and 10 Guidance for Implementing Whole Effluent Toxicity Testing Programs (Denton and Narvaez, 1996) when a mixing zone or dilution allowance for an effluent discharge is not authorized by the permitting authority. EPA's recommended procedures for calculating chronic WET permit limits or triggers when a mixing zone or dilution allowance for an effluent discharge is authorized by the permitting authority are found in Box 5-2 and Tables 5-1 and 5-2 of the TSD.

3. Chronic WET Permit Triggers

There are no chronic toxicity effluent limits for this discharge. For this discharge, a mixing zone or dilution allowance is not authorized and the chronic WET permit triggers are any one test result greater than 1.6 TUC (during the monthly reporting period), or any one or more test results with a calculated median value greater than 1.0 TUC (during the monthly reporting period). Results shall be reported in TUC, where $TUC = 100/NOEC$. The No Observed Effect Concentration (NOEC) is the highest concentration of toxicant to which organisms are exposed in a short-term chronic test that causes no observable adverse effects on the test organisms (e.g., the highest concentration of toxicant in which the values for the observed responses are not statistically significantly different from the controls). This permit requires additional toxicity testing if a chronic WET permit trigger is exceeded.

3. Chronic WET Permit Triggers

There are no chronic toxicity effluent limits for this discharge. For this discharge, a mixing zone or dilution allowance is authorized and the chronic WET permit triggers are any one test result greater than **xxx** TUC (during the monthly reporting period), or any one or more test results with a calculated average value greater than **yyy** TUC (during the monthly reporting period). Results shall be reported in TUC, where $TUC = 100/NOEC$. The No Observed Effect Concentration (NOEC) is the highest concentration of toxicant to which organisms are exposed in a short-term chronic test that causes no observable adverse effects on the test organisms (e.g., the highest concentration of toxicant in which the values for the observed responses are not statistically significantly different from the

controls). This permit requires additional toxicity testing if a chronic WET permit trigger is exceeded.

3. Chronic WET Permit Limits

There are chronic toxicity effluent limits for this discharge. For this discharge, a mixing zone or dilution allowance is not authorized and the chronic WET permit limits are any one test result greater than 1.6 TUC (during the monthly reporting period), or any one or more test results with a calculated median value greater than 1.0 TUC (during the monthly reporting period). Results shall be reported in TUC, where $TUC = 100/NOEC$. The No Observed Effect Concentration (NOEC) is the highest concentration of toxicant to which organisms are exposed in a short-term chronic test that causes no observable adverse effects on the test organisms (e.g., the highest concentration of toxicant in which the values for the observed responses are not statistically significantly different from the controls). This permit requires additional toxicity testing if a chronic WET permit limit is exceeded.

3. Chronic WET Permit Limits

There are chronic toxicity effluent limits for this discharge. For this discharge, a mixing zone or dilution allowance is authorized and the chronic WET permit limits are any one test result greater than ~~xxx~~ TUC (during the monthly reporting period), or any one or more test results with a calculated average value greater than ~~yyy~~ TUC (during the monthly reporting period). Results shall be reported in TUC, where $TUC = 100/NOEC$. The No Observed Effect Concentration (NOEC) is the highest concentration of toxicant to which organisms are exposed in a short-term chronic test that causes no observable adverse effects on the test organisms (e.g., the highest concentration of toxicant in which the values for the observed responses are not statistically significantly different from the controls). This permit requires additional toxicity testing if a chronic WET permit limit is exceeded.

4. Quality Assurance

- a. Quality assurance measures, instructions, and other recommendations and requirements are found in the test methods manual previously referenced. Additional requirements are specified, below.

The chronic instream waste concentrations and effluent dilution series specified by the permitting authority are discharge specific and are determined based on applicable water quality standards, NPDES regulations, and requirements and recommendations in the test method manuals. Note that the instream waste concentrations and dilution series specified in paragraph 4.b are based on EPA's recommendations in Technical Support Document for Water Quality-based Toxics Control (EPA/505/2-90-001, 1991; TSD) and EPA's Regions 9 and 10 Guidance for Implementing Whole Effluent Toxicity Testing Programs (Denton and Narvaez, 1996) when a mixing zone or dilution allowance for an effluent discharge is not authorized by the permitting authority. EPA's recommended procedures for specifying instream waste concentrations and a dilution series when a mixing zone or dilution allowance for an effluent discharge is authorized by the permitting authority are found in the test method manuals previously referenced and in Chapters 2 and 3 of this document.

- b. For this discharge, a mixing zone or dilution allowance is not authorized. The chronic instream waste concentrations (IWCs) for this discharge are 100% effluent and 62.5% effluent. A series of at least five effluent dilutions and a control shall be tested. At minimum, the dilution series shall include the IWCs and three dilutions below the IWCs (e.g., 100%, 62.5%, 50%, 25% and 12.5%).
- b. For this discharge, a mixing zone or dilution allowance is authorized. The chronic instream waste concentrations (IWCs) for this discharge are **XXX** % effluent and **YYY** % effluent. A series of at least five effluent dilutions and a control shall be tested. At minimum, the dilution series shall include and bracket the IWCs.

Select proper paragraph 4.c for dilution water based on test methods required in paragraph 2.

- c. Effluent dilution water and control water should be standard synthetic dilution water, as described in the test methods manual *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* (EPA/821/R-02/013, 2002). If the dilution water is different from test organism culture water, then a second control using culture water shall also be used.
- c. Effluent dilution water and control water should be prepared and used as specified in the test methods manual *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms* (EPA/600/R-95/136, 1995) and/or *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms* (EPA/821/R-02/014, 2002). If the dilution water is different from test organism culture water, then a second control using culture water shall also be used. If the use of artificial sea salts is considered provisional in the test method, then artificial sea salts shall not be used to increase the salinity of the effluent sample prior to toxicity testing without written approval by the permitting authority.
- d. If organisms are not cultured in-house, then concurrent testing with a reference toxicant shall be conducted. If organisms are cultured in-house, then monthly

- reference toxicant testing is sufficient. Reference toxicant tests and effluent toxicity tests shall be conducted using the same test conditions (e.g., same test duration, etc.).
- e. If either the reference toxicant or effluent toxicity tests do not meet all test acceptability criteria in the test methods manual, then the permittee must resample and retest within 14 days.
 - f. Following Paragraph 10.2.6.2 of the freshwater test methods manual, all chronic toxicity test results from the multi-concentration tests required by this permit must be reviewed and reported according to EPA guidance on the evaluation of concentration-response relationships found in *Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing (40 CFR 136)* (EPA/821/B-00-004, 2000).

Select proper paragraph 4.g for review of with-in test variability based on test methods required in paragraph 2.

- g. Because this permit requires sublethal hypothesis testing endpoints from Methods 1000.0, 1002.0, and 1003.0 in *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* (EPA/821/R-02/013, 2002), with-in test variability must be reviewed for acceptability and variability criteria (upper and lower PMSD bounds) must be applied, as directed under Section 10.2.8 - *Test Variability* of the test methods manual *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*. Under Section 10.2.8, the calculated percent minimum significant difference (PMSD) for both reference toxicant test and effluent toxicity test results must be compared with the upper and lower PMSD bounds variability criteria specified in Table 6 - *Variability Criteria (Upper and Lower PMSD Bounds) for Sublethal Hypothesis Testing Endpoints Submitted Under NPDES Permits*, following the review criteria in Paragraphs 10.2.8.2.1 through 10.2.8.2.5 of the test methods manual. Based on this review, only accepted effluent toxicity test results shall be reported on the DMR form. If excessive within-test variability invalidates a test result, then the permittee must resample and retest within 14 days.
- g. Because this permit requires sublethal hypothesis testing endpoints from test methods in *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms* (EPA/600/R-95/136, 1995), within-test variability must be reviewed for acceptability and a variability criterion (upper %MSD bound) must be applied, as directed under each test method. Based on this review, only accepted effluent toxicity test results shall be reported on the DMR form. If excessive within-test variability invalidates a test result, then the permittee must resample and retest within 14 days.
- g. Because this permit provides for a sublethal hypothesis testing endpoint from Method 1006.0 in *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms* (EPA/821/R-02/014, 2002), within-test variability must be reviewed for acceptability and variability criteria

- (upper and lower PMSD bounds) must be applied, as directed under Section 10.2.8 - *Test Variability* of the test methods manual *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms*. Under Section 10.2.8, the calculated percent minimum significant difference (PMSD) for both reference toxicant test and effluent toxicity test results must be compared with the upper and lower PMSD bounds variability criteria specified in Table 6 - *Variability Criteria (Upper and Lower PMSD Bounds) for Sublethal Hypothesis Testing Endpoints Submitted Under NPDES Permits*, following the review criteria in Paragraphs 10.2.8.2.1 through 10.2.8.2.5 of the test methods manual. Based on this review, only accepted effluent toxicity test results shall be reported on the DMR form. If excessive within-test variability invalidates a test result, then the permittee must resample and retest within 14 days.
- h. If the discharged effluent is chlorinated, then chlorine shall not be removed from the effluent sample prior to toxicity testing without written approval by the permitting authority.
 - i. pH drift during the toxicity test may contribute to artifactual toxicity when pH-dependent toxicants (e.g., ammonia, metals) are present in an effluent. To determine whether or not pH drift during the toxicity test is contributing to artifactual toxicity, the permittee shall conduct three sets of parallel toxicity tests, in which the pH of one treatment is controlled at the pH of the effluent and the pH of the other treatment is not controlled, as described in Section 11.3.6.1 of the test methods manual, *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* (EPA/821/R-02/013, 2002). Toxicity is confirmed to be artifactual and due to pH drift when no toxicity above the chronic WET permit limit or trigger is observed in the treatments controlled at the pH of the effluent. If toxicity is confirmed to be artifactual and due to pH drift, then, following written approval by the permitting authority, the permittee may use the procedures outlined in Section 11.3.6.2 of the test methods manual to control sample pH during the toxicity test.
5. Initial Investigation TRE Workplan

Within 90 days of the permit effective date, the permittee shall prepare and submit a copy of their Initial Investigation Toxicity Reduction Evaluation (TRE) Workplan (1-2 pages) to the permitting authority for review. This plan shall include steps the permittee intends to follow if toxicity is measured above a chronic WET permit limit or trigger and should include, at minimum:

- a. A description of the investigation and evaluation techniques that would be used to identify potential causes and sources of toxicity, effluent variability, and treatment system efficiency.
- b. A description of methods for maximizing in-house treatment system efficiency, good housekeeping practices, and a list of all chemicals used in operations at the facility.

- c. If a Toxicity Identification Evaluation (TIE) is necessary, an indication of who would conduct the TIEs (i.e., an in-house expert or outside contractor).
6. Accelerated Toxicity Testing and TRE/TIE Process
 - a. If a chronic WET permit limit or trigger is exceeded and the source of toxicity is known (e.g., a temporary plant upset), then the permittee shall conduct one additional toxicity test using the same species and test method. This test shall begin within 14 days of receipt of test results exceeding a chronic WET permit limit or trigger. If the additional toxicity test does not exceed a chronic WET permit limit or trigger, then the permittee may return to their regular testing frequency.
 - b. If a chronic WET permit limit or trigger is exceeded and the source of toxicity is not known, then the permittee shall conduct six additional toxicity tests using the same species and test method, approximately every two weeks, over a 12 week period. This testing shall begin within 14 days of receipt of test results exceeding a chronic WET permit limit or trigger. If none of the additional toxicity tests exceed a chronic WET permit limit or trigger, then the permittee may return to their regular testing frequency.
 - c. If one of the additional toxicity tests (in paragraphs 6.a or 6.b) exceeds a chronic WET permit limit or trigger, then, within 14 days of receipt of this test result, the permittee shall initiate a TRE using as guidance, based on the type of treatment facility, EPA manual *Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants* (EPA/ 833/B-99/002, 1999) or EPA manual *Generalized Methodology for Conducting Industrial Toxicity Reduction Evaluations* (EPA/600/2-88/070, 1989). In conjunction, the permittee shall develop and implement a Detailed TRE Workplan which shall include: further actions undertaken by the permittee to investigate, identify, and correct the causes of toxicity; actions the permittee will take to mitigate the impact of the discharge and prevent the recurrence of toxicity; and a schedule for these actions.
 - d. The permittee may initiate a Toxicity Identification Evaluation (TIE) as part of a TRE to identify the causes of toxicity using the same species and test method and, as guidance, EPA test method manuals: *Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I* (EPA/600/6-91/005F, 1992); *Methods for Aquatic Toxicity Identification Evaluations, Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity* (EPA/600/R-92/080, 1993); *Methods for Aquatic Toxicity Identification Evaluations, Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity* (EPA/600/R-92/081, 1993); and *Marine Toxicity Identification Evaluation (TIE): Phase I Guidance Document* (EPA/600/R-96-054, 1996).

7. Reporting of Chronic Toxicity Monitoring Results

- a. A full laboratory report for all toxicity testing shall be submitted as an attachment to the DMR for the month in which the toxicity test was conducted and shall also include: the toxicity test results—in NOEC; $TU_c = 100/NOEC$; EC25 (or IC25); and $TU_c = 100/EC25$ (or IC25)—reported according to the test methods manual chapter on report preparation and test review; the dates of sample collection and initiation of each toxicity test; all results for effluent parameters monitored concurrently with the toxicity test(s); and progress reports on TRE/TIE investigations.
- b. The permittee shall notify the permitting authority in writing within 14 days of exceedance of a chronic WET permit limit or trigger. This notification shall describe actions the permittee has taken or will take to investigate, identify, and correct the causes of toxicity; the status of actions required by this permit; and schedule for actions not yet completed; or reason(s) that no action has been taken.

8. Permit Reopener for Chronic Toxicity

In accordance with 40 CFR Parts 122 and 124, this permit may be modified to include effluent limitations or permit conditions to address chronic toxicity in the effluent or receiving waterbody, as a result of the discharge; or to implement new, revised, or newly interpreted water quality standards applicable to chronic toxicity.