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REGIONS 9 & 10 GUIDANCE FOR IMPLEMENTING WHOLE EFFLUENT TOXICITY TESTING PROGRAMS

FINAL

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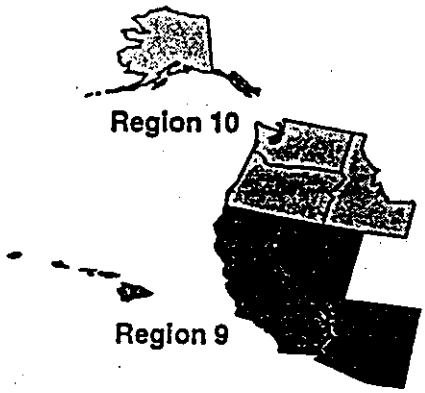

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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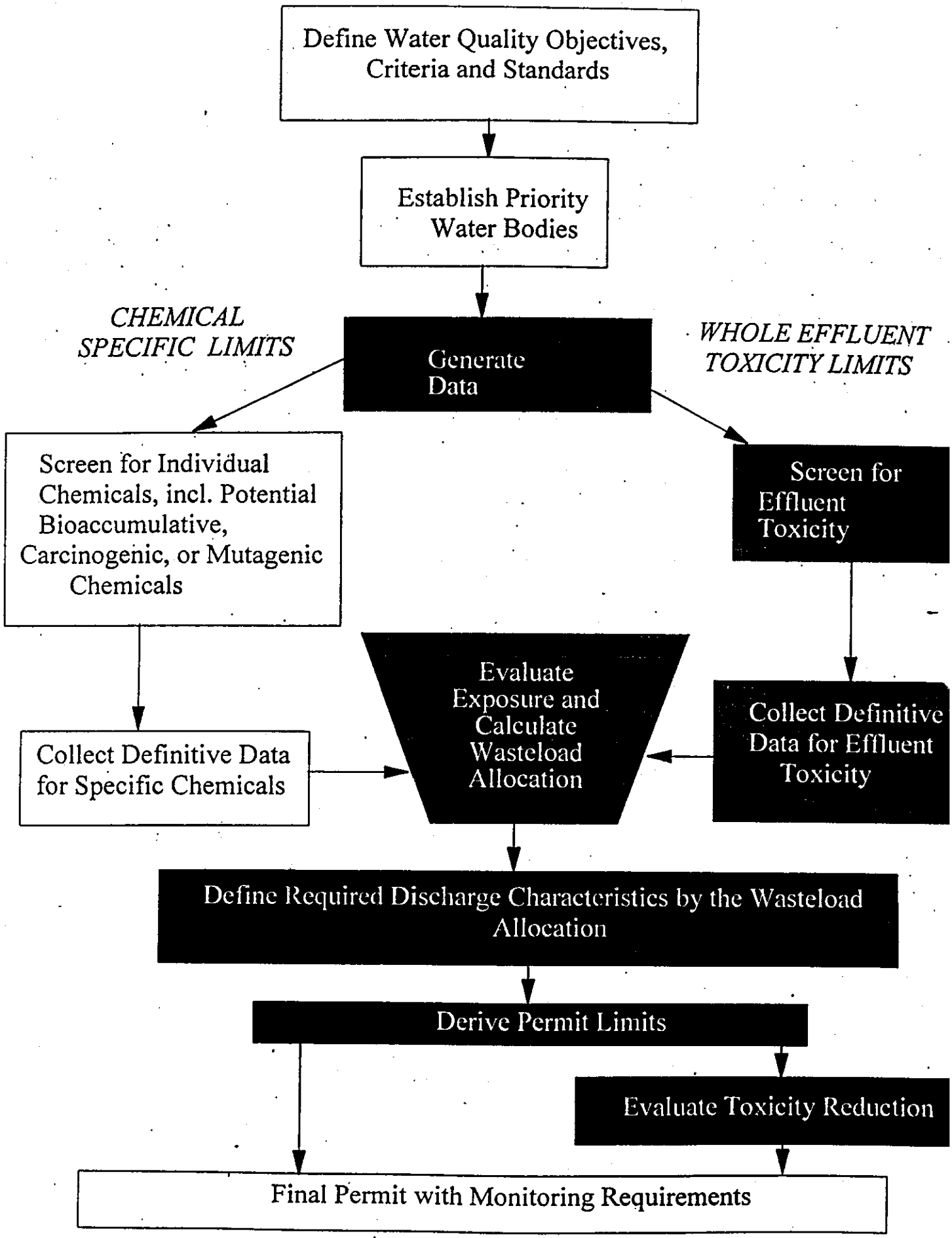
Define Water Quality Objectives, Criteria and Standards
Establish Priority Water Bodies
CHEMICAL SPECIFIC LIMITS
Generate Data
WHOLE EFFLUENT TOXICITY LIMITS
Screen for Individual Chemicals, incl. Potential Bioaccumulative, Carcinogenic, or Mutagenic Chemicals
Screen for Effluent Toxicity
Evaluate Exposure and Calculate Wasteload Allocation
Collect Definitive Data for Specific Chemicals
Collect Definitive Data for Effluent Toxicity
Define Required Discharge Characteristics by the Wasteload Allocation
Derive Permit Limits
Evaluate Toxicity Reduction
Final Permit with Monitoring Requirements

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EXECUTIVE SUMMARY

The Environmental Protection Agency (EPA), Regions 9 and 10, have developed draft guidance for implementing whole effluent toxicity (WET) programs. This guidance incorporates information on WET requirements from supporting EPA documents, such as the Technical Support Document for Water Quality-based Toxics Control (TSD, EPA/505/2-90-001) and the EPA toxicity test method manuals, in order to provide a single concise document. A collaborative effort between Regions 9 and 10, this guidance includes input from States as well as the regulated community on some non-policy issues. This interim guidance also incorporates many comments from States, EPA headquarters, and other EPA regions. Region 9 issued the second draft of the guidance to the regulated community in Region 9 states on May 18, 1995, while Region 10 distributed the second draft on June 23, 1995.

This document provides guidance to EPA permit writers and States on how best to implement EPA's National Pollutant Discharge Elimination System (NPDES) regulations regarding appropriate WET limitations and monitoring requirements in permits. It also provides guidance to the public and to the regulated community on how Regions 9 and 10 intend to exercise its discretion in implementing its regulations. This guidance is designed to implement national policy on these issues. This document does not substitute for EPA's regulations, nor is it a regulation itself. Thus, it cannot impose legally binding requirements on EPA, States, or the regulated community, and may not apply to a particular situation based upon the circumstances. EPA may change this guidance in the future, as appropriate. EPA is convening a national meeting in 1996 to discuss implementation issues with WET testing programs. This meeting will be open to public participation by the regulated community, environmentalists, laboratories, States, Tribes, EPA and other interested parties.

This guidance is not meant to supersede any established State programs, such as exist in Washington and California. This document describes many of the types of WET programs in operation and makes recommendations specific to the Regions 9 and 10 States where national guidance is extremely broad. This document also specifies that permit decisions must take into account applicable Federal, State, and Tribal laws, regulations, guidance, and standards.

The primary objective of the whole effluent toxicity testing program is to identify, characterize, and eliminate toxic effects of discharges on our aquatic resources. The permitting authorities should strive to establish NPDES WET limits and/or monitoring schedules with appropriate test methods and testing frequency to achieve the program objectives. NPDES limits and/or monitoring schedules are used to ensure that when effects are demonstrated on the aquatic organisms that the permittee will act expeditiously to identify the cause(s) of toxicity and reduce/eliminate the cause(s) to protect the aquatic resources.

Minimum requirements for a State WET testing program:

- WET monitoring data requirements: Required at all major and minor industrial categorized in the NPDES program under specific SIC codes, POTW's with pretreatment programs, POTW's with design flow ≥ 1 MGD, facilities designated under Section 304(l) of the CWA, and others where toxicity is suspected.

- Reasonable potential: The EPA statistical approach as outlined in the TSD or an approved State policy of a pre-determined number of failures. States can develop their own policy on reasonable potential.

- Type of testing: The appropriate acute or chronic toxicity testing requirement must be based on the EPA TSD statistical method, or State standards.

- Mixing zone: EPA endorses the use of mixing zones and encourages the states to include a proper mixing zone policy in their water quality standards.

- WET Limit: Required if a discharge causes, has a reasonable potential to cause, or contributes to an exceedance of applicable water quality standards, including numeric or narrative.

Permits must be written to avoid ambiguity and ensure enforceability:

- Test species/methods: Toxicity testing species and methods must be accurately referenced in the permit.

- Frequency: Where WET limits are required, frequency of toxicity testing should be monthly for majors and quarterly for minors. More frequent testing should be required on a case-by-case basis depending on the effluent variability. Less frequent testing could be allowed where no toxicity is demonstrated with an acceptable facility database covering both temporal and spatial factors. The permit writer should consider all available data when making decisions regarding testing frequency.

- Number of species: A minimum of two species must be tested for acute testing (an invertebrate and a vertebrate). A minimum of three species must be tested for chronic testing (an invertebrate, a vertebrate and a plant, or for Region 10, two invertebrates and a vertebrate), at least through the screening phase.

- Quality Assurance/Quality ControlA minimum of four replicates should be required for chronic toxicity test methods, unless the method cites a higher number.

Laboratories should calculate and report the minimum significant difference (MSD) for the reference toxicant regardless of whether the compliance endpoint is based on hypothesis testing or point estimates.

- TRE/TIE language: The permit must reference the appropriate TRE/TIE documents and TRE triggers. Limits must be written with TRE triggers. Note: if a monitoring requirement is used instead of a WET limit, then reopener language with TRE triggers must be cited in permits.

- WET limits: The permitting authority should establish permit limits using a statistical derivation procedure that adequately accounts for effluent variability. The limit should include a maximum daily permit limit (MDL) and average monthly limit (AML), unless other State standards have been adopted. Typically only a maximum daily limit is used for acute toxicity.

- Single exceedances: The initial response to a single exceedance of a WET limit, causing no known ecosystem harm, should not be a formal enforcement action with a civil penalty. In the case of inconclusive TREs, solutions should be pursued jointly with expertise from EPA and/or the States as well as the permittee.

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ACRONYMS AND INITIALS

ACR	acute-to-chronic ratio
AML	average monthly limit
AO	administrative order
ANOVA	analysis of variance
APO	administrative penalty order
BAT	best available technology
BMP	best management practices
CCC	criterion continuous concentration
CMC	criterion maximum concentration
CV	coefficient of variation
CWA	Clean Water Act of 1977 (PL 95-217)
DMR	USEPA's discharge monitoring report
EC	effect concentration
EDTA	ethylenediaminetetraacetic acid
EDW	effluent dominated waters
FAQ	frequently asked questions
IC	inhibition concentration
IWC	instream waste concentration
LC	lethal concentration
LOEC	lowest observed effect concentration
MDL	maximum daily limit

MGD	million gallons per day
MSD	minimum significant difference
MSE	mean square error
NOEC	no observed effect concentration
NOV	notice of violation
NPDES	National Pollutant Discharge Elimination System
POTW	publicly owned wastewater treatment works
QA	quality assurance
QC	quality control
SCTAG	Southern California Toxicity Assessment Group
SETAC	Society of Environmental Toxicology and Chemistry
STR	salinity/tolerance relationship
TAC	test acceptable criteria
TDS	total dissolved solids
TIE	toxicity identification evaluation
TMDL	total maximum daily loads
TRE	toxicity reduction evaluation
TSD	USEPA's Technical Support Document for Water Quality-based Toxics Control
TSERF	toxicity standardized electronic reporting format
TU	toxicity unit
USEPA	United States Environmental Protection Agency

WET	whole effluent toxicity
WLA	waste load allocations
WQBELs	water quality based effluent limitations
WQC	water quality criteria
WQS	water quality standards
WWTP	wastewater treatment plant
ZID	zone of initial dilution

REGIONS 9 AND 10 GUIDANCE FOR IMPLEMENTING WHOLE EFFLUENT TOXICITY TESTING PROGRAMS

CHAPTER 1. INTRODUCTION

BACKGROUND

When the Clean Water Act (CWA) was enacted in 1972, EPA embarked on a long term program aimed at restoring and maintaining the chemical, physical, and biological integrity of the Nation's waters. The National Pollutant Discharge Elimination System (NPDES), the centerpiece of EPA's water quality control program, was established to regulate industrial and municipal wastewater discharges.

The initial phases of the NPDES program relied on chemical-specific effluent limits and treatment technology principles to reduce discharges of toxic and conventional pollutants. Industries were required to install the best practicable control technology in order the limit the discharge of conventional pollutants such as BOD, TSS, and pH as well as some heavy metals. Publicly owned treatment works (POTWs) were required to install secondary (biological) treatment. The water quality program focused, for the most part, on conventional pollutants.

During the 1980s, industries received additional treatment technology requirements. POTWs added pretreatment programs. Even with these changes, however, many discharges remained toxic. Data gathered in the early 1980s indicated that approximately 40 percent of NPDES facilities nationwide discharged sufficient toxicity to cause water quality problems. Further reductions were necessary in order to achieve compliance with State water quality standards requirements of "no toxics in toxic amounts."

In response to these findings, EPA designed a policy to reduce or eliminate toxics discharges. The "Policy for the Development of Water Quality-based Permit Limitations for Toxic Pollutants", found at 49 FR 9016, dated March 9, 1984, introduced EPA's integrated toxics control program. This program consists of the application of both chemical-specific and biological methods to reduce toxic discharges. In support of this policy, EPA developed the **Technical Support Document for Water Quality-based Toxics Control** (TSD). First issued in 1985, this document gives specific guidance on water quality program implementation issues such as the integration of chemical and biological approaches; chemical, physical, and biological testing requirements and most importantly, whole effluent toxicity (WET) testing requirements.

On July 7, 1994, EPA issued a national policy governing the development of effluent limitations in NPDES permits to control whole effluent toxicity (WET) for the protection of aquatic life. Consisting of eight policy statements, the document reaffirmed EPA's strong continuing commitment to the existing Clean Water Act provisions and water quality permitting regulations at 40 CFR 122.44(d)(1). While EPA permit writers are expected to follow the portions of the policy that provide guidance on the implementation of statutory and regulatory requirements for

the control of WET, decisions on individual permit provisions should be made on a case-by-case basis. Thus, permit writers are expected to apply the law and regulations to specific facts and justify the decisions in the record for the permit. Nothing in the national policy should be interpreted as providing any relief from the statutory and regulatory requirements that permits include conditions as necessary to assure attainment of water quality standards. The national policy provides a general framework on which a guidance specifically applying to the Region should rest.

It has now been over ten years since EPA and states began using WET tests to assess and protect water quality. This decade-long experience has allowed for the continued refinement of test methods, and has consistently demonstrated the value of WET testing in the integrated water quality control program. In spite of this experience, however, WET testing remains contentious. The reliability and accuracy of WET testing, in particular, continues to be questioned. Regions 9 and 10 have prepared this guidance document to address the many valid concerns that remain over WET testing, and to provide detailed recommendations for complex implementation programs. Quality assurance, species selection, statistical and reasonable potential procedures, permit language, monitoring frequency, and enforcement procedures are all covered herein.

The eight statements of the national policy concern:

1. Basis for WET controls
2. Evaluation of dischargers for reasonable potential
3. Evaluating reasonable potential
4. Consequences of establishing reasonable potential
5. WET monitoring
6. Compliance schedules in NPDES permits
7. WET controls and the pollutants ammonia and chlorine
8. WET and POTWs

1-1 Eight statements of the national WET Policy.

It is the position of EPA Regions 9 and 10 that WET test methods, when closely and faithfully followed, yield reproducible and accurate results. WET testing has a vital role to play in water pollution control programs, regulating and helping to identify toxicity in both wastewater and ambient waters. It is our hope that this guidance will assist Western states, tribes, NPDES permittees, and private testing laboratories to move beyond arguments over WET test reliability and accuracy and move towards conscientious and comprehensive water quality protection.

PELLSTON 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) Pellston workshop on WET. The workshop explored the science involved in WET testing. EPA

views this as the beginning of a mid-course evaluation of a successfully implemented program. The workshop evaluated the latest science. While the proceedings will be published later this summer, the overall conclusions are listed in the box below.

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 permitees.

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 and 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.

EPA'S INTEGRATED STRATEGY

For the protection of aquatic life, the integrated strategy involves the use of three control approaches: chemical-specific control, WET control, and biological criteria/bioassessment and biosurvey. This guidance only addresses the protection of aquatic life, not human health.

Each of the three control approaches have advantages and limitations. EPA acute ambient criteria are based on protecting a minimum of eight different organisms, including fish, invertebrates, and plants. Chemical analyses can sometimes be less expensive than WET testing and biological surveys, if only a few toxicants are present. The chemical-specific approach can allow prediction of ecological impacts before they occur, since it also considers bioaccumulation and human health impacts. A limitation of the chemical-specific approach is that all toxicants in wastewaters are not known, and therefore, control requirements for all toxicants cannot be set.

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 toxic effect can be limited by limiting one parameter, i.e., WET; and ecological impacts can be predicted before they occur. The principal limitations include: only measuring and controlling toxicity to aquatic organisms; WET testing regimes usually only test two or three families, while water quality criteria are based on a minimum of eight different families; the WET test directly measures only the immediate bioavailability of a toxicant or toxicants in the test medium and cannot measure the persistence downstream and long-term cumulative toxicity of a compound.

The bioassessment approach can: directly assess the status of a waterbody, since biological communities reflect overall ecological integrity; provide a holistic measure of the aggregate impact of pollutant stressors and can measure historical trends and fluctuating environmental conditions. 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 discharger or other source.

Based on the differences of each of the three approaches, chemical-specific, whole effluent toxicity, and biological criteria/bioassessment and biosurvey, protection of aquatic life will be more thorough if all three approaches are used. The chemical-specific approach provides a high accuracy of analysis of the individual chemical constituents (while the precision of the analyses are comparable to the precision of WET analyses), has been used by regulatory authorities, and is generally lowest in cost, when there are few chemicals that need to be analyzed. However, if no chemical-specific criteria exist for the chemicals present in the effluent, the level of protection could be low or even absent. The WET approach fills this gap by measuring the aggregate effect

of all toxicants. However, even this approach can be limited by the use of insensitive or less sensitive species and protocols. Bioassessments also provide a coverage of many biological impacts and can allow for accurate historical trend analyses. Bioassessments, though, cost more than the other two approaches, and data interpretation can be extremely difficult.

It is EPA's position that the concept of "independent application" be applied to water quality-based situations [USEPA 1991(a)]. 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 122.44(d)(1) in effect require independent application of chemical-specific and whole effluent data and criteria when characterizing effluents. Few of the Region 9 and 10 states have established biocriteria, so permit writers will be relying mostly upon WET and chemical-specific data in determining limits. The TSD recommends that whenever discrepancies between the findings of the approaches occur, the regulatory agencies consider re-examining the findings to determine if simplifications or assumptions may have caused the differences. For instance, concurrent analysis of the sampling approach and analysis of the biosurvey data might be needed to see if they adequately characterize the receiving water.

SMALL COMMUNITIES CONSIDERATIONS

This guidance recognizes that the development and implementation of an extensive WET testing program may be difficult for some small municipalities. At the discretion of the permit writer, small communities may be granted effluent characterization programs or monitoring frequencies that vary from what this guidance recommends. The Reasonable Potential Section, in Chapter 2, discusses considerations for small systems in more detail.

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CHAPTER 2. DEVELOPING WET PERMIT CONDITIONS

OVERVIEW

In Chapter 1 of this guidance, the history and regulatory and statutory basis of the NPDES WET testing program were presented. Chapter 2 discusses the actual development of WET permit conditions. Subjects covered in this chapter include reasonable potential determinations, derivation and expression of WET permit limits, mixing zones, WET criteria, and acute and chronic toxicity testing parameters.

REASONABLE POTENTIAL

EPA's existing regulations require NPDES permits to include water quality-based effluent limitations (WQBELs) to control all pollutants or pollutant parameters, including WET, that the permitting authority determines are or may be discharged at a level which will cause, have the reasonable potential to cause, or contribute to an excursion above any water quality standards including numeric and narrative criteria for water quality (40 CFR Part 122.44(d)(1)). WET data are not necessary in order to assess the reasonable potential for a standards exceedance. Reasonable potential can be determined with or without facility specific effluent data, which will be discussed later in this section.

The TSD guidance recognizes that the permit writer has flexibility in assessing whether a discharge has reasonable potential to exceed water quality standards. For instance, dynamic modeling can be used. Dynamic models account for the daily variations of and relationships between flow, effluent, and environmental conditions and therefore directly determine the actual probability that a water quality standards exceedance will occur. Few facilities, though, have the quantity and quality of information available to allow the use of dynamic models. In addition, a permitting authority may decide to develop a WQBEL in the absence of facility-specific effluent monitoring data. Regardless of which approach is selected by the authority, it must satisfy all requirements of 40 CFR Part 122.44(d)(1)(ii) summarized below.

NPDES regulations at 40 CFR Part 122.44(d)(1)(i) require the establishment of an effluent limitation for any pollutant which is or may be discharged at a level that "will cause, have a reasonable potential to cause, or contribute to an excursion above any State water quality standard, including State narrative criteria for water quality." In determining the need for an effluent limitation, the permit writer must consider existing controls on other point and nonpoint sources, the variability of the pollutant or pollutant parameter in the discharge, the sensitivity of the test species (for WET) and, where appropriate, the mixing of the discharge in the receiving water [see 40 CFR Part 122.44(d)(ii)]. Effluent limitations must be included, as appropriate, for specific pollutants and/or WET.

At least three outcomes are possible when deciding whether a facility causes, has the reasonable potential to cause, or contributes to an excursion above a water quality criterion. First, a

permitting authority may determine that the WET of a facility's discharge may be at a level which causes, has the reasonable potential to cause, or contributes to an excursion above a narrative or numeric water quality criterion. In this case, the permitting authority is required to establish a WQBEL in the permit (40 CFR Part 122.44(d)(1)(ii)). This WQBEL must be for WET, unless the State does not have numeric criteria for toxicity and the permitting authority can demonstrate that chemical-specific limits are sufficient to attain and maintain applicable standards (40 CFR Part 122.44(d)(1)(v)).

Reasonable potential is shown where an effluent, in conjunction with other point and nonpoint sources, is projected to cause an excursion above the water quality criterion. This projection is based upon an analysis of available data that accounts for, among other things, limited sample size and effluent variability.

Three outcomes are possible:

- Facility discharge has reasonable potential to cause or contribute to an excursion above a WQ criterion
- Inadequate information to determine whether discharge will cause or contribute to an excursion above a WQ criterion
- Facility discharge does not cause an excursion above a WQ criterion.

2-1 Possible outcomes of an RP analysis

Second, a permitting authority may have inadequate information to determine whether a discharge causes, has the reasonable potential to cause, or contributes to an excursion of a water quality criterion. In this case, the permitting authority is not required to establish a WQBEL. **EPA does, however, recommend that the permitting authority establish appropriate monitoring requirements and a reopener clause in the permit** (see TSD, Chap. 3.3.3). A reopener clause authorizes "reopening" the permit and establishing additional permit conditions based on monitoring results or other new factors that indicate that the effluent may cause, have the reasonable potential to cause, or contribute to an excursion above water quality standards. When permits are "reopened" in this manner, permitting authorities typically impose WQBELs for WET and/or require a discharger to perform a toxicity reduction evaluation (TRE).

Third, a permitting authority may determine that WET in a facility's discharge is not discharged at a level that causes, or contributes to an excursion above a water quality criterion. Under this outcome, the permitting authority need not establish a WQBEL. **EPA recommends that monitoring be repeated at a frequency of at least once every five years** (prior to the next permit reissuance process) (see TSD, Chapter 3.3).

Where reasonable potential is not demonstrated for WET, WET limits need not be included in the permit. The tiered methodology used to evaluate reasonable potential with and without facility-specific effluent and receiving water quality data are outlined in Appendix J.

Determining the Need for Permit Limits: Without Effluent Monitoring Data at a Facility

If a regulatory authority chooses, or the situation warrants it, the permitting authority may decide to develop and impose a limit for WET without facility-specific effluent monitoring data, or prior to the generation of effluent data. In doing so, the regulatory authority must satisfy all the requirements of 40 CFR Part 122.44(d)(1)(ii). [See Appendix G, Statutory and Regulatory Considerations.] This approach is discretionary. Should the permit writer choose to impose permits limits using this approach, he/she should present a clear rationale for the approach in the permit fact sheet.

When determining whether or not a discharge causes, has the reasonable potential to cause, or contributes to an excursion of a narrative or numeric water quality criterion for individual toxicants or toxicity, the permitting authority can use a variety of factors and information where facility specific effluent monitoring data are unavailable. These factors should also be considered with available effluent monitoring data. Some of these factors are the following:

--**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, and among other factors, whether or not there is a diffuser. The lower the available dilution, the higher the potential for toxic effect. Assessment of the amount of stream dilution available should be made at the conditions required by the water quality standards, or if not specified in the standards, the 7Q10 flow (consecutive 7-day low flow with a 10 year recurrence interval) for application of the chronic criterion and 1Q10 flow (1-day low flow with a 10 year recurrence interval) for application of the acute criteria, or other comparable low flow.

--**Type of industry.** Although dischargers should be individually characterized because toxicity problems are site-specific, the primary industrial categories are of principal toxicity concern. EPA's treatment technology database generally indicates that secondary industrial categories may have less potential for toxicity than primary industries.

--**Type of POTW.** POTWs with loadings from indirect dischargers (particularly primary industries) may be candidates for toxicity limits. However, absence of industrial input does not guarantee an absence of toxicity problems. For example, commercial pesticide applicators often discharge to POTWs, resulting in pesticide concentrations high enough to cause toxicity in the POTW's effluent. Household disposal of pesticides, detergents, or other toxics may also have an effect. The types of industrial users, their product lines, and raw materials, their potential and actual discharges, as well as control equipment should be evaluated. In addition, POTWs should be evaluated for potential toxicity due to chlorine and ammonia.

--**Existing data on toxic pollutants.** Discharge monitoring reports (DMRs) and data from NPDES permit application forms 2C and 2A may provide some indication of the

presence of toxicants. The presence or absence of the 126 priority pollutants may or may not be an indication of the presence or absence of toxicity. There are thousands of toxicants not on the list of 126 priority pollutants, that are by definition "nonconventional" pollutants that may cause toxicity. Also, combinations of toxicants can produce toxicity where individual toxicants would not. EPA regulations at 40 CFR Part 122.21(j) require POTWs with design flows equal to or greater than 1 MGD and POTWs with approved pretreatment programs, or POTWs required to develop pretreatment programs, to submit the results of WET toxicity tests with their permit applications. These regulations also allow the permitting authority to request such data from other POTWs at the time of the application.

--**History of compliance problems and toxic impact.** Permitting authority 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.** Regulatory authorities may compile data on water quality. Examples of available data include reports of fish kills, State lists of priority waterbodies, and State lists of waters that are not meeting water quality standards. One source of this information is the lists of waters generated under section 304(l) of the CWA and described at 40 CFR Part 130.10(d)(6).

The presence of a combination of the 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 effluent limits including WET. If the permitting authority chooses to impose an effluent limit without facility-specific effluent monitoring data, it will need to provide adequate justification for the limit in the permit development rationale in the permit fact sheet. **EPA recommends, however, that the permitting authority obtain facility-specific WET monitoring data before permit reissuance.** The permitting authority may obtain this data through section 308 authority under the CWA, or similar State authority.

Determining the Need for Permit Limits: With Effluent Monitoring Data at a Facility

When determining the need for a chemical-specific or WET limit, the permitting authority should use all available data, together with any information like that discussed in the previous section, as a basis for a decision. While the following discussion can apply to calculation of both chemical-specific and WET limits, only WET will be addressed. **EPA emphasizes that the purpose of the data generation is to determine whether or not a WET permit limit is necessary. If the permitting authority chooses to gather WET test data through the permit, a reasonable potential determination must be made at the time the permit is reopened or reissued.**

Reasonable potential is determined using a sequential, tiered, process (see Appendix J and TSD, Chapter 3). In the first step, historical effluent data for WET and appropriate statistics derived

from those data are used to statistically estimate the maximum effluent concentration, which for WET is expressed as acute or chronic toxicity units. In practice, these statistics are used to calculate an uncertainty multiplier that adjusts the maximum observed effluent concentration to a probability-based maximum concentration (see TSD, Chapter 3.3.2). This higher concentration is then used in the mass balance equation to project the maximum resultant in-stream concentration for WET after complete mixing or at the edge of the mixing zone (see Appendix J). If the projected in-stream concentration is less than the applicable ambient WET standard, the permit writer must then exercise judgement as to whether reasonable potential exists.

In the second step, the steady-state mass balance equation is used to project the maximum resultant in-stream concentration for WET after complete mixing (or at the edge of the mixing zone) under critical flow conditions, e.g., 7Q10 and 1Q10. If the projected in-stream concentration is greater than the applicable ambient WET standard (the objective, criteria, or standard necessary to attain the designated beneficial uses), then effluent limitations must be established for WET. Reasonable potential is established if the projected in-stream concentration exceeds the ambient WET standard.[1]

PERMIT LIMIT DERIVATION

When the 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 numeric or narrative water quality criteria for toxicity, the permit must contain effluent limits controlling for WET. [40 CFR Part 122.44(d)(1)(iv)] Where state water quality standards do not contain numeric criteria for toxicity and it can be demonstrated that chemical-specific limits for the effluent are sufficient to address the observed toxicity, WET limits are not necessary. [40 CFR Part 122.44(d)(1)(v)]

There are a number of different approaches that can be used to derive permit limits for WET. This policy outlines three widely used approaches: the statistical approach; the direct application approach; and other State regulations. Both the statistical approach and the direct application approach are based on the wasteload allocation. While each of these methods is a valid approach for deriving permit limits, **EPA recommends that the permitting authority establish permit limits using a statistical derivation procedure that adequately accounts for effluent variability.** EPA believes that statistical permit limit derivation procedures will result in the most defensible and protective water quality-based permit limits. In addition, development of WET permit limits must be consistent with State or federal toxicity criteria.

Water quality-based limits are established at levels that will ensure compliance with water quality standards even during critical conditions. These requirements are generally determined by the

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[1] If there is no numeric criterion for toxicity, then the narrative criterion must be converted to a numeric one for determining reasonable potential.

wasteload allocation (WLA). The WLA defines the appropriate discharge level that the treatment facility must achieve in order protect water quality.

Two major types of water quality models are used to develop WLAs: steady-state and dynamic. Traditional single- or two-value steady-state WLA models calculate WLAs at critical conditions, using worst-case assumptions for flow, effluent, and environmental effects. Permit limits derived from a steady-state model will be protective of water quality standards at critical conditions and all environmental conditions less than critical. In general, steady-state models tend to be more conservative than dynamic models because they rely on worst-case assumptions. **EPA recommends that steady-state WLA analyses be used by permitting authorities, especially where few or no whole effluent toxicity measurements are available, or where daily water flow records are not available.**

Using steady-state models, WLA calculations are always made assuming critical conditions. To calculate acute and chronic WLAs using this approach, one must obtain values for:

- Criterion continuous concentration (CCC) [the chronic criterion]
- Chronic, fraction of 7Q10 flow available for dilution; or as specified by state water quality standards
- Criterion maximum concentration (CMC) [the acute criterion]
- Acute, fraction of 1Q10 flow available for dilution; or as specified by state water quality standards
- Effluent flow
- Background toxicity

EPA recommends that the background value of 0 (zero) should be assumed when calculating WLAs for acute and chronic toxicity. Where background toxicity is believed to exist, the permitting authority may choose to use ambient site water as dilution water for WET compliance monitoring. This practice can be useful in capturing and accounting for background toxicity.

The steady-state mass balance equation is shown below in Box 2-2.

$$Cd = \frac{[Cr(Qd + Qs)] - [(Cs(Qs)]}{Qd}$$, where

Cr =	WET criterion in toxic units (TUs)
Cd =	waste discharge WET value in TUs; the WLA
Qd =	waste discharge flow in million gallons per day (MGD), or cubic feet per second (cfs)
Cs =	background in-stream WET value in TUs above the point of discharge
Qs =	background in-stream flow above the point of discharge in MGD or cfs

2-2 Steady-state mass balance equation

Use of this mass balance equation assumes that the discharge is through a diffuser and achieves complete mix across the width of the river or stream. [Note: This language is specific to rivers and streams; the steady state model can also be used for lakes/oceans where dilution is a default value in a state's standards (for example, 10:1), or if a dilution factor can be calculated using other steady-state models (such as PLUMES).] The steady-state mass balance equation reduces to: $Cd = Cr(Qd + Qs)/Qd$, when background toxicity is set to zero. Where mixing zones are not allowed, Cd becomes the appropriate WET criterion applied at the end-of-pipe: $Cd = Cr$.

Dynamic models use estimates of effluent variability and the variability in receiving water assimilation factors to develop WLAs in terms of concentration and variability. Where circumstances dictate dynamic models that estimate dilution or fate of pollutants are available (see TSD, Chapter 4). The use of dynamic models may be a more rigorous method for calculating WLAs; however, they require large amounts of appropriate data. If these data are not available, then dynamic models can calculate inaccurate water quality projections. **EPA recommends that dynamic models be used to derive WLAs where adequate receiving water flow and effluent concentrations are available to estimate frequency distributions.**

Statistical Approach

Because effluent quality varies, EPA recommends that the permitting authority establish permit limits using a statistical derivation procedure, in conjunction with the WLA, to adequately account for variability observed in the effluent. Using this statistical approach, WLA values are first translated into long term average (LTA) values, thus ensuring that WLAs are met under critical conditions over the long-term. For either single- or two-value steady-state WLAs, the most stringent LTA is then translated into an upper bound percentile effluent quality (e.g., 99th

and 95th), and expressed as a maximum daily limit (MDL) and average monthly limit (AML). In making these translations, one must obtain values for:

- Acute to chronic ratio
- Effluent variability (coefficient of variation)
- Number of compliance monitoring samples required per month

To assist permit writers, Region 10 has developed a spreadsheet incorporating the statistical procedures necessary to derive permits using the statistical approach. (See Appendix B, References, for information on obtaining the spreadsheet.) Maximum daily limitations (MDL) and average monthly limitations (AML) required to meet the most limiting WLA are then calculated using statistical procedures outlined in Appendix K. Chapter 5 of the TSD describes the methodology in more detail. EPA has also included tables in the TSD to help permit writers determine the necessary values (TSD, Tables 5-1 and 5-2).

In cases where the effluent receives no dilution (effluent dominated waters; EDWs), low dilution, or where mixing zones are not allowed by state water quality standards, the chronic criterion will likely be more limiting than the acute criterion (provided that the ACR is greater than 6). The chronic criterion, 1.0 TUc, means that there should be no observable effect on test organisms at 100% effluent. If the statistical approach outlined above and in Chapter 5 of the TSD is used to derive a permit limit based on a criterion of 1.0 TUc where low or no dilution is available, the method would yield an average monthly limit of less than 1.0 TUc. Because a TUc value less than 1.0 is meaningless (that is, NOEC is greater than 100% effluent), and an average monthly limit of 1.0 TUc is not amenable to state water quality standards that allow compliance based on multiple samples, 1.0 TUc should be expressed as a monthly median.[2]

EPA recommends using 2.0 TUc as the maximum daily limit (twice the monthly median). This approach is supported by the TSD in Chapter 5. The MDL could also be calculated using the statistical approach outlined in the TSD; however, where the average monthly limit (or monthly median limit) has been calculated to protect the chronic criterion, the purpose of the maximum daily limit is to ensure that there are no catastrophic single-event exceedances of the chronic criterion.

The discharger may always opt for a permit limit of 1.0 TUc as a monthly median limit and 2.0 TUc as a daily maximum limit in lieu of limits calculated using the statistical approach outlined in the TSD. For example, the permittee may prefer meeting 1.0 TUc as a monthly median rather than 1.5 TUc as a monthly average.

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[2] The "median" is the middle value in a distribution, above which and below which lie an equal number of values. For example, if the results of WET testing for a month were 1.5, 1.0, and 1.0 TUc, the median value would be 1.0 TUc.

Direct Application of the WLA Approach

Another valid approach is to apply the WLA directly as a permit limit, generally as the maximum daily limit (MDL). In the absence of additional information, permit writers typically divide the MDL by 1.5 or 2.0 to derive an average monthly limit (AML)(see TSD, page 104). The factor of 1.5 or 2.0 can be further refined once additional information is obtained.

This approach is straightforward to implement and requires minimal resources. Its primary disadvantage is that the AML must be derived without information about effluent variability and the permitting authority cannot be certain that these procedures are protective of water quality criteria. Limits derived using this approach may also be overly stringent. For example, if the chronic WLA is implemented directly as the MDL, the limit will be protective against acute and chronic effects, but at the expense of being overly stringent.

Other State Regulations

In addition to the above, a State may also have technology-based requirements for WET and/or use a modified version of the WLA approaches outlined above. The State of Washington has promulgated a regulation that specifies how WET limits are to be developed and expressed. EPA-issued permits in Washington (e.g., for federal facilities) need to consider this regulation when developing WET permit limits and conditions. The State of Hawaii also has regulations that need to be considered when developing WET permit limits.

EXPRESSION OF PERMIT LIMITS

The NPDES regulations at 40 CFR Part 122.45(d) require that all permit limits be expressed, unless impracticable, as both average monthly and maximum daily values for discharges other than technology-based limits for POTWs. The maximum daily limit (MDL) is the highest allowable discharge measured during a calendar day or 24-hour period representing a calendar day. The average weekly permit limit (AWL) is the highest allowable value for the average of daily discharges obtained over a calendar week. The average monthly (AML) permit limit is the highest allowable value for the average of daily discharges obtained over a calendar month.

Water quality-based effluent limits for WET must be consistent with State water quality standards [or otherwise as the monthly or daily values using the steady-state statistical approach, or other methods as previously discussed]. At minimum, EPA recommends that both acute and chronic limits be expressed as a monthly limit (such as a monthly average) and as a maximum daily limit. In the case of EDWs, low dilution, or where State standards do not allow mixing zones, the monthly limit should be expressed as a monthly median.

MIXING ZONES

The Regions 9 and 10 endorse the use of mixing zones for whole effluent toxicity **provided use of mixing zones is authorized in state water quality standards**. Permit limits may be adjusted based on dilution allowed under State water quality standards and regulations. If mixing zones are not allowed by State regulations for acute or chronic toxicity, then the appropriate criterion (acute or chronic), must be applied at the end of the pipe.

WET CRITERIA

National criteria for toxicity have not been promulgated. As stated earlier, Regions 9 and 10 use the CCC of 1.0 TUc and CMC of 0.3 TUa as recommended by the TSD. The State of Alaska recently promulgated a water quality standard for chronic toxicity of 1.0 TUc at the edge of a mixing zone (if a mixing zone is granted). If no mixing zone is allowed, then the 1.0 TUc must be met at end-of-pipe. The California Ocean Plan objective for chronic toxicity is 1.0 TUc at the edge of the mixing zone. The other States in Regions 9 and 10 have a narrative criterion for toxicity, that is, a criterion equivalent to "no toxics in toxic amounts."

The factor of 0.3 in the CMC is used to adjust the typical LC50 point estimate (50 percent mortality) from an acute toxicity test to an LC1 value (virtually no mortality). As discussed on page 35 of the TSD, the factor of 0.3 was found to include 91 percent of observed LC1 to LC50 ratios in 496 effluent toxicity tests. This value poses a difficulty for discharges where dilution is less than 3:1. The difficulty arises because where there is no dilution, 0.3 TUa requires measuring an LC50 of greater than 300% effluent, which is impossible. As a result, whenever there is a dilution ratio of less than approximately three parts receiving water to one part effluent, the resulting WLA will be lower than the minimum level of acute toxicity that the test can measure. For this reason, EPA makes the following recommendation: **Where less than 3:1 dilution is available, the acute WET limit should be no significant difference from the control at 100 percent effluent (a t-test), applied as a monthly median of pass-fail tests, where allowed by state water quality standards.**

The following table summarizes the WET criteria for the States in Regions 9 and 10.

TABLE 2-1. STATE WHOLE EFFLUENT TOXICITY WQC

STATE	CITATION	WET WATER QUALITY CRITERION
Alaska	18 AAC 70.023 18 AAC 70.032	The discharge shall meet 1.0 chronic toxic unit at the point of discharge, or at the edge of the mixing zone boundary, based on minimum initial dilution, if a mixing zone is approved by the State. Acute aquatic life criteria apply at and beyond the boundaries of a smaller initial zone surrounding the outfall.
Arizona	R 18-11-101, 108	"Navigable waters shall be free from pollutants in amounts or combinations that: are toxic to humans, animals, plants or other organisms."
California	CA Ocean Plan and set by individual basin plan for enclosed bays, estuaries and inland waters	Ocean Plan: The discharge shall meet 1.0 chronic toxic unit at the point of discharge, or at the edge of the mixing zone boundary, based on initial dilution, if a mixing zone is approved by the State. All waters shall be maintained free of toxic substances in concentrations that are lethal to or that produce other detrimental responses in aquatic organisms.
Hawaii	HI AR Part 11-54-04, 10	"All state waters shall be free from pollutants in concentrations which exceed the test methods listed in section 11-54-04." "All state waters shall be free from pollutants in concentrations which exceed the test methods listed in section 11-54-10."

TABLE 2-1. STATE WHOLE EFFLUENT TOXICITY WQC (cont'd)

STATE	CITATION	WET WATER QUALITY CRITERION
Idaho	IDAPA 16.01.02200, 01, 02, 03	"Hazardous Material. Surface waters of the state shall be free from hazardous materials in concentrations found to be of public health significance or to impair designated beneficial uses... "Toxic Substances. Surface waters of the state shall be free from toxic substances in concentrations that impair designated beneficial uses... "Deleterious Materials. Surface waters of the state shall be free from deleterious materials in concentrations that impair designated beneficial uses..."
Nevada	NAC 445.108, 119	"Toxic materials" means any pollutant or combination of pollutants which will, on the basis of information available to the administrator, cause an organism or its offspring to die or suffer any: disease, cancer, etc. if that pollutant or combination of pollutants is discharged, and exposed to or assimilated by the organism, whether directly from the environment or indirectly through food chains. Toxicity test methods are specified.
Oregon	set by individual basin plans; OAR 340-41-xxx (4)(b)(A)(i)--acute (4)(b)(B)(i)--chronic	"The water within the mixing zone shall be free of: Materials that will cause acute toxicity to aquatic life as measured by a Department approved bioassay method." "The water outside the boundary of the mixing zone shall be: Be free of materials in concentrations that will cause chronic (sublethal) toxicity."

STATE	CITATION	WET WATER QUALITY CRITERION
Washington	section 173-201A-040 Toxic substances	"(1) Toxic substances shall not be introduced above natural background levels in waters of the state which have the potential either singularly or cumulatively to adversely affect characteristic water uses, cause acute or chronic toxicity to the most sensitive biota dependent upon those waters, or adversely affect public health, as determined by the department."
American Samoa	Am Samoa WQS Part 24.0207(a)(4)(8)	All effluents containing materials attributable to the activities of man shall be considered harmful and not permissible until acceptable bioassay tests have shown otherwise. Toxicity test methods are specified.
Guam	Guam WQS Part II A, B.12	In order to provide maximum protection for the propagation of fish and wildlife, concentrations of toxic substances: (a) shall not exceed 5 percent of the 96 hour LC50 at any time or place, nor should the 24 hour average concentration exceed 1 percent of the LC50. Toxicity test methods are specified.
Palau	24 PNC Part 3	All waters shall be maintained free of toxic substances in concentrations that are toxic to or that produce detrimental physiological responses in human, plant, animal, or aquatic life. Toxicity test methods are specified.

SMALL SYSTEMS CONSIDERATIONS

Generally, two special factors should be considered by the permitting authority when establishing WET requirements--the permittee's previous efforts at toxics control, and the limited resources of small communities. Previous efforts at toxics control may include ongoing public information campaigns that communities have implemented, such as reminding people not to dump household hazardous waste in drains; or any source or waste minimization studies conducted by the permittee. The permitting authority should also be aware that smaller systems may not be able to afford extensive monitoring requirements. For jurisdictions with small populations, but are also

listed as "major facilities"[3] (commonly found in Alaska), EPA recommends a minimum of 4 quarterly tests. If necessary, the permitting authority may consider allowing the municipality to spread this out over four years, with the tests conducted in a different season each year. For small municipalities not designated as "majors", EPA recommends that at least one suite of tests be conducted during the permit lifetime, prior to reissuance in order to assess reasonable potential.

CHRONIC AND ACUTE TOXICITY TESTING

The first decision for a permit writer to make in selecting the appropriate toxicity tests is whether to measure acute or chronic effects, as discussed earlier in this chapter. The next question to answer is whether to test with freshwater or marine species. Once that decision has been made, the following parameters should be considered when selecting the appropriate test species: taxonomic diversity; type of facility and toxicants; and seasonal and temporal effects.

Toxicity Test Methods

Chronic Test Methods

A chronic toxicity test is defined as a long-term test in which sublethal effects, such as fertilization, growth or reproduction, are usually measured in addition to lethality. Traditionally, chronic tests are full life-cycle tests or shortened tests (approximately 30 days) known as an early life stage test. 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 effluent 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 week 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 would require extensive logistical arrangements for sampling and handling of effluent. Finally, the cost of longer chronic tests would limit the feasibility of testing programs of adequate test frequency.

As a result of such considerations, EPA has developed a suite of shorter toxicity tests (short-term chronic tests) that tend 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

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[3] Major facilities, for POTWs, are defined as facilities having design flows of greater than or equal to 1 MGD and smaller facilities exhibiting certain environmentally sensitive characteristics, including effluent toxicity. For non-POTWs, majors are defined as having a rating of 85 or more points based on an EPA classification system.

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.

Acute Tests

Acute toxicity tests are used to determine the concentration of effluent or ambient water that produces an adverse effect on a group of test organisms during either a 24, 48 or 96 hour exposure. The endpoint measured is lethality. 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.

Another aspect to consider for acute testing is whether the permittee is currently conducting a chronic toxicity test which also includes a survival endpoint, such as the *Pimephales promelas* 7-day growth and survival test. In this situation, compliance with acute and chronic requirements can be jointly evaluated; the chronic toxicity at the end of the 7-day test and acute toxicity at either 48 or 96 hours into the 7-day test. Also known as a "dual endpoint" test, this is an effective use of both time and financial resources. The marine chronic test methods that could be evaluated for both acute and chronic requirements are the topsmelt, the silverside, the Pacific mysid and the Atlantic mysid. The chronic water flea test method, *Ceriodaphnia dubia*, cannot be analyzed for both acute and chronic requirements because the test design is not amenable to calculation of a lethal concentration (LC50) value as needed for the acute requirement.

Freshwater or Marine Test Methods

The decision of whether to use freshwater or marine or estuarine test methods is based on the salinity of the receiving water. As a general rule, EPA recommends the following [TSD, Chap. 3.3.6]:

1. Freshwater organisms be used when the receiving water salinity is less than 1,000 mg/L (1‰).

2. Marine organisms be used when the receiving water salinity equals or exceeds 1,000 mg/L (1‰).

Saline Effluent Discharged to Saltwater

The dissolved salts in the effluent are possible pollutants. These salts may or may not be the same as those present in the receiving water. The 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 ambient toxicity. For this reason, **marine organisms are the preferred test species.**

Saline Effluent Discharged to Freshwater

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

Freshwater Effluent Discharged to Saltwater

The lack of dissolved salts in the effluent can cause a toxic effect in the marine toxicity test organisms. In contrast to the scenarios presented above, the toxicity test does not need to measure this effect as lack of salts is not a pollutant. The marine toxicity test methods account for this by requiring the salinity of the effluent be adjusted to approximate the salinity of the receiving water. For this reason, **marine organisms are the preferred test species.**

Effluent salinity may be lower than that tolerated by the test species (see marine test method tables). Salinity adjustment is necessary when effluent concentrations to be tested are high enough to reduce test solution salinity below the acceptable range such as 34 $\pm$ 2‰ as specified in the test method. To maintain acceptable salinity, these higher test concentrations of effluent must be adjusted by adding hypersaline brine or artificial sea salts as specified in the toxicity manual. The toxicity testing laboratory should refer to the section on hypersaline brine in the chronic marine toxicity test methods.

Sometimes, marine test species such as invertebrates and plants may not be appropriate 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 tested for tests with a test salinity requirement of 34‰ (Table 2-2). Therefore, a **freshwater organism 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, because these species can tolerate a broader salinity range from 5-36‰. These fish species can be used for freshwater discharges to saltwater at 100% effluent.

Even though the greatest differences in chemical characteristics of surface waters are those between seawater and freshwater, there are not necessarily great differences in toxicity of pollutants. Marine organisms are similar in tolerance to freshwater counterparts, when both are tested in their own environments [Aquatic Toxicology, p. 144].

TABLE 2-2 Maximum effluent concentration (%) that can be tested at 34‰ without the addition of dry salts given the indicated effluent and brine salinities.

Effluent Salinity ‰	Brine 60 ‰	Brine 70 ‰	Brine 80 ‰	Brine 90 ‰	Brine 100 ‰
0	43.33	51.43	57.50	62.22	66.00
1	44.07	52.17	58.23	62.92	66.67
2	44.83	52.94	58.97	63.64	67.35
3	45.61	53.73	59.74	64.37	68.04
4	46.43	54.55	60.53	65.12	68.75
5	47.27	55.38	61.33	65.88	69.47
10	52.00	60.00	65.71	70.00	73.33
15	57.78	65.45	70.77	74.67	77.65
20	65.00	72.00	76.67	80.00	82.50
25	74.29	80.00	83.64	86.15	88.00

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. Figure 2-1 shows the decision tree to be used when selecting species for toxicity testing.

Taxonomic Diversity

In the selection of test species, EPA recommends the use of species from ecologically diverse taxa [TSD, Chap. 3.3.3]. 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. For instance, some mysids may be more sensitive to pesticides than fish [Aquatic Toxicology, p. 129]. 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 plants.

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). 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, pesticide runoff season).

Species selection for freshwater species is straightforward, since there are only one plant, one fish and one invertebrate species from which to select (Table 2-3). However, the marine tests listed in Table 2-5 have four invertebrates from which to select. 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.

Issues to address when evaluating test results with multiple species include unacceptable test results (e.g., failed test acceptability criteria (TAC)) or two or more species demonstrate the same NOEC results. If a test fails the required TAC, the permitting authority should evaluate whether or not it is necessary for a permittee to perform an additional month of screening. For example, if the species with failed TAC is a species that has demonstrated higher NOECs with the effluent (based on prior data points) than the two species with acceptable test results, there may be limited

value in having a permittee re-screen another month. If two or more species are equally sensitive with several testing events, the type of facility, potential toxicants, and seasonal impacts should be considered when selecting the most appropriate test species for monitoring.

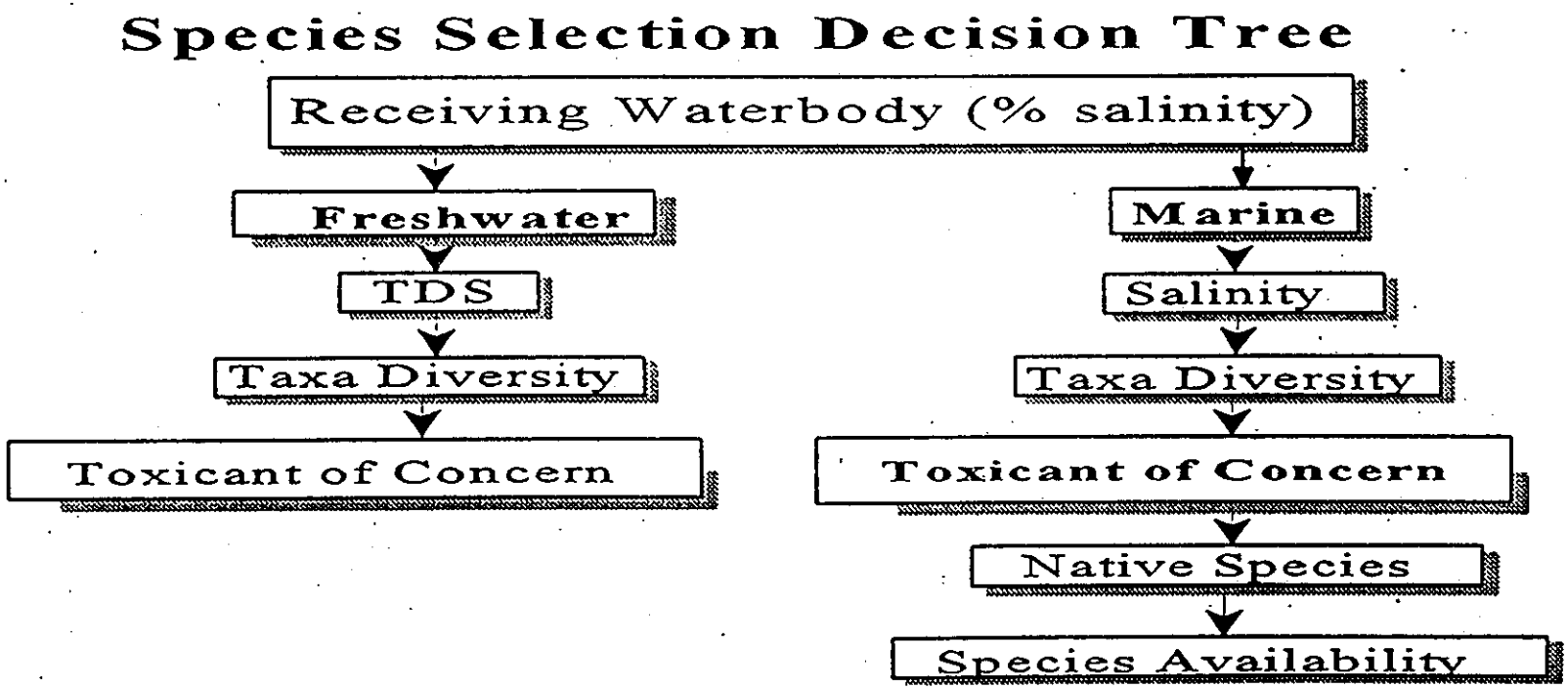

Figure 2.1 Factors to Consider When Selecting Species for Toxicity Testing

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TABLE 2-3. CHRONIC FRESHWATER TEST METHODS [Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms, EPA/600/4-91-002]

	SPECIES	TEST TYPE	TOXICANTS*	TEST ENDPOINT
Fish	Fathead minnow, *Pimephales promelas*	7-day renewal test	surfactants, ammonia	growth and survival
Invertebrate	Water flea, *Ceriodaphnia dubia*	7-day renewal test	pesticides, surfactants	reproduction and survival
Plant	Green alga, *Selenastrum capricornutum*	96-hour non-renewal	metals, herbicides	growth

* Including, but not limited to

TABLE 2-4A. CHRONIC WEST COAST MARINE TEST METHODS [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]

	SPECIES	TEST TYPE	TOXICANTS (including but not limited to)	SALINITY RANGE OF EFFLUENT DILUTIONS	TEST ENDPOINT
Fish	Topsmelt, *Atherinops affinis*	7-day renewal	ammonia	10-36‰	growth and survival
Invertebrate	Red abalone, *Haliotis rufescens*	48-hour non-renewal	metals	32-36‰	larval development
	Mussels, *Mytilus spp.*, oyster, *Crassostrea gigas*	48-hour non-renewal	metals	28-32‰	larval development
	Purple urchin, *Strongylocentrotus purpuratus* and Sand dollar, *Dendraster excentricus*	48-hour non-renewal	chlorine	32-36‰	larval development
	Purple urchin, *Strongylocentrotus purpuratus* and Sand dollar, *Dendraster excentricus*	< 1-hour non-renewal	chlorine	32-36‰	fertilization
	Mysid, *Holmesimysis costata*	7-day renewal	metals, insecticides	32-36‰	growth and survival
Plant	Giant kelp, *Macrocystis pyrifera*	48-hour non-renewal	metals, herbicides	32-36‰	germ-tube length and germination

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TABLE 2-4B. CHRONIC EAST COAST MARINE TEST METHODS [Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Marine and Estuarine Organisms, EPA/600/4-91-003]

Note: These test species and methods are only to be used when the west coast species list in Table 2- A are not available and with approval from the permitting authority.

	SPECIES	TEST TYPE	TOXICANTS*	SALINITY RANGE OF EFFLUENT DILUTIONS	TEST ENDPOINT
Fish	Inland silverside, *Menidia beryllina*	7-day renewal	surfactants, ammonia	5-36‰	growth and survival
Invertebrate	Atlantic mysid, *Mysidopsis bahia*	7-day renewal	metals	15-36‰	growth, fecundity and survival

* Including, but not limited to

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TABLE 2-5. ACUTE TEST METHODS [Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, EPA/600/4-90-027F]

	RECEIVING WATER TYPE	SPECIES	TOXICANT (including, but not limited to)	SALINITY RANGE OF EFFLUENT DILUTIONS
Fish	Freshwater	Fathead minnow, *Pimephales promelas*	ammonia	1-6‰
	Freshwater	Rainbow trout, *Oncorhynchus mykiss*	ammonia	1-2‰
	Marine	Silverside, *Menidia beryllina*	ammonia	1-36‰ Note: Can be used for end of pipe testing, if the effluent is ≥ 5‰
	Marine	Topsmelt, *Atherinops affinis*	ammonia	5-36‰ Note: Can be used for end of pipe testing, if the effluent is ≥ 5‰
Invertebrate	Freshwater	Water flea, *Ceriodaphnia dubia*	pesticides	1-3‰
	Freshwater	Water flea, *Daphnia pulex* and *Daphnia magna*	pesticides	1-6‰
	Marine	Atlantic mysid, *Mysidopsis bahia*	metals	15- 36‰
	Marine	Pacific mysid, *Holmesimysis costata*	metals, insecticides	32-36‰

NOTE: Any of these test methods can be used as either static non-renewal or static renewal tests with test durations of 24, 48, or 96 hours. Lethality is the only endpoint. In Appendix B of

the acute toxicity test methods manual, the manual contains the supplemental list of acute toxicity test species. This list specifies the test temperature, salinity for marine species and life stage to be tested. For example, the topsmelt is included as an alternate species in this list, with the temperature, salinity range, and life stage to be tested. Tests with these species should be conducted using the same protocol as for the silverside except for the parameters of test temperature, salinity and life stage.

Approved Test Methods

EPA has recently added new acute and chronic biological testing methods to the list of approved and standardized analytical methods for testing wastewater pollutants. This was published in the Federal Register as a final amendment to the 40 CFR Part 136 analytical methods. This rule became effective on November 15, 1995. For chronic toxicity testing methods, this rule only includes protocols for east coast marine and estuarine organisms.

The approved chronic methods are detailed in EPA/600/4-91/002 for freshwater and EPA/600/4-91/003 for estuarine and marine species. The approved acute methods are detailed in EPA/600/4-90/027F. These species selected by EPA for effluent testing in the NPDES program represent a "performance standard" or indicator of sensitivity to toxicity for a given phylogenetic category. They do not necessarily represent indigenous species.

EPA has stayed the effectiveness of the rule as it applies to measurements of chronic toxicity of discharges to west coast marine waters. In order to minimize disruption in the administration of existing, approved NPDES permit programs that include west coast species, permitting authorities in the west coast states may use the west coast marine species. As stated in Part 136.3, Regions 9 and 10 permitting authorities may use other approved methods for discharges to marine or estuarine waterbodies. Regions 9 and 10 may cite in NPDES permits the use of standardized west coast marine test species, instead of the east coast test species. EPA has prepared a west coast marine test methods manual [EPA/600/R-95/136] for discharges into Pacific Ocean waters.

The test methods standardized in this rule replaced unapproved test methods for NPDES permits issued after November 15, 1995, the effective date of this rule. Existing NPDES permits will not be re-opened to include test methods from this rule. However, the NPDES permittee may request that the permitting authority replace existing methods with the newly promulgated methods or the west coast chronic marine methods.

Prior to the development of the west coast method manual, many permits may include the use of two east coast species, *Mysidopsis bahia* and *Menidia beryllina*. When these permits are reissued, **EPA recommends the use of the standardized west coast marine species [EPA/600/R-95/136] for discharges in Pacific Ocean water.** For example, *Holmesimysis*

costata is recommended instead of *Mysidopsis bahia* for marine waters. Also *Atherinops affinis* is recommended instead of *Menidia beryllina* for marine waters.

During the period of transition from the use of east coast to west coast species, it may not always be possible to obtain the required test organisms. Currently, the regions are aware of several topsmelt suppliers. However, in situations when topsmelt larvae or Pacific mysid juveniles are not available, the permittee may use the species listed in Table 2-4A for that particular testing period. For example, if a permittee has a limit with *Atherinops affinis*, and there are no topsmelt larvae available from at least two different suppliers, then the permittee would test with *Menidia beryllina* with approval from the permitting authority for that particular testing event.

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 the such toxicants. For example, if a facility is discharging waste 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.

Seasonal and Temporal Effects

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

TESTING FREQUENCY

The primary reasons for WET monitoring are to:

1) determine whether or not WET limits are needed and
2) determine compliance with permit conditions and/or limitations.

2-3 Purposes for WET monitoring

Monitoring frequency is a compromise between need and cost. All toxic effects testing and exposure assessment parameters, for both individual chemicals and effluent toxicity, are associated with some degree of uncertainty. The more limited the amount of test data, the larger the statistical uncertainty. The uncertainty of an effluent's impact on receiving water quality is minimized where the following are available:

1) a complete database on the effects of acute and chronic toxicity on at least eight different indigenous species;

2) a clear understanding of ecosystem species composition and functional processes; and

3) actual measured exposure concentrations for all chemicals during seasonal changes and dilution situations.

While the uncertainty associated with such an ideal situation would be minimal, the cost to generate these data could be prohibitive to the discharger and to the permitting authority.

An example of uncertainty associated with limited monitoring data occurs when only one piece of effluent data is available (e.g., NOEC = 30%) for a facility. Effluent variability, based on the data in the TSD, could range from 20 to more than 100 percent. With only one data point available, it is impossible to determine where in this range the effluent variability really falls. To be protective, EPA recommends assuming that variability is at the high end of this range. Collection of additional data will, in most cases, result in a less conservative assumption regarding effluent variability.

Monitoring Frequency for Reasonable Potential Determinations

Tables 3-1 and 3-2 of the TSD show reasonable potential multiplying factors based on the number of samples and the effluent coefficient of variation (CV). At the default CV of 0.6 and a probability basis of 99 percent (Table 3-1 in the TSD), the multiplying factor is 13.2 with only one sample. With four samples, the factor decreases to 4.7. The fact sheet should emphasize that the more data gathered will reduce the reasonable potential factor, possibly reducing the likelihood that WET effluent limits might be needed.

Monitoring Frequency for Permit Compliance

There is no fixed guidance on establishment of monitoring frequency. As a result, the decision on the monitoring frequency is case-specific and needs to consider a number of factors, including those listed below:

- Environmental significance and nature of the pollutant or pollutant parameter
- Cost of monitoring relative to the discharger's capabilities and benefit obtained
- Compliance history
- Effluent variability
- Number of monthly samples used in developing the permit limit

EPA has observed that 10 or more samples per month provides adequate statistical likelihood that the average of monthly values will approach the true monthly long term average (LTA) value. EPA recognizes that the logistics of obtaining ten toxicity tests in a month would be difficult and extremely costly. Therefore, where WET effluent limits are required, **EPA Regions 9 And 10 recommend the following testing frequency for the first year: monthly for majors, and quarterly for minors.**

The rationale for this is that majors, given such factors as type, size and variability of the discharge, and receiving waters discharged into, 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.

In some cases, the available effluent data may not actually project an excursion above the acute or chronic toxicity criterion, but may be close to the criterion. Under these conditions, EPA recommends that toxicity tests be repeated at a minimum of quarterly for majors and annually for minors. If no reasonable potential exists for excursions above the acute or chronic toxicity criterion, EPA recommends that the toxicity tests be repeated at least once before permit reissuance, especially if there have been any significant changes at the facility. Where these recommended frequencies are not followed, the fact sheet should explain why some other frequency was proposed.

Testing frequency may be reduced based on the results of one year's worth of testing, where no previous data are available. EPA recommends that frequency be reduced if no individual toxicity test results in a value greater than the WET limit or trigger divided by the reasonable potential factor. The reasonable potential factor, from Table 3-1 of the TSD, is based on the number of samples and CV. The reasonable potential factor decreases with increased number of samples. If WET limits are required, though, the minimum monitoring frequency allowed by the regulations at 40 CFR 122.44(i)(2) is annual.

In addition, the frequency of testing may be adjusted in accordance with historical monitoring data for a particular discharge. Generally, monitoring data covering a period of two years with multiple tests for each year should be required before reducing the recommended monitoring frequencies prior to permit issuance. If the data have met TAC and data are within the permit limit or monitoring requirement, as described above, then the permitting authority may consider a less frequent testing frequency. The frequency of multiple species testing may be reduced if the effluent testing demonstrates no toxicity with multiple species testing covering potential temporal and spatial toxicants. However, if there are any facility changes which potentially alter effluent toxicity or addition of new chemicals, then the facility will have to re-screen with multiple species or demonstrate a continued lack of toxicity with these changes.

As discussed earlier under "Small Systems Considerations", the permit writer should factor in the small communities' limited resources as well as other information when establishing monitoring frequency. Finally, permitting authorities may want to reduce the effluent monitoring frequency in return for increased ambient monitoring.

SAMPLE COLLECTION

Effluent samples should be collected as either 24-hour composite or grab samples. The most frequently used sampling is the 24-hour composite. The decision on whether to collect grab or composite samples is based on the objectives of the test and an understanding of the short and long-term operations and schedules of the discharger. If the effluent quality varies considerably with time, which can occur where holding times within the treatment facility are short, grab samples may be preferable because of the ease of collection and the potential of observing peaks (spikes) in toxicity. Grab samples may need to be used for stormwater testing and power plants. However, the sampling duration of a grab sample is so short that full characterization of an effluent over a 24-hour period would require a prohibitive number of separate samples and tests. Grab samples are also appropriate where the effluent varies little with time (for instance, long holding times).

Composite samples (for example, flow-proportional or timed composites) should be collected using an iced or refrigerated collection device. Effluent samples must be maintained at 4 ± 2 °C from collection until utilized in the toxicity testing procedure. The single allowable exception is when a grab sample is collected and delivered to the performing laboratory for test initiation no later than 4 hours following the time of collection. All other samples must be received by the laboratory at a temperature at 4 °C or the sample should be considered invalid. See the Handbook for Sampling and Sample Preservation of Water and Wastewater, EPA/660/4-82/029, Table 2.5 for a discussion of the advantages and disadvantages of composite methods, as well as a discussion of sampling techniques and equipment considerations.

The maximum elapsed time between the collection of a sample and its first use is 36 hours for offsite testing . The composite sample begins at time zero when the last composite in a 24-hour composite is collected. EPA believes that 36 hours is adequate time to deliver the sample to the laboratory performing the test in most cases. In the isolated cases, where the permittee can document that this delivery time cannot be met, the permitting authority can allow an option for an extension of shipped sample holding time such as for overseas shipping. The request for a variance in sample holding time must include supporting data which show that the toxicity of the effluent sample is not reduced (e.g., because of volatilization and/or sorption of toxics on the sample container surfaces) by extending the holding time beyond 36 hours.

The sampling site should be located below the last waste treatment process, including disinfection. There may be no removal of chlorine or any other effluent constituent by either chemical or

physical methods prior to testing without approval from the permitting authority. The collection container should be filled with no headspace and closed immediately to minimize loss of volatiles.

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. These objectives are:

(1) 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, a standard laboratory dilution water as defined in each test method is used.

(2) 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.

(3) 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, dilution water collected immediately upstream or outside the influence of the outfall.

In Region 10, the United States Fish and Wildlife Service (USFWS) frequently requests that receiving water be used for dilution water. As stated above, receiving water can be an acceptable dilution water, as long as the controls meet all the TAC (TAC).

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

Freshwater Tests

The following are circumstances when using receiving water as the dilution water may not be allowed:

1. Where the toxicity tests are conducted on effluent discharges to receiving waters that are classified as intermittent streams, or where there is no receiving water available due to zero flow conditions, the permittee shall:

(a) Substitute a synthetic dilution water that has a pH, hardness, and alkalinity similar to that of the closest downstream perennial water unaffected by the discharge, or

(b) utilize the closest downstream perennial water unaffected by the discharge.

2. If the receiving water is unsatisfactory as a result of pre-existing instream toxicity (e.g., dilution controls fail the required TAC), the permittee may substitute synthetic dilution water for receiving water in all subsequent tests provided the unacceptable receiving water test meets the following stipulations:

   (a) In addition to the receiving water control, a synthetic laboratory water control was performed which fulfills the TAC;

   If the test using receiving water met the TAC, then its results are reported. If the receiving water has an unacceptable control response, then the results from the laboratory water are reported (provided these results meet TAC). A footnote to the DMR should indicate which source of dilution water was used for the reported test results.

   (b) the test indicating receiving water toxicity was carried out to completion of the test duration (e.g., 7 days);

   (c) the permittee submits all test results indicating receiving water toxicity with the testing reports.

The permittee may substitute other appropriate dilution water with chemical and physical characteristics similar to that of the receiving water upon approval by the permitting authority.

In estuarine or marine testing, a concentrated brine solution or a synthetic sea salt may be used with the dilution water to achieve the required salinity for the test method. In that case, a brine control is required.

Marine and Estuarine Tests

If the receiving water is unsatisfactory as a result of ambient toxicity (i.e., dilution controls fail the required TAC), the permittee should proceed as follows:

1. The receiving water should be re-sampled. This establishes whether an ambient toxicity problem is recurring at that site or was a one time incident. When it is demonstrated that the problem is recurring, then an alternative site may be chosen.

2. An alternative receiving water source, more remote from the discharge site, may be used.

3. If the alternative receiving water source also demonstrates ambient toxicity, the permittee may substitute laboratory seawater (filtered or reconstituted) or may substitute a known "clean site" in all subsequent tests provided the unacceptable receiving water test meets the following stipulations:

   (a) In addition to the receiving water control, a synthetic laboratory seawater control was performed which fulfills the TAC;

   If the test using receiving water met the TAC, then its results are reported. If the receiving water has an unacceptable control response, then the results from the laboratory water are reported (provided these results meet TAC). A footnote to the DMR should indicate which source of dilution water was used for the reported test results.

   (b) the test indicating receiving water toxicity was carried out to completion of the test duration (e.g., 7 days);

   (c) the permittee submits all test results indicating receiving water toxicity with the testing reports.

The permittee may substitute other appropriate dilution water with chemical and physical characteristics similar to that of the receiving water upon approval by the permitting authority.

SELECTION OF DILUTION SERIES FOR TESTING

It is important to calculate the dilution at the edge of the mixing zone in order to determine whether or not the results of the toxicity testing indicate toxicity. The instream waste concentration (IWC) is the inverse of the dilution factor.

Compliance with NOEC Endpoint

One of the five effluent treatments must be a concentration of effluent mixed with dilution water which corresponds to the facility's IWC. At least two of the effluent treatments must be of lesser effluent concentration than the IWC. No concentration should be greater than two times that of the next lower concentration.

Example:

- IWC = 45%; possible dilution series is 22.5%, 35%, 45%, 70% and 90%.
- IWC = 100%; possible dilution series is 12.5%, 25%, 50%, 75% and 100%.

2-4 Examples of dilution series

Compliance with a Point Estimate (LC/IC/ECp) Endpoint

The toxicity tests shall be performed with a minimum of five treatments and a control.

Compliance with a t-test

Toxicity tests shall be performed with the IWC and a control.

SELECTION OF TEST DURATION

The test duration for the chronic tests range from 40 minutes to 7 days. The chronic test methods specify the duration of the test, such as 48 hours for the red abalone larval development test. The acute test methods can be conducted as either 24, 48 or 96 hours in duration. If the toxicant is fast acting, then select either a 24 or 48 hour duration. These tests are usually conducted as static non-renewal tests. Non-renewal testing is important when it may be difficult to collect effluent renewals such as stormwater or overseas samples. If the mode of toxicant is unknown as is the case with most effluents, then select a 96-hour test with a renewal at 48 hours.

SELECTION OF TEST TYPE

Tests may be conducted as static (static non-renewal or static renewal) or flow-through.

1. Static non-renewal tests: The test organisms are exposed to the same test solution for the duration of the test.

2. Static renewal tests: The test organisms are exposed to a fresh test solution of the same concentration of sample every 24-hour or other prescribed interval, either by transferring the test organism from one test chamber to another, or by replacing all or a portion of solution in the test chambers.

3. Flow-through tests: (1) sample is pumped continuously from the sampling point directly to the dilutor system; or (2) grab or composite samples are collected periodically, and then placed in a tank to the dilutor system.

Static non-renewal:	red abalone larval development test
Static renewal:	topsmelt survival and growth test

2-5 Methods developed for specific test types

The chronic test methods specify whether the test is to be conducted as static non-renewal or as static renewal.

The acute test methods can be conducted as either static non-renewal, static renewal or flow through tests. See Diamond et al., 1995

for a description of a flow-through system design using larval fish. The acute test manual highlights some advantages and disadvantages of the test types to consider when determining whether to use static non-renewal, static renewal or flow through for acute toxicity testing [EPA/600/4-90/027F, p.45].

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CHAPTER 3. STATISTICS AND QUALITY ASSURANCE

OVERVIEW

This chapter is designed to provide the permit writer a background for evaluating and reviewing whole effluent toxicity (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.

STATISTICAL ENDPOINTS AND ANALYSIS

This statistical section will highlight some of the statistical discussions covered in the EPA acute [USEPA 1993] and chronic test methods [USEPA 1994a, 1994b, 1995]. The objective of a toxicity test is to estimate the highest "safe" or "no-effect concentration" of wastewaters. When a single WET test is conducted; the observed toxicological measurement 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. The no observed effect concentration (NOEC) is determined by hypothesis testing. The NOEC is the highest concentration of toxicant to which organisms are exposed in a full life-cycle or partial life-cycle (short-term) 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", but that only no statistically significant effect was observed. Point estimation 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 can be reported as the effective concentration (EC), the lethal concentration (LC), or the inhibition concentration (IC). When mortality is the measure of toxicity, LCp is used, and ECp is used to determine the toxicity measure for quantal data such as survival or fertilization. The inhibition concentration, ICp, is generally used for tests where the percent reduction is a nonquantal continuous measurement such as length, weight, or reproduction.

Chronic Statistical Analysis

The USEPA [1994a, 1994b, 1995] recommends statistical procedures for analyzing the test results. The methods allow the choice of hypothesis testing (e.g., NOEC from Dunnett's) or point-estimation techniques (e.g., ECp and confidence limits on the ECp from Probit model).

Hypothesis Testing

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

(1) the results can provide statistical information regarding test variability (e.g., minimum significant difference (MSD));
(2) the results inform the regulator of the no-observed effect level;
(3) the researcher can use the same statistical methods for many different test methods and endpoints;
(4) the researcher can test just the instream waste concentration (IWC) vs. the control (by using a standard t-test); and
(5) the researcher can use routine statistical analyses [USEPA 1993, 1994a, 1994b, 1995].

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 beta (see 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 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 lowest observed effect concentration (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 controlled and ideally the concentrations should bracket the IWC or include the IWC as one of the test concentrations. Another way to address concerns over test variability is to establish a test sensitivity criterion, such as an MSD that must be met when using hypothesis testing.

Defining false positives and false negatives. 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 treatment and the control (i.e., the effluent or ambient water is toxic). Table 3-1 presents the possible outcomes and decisions that can be reached in hypothesis testing.

Table 3-1. COMPARISON OF TYPE I AND TYPE II STATISTICAL DECISION ERRORS. 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).

DECISION:	TRUE CONDITION Treatment = Control	TRUE CONDITION Treatment > Control
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)

Note: *Table entries correspond to the probability decision given in parentheses.*

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 alpha (α), environmental researchers have ignored beta (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).

Alpha and beta are dependent on each other (as alpha increases, beta decreases), assuming that sample size (number of treatments, number of replicates), the amount of difference to detect and the variability are held fixed. Increasing alpha level of a statistical test increases the power of the test, if all other factors are held constant. Selection of the appropriate alpha level of a test is a function of the costs associated with making Type I errors. For a given alpha, beta decreases (power increases) as the sample size increases and the variance[1] 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 the decision to not reject is questioned, and the possibility exists that a false negative occurs.

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[1] Variance is the average of the squared deviations around the mean for a data set.

Although the USEPA effluent test manuals [USEPA 1993, 1994a, 1994b] require an alpha of 0.05 (5%), a level of beta is not required. If beta is not specified, then we might not detect toxicity when, in fact, an effluent is toxic. Without specifying the level of beta, there is little incentive for a testing laboratory to produce precise test results (i.e., limit test variability). Therefore, alpha and beta should be specified for each method to achieve an acceptable level of toxicity that can be detected.

Test sensitivity and minimum significant difference. To limit the degree of test variability, USEPA (1995) included an MSD criterion that must be achieved in the seven west coast test methods. The MSD is a measure of the within-test variability and represents the amount of difference from the control that can be detected statistically. A difficulty arises with the calculation of an MSD criterion for data with either non-normal distribution and or heterogenous variances. While the MSD can be estimated, it may be biased and further evaluation is necessary to determine the magnitude of the bias.

The following formula is used to calculate MSD (as recommended by USEPA 1995):

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

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 = MSD/control mean X 100). A level of test sensitivity has been used by the State of California (Anderson et al. 1990) that sets a maximum allowable mean square error term (MSE) for each test method. A limitation of the MSE is that it only reflects test variability. The MSD, though, incorporates alpha (α) and number of experimental units, 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 per 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.

Hypothesis testing procedures. Hypothesis testing procedures, such as the Dunnett test are used to determine the NOEC (see Figure 3.1).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 are 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 (normally or homogeneity of variance) fail, 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 are not equal, then Wilcoxon Rank Sum Test with Bonferroni's adjustment should be used.

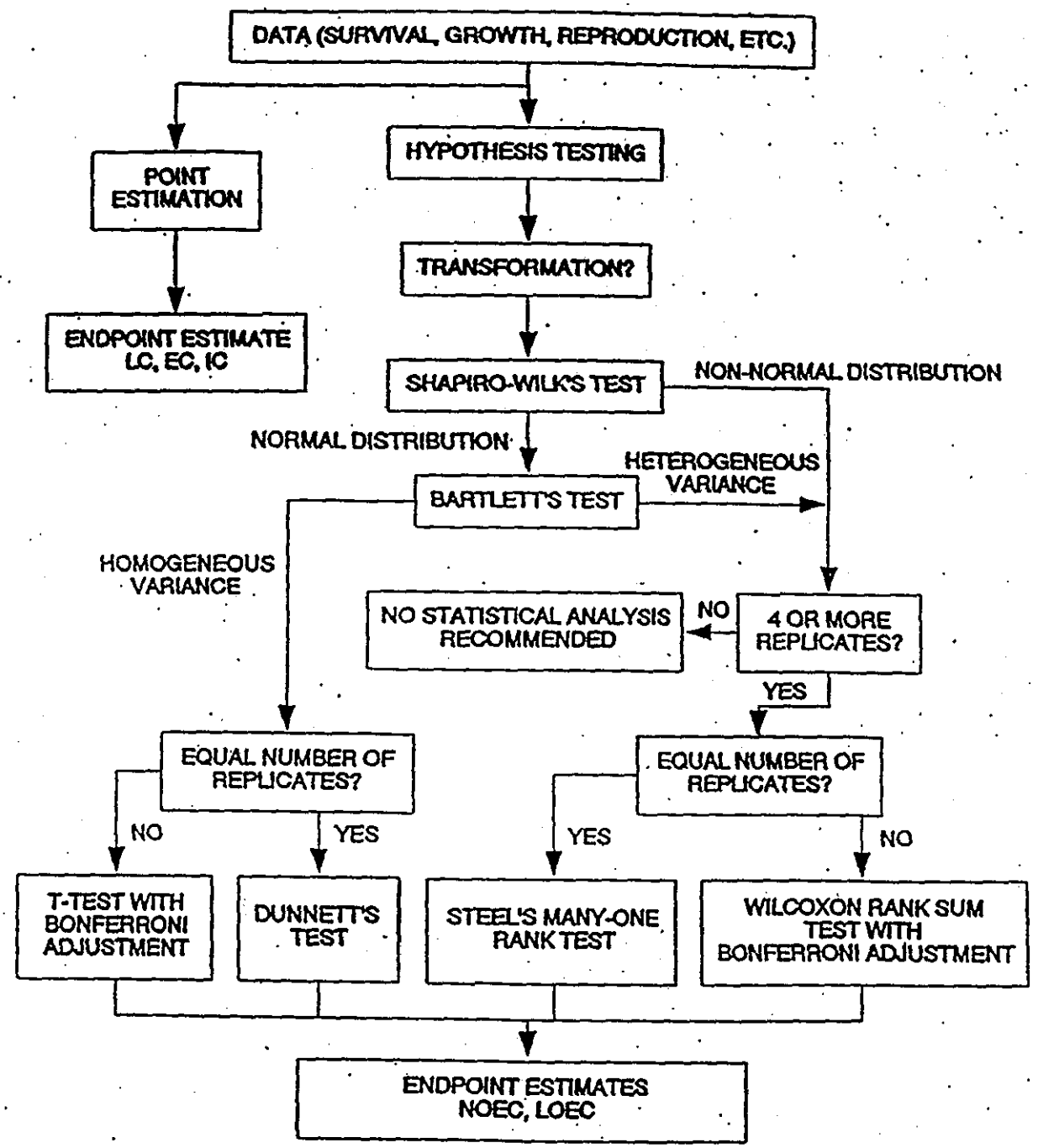

Figure 3.1 Flowchart for statistical analysis of test data.

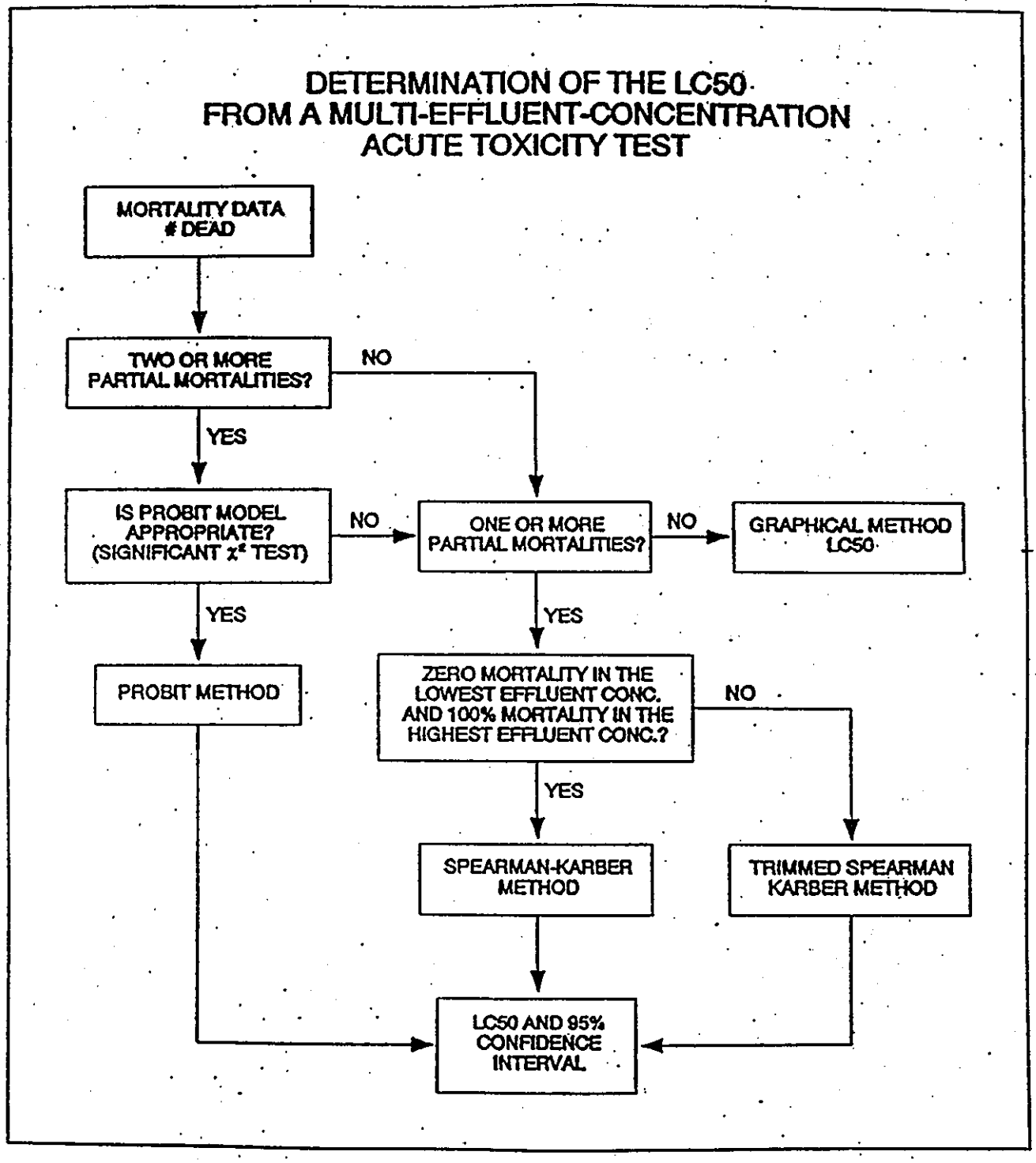

Figure 3.2 Flowchart for determination of the LC50 for multi-effluent-concentration acute toxicity tests.

If in the calculation of a NOEC, two tested concentrations caused statistically significant adverse effects, but an intermediate concentration did not cause statistically significant effects, the **results should be used with extreme caution**. For example: 6.25, 12.5, 25, 50 and 100% effluent concentrations are tested using hypothesis testing. The 12.5 and 50% concentrations are statistically significant (LOECs) but 25% is not significant. **The Regions recommend that the test should be repeated or the NOEC is the lowest no observed effect concentration (i.e., NOEC = 6.25%).**

Point Estimate Techniques

Most point estimate endpoints, such as the LC, EC, or IC are derived from a mathematical model that assumes a continuous dose-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.

Point estimation methods have many benefits different from hypothesis testing. These types of methods can:

(1) use all information from a dose-response relationship;
(2) minimize the importance of the effects at the IWC;
(3) quantify the precision between and among testing laboratories;
(4) confidence intervals can be obtained; and
(5) avoid having power of the test be as dependent on experimental design as is the case with hypothesis testing.

As with hypothesis testing, point estimation techniques also have some criticisms. They include:

(1) The point-estimate is model dependent, especially for small levels of p in ECp.
(2) The data from a single toxicity test might give very little information as to which model is appropriate.
(3) The appropriate model might vary with effluent sample, species, concentrations tested, the amount of toxicity present and the type of dilution water used (Fulk et al., 1993).

For simple linear curve fitting models for point estimation, typical data can depart from the models for several reasons. A hormesis like-effect can occur where the response is greater at the higher concentration than the control. Nonsymmetry can occur where the slope up to the 50%

effect level is less/more steep than at the higher concentrations. In addition, a no-dose response[2] or an extremely irregular data set (Noppert et al., 1994) can occur.

The primary question in applying the point estimation techniques has been what effect level (e.g., ECp) should be reported for compliance purposes? In 1991, the USEPA evaluated existing data for two freshwater test species methods, *C. dubia* and *P. promelas* and three east coast marine test methods, *Arbacia punctulata*, *Cyprinodon variegatus*, and *C. parvula*. In the comparisons of both types of data, EPA indicated that an NOEC derived using the IC25 is approximately the analogue of an NOEC derived using hypothesis testing [USEPA 1991].

With the development of the standardized west coast marine toxicity test methods, an evaluation was conducted to evaluate what "p" value is approximately equivalent to the NOEC. Quantal endpoints (e.g., survival and fertilization) were determined using the USEPA Probit Model [USEPA 1993] and nonquantal endpoints (e.g., weight, length, number of offspring) were determined using linear interpolation with the ICPIN program [USEPA 1995]. Quantal endpoints were estimated by interpolation, using the slope and intercept from the Probit model to generate the point estimation corresponding to the NOEC. The nonquantal endpoint estimates were grouped categorically (e.g., IC values 0 to < 5%, 5 to < 10%, 10 to < 15% and 15 to < 20%) and then compared to the corresponding NOEC value. For all the test methods analyzed, the approximate "p" value was below an EC25 [Denton et al., 1994]. A substantial number of the dose-response curves did not fit the Probit model (e.g., significant lack of fit). It is not desirable to use different ECp's for every test guideline, but if necessary, then the rationale for doing this must be succinct and defensible.

In order to adopt the ECp approach, dose-response models are needed, and the value of p should be selected so that the ECp estimate is not too model dependent. The ECp approach is advantageous because the ECp value is not restricted to being a test concentration, the precision can be quantified, the ECp values are comparable, confidence intervals may be calculated, and the acute and longer term studies use the same basic approach for data analysis. However, as difficulties arise when choosing a model, the confidence intervals may be very wide for low or high percentages. The use of the ECp in place of the NOEC requires the value of p to be specified and the selection of the p value may be arbitrary.

Point estimate models. The statistical models are highlighted in the EPA test method manuals flowchart (see figure 3.2). 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, or larval development). The analysis consists of adjusting the data for mortality in

[2] Almost no effect, even at the highest concentration.

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.

The assumption upon which the use of Probit analysis is contingent is 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 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.

In cases where Probit analysis is not appropriate, the LC50 may be estimated by Spearman-Karber method or the trimmed Spearman-Karber for acute toxicity only. If a test results in 100% survival and 100% mortality in adjacent treatments (all or nothing effect), the LC50 may be estimated using the Graphical method. For chronic toxicity endpoints the Linear Interpolation method should be used when Probit analysis is not appropriate, since the effect needed to be observed is less than a 25 percent 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., ≤ 25 percent effect) in continuous endpoints (e.g., reproduction or growth). Use of the Linear Interpolation method is based on the assumptions that 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)
- follow a piece-wise linear response function, and
- are from a random, independent, and representative sample of test data.

The assumption for piece-wise linear response cannot be tested statistically, and no defined statistical procedure is provided to test the assumption for monotonicity. Where the observed means are not strictly monotonic by examination, they are adjusted by smoothing. In cases where the responses at the low toxicant concentrations are much higher than in the controls, the smoothing process may result in a large upward adjustment in the control mean.

Acute Statistical Analysis

Hypothesis Testing

The two hypothesis testing statistical endpoints are either the no observed adverse effect concentration (NOAEC) for multi-concentration tests and the t-test (pass or fail) for single-concentration tests. The NOAEC is the lowest concentration at which survival is not significantly different from the control. In the pass/fail tests, the objective is to determine if the survival in the single treatment (effluent or receiving water or a combination) is significantly different from the control survival.

NOAEC endpoint. The assumptions when using ANOVA are that the data are distributed normally as tested by Shapiro-Wilk's Test and that the group variances are homogenous as tested by Bartlett's Test. The first step in these analyses is to transform the responses, expressed as the proportion surviving, by the arc-sine-square-root transformation. This transformation is commonly used on proportionality data to stabilize the variance and satisfy the normality requirement. In cases where the number of replicates for each concentration are not equal, a test may be performed with Bonferroni's adjustment for multiple comparisons, instead of using Dunnett's procedure. If either of the statistical assumptions (nornality or equal variances) fail, then the Steel's Many-one Rank Test should be used if there are four replicates per test concentration. If the number of replicates are not equal, then Wilcoxon Rank Sum Test with Bonferroni's adjustment should be used.

If in the calculation of a NOAEC, two tested concentrations caused statistically significant adverse effects, but an intermediate concentration did not cause statistically significant effects, the **results should be used with extreme caution**. For example: 6.25, 12.5, 25, 50 and 100% effluent concentrations are tested using hypothesis testing. The 12.5 and 50% concentrations are statistically significant (LOECs), but 25% is not significant. The Regions guidance is that the test should be repeated or the NOAEC is the lowest no observed effect concentration (i.e., NOEC = 6.25%).

Single concentration endpoint. After the data have been transformed, test the assumption of normality 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% concentration is significantly less than the survival in the control.

Point Estimate Techniques

The method used to estimate the LC50 from multi-concentration acute toxicity tests depends on the shape of the tolerance distribution and how well the effluent concentrations chosen characterize the cumulative distribution function for the tolerance distribution (i.e., the number of partial mortalities). The four statistical methods for estimating the LC50 are the Graphical Method, the Spearman-Karber Method, the Trimmed Spearman-Karber Method, and the Probit Method. The acute test methods manual [USEPA 1993] provides a description of the calculations involved for each method and an example of the calculations.

EPA Regions 9 and 10 recommend the statistical endpoint of LC50 be calculated with point estimate techniques or the statistical endpoint of pass/fail test calculated with a t-test.

Evaluation of Toxicity Data

Chronic Toxicity Data

1. Examine the test results to verify that the laboratory is using the test method and dilution series as required in the NPDES permit. Note: This may only need to be performed after a permit has been first issued.

2. Evaluate the test results to verify that the laboratory met the test acceptability criteria (TAC) as specified in the test method.

   Example: A laboratory conducts the chronic reproduction and survival water flea, *Ceriodaphnia dubia* test. The following criteria must be achieved for both the reference toxicant and effluent test:

   a) Survival in the controls must be at least 80%;
   b) Reproduction in the controls must average 15 or more young per surviving female;
   c) The laboratory must report the MSD value.

3. Examine the chemical and physical parameters of the test:

   a) Minimum and maximum pH, temperature and dissolved oxygen for the test. Note: The test method specifies that the temperature should be ~~20~~ 25 ± 1 °C. The data reviewer should evaluate these parameters on best professional judgement. For example, the test met the required TAC, and the data demonstrates a normal dose response curve, but the temperature minimum was ~~18.5~~ 23.5 °C and maximum was ~~20.0~~ 25.0 °C. This should be an acceptable test result.

4. Examine the statistical results to verify the following:

   a) Did the laboratory use the correct statistical programs (see Appendix B, [USEPA 1994a, 1994b, 1995])?

   b) Did the laboratory perform the necessary number of replicates?

   c) Do the data indicate a good dose response curve? Note: Reference toxicant tests should have good dose response curves, but this may not be the case with effluent tests.

5. Calculate the TUc and compare with permit limit.

   NOEC = 50% effluent

   TUc = 100/50 = 2.0 TUc

Acute Toxicity Data

1. Examine the test results to verify that the laboratory is using the test method and dilution series required in the NPDES permit. Note: This may only need to be performed after a permit has been first issued.

2. Evaluate the test results to verify that the laboratory met the TAC as specified in the test method.

   The only TAC for all acute test methods is the following for both the reference toxicant and the effluent test:

   a) Survival in the controls must be at least 90%.

3. Examine the chemical and physical parameters of the test:

   a) Minimum and maximum pH, temperature and dissolved oxygen for the test. Note: The test method specifies that the temperature should be 20 ± 1 °C. The data reviewer should evaluate these parameter on a best professional judgement. For example, the test met the required TAC, and the data demonstrates a normal dose response curve, but the temperature minimum was ~~23.5~~ °C and maximum was ~~25~~.0 °C. This should be an acceptable test result.

4. Examine the statistical results to verify the following:

   a) Did the laboratory use the correct statistical programs (see Appendix B, [USEPA 1993])?

   b) Did the laboratory perform the necessary number of replicates?

   c) Do the data indicate a good dose response curve? Note: Reference toxicant tests should have good dose response curves, but this may not be the case with effluent tests.

5. Calculate the TUa and compare with permit limit.

   LC50 = 67% effluent

   TUa = 100/67 = 1.49 TUa

QUALITY ASSURANCE (QA) PROCEDURES

This quality assurance (QA) section will only highlight the general discussions from the testing manuals, such as the use of reference toxicants, dose response curves and test acceptability criteria. 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:

(1) Appoint a QA officer with the responsibility and authority to develop and maintain a QA program;
(2) Prepare a quality assurance plan with stated data quality objectives;
(3) 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
(4) Provide an adequate, qualified technical staff for culturing and toxicity testing the 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:

(1) effluent sampling and handling
(2) source and condition of the test organisms
(3) condition of equipment
(4) test conditions
(5) instrument calibration
(6) replication
(7) use of reference toxicants
(8) record keeping
(9) data evaluation

Additional QA requirements have been developed to provide further guidance for consistency among testing laboratories. The chronic marine west coast methods [USEPA 1995] require a specific reference toxicant and test concentrations for each test method. This level of detail was encouraged by the regulated community. This guidance can be helpful for many reasons, including ease of comparison of control charts and quantifying precision among laboratories when using a uniform reference toxicant. These types of statistical and QA issues have evolved from discussions with the Southern California Toxicity Assessment Group (SCTAG). SCTAG is composed of dischargers, consulting laboratories, academia and government scientists and managers that meet to discuss and resolve technical aspects of the WET program (e.g., guidance on selection of reference toxicants and statistical applications). We believe these types of forums are important to ensure a successful WET program.

Reference Toxicants

Reference toxicant tests indicate the sensitivity of the test organisms being used and demonstrate a laboratory's ability to obtain consistent results with the method. It is the laboratory's responsibility to demonstrate its ability to obtain consistent, precise results with reference toxicants before it performs toxicity tests with effluents for permit compliance purposes. To meet this requirement, the intra laboratory precision, expressed as percent coefficient of variation (CV = standard deviation/mean x 100) should be determined by performing five or more tests with different batches of test organisms, using the same reference toxicant, at the same concentrations under the same test conditions (i.e., the same test duration, type of dilution water, age of test organisms, feeding, etc.), and the same statistical analysis.

When to conduct reference toxicant tests

1. If the laboratory obtains the test organisms from an outside source (e.g., organism supplier) then a reference toxicant test must be conducted concurrently with the effluent test to determine the sensitivity of the test organisms.

2. If the laboratory maintains in-house cultures, a reference toxicant test must be conducted at least once a month. It is preferred, that this reference toxicant test be performed concurrently with an effluent toxicity test. However, if a given species of test organism produced by in-house cultures is used only monthly, or less frequently in toxicity tests, a reference toxicant must be performed concurrently with each short-term chronic effluent toxicity test.

Which reference toxicant to use

The test methods for chronic freshwater organisms [USEPA 1994a, 1994a] and chronic marine east coast organisms [USEPA 1994b], and the acute freshwater and marine organisms [USEPA 1993] do not specify a particular reference toxicant and dilution series. There are currently several possible reference toxicants recommended for testing such as sodium dodecyl sulfate (SDS), copper sulfate ($CuSO_4$), sodium chloride (NaCl), potassium chloride (KCl_2), or cadmium chloride ($CdCl_2$). Standard reference toxicants can be obtained from a commercial supply company, or can be prepared in-house using reagent grade chemicals. Reference toxicant information is currently under evaluation to determine acceptable reference toxicants and dilution series for these test methods.

However, the test methods for chronic west coast marine organisms [USEPA 1995] do specify a particular reference toxicant and dilution series, such as the red abalone larval development test method requires zinc sulfate to be tested at 10, 18, 32, 56 and 100 µg/L.

Test Precision

Precision is a measure of test consistency or repeatability both within a laboratory (intra laboratory) and among several laboratories (interlaboratory). Precision is quantified by a variety of measures including the coefficient of variation (CV = standard deviation/mean x 100) 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.

The USEPA Technical Support Document (TSD) [USEPA 1991] 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) discuss 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 coefficients of variation (CV) were obtained in their study was because the method was clearly defined and uniformly followed by all laboratories. More recently, Anderson (1991) and 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 rufescans*, 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. Examples of how to improve these include using a well-defined test method [USEPA 1993, USEPA 1994a, 1994b, USEPA 1995], controlling test sensitivity (e.g., MSD) and maintaining communication with the regulated community regarding test method details, data analysis, and interpretation of test results. For example, one area of inconsistency arises when laboratories analyze the data for the chronic *Ceriodaphnia dubia* test when males are identified as present. When males are produced, this is often a sign of a stressed culture, but if the percent of males is less than 5%, the data might be informative. Yet problems arise when the data is analyzed and some laboratories include males in the calculation of survival while other laboratories do not. Incorporation of the data into the survival estimate or excluding it may drastically alter the results reported. Another problem area is the lack of consistency in use of the statistical programs. The proliferation of statistical packages has been helpful in data analysis, however, they have also resulted in misapplication of the methods and in many instances, additional statistical tests have been added which can easily lead to confusion on the part of the users. These are but a few of examples of where frequent and open communication with the testing community to resolve issues is essential.

Variability in Toxicity Test Results

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 manual) 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 toxicant test concentration

Factors which can affect test success and precision include:

(1) the experience and skill of the laboratory analyst;
(2) test organism condition and sensitivity;
(3) dilution water quality;

(4) chemical and physical water quality parameters (e.g., temperature, DO); and
(5) quality and quantity of food provided.

These are also some additional factors to consider for the possible differences in test results:

(1) Effluent variability is caused by changes in the composition of the effluent. Virtually all effluents vary in chemical composition and concentration over time.

(2) Exposure variability is caused by changes in flow rates of both effluent and receiving water. There are variable receiving water parameters that may be independent of flow, such as background toxicant levels, pH, salinity, tides, suspended solids, hardness, dissolved oxygen and temperature, that can be important in assessing impact.

(3) Species sensitivity differences are caused by the difference in response to toxicants between species. For example, the water flea, *Ceriodaphnia dubia*, is more sensitive to pesticides (e.g., diazinon) than fathead minnows or the green alga, *Selanastrum capricornutum*.

Dose Response Curves

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 the greater the biological response is measured (e.g., increase in lethality, growth or reproduction).

However, it is common for the lowest concentration to sometimes demonstrate an effect that is greater than the control. The apparent enhancement of a physiological process by low toxicant doses is well known in pharmacology and toxicology [Laughlin et. al., 1981]. This is referred to as hormesis, mechanistically, it has been attributed to transient overcorrections by control mechanisms to inhibitory challenges well within its capacity to counteract [A.D. Stebbing, 1979].

Test Acceptability Criteria (TAC)

Test acceptability criteria set minimum requirements for performing toxicity tests. These minimum requirements are clearly identified in the toxicity testing methods. Both effluent and reference toxicant tests must meet these TAC. As 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.

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).

Additional Testing Requirements

EPA Regions 9 and 10 recommend that the following additional QA requirements be implemented to enhance the current QA procedures:

1) A requirement that a minimum of four replicates be required for the chronic toxicity test methods, unless the method cites a number of replicates higher than four. This is necessary in order to perform non-parametric statistics when conducting hypothesis testing.

2) A requirement that laboratories must calculate and report the Minimum Significant Difference (MSD) for the reference toxicant regardless of whether the compliance endpoint is based on hypothesis testing or point estimates.

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CHAPTER 4. TOXICITY REDUCTION EVALUATIONS

OVERVIEW

Where monitoring indicates unacceptable effluent toxicity (i.e., effluent toxicity exceeds the whole effluent toxicity (WET) limit or some other trigger), the principal mechanism for bringing a discharger into compliance with a water quality-based WET requirement is a toxicity reduction evaluation (TRE). The purpose of a TRE is to investigate the causes of and to identify corrective actions for difficult effluent toxicity problems. The first step is to define clearly and understand the objectives of the TRE and to establish appropriate intermediate goals. The TRE's objectives should be specified in the permit, in applicable State regulations, and where necessary, in the administrative letter requiring submittal of the study plan.

A TRE is a site-specific study conducted in a stepwise process to narrow the search for effective control measures for effluent toxicity. TREs are designed to identify the causative agents of WET, evaluate the effectiveness of the toxicity control options, and then confirm the reduction in effluent toxicity. Ultimately, the object of a TRE is to have the discharger achieve compliance with the limits or other permit requirements for WET contained in the permit, thus attaining and maintaining compliance with water quality standards. TREs can vary widely in complexity, ranging from simply changing housekeeping procedures to conducting toxicity identification evaluations (TIEs). Figure 4.1 is a flowchart showing Tiers I and II of the TRE process.

EPA has published guidance documents for conducting TREs and TIEs (which can be part of a TRE, as explained below). A list of those documents can be found at the end of this chapter. The documents recommend, for successful completion of TREs, that a systematic, stepwise approach that eliminates possible causes or sources of toxicity be used until a solution or control method is determined. While TREs and TIEs are generally site-specific and the TRE's details can only be determined once it has been triggered, generic TRE plans can be made ahead of time. Where the permitting authority includes a TRE provision in the permit, EPA recommends that the discharger be required to submit, within 60 to 90 days of the effective date of the permit, a plan for responding to noncompliance with the WET limit or permit requirement. An implementation schedule should also be developed if noncompliance occurs.

EPA recommends that the permitting authority only approve the implementation schedule, rather than stating its approval or disapproval of the plan itself. Furthermore, EPA recommends that the permitting authority only review and comment on the plan itself. If the permitting authority approves the plan, there is the possibility that the discharger may believe that if the plan is not successful, no more effort is required by the discharger to come into compliance with the WET limit or permit conditions. Many of the elements discussed below parallel best management practices (BMP) plan and stormwater requirements. To prevent duplication of effort, evidence of complying with those requirements may be sufficient to comply with TRE requirements.

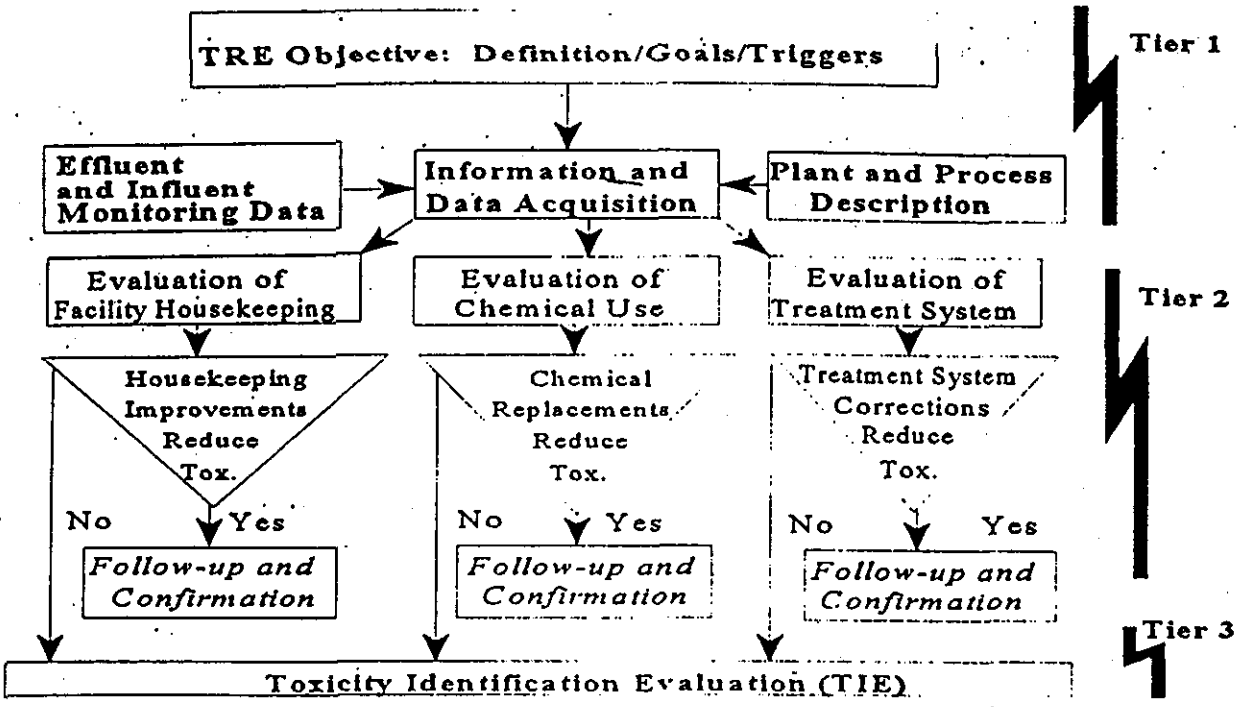

Figure 4.1 **Toxicity Reduction Evaluation (TRE) Flowchart**

Because the TRE workplan is required prior to any actual exceedances of the WET limits or criteria, the final TRE plans will be variable and site specific. An acceptable final plan should be comprehensive and cover all the work which might need to be performed to complete a successful TRE. Some TRE plans have been developed to focus upon a suspected toxicant when the actual toxicant had not been confirmed. To the extent possible, the plan should also completely describe the work that will be performed if the suspected toxicant is not confirmed.

The approaches or methods to be used should be described to the extent possible prior to reaching the decision points without the data and results that will be collected in the initial steps of a TRE. All proposed actions should be thoroughly justified and the rationale for the proposed course of action must be presented.

Also, in some cases, the results of initial TRE tiers could alter the proposed work. The initial plan must contain assurances that appropriate detailed proposals will be developed as necessary. Where possible, any notice of proposed work should be incorporated into the quarterly progress reports.

Reasonable time should be allowed for each aspect of the study. Proposed time frames for completion of each phase should be clearly presented and justified (to the extent possible in the

initial workplan). The final TRE report, progress reports, subsequent proposals and meetings with the permitting authority should be included as part of the schedule. The plan should also specify the information and data that will be included in progress reports and the final report.

EPA recommends a generalized process, consisting of six tiers, for performing a TRE. Tier I includes the acquisition of available data and facility specific information. The available information can usually be divided into three categories: regulatory information, effluent and influent monitoring data, and facility information.

Tier II evaluates general housekeeping, optimization of treatment plant operation, and the selection and use of process and treatment chemicals as a means of reducing final effluent toxicity. If the efforts of Tiers I and II do not reduce effluent toxicity to acceptable levels, then Tier III, a TIE is initiated. The objective of the TIE is to characterize and identify the cause(s) of final effluent toxicity.

Following successful identification or characterization of the toxicant(s), the TRE process can proceed in either of two directions. One approach is to evaluate options for treating the final effluent (Tier IV). The other approach is to identify the source(s) of the toxicant(s) and evaluate within plant options or modifications (Tier V). The two approaches can be pursued simultaneously in some cases. If they are, then the most technologically and/or economically attractive option may be selected.

Tier VI consists of follow-up and confirmation. This step occurs after the toxicity control method has been selected and implemented. It must be designed such that it will assure that the objectives of the TRE have been achieved and that they are maintained over time.

The **Technical Support Document for Water Quality-based Toxics Control** (EPA/505/2-90-001, PB91-127415, March 1991) (TSD) recommends that in cases where toxicity is repeatedly or periodically present above effluent limits (or other trigger levels) more than 20 percent of the time, a TRE should be required. In order to determine if effluent toxicity is in fact repeated or periodic, EPA Regions 9 and 10 require accelerated testing, consisting of 6 tests to be conducted during the following 12 weeks, after the first exceedance of a permit requirement. Regions 9 and 10 consider this accelerated testing to be the first step of the TRE. If any of the tests during the accelerated testing period show toxicity as defined by the permitting authority, then the TIE requirement is triggered. This scenario is comparable to the recommendation in the TSD, since 20 percent of 7 tests (the first one and then the 6 accelerated tests) is 1.4 tests. Therefore, two tests indicating toxicity comprise more than 20 percent of the time. The TSD, in recommending that a TRE be triggered, anticipates that all six tiers of the TRE process will commence. By requiring the first steps of the TRE to be accelerated testing and review of the facility's TRE workplan, a TRE may be ended in its early stages.

INFORMATION AND DATA ACQUISITION

As recommended by EPA's guidance documents, the starting place for investigations of toxicity sources and reduction is a thorough information-gathering phase. This is the stage where preliminary issues should be investigated and information evaluated for potential sources or causes of toxicity. In most cases, this can be done prior to the time when toxicity has been indicated (i.e., by exceedance of the toxicity limits or triggers during accelerated testing) and should be the major component of the TRE workplan submitted soon after the effective date of the permit. Table 4.1 shows the information needed for this tier.

The importance of this initial information-gathering phase cannot be exaggerated, either in terms of the TRE's outcome or of the efficiency with which the outcome of the TRE is produced. In certain instances, it is likely that sources of toxicity can be targeted or eliminated by simple calculation rather than by further testing, thus greatly reducing the cost of and time for the investigation. This information-gathering phase may be conducted by the permittee prior to contact with any paid consultants (which will further reduce the costs when consultants are hired) and before any actual testing takes place. 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.

TABLE 4.1. TYPES OF INFORMATION NEEDED FOR TIER I

Type of Facility	Information Needed for Tier I
Municipal POTWs	NPDES permit Treatment system design criteria, flow diagrams, descriptions of treatment elements Influent and effluent flow data Effluent toxicity data and trends Process control and operational data and histories In-plant chemical usage (e.g., polymers, coagulants, chlorine, sodium bisulfite) Pretreatment information (where applicable) Industrial waste surveys Industrial user self-monitoring reports Industrial user operational schedules and flow patterns Waste hauler monitoring and manifests Hazardous waste inventories
Industrial wastewater treatment plants (WWTP)	NPDES permit Process and wastewater generating process diagrams and descriptions Diagrams and descriptions of non-process wastewater sources (e.g., cooling towers, boilers, floor drains) In-plant flow records and water usage Chemical inventories and usage records Chemical labels, MSDS, and toxicity information Operating schedules with emphasis on how these schedules affect wastewater flow/composition WWTP operational data and histories Wastewater monitoring records (chemical and toxicity).

Industrial facilities should identify and regulate all possible contributions to the wastewater systems, including floor drains. Unwanted materials may have been added to the system without the wastewater staff's knowledge. In addition to the obvious process waste streams, side streams such as cooling tower discharge, boiler blowdown, or airwash discharges should also be reviewed for the presence of toxic chemicals. Additional useful aquatic toxicity information is available for some of the commonly used biocidal compounds used in treatments of, for example, cooling water discharges. Many MSDSs (material safety data sheets) now include toxicity data using daphnids or fathead minnows, such as are used for compliance testing, instead of data gathered using bluegills. MSDS data using daphnids instead of blue gills are more appropriate since compliance with WET limits and conditions is generally determined using a more sensitive species than bluegills.

POTWs should investigate the toxicity of added treatment chemicals and should attempt to correlate effluent toxicity and use records of such chemicals. North Carolina's Department of Environment, Health and Natural Resources (DEHNR) has found the two most common causes of effluent toxicity to be chlorine and ammonia.

GOOD HOUSEKEEPING/BEST MANAGEMENT PRACTICES (BMPs)

Waste treatment efficiency must be maximized in order that it does not become another variable in the TRE process. The objective of this step is to identify plant practices and operations which may directly or indirectly affect effluent quality. The effort required to perform the housekeeping evaluation will vary among facilities.

TREATMENT PLANT OPTIMIZATION

After information gathering, emphasis should be placed on maximizing in-house treatment efficiency and assuring that housekeeping practices are not contributing unnecessarily to final effluent toxicity. The objective of this stage is to assure that the existing wastewater treatment system is operating in optimal fashion with respect to its design parameters. This will maximize the probability that toxicity will be removed. In some cases, the plant was not designed to remove the constituents causing toxicity. It is important to document the plant design information.

This description should include the specific treatment units and how they are linked, design capacity and loading rates, and what the plant was intended to treat. In the study plan, specific sources of information or methods for obtaining the information should be identified. Details of the analysis procedure and design performance criteria should be in the plan. Methods for identification and implementation of corrective actions, if needed, should also be discussed in as much detail as possible. Should corrective actions be implemented, a follow-up and confirmation study would need to be performed.

CHEMICAL OPTIMIZATION

Chemical optimization is a process that can be performed in conjunction with the housekeeping and treatment plant optimization parts of the TRE. The goal of this process is to identify simple solutions to toxicity problems by evaluating and possibly modifying chemical use at the facility. For POTWs, excess variation in chlorination and over-chlorination should be high on the list as potential toxicity problems. One POTW in California found that they had been over-dosing sodium bisulfite in their dechlorination program.

EPA's TSD includes a list of evaluation criteria for TRE plans that could be helpful in designing or evaluating a plan submitted by a consultant. The list is as follows:

- Are the objectives or targets of the TRE stated clearly and accurately?
- Are the final TRE report, progress reports, and meetings with the regulatory authority included as part of the schedule?
- Are the approaches or methods to be used described to the extent possible prior to beginning the TRE?
- Has available EPA guidance been used in designing the TRE and developing the TRE plan (or if other methods are proposed, have they been sufficiently documented?)
- Does the TRE plan specify what results and data are to be included in the interim and final reports?
- Does the TRE plan provide for arrangements for any inspections or visits to the facility or laboratory that are determined to be necessary by the permitting authority?
- Are the toxicity test methods and endpoints to be used described or referenced?
- Does the approach described build on previous results and proceed by narrowing down the possibilities in a logical progression?
- Does the plan provide for all test results to be analyzed and used to focus on the most effective approach for any subsequent source investigations, treatability studies, and control evaluations?
- Are optimization of existing plant/treatment operations and spill control programs part of the initial steps of the TRE?
- Does the TRE plan allow a sufficient amount of time and appropriate level of effort for each of the components of the study plan?
- Does the TIE use broad characterization steps and consider quantitative effluent variability?
- Is toxicity tracked with aquatic organism toxicity tests throughout the analyses?
- Is the choice of tests for the TRE logical and will correlations be conducted if the species used are different from those used for biomonitoring?

-- Is the laboratory analytical capability and the expertise of the investigator broad enough to conduct the various components of the evaluation?

In summary, the overall goal of a TRE is to reduce or eliminate the observed toxicity in an effluent. At the same time, the permitting authority should encourage the use of the most efficient means of attaining this goal, so that unnecessary tests and costs are not incurred. Requiring the plan to be developed prior to finding unacceptable toxicity will help both the permittee and the permitting authority.

TOXICITY IDENTIFICATION EVALUATIONS (TIEs)

Overview

In the suggested permit language included as Appendix A, accelerated monitoring is initiated upon exceedance of the WET limit (such as a daily maximum, monthly average, or monthly median) or other trigger. If implementation of the generic workplan locates the source of the toxicity (for example, a plant upset), then only one further test, to show that the toxicity is gone, is necessary. Otherwise, the accelerated monitoring program must continue. **EPA Regions 9 and 10 recommend that six bi-weekly tests be conducted over twelve weeks.** These follow-up tests are not used to confirm toxicity, but to establish the presence of consistent toxicity. If toxicity is detected in any of the follow-up WET tests, then the facility must begin a toxicity identification evaluation (TIE) to determine the cause of the toxicity. If toxicity is detected in any of the tests prior to the sixth one, the remaining tests do not need to be completed before starting the TIE.

The goal of a TIE is to identify the toxicant(s) causing toxicity in an effluent. EPA methods use the responses of organisms to detect the presence of toxicity in the first stages of a TIE. There are two main objectives in the first step of this approach. First, characteristics of the potential toxicants, such as solubility or volatility, must be established. Then they can be separated from other non-toxic constituents to simplify analyses and enhance interpretation of the analytical data. Secondly, throughout the TIE, one must establish whether or not toxicity is consistently caused by the same substances.

The EPA manuals describe three phases of a TIE: characterization (Phase I), toxicant identification (Phase II), and toxicant confirmation (Phase III). Figure 4.2 is a flowchart showing Tiers 3-6 of the TRE process. The purpose of this section is to summarize the general tests of a Phase I TIE and to help a permit writer begin analyzing TIE plans or the initial results of TIE studies submitted to the permitting agency.

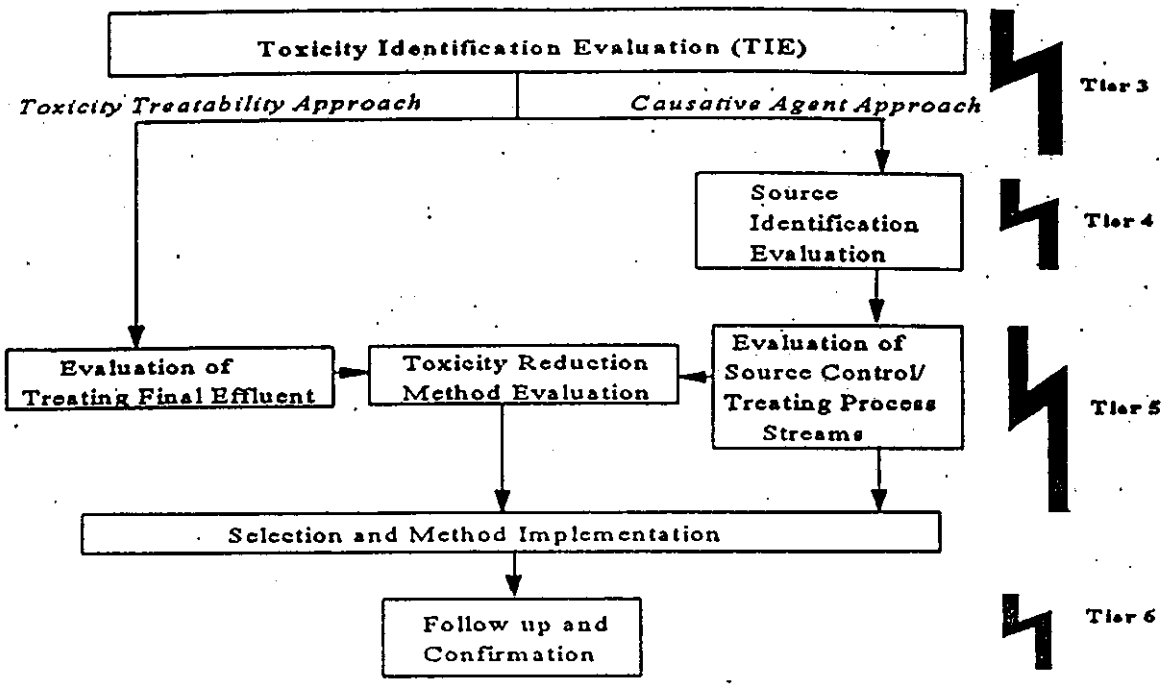

Figure 4.2 Toxicity Identification Evaluation (TIE) Flowchart

The permit writer should consult the manuals themselves for more in-depth discussions of the test manipulations and interpretation of results. Phase I tests characterize the physical/chemical properties of the effluent toxicant(s) using effluent manipulations and accompanying toxicity tests. Each test in Phase I is designed to alter or render biologically unavailable a group of toxicants such as oxidants, cationic metals, volatiles, non-polar organics or chelatable metals. Aquatic toxicity tests, performed on the effluent before and after the individual characterization treatment, indicate the effectiveness of the treatment and provide information on the nature of the toxicant(s).

By repeating the toxicity characterization tests using samples collected over time, these screening tests will provide information as to whether the characteristics of these compounds causing toxicity remain consistent. However, these tests will not provide information on the variability of toxicants within a characterization group. Categorizing the toxicants classes during Phase I as to chemical and physical properties can lead to further identification during Phase II using similar techniques. With successful completion of Phase I, the toxicants can be tentatively categorized as:

- *cationic metals*
- *non-polar organics*
- *oxidants*

- *substances whose toxicity is pH-dependent*
- *others*

The physical/chemical characteristics of the toxicants that are evaluated include filterability, degradability, volatility and solubility. One of two choices can result from Phase II of testing, i.e., treatability or toxicant identification.

Phase II involves several steps, all of which rely on carefully tracking the toxicity of the effluent throughout the analytical procedure. Although effluent toxicants are partially isolated during Phase I, further separation from other compounds present in the effluent is usually necessary. Phase II procedures, unlike Phase I procedures, will be toxicant-specific, rather than simply isolating classes of compounds.

Phase II Identifies
• Non-polar organics
• EDTA-chelatable metals
• Ammonia
• Surfactants

4.1 Major Phase II analyses

Once the toxicants have been adequately isolated from other compounds in the effluent and tentatively identified as the causative agents, final confirmation (Phase III) can begin. As in Phase I, Phase III tests use methods generic to all toxicants. As a result of this, no single test provides irrefutable proof that a certain chemical is the cause of the toxicity. In this case, the combined results of the confirmation tests are used to provide the"weight of evidence" that the toxicant has been identified. TIEs require that toxicity be present frequently enough and be persistent (i.e., not rapidly degraded in storage) so that repeated testing can characterize, and subsequently identify and confirm the toxicants in Phases I and II. Therefore, sufficient testing must be done in order to assure consistent presence of toxicity before TIEs are initiated. No minimum amount of toxicity needed to perform a TIE has been established. However, low levels of toxicity may require more time and analyses to identify the cause of toxicity.

TIEs
• Must be conducted on treated effluent
• Must contain sufficient testing to establish the frequency and persistence of toxicity
• Must be conducted by multi disciplinary teams whose members interact daily

4.2 Components of a successful TIE

Phase I Interpretation of Results

Nine categories of toxicity tests must be conducted to complete Phase I of a TIE. These are: initial toxicity test, baseline test, pH adjustment test, pH adjustment/filtration test, pH adjustment/aeration tests, pH adjustment and C_{18} solid phase extraction (SPE) test, EDTA addition test, sodium thiosulfate addition test, and graduated pH test. No Phase I characterization test should be dropped from use on the basis that the toxicants it is designed to target are not likely to be present in the effluent. The investigator should approach effluent characterization without a preconceived notion as to the cause of toxicity. On the other hand, if one only wants to know whether a certain chemical is the toxicant, for example, ammonia, then the tests can be selected to accomplish that goal. It is also important to realize that the analysis scheme can be designed in order to implicate a certain toxicant. The choice may be based on the laboratory's expertise in conducting TIEs or whether or not criteria exist for a certain pollutant. For example, some facilities may not have limits for toxicants, such as ammonia, because there is no criterion for it. These facilities may find that ammonia is a cause of toxicity and erroneously conclude that no further work is necessary, because there are no limits for ammonia. In cases such as these, the study should conclusively show that ammonia, etc. is the overriding cause of toxicity. The facility would still be under the obligation to reduce its toxicity, in order to comply with the WET limit or requirement.

Following are various examples of Phase I results that may be expected for certain categories of pollutants. These should only be used as guides and not as definitive diagnostic characteristics. The EPA manuals advocate using a weight of evidence approach while being aware that artifacts at this point cannot always be identified.

Indicators of Non-Polar Toxicants	
All toxicity in the post-C_{18} SPE column effluent was removed.	The toxicity removed was recovered in the methanol elution of the SPE column.

4.3 Non-Polar Toxicants Indicators

Non-polar organic toxicants

Toxicants other than non-polar organic compounds, such as metals, may be retained by the SPE column, but they are less likely to be eluted sharply. Some toxicants (types of surfactants) may not elute from the SPE column with methanol. Thus, the failure to recover toxicity in the eluent does not exclude the possibility of a non-polar organic toxicant.

Total Dissolved Solids (TDS)

A group of common cations and anions (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, SO_4^{-}, NO_3^{-}, Cl^{-}, CO_3^{-}) comprise TDS. In some parts of the United States, this water quality characteristic is called "salinity". TDS is usually measured by conductivity, density, or refraction, none of which measure specific

compounds or ions. The toxicity of any given amount of TDS will depend on the specific ionic composition. TDS behaves like a mixture of toxicants which do not cause toxicity through osmotic stress. Evidence of this is that the LC50s of the individual salts expressed in moles, are quite different. If osmotic stress were the mode of action, the concentration in moles at the LC50s would be similar. In addition, marine organisms cannot be used to eliminate the TDS effect unless NaCl is by far the dominant salt. Like freshwater organisms, marine organisms regulate Na^+ and Cl^-, but are sensitive to non-NaCl TDS.

For these reasons, only very general relationships exist between toxicity and TDS salts. Because of their different properties, they do not sort out clearly in Phase I. Unless conductivity is very high (e.g., 10,000 umhos/cm), TDS might be suspected only when nothing else is indicated. For example, if high TDS were caused by calcium sulfate ($CaSO_4$), toxicity is likely to be removed by the adjustment to pH 11 or certainly by the pH 11 adjustment/filtration manipulation. If the TDS were due to NaCl, toxicity would likely not be affected. (For chronic tests, the appropriate pH to look at would be pH 10.)

As a general guide, when conductivity exceeds 3,000 and 6,000 umhos/cm at the LC50 for *Ceriodaphnia* and fathead minnows, respectively (for chronic tests, 1,000 and 3,000 umhos/cm) at the effect concentration, TDS toxicity could be considered. It should be noted that the relevant reading is the conductivity at the concentrations bracketing the effluent LC50, not the conductivity at 100 percent effluent. For chronic tests, the relevant reading for the conductivity is bracketed between the no effect and the lowest observed effect concentrations. The following table summarizes some of the Phase I tests indicators for TDS toxicants.

TABLE 4.2. PHASE I INDICATORS OF TDS TOXICITY

Select Phase I general indicators that TDS is a suspect
No pH adjustments changed the toxicity, unless a visible precipitate occurs upon pH adjustment, pH adjustment/filtration and pH adjustment/aeration.
No loss of toxicity in the post C_{18} column effluent, or a partial loss of toxicity with no change in conductivity reading.
No change in toxicity with EDTA additions, thiosulfate additions, or in the graduated pH test.

In addition, two tests not included in Phase I but which are discussed in the Phase I manuals, can be used. These are the use of an acid/base ion exchange test and an activated carbon filter. With the use of an acid/base ion exchange resin, if toxicity is removed or reduced, the toxicity could be due to TDS. If an activated carbon filter is used to remove toxicity, and if no toxicity is removed by passing the effluent over the carbon, TDS could be responsible for toxicity. Where TDS is

marginally high, the conductivity of the solutions should be monitored closely before and after pH adjustment to avoid producing artifactual TDS toxicity.

Surfactants

Surfactants are surface active agents that have a molecular structure that includes a polar, hydrophilic segment (either ionic or non-ionic) and a relatively large, non-polar hydrophobic, hydrocarbon segment. There are three main groups of surfactants and/or flocculants (anionic, cationic, and non-ionic) that may occur in effluents. The following table summarizes potential indicators of surfactant toxicity.

TABLE 4.3. INDICATORS OF SURFACTANT TOXICITY

General Phase I results implicating surfactants as the toxicants
Toxicity is reduced or removed in the filtration test.
Toxicity is reduced or removed by the aeration test. In some cases, the toxicity is recoverable from the walls of the aeration vessel after removing the aerated effluent sample.
Toxicity is reduced or removed in the post C_{18} 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 the extraction of the glass fiber filter.
Toxicity degrades over time as the effluent sample is kept in cold storage (4 °C). Degradation is slower when effluent is stored in glass containers rather than plastic container.

Ammonia

Indicators of Ammonia Toxicity

The concentration of total ammonia is 5 mg/L or more

In the graduated pH test, the toxicity increases as the pH increases

The effluent is more toxic to fathead minnows than to *Ceriodaphnia*

4.4 Ammonia toxicity indicators

Ammonia concentrations can be measured easily. Since it is such a common effluent constituent, determining the total ammonia concentration is a recommended first step. If more than 5 mg/L of total ammonia is present, additional evaluations should be conducted. Sole dependence upon chemical analyses is not advisable because the chronic (and acute) affects of ammonia and ammonia in combination with some other toxicants (e.g., surfactants), are not well known. Even though the ammonia concentration is sufficient to cause toxicity, other chemicals may be present to cause toxicity if the ammonia is removed.

Oxidants

In effluents, oxidants other than chlorine may be present. Measurement of a chlorine residual (TRC) is not enough to conclude that the toxicity is due to an oxidant.

Indicators of Oxidant Toxicity
Toxicity is reduced/removed by the sodium thiosulfate test
Toxicity is reduced/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
Ceriodaphnia are more sensitive than fathead minnows

4.5 Indicators of oxidant toxicity

However, TRC greater than 0.1 mg/L in 100 percent effluent might indicate chlorine as an oxidant causing the toxicity. In addition, the dechlorination with SO_2 provides additional evidence of chlorine toxicity in the same manner as the sodium thiosulfate addition test.

Cationic Metals

No single characteristic 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 low or high pH depending upon the particular metals involved. This characteristic for chronic toxicity, though, has not yet been demonstrated to the extent it has been for the acute toxicity of several metals.

Cationic Metals Indicators
Toxicity is reduced/removed in:
EDTA addition test
post C_{18} SPE column test
the filtration test, especially when pH adjustments and filtration are combined
Erratic dose response curve observed

4.6 These test results indicate the presence of cationic metals toxicants

TABLE 4.4. SUMMARY OF PHASE I TOXICITY CHARACTERIZATION PROCEDURES

TREATMENT	COMPOUNDS DELETED
pH adjustment	acids, bases, metals
Aeration	volatile/oxidizable compounds
Filtration	filterable material
C_{18} solid phase extraction (SPE)/elution	nonpolar organics (NPOs) and metal chelates
Oxidant reductions	disinfection compounds; bromine, iodine, manganous ions, electrophile organic chemicals
EDTA chelation	cationic metals
Graduated pH	ammonia

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CHAPTER 5. ENFORCEMENT GUIDELINES FOR WET VIOLATIONS

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 the 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.

In a joint memorandum issued by EPA Headquarters Offices of Regulatory Enforcement and Wastewater Management on August 22, 1995, 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 exceedances of WET limits, and 2) inconclusive toxicity reduction evaluations (TREs).

Section 309(a) of the Clean Water Act (CWA) states that any violation of a permit condition or limitation is subject to enforcement. Through EPA's "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 guidance follows the EMS.

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 exceedances 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."

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. EPA Headquarters has committed to providing support in "highly unusual cases" and is in the process of determining the number of facilities nationwide that fit in the category.

The primary corrective action required for violations of WET limits is completion of a, including, if necessary, a Toxicity Identification Evaluation (TIE). This requirement is being incorporated into the Regions' NPDES permits. The permit language addressed in this guidance contains provisions requiring the permittee to: implement the generic TRE plan; increase the testing frequency following a violation if necessary; and, if also necessary, initiate a facility-specific TRE and a TIE following additional violations during the accelerated monitoring period. The permits require permittees to develop and submit a generic TRE workplan within 90 days of permit issuance.

Table 5.1 summarizes the Regions' WET enforcement guidance. The following sections discuss the types of noncompliance and the appropriate enforcement responses in more detail. Appropriate EPA or State laws, policy and enforcement personnel must be consulted prior to a determination of noncompliance or initiation of enforcement actions.

TYPES OF NONCOMPLIANCE

Noncompliance with the NPDES permit and the Clean Water Act (CWA) includes:

(a) violation of the numeric WET permit limits;

(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;
  (2) initiation of a TRE within 15 days of a violation;
  (3) initiation of a TIE following a subsequent violation during the accelerated monitoring period;
  (4) 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).

TYPES OF ENFORCEMENT ACTIONS

In ascending order of severity, the enforcement actions available to EPA include Notice of Violations (NOVs) and Administrative Orders for Compliance (AOs)[1], Administrative Penalty Orders (APOs), Civil Referral/Litigation, and criminal prosecution. Similar State actions are available to each delegated 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-based violations, that is, the need to compel or expedite a discharger'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 discharger's degree of control over the causative agent of toxicity;

(d) a discharger's history of violations/recalcitrance; and,

(e) the economic benefit gained from noncompliance.

Notice of Violation and Administrative Order for Compliance

An AO or its equivalent, issued in conjunction with an 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 discharger failed to conduct the required WET tests on one or more occasions;

(b) after a WET limit violation, a discharger failed to initiate a TRE and/or TIE, or failed to increase the testing frequency;

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[1] 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.

(c) a discharger 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 discharger 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;[2]

(e) a discharger's TRE efforts are inadequate, the corrective actions are inadequate, or the time frames for completing corrective actions are unacceptable;

(f) a discharger 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 discharger has a history of recalcitrance);

(g) WET violations resulted in documented environmental impacts; and,

(h) the discharger has not eliminated or reduced the toxicity within a reasonable amount of time, and the violations are ongoing, whether continuously or sporadically.

Administrative Penalty Order

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 referral should be considered. EPA/State should consider issuing an APO, or its equivalent, for the following situations:

(a) a discharger 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 discharger repeatedly failed to comply with any narrative WET condition or repeatedly failed to provide valid test results;

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[2] In most cases, an AO would be issued if technical assistance by EPA or the State does not resolve the problems.

(c) a discharger repeatedly failed to conduct WET tests;

(d) the WET limit violation(s) was caused by negligence,[3] poor O&M practices, a poor pretreatment program, or other circumstances within the control of the discharger which could have reasonably been avoided;

(e) the WET violation(s) resulted in, or contributed to, significant adverse environmental impacts;

(f) the discharger gained significant economic benefit from noncompliance;

(g) the discharger demonstrated recalcitrance in initiating or completing corrective actions; and,

(h) the penalty calculation, which is based on economic benefit and gravity, is less than $125,000.

Civil Referral

A civil referral is appropriate under circumstances similar to 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 discharger'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 discharger's repeated failure to conduct a TRE in an aggressive or good faith manner, or to otherwise eliminate or reduce toxicity;

(c) a discharger's failure to adequately comply with an AO;

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[3] 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.

(d) situations where extensive corrective action is required, especially extensive construction, or where a discharger 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 $125,000.

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.

WHEN TO TAKE ENFORCEMENT ACTION

In comparison to chemical-based effluent 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.

Violation of Numeric WET Limitations

In general, EPA or the State should not take enforcement action following a violation of WET limitations if the discharger 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;[4] 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 discharger 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.

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.

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.

Ammonia Toxicity

Due to the high capital costs associated with ammonia removal, enforcement actions based on ammonia toxicity can be controversial, especially in those cases where the facility is in compliance with chemical-based limits. It is EPA's national policy that WET violations caused by ammonia be treated in the same manner as WET violations caused by other toxics. As a result, corrective actions may be necessary based solely on ammonia toxicity. However, prior to requiring such

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[4] In this case, there will probably be violations of chemical-based effluent limits as well.

potentially costly corrective actions, EPA or the State should first assist the permittee in pursuing all other possible solutions, such as mixing zones.

Total ammonia is a compound frequently present in municipal and industrial effluents. Its toxicity varies with pH, temperature and dissolved oxygen concentrations. The toxicity of total ammonia can increase by an order of magnitude between pH 7 and pH 9 [EPA 1985]. Ammonia acts as a basic compound in water. Total ammonia is measured in effluent and receiving water samples where toxicity is largely contributed by un-ionized ammonia. The concentration of un-ionized (free) ammonia in a sample is a function of temperature and pH, and at normal pH ranges is only a small fraction of the total ammonia present.

Since pH and temperature have an influence on ammonia toxicity, it may be important to consider the impact of these factors on toxicity test results. During testing, the pH of effluent samples may increase by 1 to 2 units. If ammonia is present in sufficient concentrations, an increase in pH may convert a sufficient amount of the ammonia to the un-ionized form that causes a toxic response. This shift in pH and toxic response may not mimic ambient conditions. Thus it may be important to control test conditions so as to avoid creating artifactual toxicity. As temperature also affects dissociation of ammonia, temperature should be held constant during testing as specified in the test method procedures.

The discharger must demonstrate the effluent toxicity is caused by ammonia because of increasing test pH when conducting the toxicity test. It is important to distinguish the potential toxic effects of ammonia from other pH sensitive chemicals, such as certain heavy metals, sulfide and cyanide. The following may be steps to demonstrate the toxicity is caused by ammonia and not other toxicants before the permitting authority would allow to control for pH in the test.

(1) There is consistent toxicity at the IWC and the maximum pH in the toxicity test are in the range to cause toxicity due to increased pH.

(2) Chronic ammonia concentrations at the IWC are greater than 4 mg/L total ammonia. The level of detection for total ammonia generally need not be below 0.5 - 1.0 mg/L, since concentrations $\leq$ 1.0 mg/L of total ammonia have not been found to be toxic to fathead minnows and *Ceriodaphnia dubia*. Acute ammonia LC50 values of 3 mg/L and 1 mg/L for *Ceriodaphnia dubia* and fathead minnows, respectively, at pH 8.0. Then,

(3) Conduct the graduated pH tests as specified in the toxicity identification evaluation methods. For example, mortality should be higher at pH 8 and lower at pH 6 [EPA 1989a, 1989b, 1989c, 1991a, 1991b].

(4) Treat the effluent with a zeolite column to remove ammonia. Mortality in the zeolite treated effluent should be lower than the non-zeolite treated effluent. Then add ammonia back to the zeolite-treated samples to confirm toxicity due to ammonia.

After it has been demonstrated that toxicity is due to ammonia, pH may be controlled using appropriate procedures which do not significantly alter the nature of the effluent with permission from the permitting authority. Note: This is an appropriate procedure that is not in conflict with Part 136 regulations. For example, any procedure which removes ammonia (such as treatment with zeolite) would not routinely be allowable. Controlling the carbon dioxide (CO_2) environment may be acceptable, if carbon dioxide can be delivered directly into test chambers with airline tubing and a pipette or by using a complex solenoid system (on demand only). The use of CO_2 is the preferable method because less alternation of normal test solution chemistry and use of a natural buffer system to achieve pH control [Mount D.R. and D.I. Mount, 1992]. Another alternative is to maintain a closed carbon dioxide environment, delivering a solution of CO_2 in oxygen to the closed system. The amount of CO_2 required will vary depending on the amount of adjustment needed and the buffering capacity of the effluent.

Total Dissolved Solids (TDS)

TDS is a measure of the dissolved organic and inorganic constituents in a sample. In most cases the biggest contributors to TDS are the major ions: sodium, potassium, calcium, magnesium, chloride, sulfate and bicarbonate. For toxicity caused by TDS, the ratios and concentrations of the major cations and anions can be measured analytically.

The effects of TDS on test organisms may be toxic at certain levels. However, a simple measurement of TDS is inadequate to predict biological impacts. The distribution of ions which make up TDS is of critical importance. To predict impacts, it is necessary to thoroughly characterize the ions in a sample. Once this characterization has been carried out, a model like the Salinity/Toxicity Relationship (STR) model can be used to predict toxicity. Also, conducting supplemental testing with a "mock" effluent (laboratory water reconstituted to the same ion concentrations) is an important confirmation step.

Research conducted to characterize the toxicity of common ions to freshwater organisms has resulted in the development of predictive toxicity models (FW STR) for three freshwater species: *Ceriodaphnia dubia*, *Pimephales promelas*, and *Daphnia magna* [Tietge, et al., 1995]. These freshwater models, used in conjunction with toxicity identification evaluation phase I procedures offer, a powerful combination of techniques to discriminate between toxicity caused by common ions and other compounds.

Confounding Pollutants

Certain pollutants defy traditional approaches at reduction or removal. Such pollutants usually persist in POTW effluents despite implementation of normal pretreatment program controls and operation of standard end-of-pipe wastewater treatment, resulting in effluent toxicity. Such "confounding pollutants" that the Regions are most familiar with include diazinon and total dissolved solids (TDS).

The Regions' recommended approach for addressing the presence of these pollutants is for the POTW to characterize the pollutant(s) and its source(s), and to then implement a series of measures to control those sources and/or treat the effluent so as to achieve the permit's WET requirements. The sequence of events which should follow failure of a WET test due to a "confounding" pollutant is as follows:

1. Conduct research to determine chemical nature and origin(s) of the confounding pollutant. Such research shall include conducting TREs and TIEs, as necessary, as well as going upstream in the collection system to identify individual sources or characterize the pervasiveness of the pollutant. It may also be appropriate to investigate the environmental effects of the pollutant, including its fate and transport in the receiving water, so as to determine the severity of its impact upon the environment.

2. Develop, prioritize and implement control measures sufficient to achieve the permit's WET conditions. Such measures should initially be aimed at source reduction or control. Included in these may be public education programs on responsible use and disposal of the pollutant (especially for pesticides). Alternatives to its use, or broader efforts such as restrictions on distribution or application of products containing the pollutant should also be considered. For pollutants such as minerals originating in groundwater or metals leaching from piping, etc., alternative water sources or distribution systems should be considered and schedules developed for their gradual substitution/phasing in.

   It would be useful for information gathered by the POTW at this stage in the process to be provided to EPA, for use by our regional or national programs aimed at developing water quality criteria and/or regulating toxic substances by means of disposal measures, bans, etc. In this way, a more generic solution to particularly prevalent or intractable problems may be developed, if necessary, with a maximum of input from localities and effected populations.

3. If source reduction alone does not achieve sufficient control of the pollutant, the POTW must then consider and implement other measures, including best management practices (BMPs) and, if necessary, additional treatment, to eliminate WET.

A particularly sensitive issue to be resolved by each permitting authority faced with this problem is at what point in the sequence of events described above to impose chemical-specific effluent limitations for pollutants. Re-openers contained in most permits with WET provisions allow the permitting authority to modify permits when information becomes available which provides a basis for imposing new requirements. Factors to consider in deciding whether to modify a permit, and when in the process to do so, include:

1) The severity of environmental impacts.

2) The ability of the POTW and other interested parties to reduce or eliminate the pollutant.

3) Whether State WQS allow for compliance schedules, and of what duration? If they do, can a phased control approach, starting with source control and only culminating in the construction of additional treatment facilities if necessary, be accommodated by the State?

TABLE 5.1 ENFORCEMENT RESPONSE SUMMARY

NONCOMPLIANCE	INITIAL RESPONSE	ELEVATED RESPONSE FOLLOWING REPEATED OR SUSTAINED VIOLATIONS
Limit Violations	None[5]	NOV/AO; APO; REFERRAL
Failure to Conduct TRE, TIE, or Accelerate Testing	NOV/AO	APO; REFERRAL
Failure to Test	NOV/AO	APO
Invalid Results - Good Faith Effort - Lack of Good Faith - Failure to Re-Test	 Tech. Assist. NOV/AO NOV/AO	 NOV/AO; APO APO APO
Failure to Comply with Narrative Conditions	NOV/AO	APO

REFERENCES

Mount, D.R. and D.I. Mount. 1992. A simple method of pH control for use in aquatic toxicity tests. Environ. *Toxicol. Chem.* 11:609-614.

Tietge, J.E., J.R. Hockett, and J.M. Evans. 1995. Discrimination of ion toxicity in six produced waters using the freshwater salinity toxicity relationships and TIE procedures. Society of Petroleum Engineers. pp. 393-402.

U.S. Environmental Protection Agency. 1985. Ambient water quality criteria for ammonia. EPA/440/5-85/001.

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[5] Provided the permittee increases the testing frequency and initiates a TRE/TIE in accordance with permit requirements.

U.S. Environmental Protection Agency. 1989a. Methods for Aquatic Toxicity Identification Evaluations: Phase II. Toxicity Identification Procedures. Edited by Mount, D.I., and L. Anderson-Carnahan. EPA/600/R-92081. Office of Research and Development, Duluth, MN.

U.S. Environmental Protection Agency. 1989b. Methods for Aquatic Toxicity Identification Evaluations: Phase III. Toxicity Confirmation Procedures. Edited by Mount, D.I. EPA/600/3-88/036. Office of Research and Development, Duluth, MN.

U.S. Environmental Protection Agency. 1989c. Toxicity Reduction Evaluation Protocol for Municipal Wastewater Treatment Plants. Edited by Botts, J.A., J.W. Braswell, J. Zyman, W.L. Goodfellow, and S.B. Moore. EPA/600/2-88/062. Office of Research and Development, Cincinnati, OH.

U.S. Environmental Protection Agency. 1991a. Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I. Edited by Norberg-King, T.J., D.I. Mount, J.R. Arnato, D.A. Jensen, and J.A. Thompson. EPA/600/6-91-005F. Office of Research and Development, Duluth, MN.

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U.S. Environmental Protection Agency. 1995. "National Policy Regarding Whole Effluent Toxicity Enforcement". Memorandum from Van Heuvelen, R. and Cook, M. To Water Management Directors, Regional Counsels (Regions I-X), and State NPDES Directors. Offices of Regulatory Enforcement and Wastewater Management.

APPENDICES

A. Sample Permit Language
B. References
C. List of Contacts
D. Definition of Terms
E. WET Test Costs
F. Frequently Asked Questions (FAQs)
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APPENDIX A--SAMPLE PERMIT LANGUAGE

This appendix contains suggested language and format for including whole effluent toxicity testing requirements and/or limits in permits. Items marked in redline are individual decisions that need to be made by the permit writer. Information and guidance on making those decisions are discussed in the previous sections of this document. **NOTE TO EPA PERMIT WRITERS: "or subsequent editions" refers to editions of manuals available at the time of permit issuance.**

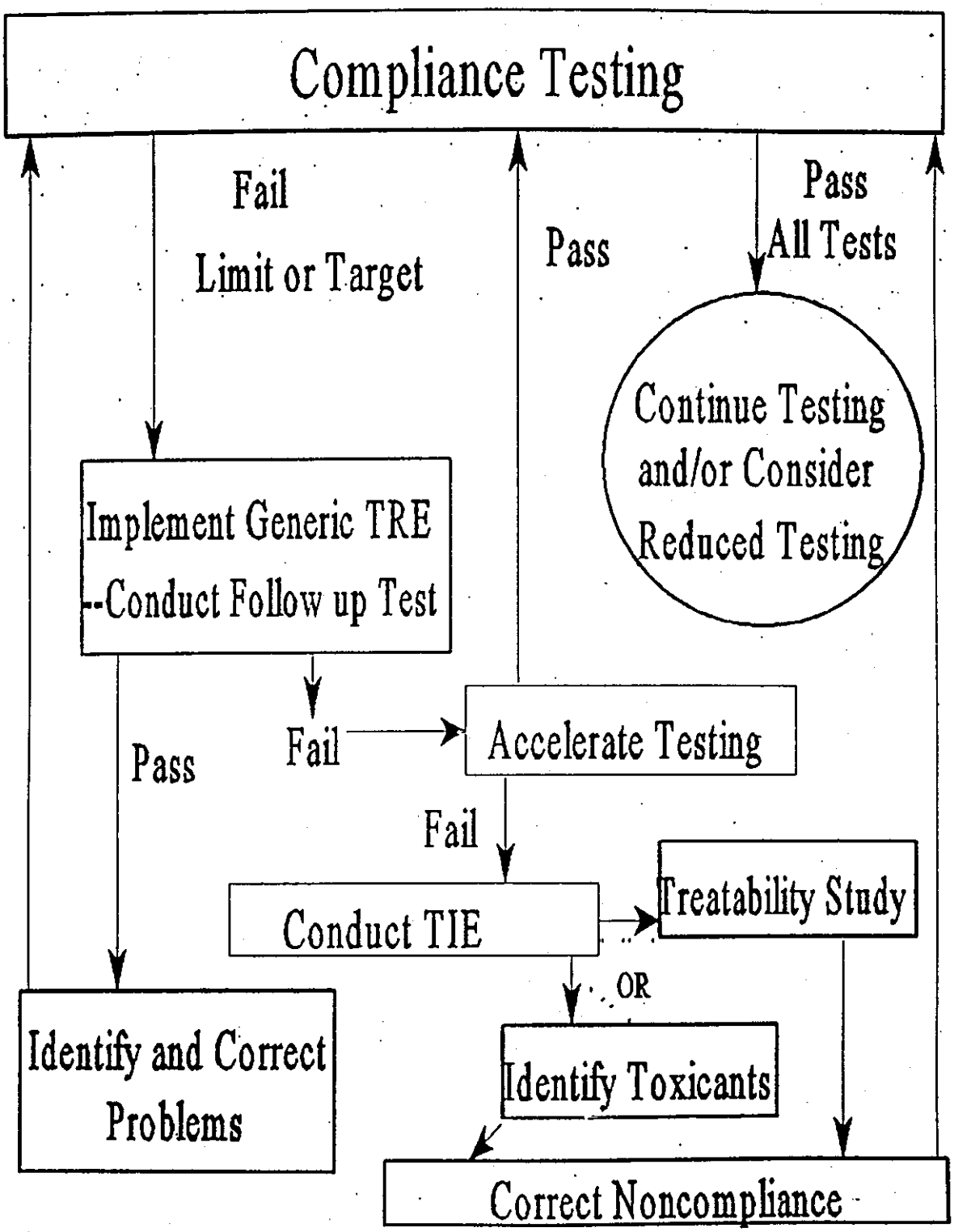

Figure 1 **Flowchart for Whole Effluent Toxicity Compliance Testing**

I. ACUTE TOXICITY

WHOLE EFFLUENT TOXICITY TESTING

The permittee shall conduct monthly/quarterly/semi-annual/annual toxicity tests on grab/24-hour composite effluent samples. Samples shall be taken at the NPDES sampling location. If, after one year of testing, the maximum measured toxicity is less than or equal to {target/reasonable potential factor} TUa[1], then monitoring frequency shall be reduced to annual/once more before permit issuance. {If a WET limit is required, monitoring frequency shall be reduced to no less than once per year.}

1. Test Species and Methods:

NOTE: CHOOSE EITHER FRESHWATER OR MARINE

Freshwater

a. The permittee shall conduct 48-hour non-renewal/96-hour static renewal tests with an invertebrate, the water flea, *Ceriodaphnia dubia*/Daphnia pulex or *Daphnia magna* and a vertebrate, the fathead minnow, Pimephales promelas/rainbow trout, *Oncorhynchus mykiss*[2] for the first three suites[3] of tests. After this screening period, monitoring shall be conducted on the most sensitive species.

b. Every year, the permittee shall re-screen once with the two species listed above and continue to monitor with the most sensitive species. Rescreening shall be conducted at a different time of year from the previous year's screening.

c. The presence of acute toxicity shall be determined as specified in **Methods for Measuring the Acute Toxicity of Effluents to Freshwater and**

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[1] "Target" is the trigger for toxicity when a WET limit is not required. If a limit is required, then the target is the limit. The reasonable potential factor is found in Table 3-1 of the TSD, page 57. It is based on the CV and number of samples taken.

[2] Any freshwater species listed in Appendix B, "Supplemental List of Acute Toxicity Test Species", may be used in place of the foregoing.

[3] "Suites of tests" means the two or three species used for testing during the permit term.

Marine Organisms, Fourth Edition, EPA/600/4-90/027F, August 1993. Or subsequent editions

Marine/Estuarine

a. The permittee shall conduct 48-hour non-renewal/96-hour static renewal tests with the invertebrate Pacific mysid, *Holmesimysis costata*/Atlantic mysid, *Mysidopsis bahia* and a vertebrate, the topsmelt, *Atherinops affinis*/ inland silverside, *Menidia beryllina* for the first three tests. After this screening period, monitoring shall be conducted using the most sensitive species.

b. Every year, the permittee shall re-screen once with the two species listed above for one month and continue to monitor with the most sensitive species. Rescreening shall consist of one test conducted at a different time than the previous year's test.

c. The presence of acute toxicity will be determined as specified in **Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms**, Fourth Edition, EPA/600/4-90/027F, August 1993 or subsequent editions.

2. Limits/Definition of Toxicity[4]

If greater than 3:1 dilution is available, sections 2a & 2b apply; if less than 3:1, sections 2c & 2d apply.

a. For the purposes of this permit, acute toxicity is defined as an LC50 < {trigger, etc. to be specified}, determined using the test organisms and statistical procedures required in Part ____ of the permit. [When a limit for acute toxicity is appropriate, put it in the limits section. If a permit limit is not appropriate, then this section should be called "Definition of Toxicity".]

b. Where the LC50 is calculated, results shall be reported in TUa, where TUa = 100/LC50 (in percent effluent).

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[4] If a WET limit is appropriate, then this section should be contained in the Effluent Limitations section of the permit, and not in the monitoring requirements sections.

c. Acute toxicity is significantly reduced survival at 100 percent or at the instream waste concentration (IWC) compared to a control, using a t-test. The IWC is the effluent concentration at the edge of the mixing zone.

d. Results shall be reported as pass (P) or fail (F) when using a t-test.

3. Quality assurance

NOTE: CHOOSE ONE, LC50 or t-test

a. A series of five dilutions and a control will be tested. The series shall include the instream waste concentration (IWC) (permit writer should insert the actual value of the IWC), two dilutions above the IWC, and two dilutions below the IWC.

a. Two dilutions shall be used, i.e., 100 percent or the IWC and a control (when t-test is used instead of an LC50).

b. If organisms are cultured in-house, reference toxicant tests shall be run monthly. Otherwise, concurrent testing with reference toxicants shall be conducted.

c. If either of the reference toxicant tests or the effluent tests do not meet all test acceptability criteria as specified in the test methods manual, then the permittee must re-sample and re-test within 14 days of receiving the results of the failed test/as soon as possible.

d. Reference toxicant tests shall be conducted using the same test conditions as the effluent toxicity test (i.e., same test duration, etc.).

e. Control and dilution water should be receiving water or lab water, as appropriate, as described in the manual[5]. If the dilution water is different from the culture water, a second control shall be used, using culture water.

f. Chemical testing for the parameters for which effluent limitations exist shall be performed on a split of each sample collected for WET testing. To the extent that the timing of sample collection coincides with that of the sampling required in Part ____ of this permit, chemical analysis of the split sample will fulfill the requirements of that Part as well.

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[5] The manuals describe various situations in which either receiving water or lab water should be used for control and dilution water. Depending upon the objective of the test, either lab water or receiving water may be used.

4. Preparation of Generic TRE Workplan

The permittee shall submit to EPA a copy of the permittee's toxicity reduction evaluation (TRE) workplan [1-2 pages] within 90 days of the effective date of this permit. This plan shall describe the steps the permittee intends to follow in the event that toxicity is detected, and should include at a minimum:

(a) A description of the investigation and evaluation techniques that would be used to identify potential causes/sources of toxicity, effluent variability, treatment system efficiency;

(b) A description of the facility's method of maximizing in-house treatment efficiency, good housekeeping practices, and a list of all chemicals used in operation of the facility;

(c) If a toxicity identification evaluation (TIE) is necessary, who will conduct it (i.e., in-house or outside consultant)

5. Reporting

a. The permittee shall submit the results of the toxicity tests in TUs with the discharge monitoring reports (DMR) for the month in which the tests are conducted.

b. The full report shall be submitted by the end of the month in which the DMR is submitted.

c. The full report shall consist of: (1) the toxicity test results; (2) the dates of sample collection and initiation of each toxicity test; (3) the type of production; (4) the flow rate at the time of sample collection; and (5) the results of the effluent analyses for chemical/physical parameters required for the outfall(s) as defined in Part ___ of the permit.

d. Test results for acute tests shall be reported according to the acute methods manual chapter on Report Preparation, and shall be attached to the DMR. Where possible, the permittee shall submit the data on an electronic disk (3.5") in the Toxicity Standardized Electronic Reporting Form (TSERF).

e. Evaluation results--the permittee shall notify EPA and the State in writing within fifteen (15) days of receipt of the results of the exceedance of the limit/TRE trigger of

(1) The finding of the TRE or other investigation to identify the cause(s) of toxicity;

(2) Actions the permittee has taken or will take to mitigate the impact of the discharge, to correct the noncompliance and to prevent the recurrence of toxicity;

(3) Where corrective actions including a TRE have not been completed, an expeditious schedule under which corrective actions will be implemented; and

(4) If no actions have been taken, the reason for not taking action.

6. Accelerated Testing

a. If acute toxicity is greater than _____ TUa in any test [i.e., the permit limit or the TRE trigger when no permit limit], then the permittee shall conduct six more tests, bi-weekly (every two weeks), over a twelve-week period, beginning within two weeks of receipt of the sample results of the exceedance.

b. If implementation of the generic TRE workplan indicates the source of toxicity (for instance, a temporary plant upset), then only one additional test is necessary. If toxicity is detected in this test, then Part 5a. shall apply.

c. If any of the six additional tests indicate acute toxicity greater than _____ TUa [i.e., the permit limit when a limit is used, or the trigger when no permit limit for WET is used] then, in accordance with EPA manuals EPA/600/2-88/070 (industrial) or EPA/600/4-89/001A (municipal) and the permittee's TRE workplan , the permittee shall initiate a TRE within fifteen (15) days of receipt of the sample results of the exceedance.

d. If none of the six tests indicates toxicity, then the permittee may return to the routine testing frequency.

7. Toxicity Identification Evaluation (TIE)

a. If acute toxicity is detected in any two of the six bi-weekly tests, the permittee shall, in accordance with EPA acute and chronic manuals EPA/600/6-91/005F (Phase I), EPA/600/R-92/080 (Phase II), and EPA-600/R-92/081 (Phase III), initiate a TIE within 15 days.

b. If a TIE is triggered prior to completion of the accelerated testing, the accelerated testing schedule may be terminated, or used as necessary in performing the TIE.

8. Reopener

This permit may be modified in accordance with the requirements set forth at 40 CFR Parts 122 and 124, to include appropriate conditions or limits to address demonstrated effluent toxicity based on newly available information, or to implement any EPA-approved new State water quality standards applicable to effluent toxicity.

II. CHRONIC TOXICITY

WHOLE EFFLUENT TOXICITY TESTING

The permittee shall conduct monthly/quarterly/semi-annual/annual toxicity tests on grab/24-hour composite effluent samples. Samples shall be taken at the NPDES sampling location. If, after one year of testing, the maximum measured toxicity is less than or equal to {target/reasonable potential factor} TUc[1], then monitoring frequency shall be reduced to annual/once more before permit issuance. {If a WET limit is required, monitoring frequency shall be reduced to no less than once per year.}

1. Test Species and Methods:

NOTE: CHOOSE EITHER FRESHWATER OR MARINE LANGUAGE

Freshwater

a. The permittee shall conduct short-term tests with the water flea, *Ceriodaphnia dubia* (survival and reproduction test), the fathead minnow, *Pimephales promelas* (larval survival and growth test) and the green alga, *Selanastrum capricornutum* (growth test) for the first three suites[2] of tests. After this screening period, monitoring shall be conducted using the most sensitive species.

b. Every year, the permittee shall re-screen once with the three species listed above and continue to monitor with the most sensitive species. Re-screening shall be conducted at a different time of year from the previous year's re-screening

c. The presence of chronic toxicity shall be estimated as specified in **Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms**, Third Edition, EPA/600-4-91-002, July 1994. or subsequent editions

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[1] "Target" is the trigger for toxicity when a WET limit is not required. If a limit is required, then the target is the limit. The reasonable potential factor is found in Table 3-1 of the TSD, page 57. It is based on the CV and number of samples taken.

[2] "Suites of tests" means the two or three species used for testing during the permit term.

d. The permittee may also determine compliance with acute fathead minnow test based on the mortality data from chronic test data.

Marine/Estuarine

Test Species and Methods:

a. The permittee shall conduct tests with a vertebrate, an invertebrate, and a plant, as follows for the first three suites of tests. For Region 10: The permittee shall conduct tests with a vertebrate and two invertebrates, as follows for the first three suites of tests. After the screening period, monitoring shall be conducted using the most sensitive species.

For Region 9 only:

Plant: Giant kelp, *Macrocystis pyrifera* (germination and germ-tube length test).

For both Regions 9 and 10:

Vertebrate: Inland silverside, *Menidia beryllina (survival and growth)*/topsmelt, *Atherinops affinis (survival and growth)*.

Invertebrate: (select one for Region 9 and two for Region 10)

1. Atlantic mysid, *Mysidopsis bahia* (survival, growth and fecundity test)/Pacific mysid, *Holmesimysis costata* (survival and growth test);

2. Bivalve species, mussel, *Mytilis spp.* or Pacific oyster, *Crassostrea gigas* (larval development test),

3. Purple urchin, *Strongylocentrotus purpuratus* and sand dollar, *Dendraster excentricus* (fertilization test),

4. Purple urchin, *Strongylocentrotus purpuratus* (larval development test), and sand dollar, *Dendraster excentricus* (larval development test).

5. Red abalone, *Haliotis rufescens* (larval development test).

b. Every year, the permittee shall re-screen once with the three species listed above and continue to monitor with the most sensitive species. Re-

screening shall be conducted at a different time of year from the previous year's re-screening.

c. The chronic toxicity of the effluent shall be estimated as specified in **Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms**[3], EPA-600-4-91-003, July 1994, to be specified when using *Menidia* or *Mysidopsis* species and/or **Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms**,[3] EPA/600/R-95/136, August 1995. to be specified when using West Coast marine organisms.

2. Limits/Definition of Toxicity[4]

a. Chronic toxicity measures a sublethal effect (e.g., reduced growth, reproduction) to experimental test organisms exposed to an effluent or ambient waters compared to that of the control organisms. [When a permit limit is appropriate, the following shall apply]: The chronic toxicity limitation is: 1 TUc based on any monthly median (where there is not a mixing zone), or the value calculated using the statistical method, expressed as a monthly average (where mixing zones are allowed), or any one test result with a daily maximum greater than 2.0 TUc at the edge of the mixing zone. (or other language, based on State wqs.) If a permit limit is not appropriate, then this section should be called "Definition of Toxicity".

b. Results shall be reported in TUc, where TUc = 100/NOEC or 100/ICp or ECp (in percent effluent). The no observed effect concentration (NOEC) is the highest concentration of toxicant to which organisms are exposed in a chronic test, that causes no observable adverse effect on the test organisms (e.g., the highest concentration of toxicant to which the values for the observed responses are not statistically significant different from the controls)[5]. The inhibition concentration, IC, is a point estimate of the

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[3] Or subsequent editions

[4] If a WET limit is appropriate, then this section should be contained in the Effluent Limitations section of the permit, and not in the monitoring requirements sections.

[5] If in the calculation of a NOEC, two tested concentrations cause statistically adverse effects, but an intermediate concentration did not cause statistically significant effects, the test should be repeated or the lowest concentration must be

toxicant concentration that causes a given percent reduction (p) in a non-quantal biological measurement (e.g., reproduction or growth) calculated from a continuous model (the EPA Interpolation Method). The effective concentration, EC, is a point estimate of the toxicant concentration that would cause a given percent reduction (p) in quantal biological measurement (e.g., larval development, survival) calculated from a continuous model (e.g., Probit).

3. Quality assurance

a. A series of five dilutions and a control shall be tested. The series shall include the instream waste concentration (IWC) (permit writer should insert the actual value of the IWC), two dilutions above the IWC, and two dilutions below the IWC. The IWC is the concentration of effluent at the edge of the mixing zone. If there is no mixing zone, then the dilution series would be the following concentrations: for example, 12.5, 25, 50, 75 and 100 percent effluent.

b. If organisms are not cultured in-house, concurrent testing with reference toxicants shall be conducted. Where organisms are cultured in-house, monthly reference toxicant testing is sufficient.

c. If either the reference toxicant tests or the effluent tests do not meet all test acceptability criteria as specified in the test methods manual, then the permittee must re-sample and re-test within 14 days/as soon as possible.

d. Reference toxicant tests shall be conducted using the same test conditions as the effluent toxicity test (i.e., same test duration, etc.).

e. Control and dilution water should be receiving water or lab water, as appropriate, as described in the manual[6]. If the dilution water used is different from the culture water, a second control, using culture water shall also be used.

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used. For example: 6.25, 12.5, 25, 50 and 100% effluent concentrations are tested. The 12.5 and 50% concentrations are statistically significant, but 25% is not significant. If the test is not repeated, then the NOEC is 6.25%.

[6] The manuals describe various situations in which either receiving water or lab water should be used for control and dilution water. Depending upon the objective of the test, either lab water or receiving water may be used.

f. Chemical testing for the parameters for which effluent limitations exist shall be performed on a split of each sample collected for WET testing. To the extent that the timing of sample collection coincides with that of the sampling required in Part ____ of this permit, chemical analysis of the split sample will fulfill the requirements of that Part as well.

4. Preparation of Generic TRE Workplan

The permittee shall submit to EPA a copy of the permittee's toxicity reduction evaluation (TRE) workplan [1-2 pages] within 90 days of the effective date of this permit. This plan shall describe the steps the permittee intends to follow in the event that toxicity is detected, and should include at a minimum:

(a) A description of the investigation and evaluation techniques that would be used to identify potential causes/sources of toxicity, effluent variability, treatment system efficiency;

(b) A description of the facility's method of maximizing in-house treatment efficiency, good housekeeping practices, and a list of all chemicals used in operation of the facility;

(c) If a toxicity identification evaluation (TIE) is necessary, who will conduct it (i.e., in-house or other)

5. Reporting

a. The permittee shall submit the results of the toxicity tests, including any accelerated testing conducted during the month, in TUs with the discharge monitoring reports (DMR) for the month in which the tests are conducted. If the generic TRE workplan is used to determine that accelerated testing is unnecessary, then those results shall also be submitted with the DMR for the month in which the investigation occurred.

b. The full report shall be submitted by the end of the month in which the DMR is submitted.

c. The full report shall consist of: (1) the toxicity test results; (2) the dates of sample collection and initiation of each toxicity test; (3) the type of production; (4) the flow rate at the time of sample collection; and (5) the results of the effluent analyses for chemical/physical parameters required for the outfall(s) as defined in Part ___ of the permit.

d. Test results for chronic tests shall be reported according to the chronic manual chapter on Report Preparation, and shall be attached to the DMR. Where possible, the results shall also be submitted on electronic disk (3.5") in the TSERF format.

e. Evaluation results--the permittee shall notify EPA and the State in writing within fifteen (15) days of receipt of the results of the exceedance of the limit/TRE trigger of

(1) The finding of the TRE or other investigation to identify the cause(s) of toxicity;

(2) Actions the permittee has taken or will take to mitigate the impact of the discharge, to correct the noncompliance and to prevent the recurrence of toxicity;

(3) Where corrective actions including a TRE have not been completed, an expeditious schedule under which corrective actions will be implemented; and

(4) If no actions have been taken, the reason for not taking action.

6. Accelerated Testing:

a. If chronic toxicity as defined [i.e., the permit limit or the TRE trigger, when there is no limit] is detected, then the permittee shall conduct six more tests, bi-weekly (every two weeks), over a twelve-week period. Testing shall commence within two weeks of receipt of the sample results of the exceedance.

b. If implementation of the generic TRE workplan indicates the source of toxicity (for instance, a temporary plant upset), then only one additional test is necessary. If toxicity is detected in this test, then Part 5a. shall apply.

c. If chronic toxicity as defined [i.e., the permit limit or the TRE trigger, when there is no limit] is detected in any of the six additional tests, then, in accordance with the permittee's TRE workplan and, at a minimum, EPA manuals EPA/600/2-88/070 (industrial) or EPA/600/4-89/001A (municipal), the permittee shall initiate a TRE within fifteen (15) days of receipt of the sample results of the exceedance.

d. If none of the six tests indicates toxicity, then the permittee may return to the normal testing frequency.

7. Toxicity Identification Evaluation (TIE)

a. If chronic toxicity is detected in any two of the six bi-weekly tests, then the permittee shall, in accordance with EPA acute and chronic manuals EPA/600/6-91/005F (Phase I), EPA/600/R-92/080 (Phase II), and EPA-600/R-92/081 (Phase III), initiate a TIE within 15 days.

b. If a TIE is triggered prior to completion of the accelerated testing, the accelerated testing schedule may be terminated, or used as necessary in performing the TIE.

8. Reopener

This permit may be modified in accordance with the requirements set forth at 40 CFR Parts 122 and 124, to include appropriate conditions or limits to address demonstrated effluent toxicity based on newly available information, or to implement any EPA-approved new State water quality standards applicable to effluent toxicity.

APPENDIX B

REFERENCES

REFERENCES

TEST METHODS, TRE AND TIE DOCUMENTS

Acute toxicity test methods.

USEPA Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms (EPA/600/4-90-027F). Note, see Appendix B of the acute toxicity test manual for the supplemental list of acute test species.

Freshwater tests

Vertebrates:
- Fathead minnow, *Pimephales promelas*
- Rainbow trout, *Oncorhynchus mykiss*
- Brook trout, *Salvelinus fontinalis*

Invertebrates:
- Water flea, *Ceriodaphnia dubia*
- Water flea, *Daphnia pulex and D. magna*

Marine tests

Vertebrates:
- Inland silverside, *Menidia beryllina*
- Topsmelt, *Atherinops affinis*

Invertebrates:
- Atlantic mysid, *Mysidopsis bahia*
- Pacific mysid, *Holmesimysis costata*

Chronic toxicity test methods

Freshwater tests

USEPA Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms (EPA/600/4-91-002).

Vertebrate:
- Fathead minnow, *Pimephales promelas*

Invertebrate:
- Water flea, *Ceriodaphnia dubia*

Plant:
- Green alga, *Selenastrum capricornutum*

Marine tests

USEPA Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms (EPA/600/4-91-003).

USEPA 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, August 1995).

Vertebrates:

- Inland silverside, *Menidia beryllina* (EPA/600/4-91-003)
- Topsmelt, *Atherinops affinis* (EPA/600/R-95/136)

Invertebrates:

- Atlantic mysid, *Mysidopsis bahia* (EPA/600/4-91-003)
- Red abalone, *Haliotis rufescens* (EPA/600/R-95/136)
- Bivalves, *Crassostrea gigas* and *Mytilus spp.* (EPA/600/R-95/136)
- Purple urchin, *Strongylocentrotus purpuratus* and Sand dollar, *Dendraster excentricus* (EPA/600/R-95/136)

Plants:

- Giant kelp, *Macrocystis pyrifera* (EPA/600/R-95/136)

Toxicity reduction/identification evaluation methods

TRE

USEPA Toxicity Reduction Evaluation Protocol for Industrial Treatment Plants (EPA/600/2-88/070).

USEPA Toxicity Reduction Evaluation Protocol for Municipal Wastewater Treatment Plants (EPA/600/2-88/062).

TIE

USEPA Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I (EPA/600/6-91-05F).

USEPA Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity (EPA/600/R-92-080).

USEPA Methods for Aquatic Toxicity Identification Evaluations: Phase III Toxicity Confirmation Procedures (EPA/600/R-92-81).

Other documents

USEPA Technical Support Document for Water Quality-based Toxics Control (EPA/505/2-90-001). Office of Water. Washington, DC.

USEPA Manual for the Evaluation of Laboratories Performing Aquatic Toxicity Tests (EPA/600/4-90-031).

USEPA Methods for Chemical Analysis of Water and Wastes (EPA/600/4-79/020). Revised March, 1983.

STATISTICAL PROGRAMS

Dunnett Program (Version 1.5)

Inhibition Concentration (ICp) Approach (Version 2.0).

Probit Analysis (Version 1.5)

Trimmed Spearman-Karber (Version 1.5)

Note: **If you are interested in obtaining any of these statistical programs, please send a formatted 3.5" disk to James Lazorchak, EPA EMSL-Ci, 3411 Church Street, Cincinnati, OH 45244.**

SPREADSHEETS

Contact: Madonna Narvaez, USEPA, Region 10, OW-130, 1200 Sixth Avenue, Seattle, WA 98101. Telephone: (206) 553-1774; FAX: (206) 553-1280.

VIDEOS

USEPA Freshwater Culturing Methods for *Ceriodaphnia dubia* and *Pimephales promelas*.

USEPA Test Methods for Freshwater Effluent Toxicity Tests.

USEPA Culturing and Toxicity Test Methods for Marine and Estuarine Effluents for *Mysidopsis bahia.*

Note: If you are interested in obtaining these three videos at a cost, please call The National Audiovisual Center at (800) 788-6282.

DATABASES

AQUIRE - (AQUatic Information REtrieval database)
ASTER - (ASsessment Tools for the Evaluation of Risk)

The AQUIRE database now contains more than 127,000 individual test records for 5,525 chemicals and 2,791 freshwater and marine organisms. Over 9,000 publications have been reviewed for AQUIRE. These data are also available from the ASTER Database System. Both AQUIRE and ASTER now have the electronic capability of sending help text and reports to an internet address.

For information about logging onto these databases, contact the Environmental Research Laboratory-Duluth at (218) 720-5602; fax (218) 720-5539; and internet at outreach@du4500.dul.epa.gov.

APPENDIX C

LIST OF CONTACTS

Please identify your state WET coordinator with a phone, fax numbers and address.

REGION 9 CONTACTS

LOCATION:	NAME AND PHONE:	ADDRESS:
EPA Region 9 WET Coordinator	Debra Denton p (415) 744-1919 f (415) 744-1873	75 Hawthorne St (W-5-1) San Francisco, CA 94105-3901
Arizona Arizona Department of Environmental Quality	Sam Rector p (602) 207-4536 f (602) 207-4528	3033 North Central Phoenix, AZ 85012
California State Water Resources Control Board	Victor deVlaming p (916) 657-0795 f (916) 657-2388	Division of Water Quality PO Box 944213 Sacramento, CA 94244-2130
Hawaii Hawaii State Dept of Health	Alec Wong p (808) 586-4309	Clean Water Branch 919 Ala Moana Blvd Room 301 Honolulu, HI 96814
Nevada Department of Conservation and Natural Resources	Leo Drozdoff p (702) 687-5870, ext. 3142 f (702) 687-5856	Division of Environmental Protection Capitol Complex 333 W. Nye Lane Carlson City, NV 89710

REGION 10 CONTACTS

LOCATION:	NAME AND PHONE:	ADDRESS:
EPA Region 10 WET Coordinator	Madonna Narvaez p (206) 553-1774 f (206) 553-1280	1200 Sixth Avenue (OW-130) Seattle, WA 98101
Alaska	Katie McKerney p (907) 465-5018 f (907) 465-5274	AWQ/WQTS 410 Willoughby Avenue, Ste 105 Juneau, AK 99811-1800
Idaho		
Oregon	Judy Johndohl p (503) 229-6896 f (503) 229-6037	OR DEQ Water Quality 811 SW 6th Avenue Portland, OR
Washington	Randall Marshall p (360) 407-6445 f (360) 6426	WA DOE PO Box 47696 Olympia, WA 98504

APPENDIX D

DEFINITIONS

DEFINITIONS

ACUTE TOXICITY is a test to determine the concentration of effluent or ambient waters that produces an adverse effect on a group of test organisms during a short-term exposure (e.g., 24, 48 or 96 hours). The endpoint is lethality. Acute toxicity is measured using statistical procedures (e.g., point estimate techniques or a t-test).

ACUTE-to-CHRONIC RATIO (ACR) is the ratio of the acute toxicity of an effluent or a toxicant 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.

ADDITIVITY is the characteristic property of a mixture of toxicants that exhibits a total toxic effect equal to the arithmetic sum of the effects of the individual toxicants.

AMBIENT TOXICITY is measured by a toxicity test on a sample collected from a receiving waterbody.

BIOASSAY is a test used to evaluate the relative potency of a chemical or a mixture of chemicals by comparing its effect on a living organism with the effect of a standard preparation on the same type of organism. Bioassays frequently are used in the pharmaceutical industry to evaluate the potency of vitamins and drugs.

CHRONIC TOXICITY is defined as a long-term test in which sublethal effects (e.g., reduced growth or reproduction) are usually measured in addition to lethality. Chronic toxicity is defined as TUc = 100/NOEC or TUc = 100/ECp or TUc = 100/ICp). The ICp and ECp value should be the approximate equivalent of the NOEC calculated by hypothesis testing for each test method.

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. Coefficient of variation is a measure of precision within (intralaboratory) and among (interlaboratory) laboratories.

CRITERIA CONTINUOUS CONCENTRATION (CCC) is the EPA national water quality criteria recommendation for the highest instream concentration of a toxicant or an effluent to which organisms can be exposed indefinitely without causing unacceptable effect.

CRITERIA MAXIMUM CONCENTRATION (CMC) is the EPA national water quality criteria recommendation for the highest instream concentration of a toxicant or an effluent to which organisms can be exposed for a brief period of time without causing an acute effect such as lethality.

CRITICAL LIFE STAGE is the period of time in an organism's lifespan in which it is the most susceptible to adverse effects caused by exposure to toxicants, usually during early development (egg, embryo, larvae). Chronic toxicity tests are often run on critical life stages to replace long duration, life-cycle tests since the most toxic effect usually occurs during the critical life stage.

EFFECT CONCENTRATION (EC) is a point estimate of the toxicant concentration that would cause an observable adverse effect (e.g., survival, fertilization) in a given percent of the test organisms, calculated from a continuous model (e.g., EPA Probit Model).

HYPOTHESIS TESTING is a technique (e.g., Dunnetts test) that determines what concentration is statistically different from the control. Endpoints determined from hypothesis testing are NOEC and LOEC.

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-quantal biological measurement (e.g., reproduction, growth) calculated from a continuous model (i.e., EPA Interpolation Method).

INSTREAM WASTE CONCENTRATION (IWC) is the concentration of a toxicant in the receiving water after mixing. The IWC is the inverse of the dilution factor.

LC50 is the toxicant concentration that would cause death in 50 percent of the test organisms.

LOWEST OBSERVED EFFECT CONCENTRATION (LOEC) is the lowest concentration of toxicant to which organisms are exposed in a test, which causes statistically significant adverse effects on the test organisms (i.e., where the values for the observed endpoints are statistically significant different from the control).The definitions of NOEC and LOEC in the method manuals assume a strict dose-response relationship between toxicant concentration and organism response. If this assumption were always the case, there would be no issue concerning the endpoint definitions because the NOEC would always be a lower concentration level than the LOEC. However, this strict dose-response relationship does not exist with all toxicants. When this occurs the test must be repeated or the lowest NOEC should be reported for compliance purposes.

MINIMUM SIGNIFICANT DIFFERENCE (MSD) is the magnitude of difference from control where the null hypothesis is rejected in a statistical test comparing a treatment with a control.

MIXING ZONE is an area where an effluent discharge undergoes initial dilution and may be extended to cover the secondary mixing in the ambient waterbody. A mixing zone is an allocated impact zone where water quality criteria can be exceeded as long as acutely toxic conditions are prevented.

MONTHLY MEDIAN is the middle value in a monthly distribution above and below which lie an equal number of values. If the number of values are even, then the monthly median is the average of the middle two measurements.

NO OBSERVED EFFECT CONCENTRATION (NOEC) is the highest tested concentration of toxicant to which organisms are exposed in a full life-cycle or partial life-cycle (short-term) test, 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 significant different from the controls).

POINT ESTIMATE TECHNIQUES such as the EPA Probit model, Interpolation method, Spearman-Karber method are used to determine the effluent concentration at which adverse effects such as fertilization, growth or survival have occurred. For example, concentration at which a 25 percent reduction in fertilization occurred.

REFERENCE TOXICANT TEST indicates the sensitivity of the organisms being used and demonstrate a laboratory's ability to obtain consistent results with the test method. Reference toxicant data are part of routine QA/QC program to evaluate the performance of laboratory personnel and test organisms.

SIGNIFICANT DIFFERENCE is defined as statistically significant difference (e.g., 95% confidence level) in the means of two distributions of sampling results.

TEST ACCEPTABILITY CRITERIA (TAC) For toxicity tests results to be acceptable for compliance, the effluent and the reference toxicant must meet specific criteria as defined in the test method (e.g., *Ceriodaphnia dubia* survival and reproduction test, the criteria are: the test must achieve at least 80% survival and an average of 15 young/female in the controls).

t-TEST is a statistical analysis comparing only two test concentrations (e.g., a control and 100% effluent). The purpose of this test is determine if the 100% effluent concentration is different from the control (i.e., the test passes or fails).

TOXICITY TESTS are laboratory experiments which employ the use of standardized test organisms to measure the adverse effect (e.g., growth, survival or reproduction) of effluent or ambient waters.

TOXIC UNIT ACUTE (TUa) is the reciprocal of the effluent concentration that causes 50 percent of the organisms to die by the end of the acute exposure period (i.e., TUa = 100/LC50).

TOXIC UNIT CHRONIC (TUc) is the reciprocal of the effluent concentration that causes no observable effect on the test organisms by the end of the chronic exposure period (i.e., TUc = 100/NOEC or TUc = 100/ECp).

TOXIC UNITS (TUs) are a measure of toxicity in an effluent as determined by the acute toxicity units or chronic toxicity units. Higher TUs indicate greater toxicity.

TOXICITY IDENTIFICATION EVALUATION (TIE) is a set of procedures to identify the specific chemical(s) responsible for effluent toxicity. TIE is a subset of the TRE.

TOXICITY REDUCTION EVALUATION (TRE) is a site-specific study conducted in a stepwise process designed to identify the causative agents of effluent toxicity, isolate the sources of toxicity, evaluate the effectiveness of toxicity control options, and then confirm the reduction in effluent toxicity.

WHOLE EFFLUENT TOXICITY is the total toxic effect of an effluent or receiving water measured directly with a toxicity test.

APPENDIX E

WET TEST COSTS

WET TEST COSTS

An informal survey of Region 9 WET testing laboratories yielded the following information. Costs for definitive freshwater acute non-renewal tests range from $250-$500, while marine acute non-renewal test costs range from $250-$750 (the higher cost was for *Mysidopsis bahia*). Costs for definitive freshwater chronic renewal tests range from $950-$1250. Costs for definitive marine chronic renewal tests range from $800-$2250 (the higher cost was for *Mysidopsis bahia* since this test has three endpoints). Costs depend on: (1) the organism supplies, costs and availability, (2) ease of working with test organisms, and (3) amount of time in calculating test endpoint (e.g., microscope time), etc.

W.H. Peltier of EPA Region 4 (Atlanta, Georgia) compiled costs as of May 1989 for freshwater and marine acute and chronic WET tests. He found that costs could be decreased by the number of tests contracted for. He expects that this cost comparison will be updated by summer 1995. Costs for definitive freshwater acute non-renewal tests ranged from $225-$500, while marine acute non-renewal test costs ranged from $225-$600. Costs for definitive freshwater chronic renewal tests ranged from $825-$1500. Costs for definitive marine chronic renewal tests ranged from $1020-$1500. The following tables summarize the information from both regions.

Acute Toxicity Test Costs:

TEST SPECIES	RANGE OF COSTS
Ceriodaphnia dubia, *Daphnia pulex*, *Daphnia magna*, *Pimephales promelas*, *Oncorhynchus mykiss*, *Menidia beryllina*	$225 - 500
Mysidopsis bahia	$600 - 750

Chronic Toxicity Test Costs

TEST SPECIES	RANGE OF COSTS
Selenastrum capricornutum growth test	$600 - 950
Ceriodaphnia dubia survival and reproduction, *Pimephales promelas* survival and growth, *Menidia beryllina* survival and growth, *Atherinops affinis*, survival and growth	$1000 - 1250
Mytilus spp. and *Strongylocentrotus purpuratus* larval development	$800 - 1100
Strongylocentrotus purpuratus and *Dendraster excentricus* fertilization	$500 - 1100
Haliotis rufescens larval development test	$1000 - 1250
Macrocystis pyrifera germination and germ-tube length	$1000 - 1250
Mysidopsis bahia survival, fecundity and growth	$1100 - 2250*

* The fecundity endpoint can be optional, since there are two sublethal endpoints (growth and fecundity). This must be approved by the permitting authority.

APPENDIX F

FREQUENTLY ASKED QUESTIONS (FAQs)

STATISTICS

Q: The EPA definition of the NOEC is "highest concentration of toxicant to which organisms are exposed in a full life-cycle or partial life-cycle test, that causes no observable adverse effects on the test organisms." How should the NOEC be reported for the following example: dilution concentrations 6.25, 12.5, 25, 50 and 100 percent effluent. The concentrations 12.5, 50 and 100 percent were statistically different from the control. What is the LOEC?

A: The LOEC is the concentration of 12.5, therefore, the NOEC is 6.25. The definitions of NOEC and LOEC in the methods manuals assume a strict dose-response relationsh between toxicant concentration and organism response. If this assumption were alway the case, there would be no issue concerning the endpoint definitions because the NOEC would always be a lower concentration level than the LOEC. However, this strict dose-response relationship does not exist with all toxicants. When this occurs the test must be repeated or the lowest NOEC should be reported for compliance purpose

Q: Is it appropriate to analyze toxicity data for compliance reporting using statistical tools other than those identified in the EPA flowcharts for statistical analysis?

A: Section 11.1.4 of the most recent edition of the acute manual (1993) states: "The data analysis methods recommended in the EPA toxicity testing methods manuals were chosen primarily because they are (1) well-tested and well-documented, (2) applicable to most types of test data sets for which they are recommended, but still powerful, and (3) most easily understood by non-statisticians. Many other methods were considered in the selection process, and it is recognized that the methods selected are not the only possible methods of analysis." The appropriateness of other methods for use on acute and chronic toxicity test results, however, must be determined with a careful evaluation of a complete array of possible toxicity test results on which the method might be used.

Q: How are males in the *Ceriodaphnia dubia* survival and reproduction test calculated for the survival endpoint?

A: Males are included for the survival analysis as either dead or alive the same as females.

Q: In the chronic tests with survival endpoints (e.g., *Pimephales promelas* survival and growth test) can the survival be used for acute test results?

A: Yes, it is recommended to report both 7 day survival results, in addition to either the 48 or 96-hour survival results. This reduces the costs of compliance testing for requirements of acute and chronic testing.

Q: According to the recommended test conditions section the number of replicates per concentration is four (minimum of three). When a test is conducted with only three replicates and the data fails the assumptions of parametric testing, what analysis should be performed?

A: If the data fails the assumptions of parametric testing, then non-parametric statistics would be performed, however a minimum of four replicates are necessary. In the situation described above the data could be forced through parametric tests and the results would be interpreted with caution. Ideally, the test should be repeated and the laboratory should use a minimum of four replicates.

DATA SUBMISSION

Q: Should test results that fail the required test acceptability criteria (e.g., ≥ 90% survival in the controls for the acute toxicity methods) be reported to the permitting authority?

A: It is the permittee's responsibility to determine if the results of toxicity tests fulfill test requirements and, therefore, should be submitted. The permitting authority will reject data that do not meet test method specifications.

TEST ORGANISMS

Q: What type of documentation and level of effort is appropriate to demonstrate a laboratory's effort to obtain organisms for a test?

A: A laboratory should make best effort to obtain spawnable test organisms from two organism suppliers. Documentation should consist of order forms or verification of order placed by phone (signed and dated entries in a bound notebook).

SALINITY ISSUES

Q: Should salinities of effluent be matched to ambient salinity or to a "typical" ambient salinity?

A: The test must be conducted at a salinity that is acceptable for the particular test species (e.g., the red abalone test must be conducted at 34 ê 2‰). However, when conducting ambient toxicity tests the salinities should be matched to ambient salinities, not to a "typical" ambient salinity.

Q: If there are difficulties with commercial brine, what is the preferred source of salt?

A: Brine such as commerical salts or hypersaline brine are used to achieve the required method salinity. The preferred source of brine is to use clean seawater that has been

concentrated by evaporation or freezing procedures. See the section on hypersaline brine additions in the marine chronic test method manuals.

TESTING CONDITIONS

Q: Should temperature be held constant during testing if the test temperature is higher th ambient temperature?

A: The test must be conducted at the test temperature as specified in the toxicity test manual for that specific test species.

ENFORCEMENT

Q: If conducting tests with two or more species, how is compliance determined? Looking at all test results together, regardless of species, or looking at results on a species by species basis?

A: Look at species by species basis: compliance would be based on the endpoint per species with the lowest NOEC value or point estimate value (EC 25) as specified in the permit per test endpoint per test species.

Q: The laboratory reports the NOEC and LOEC as percent effluent for both survival and growth with the chronic fathead minnow and both survival and reproduction with the chronic *Ceriodaphnia dubia*. What should be entered onto the DMR?

A: Report the lowest NOEC value of either the survival or growth for the fathead minnow test and the lowest NOEC value of either the survival or reproduction for *Ceriodaphnia dubia* test.

Q: When both a brine and dilution water control are used for the marine toxicity test methods, which control should be used to compare to the treatments?

A: First, a t-test is conducted to compare the brine control to the dilution water control. If there is no statistical difference between the controls, then use the dilution water control for all the treatments. If there is a statistical difference between the controls, then use the dilution water control for the treatments without brine addition and the brine control for the treatment with brine addition.

APPENDIX G

STATUTORY AND REGULATORY CONSIDERATIONS

STATUTORY AND REGULATORY CONSIDERATIONS

A primary objective of the NPDES and water quality standards programs is to control the discharge of toxics. The CWA and EPA regulations authorize and require the use of the "integrated strategy" to achieve and maintain water quality standards. Relevant provisions that provide statutory authority for using toxicity testing and WET limitations include the following:

- Section 101(a) of the CWA sets forth the "goal of restoring and maintaining the chemical, physical, and biological integrity of the Nation's waters" and, at section 101(a)(3), prohibits "the discharge of toxic pollutants in toxic amounts."

- Section 502(15) of the CWA defines biomonitoring as the "determination of the effects on aquatic life, including accumulation of pollutants in tissue, in receiving waters due to the discharge of pollutants (A) by techniques and procedures, including sampling of organisms representative of appropriate levels of the food chain appropriate to the volume and physical, chemical, and biological characteristics of the effluent, and (B) at appropriate frequencies and locations."

- Section 304(a)(8) requires EPA to "...develop and publish information on methods for establishing and measuring water quality criteria for toxic pollutants on other bases than pollutant-by-pollutant criteria, including biological monitoring and assessment methods."

- Section 303(c)(2)(B) states, in part, "Nothing in this section shall be construed to limit or delay the use of effluent limitations or other permit conditions based on or involving biological monitoring or assessment methods..."

- Section 302(a) provides authority to EPA and the States to establish water quality-based effluent limitations on discharges that interfere with the attainment or maintenance of that water quality which shall assure protection of public health, public water supplies, and the

STATUTORY CONSIDERATIONS

- Section 101(a) - states national goals
- Section 502(15) - defines biomonitoring
- Section 304(a)(8) - develops biomonitoring methods
- Section 303(c)(2)(B) - outlines biological methods for standards
- Section 302(a) - requires effluent limits to protect aquatic life
- Section 301(b)(1)(C) - requires limits necessary to meet water quality standards including narrative
- Section 308(a) - provides authority to require permittees to use biological methods
- Section 402 - sets out requirements of NPDES permits program
- Section 510 - requires states to adopt standards at least as stringent as those in effect under the Act

G.1 Statutory basis for WET controls

protection and propagation of a balanced population of shellfish, fish and wildlife.

- Sections 301(b)(1)(C) and 402 require that all NPDES permits must comply with any more stringent limitations necessary to meet applicable water quality standards, whether numeric or narrative. Section 301(b)(1)(C) states that "In order to carry out the objective of this Act there shall be achieved...any more stringent limitations including those necessary to meet water quality standards..., or required to implement any applicable water quality standard...".

- Sections 308(a) and 402 provide authority to EPA or the State to require that NPDES permittees/applicants use biological monitoring methods and provide chemical toxicity and instream biological data when necessary for the establishment of effluent limits, the detection of violations, or the assurance of compliance with water quality standards. Section 308(a) states "whenever required to carry out the objective of this Act, including but not limited to (1) developing or assisting in the development of any effluent limitation...(2) determining whether any person is in violation of any such effluent limitation...(A) the Administrator shall require the owner or operator of any point source to...(iii) install, use, and maintain such monitoring equipment or methods (including where appropriate, biological monitoring methods)...".

- Section 510 provides authority for States to adopt or enforce any standards or effluent limitations for the discharge of pollutants only on the condition that such limitations or standards are no less stringent than those in effect under the CWA.

On May 26, 1989, the EPA Deputy Administrator signed regulations that implemented section 304(l) of the CWA (54 FR 23868, June 2, 1989). Commonly referred to as the 304(l) regulations, these regulations did more than implement section 304(l). While 40 CFR Parts 130.10 and 123.46 were modified specifically for 304(l) requirements, 40 CFR Part 122.44(d) was modified to clarify and reinforce EPA's existing regulations governing water quality-based

REGULATORY CONSIDERATIONS

- **40 CFR Part 122.44(d)(1)(i)** - reflects EPA's water quality-based approach
- **40 CFR Part 122.44(d)(1)(ii)** - presents procedures for water quality-based limits considerations
- **40 CFR Part 122.44(d)(1)(iv)** - requires WET limits where WET standards are exceeded
- **40 CFR Part 122.44(d)(1)(v)** - requires WET limits if the narrative standard is exceeded
- **40 CFR Part 122.44(d)(1)(vii)** - requires permit conditions to assure compliance with water quality standards and WLAs
- **40 CFR Part 122.21(j)** - requires POTWs to submit biomonitoring data with permit application
- **40 CFR Part 130.7** - requires TMDLs using specific pollutants or biomonitoring approach

G.2 Regulatory basis for WET controls

permitting. The following parts of 40 CFR Part 122.44(d) pertain to the requirements for WET limits in NPDES permits.

- 40 CFR Part 122.44(d)(1)(i) was expanded to reflect EPA's approach to water quality-based permitting, an approach that includes all parameters (conventional, nonconventional, and toxics) and all applicable standards, both narrative and numeric.

- 40 CFR Part 122.44(d)(1)(ii) discusses procedures to be used to determine if a discharge causes, has a reasonable potential to cause, or contributes to an excursion of a water quality standard. The procedures include consideration of four general factors: "...existing controls on point and nonpoint sources...variability of the pollutant...in the effluent, the sensitivity of the species to toxicity testing...and...the dilution of the effluent in the receiving stream."

- 40 CFR Part 122.44(d)(1)(iv) requires effluent limits for whole effluent toxicity when it has been shown that a discharge causes, has a reasonable potential to cause, or contributes to an excursion of a numeric WET criterion.

- 40 CFR Part 122.44(d)(1)(v) requires limits for WET when it has been shown that a discharge causes, has a reasonable potential to cause, or contributes to an excursion of a narrative WET criterion. However, WET limits are not necessary if it can be demonstrated satisfactorily that chemical specific limits are sufficient to maintain all applicable standards.

- 40 CFR Part 122.44(d)(1)(vii) requires that all permit limits and conditions assure compliance with water quality standards and wasteload allocations.

The regulations described above were subsequently challenged and upheld. In the Natural Resources Defense Council, Inc. v. EPA, court case, at 859 F.2d 156 (D.C. Cir., 1989), several issues with regard to WET implementation were reviewed. The Court held that EPA has the authority to express permit limitations in terms of toxicity as long as the limits reflect the appropriate requirements of the CWA, as provided in 40 CFR 125.3(c)(4). [More detail on this case can be found in Appendix B-6 of the TSD.]

In addition to the May 1989 changes to 40 CFR Part 122.44(d)(1), on July 3, 1990, the EPA Administrator signed final regulations that modified the permit application regulations at (55 FR 30082, July 24, 1990) 40 CFR Part 122.21(j). This section now requires large publicly-owned treatment works (POTWs) to provide the results of valid whole effluent biological toxicity testing with their application for a permit. This requirement applies to the following POTWs:

-All POTWs with a design flow of greater than or equal to 1 MGD (major facilities)

-All POTWs with approved pretreatment programs or POTWs required to develop a pretreatment program, and
-Any other POTW as determined by the State Director

Further regulations at 40 CFR Part 130.7 require total maximum daily loads (TMDLs) and wasteload allocations (WLAs) be developed for water quality-limited stream segments. A pollutant-by-pollutant or biomonitoring approach may be used to establish TMDLs.

APPENDIX H

TRE/TIE CASE STUDIES

INDUSTRIAL CASE STUDIES

These are a few industrial TRE case studies prepared by the Texas Natural Resource Conservation Commission (TNRCC). The TNRCC does not mandate that permittees utilize any particular TRE protocol. They found that most permittees began the TRE process using EPA protocols and later modify these protocols as necessary to accommodate the TRE findings. Overall, the TNRCC's experience monitoring TREs has been educational and positive. They observed several complicating events or planning problems in many of these TREs. The following list of TRE shortcomings/complications will be useful to environmental managers and consultants involved in future TREs.

- Failure to collect adequate sample volume necessary to perform chemical analysis and characterization tests in the event that a biomonitoring sample is toxic.
- Failure to follow-up with characterization tests when an effluent sample is acutely or chronically toxic.
- Failure to correlate the presence or absence of toxicity with operational changes.
- Inability to interpret multiple characterization test results.
- Devoting unnecessary time and effort to studies of potential surrogate test species.
- Complications due to infrequent toxicity.
- Limiting the TRE effort to routine biomonitoring tests.
- Failure to utilize abbreviated screening tests to track effluent toxicity when routine biomonitoring tests are not required.
- Failure to recognize patterns of toxicity.
- Failure to scrutinize artificial sea salts for toxic contaminants.

Phillips Petroleum Company

This refinery and petrochemical complex is located near Sweeny in Brazoria County, Texas. The permit issued on September 27, 1990, required the permittee to conduct the chronic 7-day survival and reproduction test with the water flea, *Ceriodaphnia dubia* and the chronic 7-day larval survival and growth test with the fathead minnow, *Pimephales promelas* using samples from outfall 001. A September 15, 1991, permit amendment retained this requirement. Treated

process wastewater is discharged at this outfall. The discharge enters Linnville Bayou which flows to Caney Creek. The defined critical dilution is 98% effluent. The dilution series defined in the permit is 6,12, 25, 49 and 98% effluent.

This facility began a TRE effort in December 1989 as a requirement of its NPDES permit. Both test organisms demonstrated sensitivity to the effluent. From October 1990 through May 1993, 13 of 29 *Ceriodaphnia dubia* tests demonstrated statistically significant mortality at the critical dilution. Of the failed tests, survival NOEC values ranged between 12 and 50% effluent. Where survival was not affected at the critical dilution, 15 out of 16 *C. dubia* tests demonstrated statistically significant reproduction effects at the critical dilution.

From October 1990 through May 1993, 19 of 28 fathead minnow tests demonstrated statistically significant mortality at the critical dilution. Of the failed tests, survival NOEC values ranged between 6 and 50% effluent. Where survival was not affected at the critical dilution, 4 out of 9 tests demonstrated statistically significant growth effects at the critical dilution.

Characterization tests conducted between November 1991 and March 1992 indicated that effluent toxicity was attributable to three sources: (1) chloride, (2) ammonia, (3) one or more organic chemicals. Continued *Ceriodaphnia* reproduction effects were attributed to effluent chloride levels (approximately 700 - 800 mg/L). For this reason, Phillips is now beginning an effort to evaluate the ionic makeup of the effluent. In recent characterization studies, effluent toxicity to fathead minnow was removed by solid phase extraction with a C_{18} resin. Phillips has considered napthenic acids as a possible cause of toxicity although information thus far has not been conclusive. A powdered activated carbon treatment pilot plant test and powdered activated carbon tests effectively controlled the toxicity due to the unknown organic constituent(s).

Effluent toxicity and ammonia levels have decreased over the past year. Phillips attributes this success to a number of waste improvement projects throughout the refinery. Additionally, Phillips began operating a new waste water treatment system in April 1993 (2-staged activated sludge system with a ZIMPRO powdered activated carbon process).

Bell Helicopter Textron, Inc.

This facility manufactures components for the aircraft industry and assembles complete helicopters in Fort Worth, Texas. The permit issued on November 14, 1991, required the permittee to conduct the chronic 7-day survival and reproduction test with the water flea, *Ceriodaphnia dubia* and the chronic 7-day larval survival and growth test with the fathead minnow, *Pimephales promelas* using samples from outfall 001. Waste streams permitted at this outfall include air conditioning condensate and stormwater runoff. The discharge enters a railroad ditch which enters Valley View Branch, which flows to Walker Branch, which enters the West

Fork of the Trinity River. The defined critical dilution is 76% effluent. The dilution series defined in the permit is 59, 67, 76, 86 and 98% effluent.

The November 1991 permit recognized that Bell Helicopter had already initiated the TRE process since the NPDES permit effective in September 1991, specified a WET limit. The *Ceriodaphnia* was the most sensitive species tested. NOEC values for *Pimephales* survival ranged between <6 and 98% for 22 tests between October 1991, and June 1993. The TNRCC database reflects only one statistically significant survival failure at the critical dilution. However, Bell Helicopter's historical biomonitoring data collected as a result of earlier federal requirements was not reflected in the TNRCC database. Six fathead minnow tests demonstrated statistically significant growth effects at the critical dilution. For tests that were conducted from October 1991 through June 1993, only 1 of 21 *Ceriodaphnia dubia* survival tests revealed statistically significant effects at the critical dilution.

Under the TRE effort, Bell Helicopter implemented rigorous outside housekeeping improvements. Bell Helicopter began washing fleet vehicles off-site, plugged storm drains near potential contamination sources such as chemical and hazardous waste storage areas, improved housekeeping and containment for raw material drum storage areas, and covered and installed containment sumps. Bell Helicopter has recently implemented a stormwater pollution prevention plan. Statistically significant effluent toxicity has not been demonstrated for a year and a half. A single EDTA characterization chelation test performed in January 1992 failed to yield significant information as about the effluent toxicity. A permit amendment issued on July 30, 1993, specifies a WET limit that goes into effect in July 1994.

Intercontinental Terminals Company (ITC)

This bulk liquids storage terminal and commercial waste water treatment facility is located in Deer Park. The permit issued on March 21, 1990, required the permittee to conduct the acute static renewal 48-hour test with the Atlantic mysid, *Mysidopsis bahia* and the acute static renewal 48-hour with the sheepshead minnow, *Cyprinodon variegatus* using samples from outfall 002. Treated industrial wastewater is discharged at this outfall. The discharge enters drainage ditches that flow to Tucker Bayou which enters the Houston Ship Channel. The defined critical dilution is 30% effluent. The dilution series defined in the permit is 11, 18, 30, 50 and 83% effluent.

This facility began the TRE effort in January 1991. The mysid has been the most sensitive species tested. From June 1990 through June 1993, 31 of 43 *Mysidopsis bahia* tests demonstrated statistically significant mortality at the critical dilution. The majority of the NOEC values were less than 11% effluent. Since, October 1992, the *Mysidopsis bahia* test for survival passed at the 30% critical dilution (NOECs of 50 and 83% effluent). From June 1990 through June 1993, only 3 of 41 *Cyprinodon variegatus* tests demonstrated statistically significant mortality at the critical dilution.

Five initial characterization efforts in 1991 were inconclusive. No particular class of chemicals was implicated as a probable cause of effluent toxicity. ITC then launched a program of source segregation where various waste streams were routed away from the treatment system to determine if elimination of the segregated stream resulted in a reduction of effluent toxicity. ITC isolated various third party streams and in-plant wastewaters. The program revealed that a particular third party stream treated at ITC's facility was highly toxic. ITC ceased accepting this third party stream in June 1992. Since then, test results have demonstrated a continuous reduction in effluent toxicity. ITC reports that there have been no other operational changes since removal of the suspected third party stream.

Central Power and Light - J.L. Bates Station

This steam electric station is located near the City of Mission in Hidalgo County, Texas. The permit issued on March 22, 1988, required the permittee to conduct the chronic 7-day survival and reproduction test with the water flea, *Ceriodaphnia dubia* and the chronic 7-day larval survival and growth test using the fathead minnow, *Pimephales promelas*, using samples from outfall 001. Waste streams permitted at this outfall include cooling tower blowdown, low volume wastewater, metal cleaning wastes, and storm water runoff. The discharge enters a drainage ditch which flows to the Arroyo Colorado. The defined critical dilution is 100% effluent. The dilution series defined in the permit is 6.25, 12.5, 25, 50 and 100% effluent.

This facility began the TRE effort in June 1989. Effluent toxicity based on survival was intermittent throughout this TRE effort. The water flea was the most sensitive species tested. *Ceriodaphnia dubia* survival NOEC values ranged between 6 and 100% effluent for 34 tests between June 1988, and December 1992. Eleven *Ceriodaphnia* tests demonstrated statistically significant mortality at the critical dilution. Thirty *Ceriodaphnia* tests demonstrated statistically significant reproduction effects at the critical dilution. Test results have revealed statistically significant mortality for only 2 of 26 fathead minnow tests conducted between June 1988 and August 1990. Growth effects at the critical dilution were indicated in 3 of these tests.

This TRE has been complicated by intermittent lethal toxicity sometimes associated with turn-around events. Recent TRE findings have indicated several probable effluent toxicants. Probable sources of toxicity include: 1) tributyltin (TBT) used in periodic cooling tower treatment, 2) water treatment process polymers, and 3) copper originating within the steam cycle system.

A January 1993, effluent sample revealed significant lethality to *Ceriodaphnia*. Subsequent investigation revealed that the cooling tower was treated with TBT in December 1992, and that the Unit 1 cooling tower was drained while the January 1993, biomonitoring sample was collected. Chemical analyses of the effluent indicated a whole effluent TBT concentration of 1.696 ppb. Interestingly, the TBT concentration determined in a filtered effluent sample was 0.541 ppb. Characterization tests revealed that toxicity was removed by filtration at every pH.

Toxicity was also reduced in samples passed through the C_{18} Solid Phase Extraction (SPE) column. Past TBT treatments appear to correlate well with past toxicity events.

Methanol elution of the SPE column failed to recover a toxic fraction. CP&L believes that this characteristic is indicative of surfactant or polymer behavior. CP&L reports that it is possible that the January 1993 effluent sample contained one of two polymers used for water treatment. These are Chemlink IPC 6115 (which contains formaldehyde as a component) and Betz Polymer 1192. CP&L indicated that backwash from the water treatment filtration unit may accumulate in the cooling tower Unit 1 basin when the unit is not operating.

Based on this information, CP&L performed 48-hour acute range-finding tests on non-toxic effluent dosed with the suspect polymers. No acute toxicity was demonstrated with IPC6115. At concentrations of 10 and 100 mg/L, Betz polymer 1192 was acutely toxic to *C. dubia*. Reproduction effects were apparent at concentrations above 0.63 mg/L. CP&L suspects that the maximum expected effluent concentration for this polymer should be somewhere between 1 and 10 mg/L.

CP&L recently conducted waste stream surveys to investigate sources of copper within the plant. Primary copper sources are indicated within the boiler and boiler cooling circuits. Unit 1 copper levels are consistently higher that those associated with Unit 2. Because the condenser for the Unit 1 boiler contains brass tubes (copper and nickel), CP&L representatives speculate that copper may readily go into solution at the low pH (6.7) of the cooling water. Since nickel and zinc are consistently present in the final effluent, CP&L continues to evaluate their potential contribution to the overall effluent toxicity.

MUNICIPAL CASE STUDIES

The California San Francisco Regional Water Quality Control Board (Regional Water Board) in Oakland, California supplied information for various POTWs in the San Francisco Bay Area. The Regional Water Board has revised many of the NPDES permits for POTWs and some industries to include self-implementing TIE language. Permittees are required to call the Regional Water Board if they have any violations and then they are to follow up the call by letter or by including the notice with their discharge monitoring reports (DMRs). The Regional Water Board has found generally had good cooperation from the facilities. About eight POTWs and more than four refineries have performed at least a Phase I TIE. One POTW has completed a Phase III, confirmation study. The various studies conducted at facilities in Regional Board indicated probable causes of toxicity as the pesticide diazinon, ammonia, possible poor lab quality assurance, hardness, and methods used for culturing test organisms.

Central Contra Costa Sanitary District (CCCSD)

CCCSD began a TIE investigation in early 1992, and completed the Phase III confirmation study in early 1994. The primary cause of toxicity was found to be diazinon. As an effort to reduce the toxicity from diazinon, the district recently began a public information campaign describing how homeowners and others should use and dispose of diazinon to lessen the environmental impacts.

In performing the studies, the CCCSD found that if high conductivity (or TDS) is a suspected toxicant, then it is useful to compare the toxicity of nitrified samples to de-nitrified samples. If the toxicities of nitrified and denitrified samples were not different, then TDS would not account for the difference in toxicity. In addition, as a control for conductivity effects, CCCSD increased the conductivity of the lowest concentration of the combined effluent to the level found in the 100 percent concentration of the combined solution. Then CCCSD compared the conductivity of the concurrent reference toxicant tests to the 100 percent effluent concentration. If the values in the reference toxicant tests were well above the 100 percent effluent concentration, conductivity was eliminated as a suspect toxicant.

Also a metabolic inhibitor, piperonyl butoxide was used to prevent the activation of indirect acting organophosphates (OPs) such as diazinon to their toxic form. This is one test to help identify the presence of OP toxicity in effluents. CCCSD also concluded that analytical methods with detection limits under 0.1 ug/L are needed to detect OPs in effluent matrix.

City of South San Francisco

The City of South San Francisco initiated a Phase I TIE in September 1992. Their contractor modified the EPA TIE methods by using a C_8 instead of C_{18} column for the SPE tests. The contractor had previously found that some of the nonpolar organics do not elute from C_{18} columns even with 100 percent methanol. After performing the initial Phase I tests, the contractor identified that toxicity may have been related to the sodium meta-bisulfite used to dechlorinate the effluent. The facility adjusted their dosing of the bisulfite and came back into compliance with their toxicity limit.

East Bay Dischargers Authority (EBDA)

The results of an initial Phase I TIE study for EBDA indicated nonpolar organics as possible causes of toxicity. Because of the high level of toxicity to *Ceriodaphnia dubia*, EBDA concluded that any further chemical analyses should also target nitrogen, OPs, and sulfur-based pesticides.

City of Palo Alto

After submitting and following a detailed TIE study plan, the facility identified that toxicity was caused by hardness effects on the green alga *Selenastrum capricornutum*. The study consisted of toxicity characterization, POTW performance evaluation, TIE, toxicity source evaluation, in-plant control evaluation, toxicity control selection, and control implementation with follow up monitoring.

In exploring the toxicity to *Selenastrum*, the facility found that metals, anions and elevated hardness play major roles. Other tests performed suggested that zinc was the prime suspect in metal toxicity. In performing the aeration tests, the facility found reduced toxicity at pH 11. Toxicity was eliminated at pH 3. This could mean that toxicity was caused by compounds volatile under acidic conditions (e.g., hydrogen sulfide) or by short chain acidic organics. Alternatively, the results could mean that under acidic conditions, insoluble precipitates are formed and that this reaction is catalyzed by the mixing associated with aeration.

In addition, a loss of toxicity via aeration may also be caused by surfactants. In order to evaluate that possibility, the facility redissolved residual materials in the aeration vessels in clean water and then tested for toxicity. Upon finding no toxicity, the facility concluded that surfactants were not a cause of toxicity.

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APPENDIX I

AMBIENT TOXICITY TESTING PROGRAMS

The following contains a brief summary of the uses and findings of selected laboratory toxicity testing projects with ambient water samples collected in California during the last eight years with the objectives of screening for and identifying water quality problems. Ambient water toxicity testing has been used by Regional and State Water Boards, not as a compliance measure, but rather as a screening tool which can be followed up with Toxicity Identification Evaluations (TIEs) and analytical chemistry procedures to identify the specific chemical causes of water quality problems. There is no officially designated ambient water toxicity testing program in California.

COLUSA BASIN DRAIN--PESTICIDES USED IN RICE CULTIVATION

In the spring Colusa Basin Drain (CBD) receives large quantities of tailwater discharged from rice field floodings. CBD, in turn, discharges into the Sacramento River and, during this time, can constitute up to one third of the river flow.

Acute toxicity tests were conducted with water samples collected from CBD before, during, and after the release of tailwater from rice fields. Tests organisms were *Ceriodaphnia*, *Neomysis*, and striped bass larvae and eggs. These toxicity tests clearly identified toxicity associated with the discharge of tailwater from rice fields. TIEs and associated chemical analyses specifically identified some of the pesticides used in rice cultivation as the causes of toxicity to *Ceriodaphnia* and *Neomysis*.

As a result of these findings, the Central Valley Regional Water Quality Control Board and the Department of Pesticide Regulation (DPR) initiated actions which resulted in alterations of irrigation practices on rice fields (e.g., increased holding times of irrigation water following the application of pesticides). The increased on-field holding times resulted in decreased frequency and magnitude of toxicity, as well as lower concentrations of pesticides, in CBD and Sacramento River water samples during the release of rice irrigation tailwater. Water quality of the CBD discharge was clearly improved as a consequence of the information gained from toxicity testing and TIE data.

IMPERIAL COUNTY -- ALAMO RIVER

There is extensive irrigation of Imperial County agriculture with Colorado River water via the All-American Canal. The Alamo River, which discharges into the Salton Sea, consists primarily of agricultural irrigation tailwater. For over two years, water samples have been collected at up to 11 stations along the 50 mile course of the Alamo River. These samples have been screened for water quality using 96-hour acute toxicity tests with *Ceriodaphnia* and *Neomysis*.

Although the head water of the Alamo River in the United States has never tested toxic, frequent and high magnitude acute lethality has been seen in water samples taken along the entire length of

the river which receives irrigation tailwater. TIEs and associated analytical chemistry have clearly demonstrated that five pesticides, chlorpyrifos, diazinon, carbofuran, malathion, and carbaryl, are major contributors to the toxicity in many of the Alamo River toxic samples.

Staff from the Colorado River Basin Regional Water Quality Control Board and the State Water Resources Control Board (SWRCB) and DPR have agreed to work cooperatively to reduce pesticide concentrations in the Alamo River. These three agencies, along with the Imperial Irrigation District, will attempt to convene an Interagency Coordinating Committee (ICC) of interested parties to assist in the development of practices aimed at reducing pesticides in the Alamo River to nontoxic levels. This ICC could include the above entities, as well as the Imperial County Agricultural Commissioner, the Farm Bureau, grower organizations, pesticide advisors, applicators organizations, and the Soil Conservation District.

SAN JOAQUIN RIVER WATERSHED

The San Joaquin River has the second largest watershed in California and, due to extensive hydrological manipulations, this river now receives large volumes of agricultural tile drain water, as well as irrigation tailwater. The San Joaquin and its tributaries were extensively sampled from February 1988 through June 1990. The samples were screened using the EPA chronic freshwater - three species methods, *Ceriodaphnia*, *Pimephales*, and *Selenastrum*.

A pattern of frequent and high magnitude acute mortality to *Ceriodaphnia* was demonstrated in a 43 mile stretch of the San Joaquin River between its confluence with the Merced and Stanislaus Rivers. Based on chemical analyses of the toxic samples, the primary causes of the toxicity water quality problem were attributed to pesticides, including diazinon, chlorpyrifos, carbofuran, carbaryl and parathion. The US Geological Survey and DPR performed subsequent studies on the San Joaquin River which confirmed extensive pesticide contamination.

Although no regulatory actions have been initiated to address these water quality problems, the San Joaquin County Agricultural Commissioner has been conferring with the Central Valley Regional Water Quality Control Board staff regarding these problems.

SACRAMENTO-SAN JOAQUIN DELTA ESTUARY

The Sacramento-San Joaquin delta estuary is of monumental ecological, aesthetic, and economic significance in California. Over the past 21 months there has been extensive sampling (approximately 24 sites, sampled monthly) in the delta estuary. These samples have been screened with the EPA chronic freshwater three species methods, *Ceriodaphnia*, *Pimephales*, and *Selenastrum*. The data collected to date demonstrate periodic and widespread water quality problems in this critical area.

Although the causes of the toxicity have not been completely identified, TIEs and chemical analyses reveal that chlorpyrifos, diazinon, and carbofuran contribute to the toxicity seen at some times during the year. These data are currently being incorporated into a draft report which will be circulated for technical review.

ORCHARD RUNOFF IN THE CENTRAL VALLEY

Considerable acreage in the Sacramento, Feather, and San Joaquin River watersheds is devoted to fruit and nut growing. Acute toxicity screening tests of water samples collected at multiple sites throughout these watersheds indicated water quality problems during January and February. Specifically, many of the samples collected during this time yielded *Ceriodaphnia* mortality.

Follow up analytical chemistry and immunosorbant analyses pointed to diazinon, a pesticide applied to dormant orchards during December and January for the control of a bud boring insect, as a water quality problem in these watersheds. The concentrations of diazinon measured in samples collected during this period frequently exceeded the acute mortality LC50 of several aquatic species. These studies also suggested that certain orchard areas surrounding the Feather, Sacramento, and San Joaquin Rivers were the geographic source of diazinon.

To date no regulatory actions have been taken to control the offsite movement of this pesticide. However, DPR, UC Davis Extension (the BIOS project) and Ciba-Geigy Corporation (a manufacturer of diazinon) have conducted some exploratory studies on practices which could reduce the offsite movement of diazinon. These studies included the voluntary cooperation of growers.

REVOLON SLOUGH/MUGU LAGOON

Mugu Lagoon is considered a significant ecological area which may be at high risk. Revolon Slough, in Ventura County, receives large volumes of agricultural irrigation tailwater. Water was collected at sites on this slough over the course of a year and screened with the EPA chronic freshwater three species methods, *Ceriodaphnia*, *Pimephales*, and *Selenastrum*.

Data from this study revealed periodic toxicity to each of the three species. Based on these initial data, another year of testing has been initiated which will include TIEs and chemical analyses to identify the causes of water quality problems.

ANAHEIM/NEWPORT BAYS

Four freshwater streams and channels discharging into the sensitive Anaheim and Newport Bays were sampled. Four sites were sampled twice between November through February. Water

quality in these samples was screened using the EPA chronic freshwater three species methods, *Ceriodaphnia*, *Pimephales*, and *Selenastrum*.

Periodic acute and chronic toxicity were detected in these samples. Discharges into Newport Bay were primarily toxic to *Ceriodaphnia*. TIEs suggested that the toxicants were organic chemicals and, although pesticides were detected in these toxic samples, there was no confirmation as to the causes of toxicity. Freshwater discharges into Anaheim Bay proved to be toxic to all three test species, but there was no identification of the causative chemicals. Funds were not available to specifically identify the causes of toxicity or to follow up these initial findings.

FINAL COMMENTS

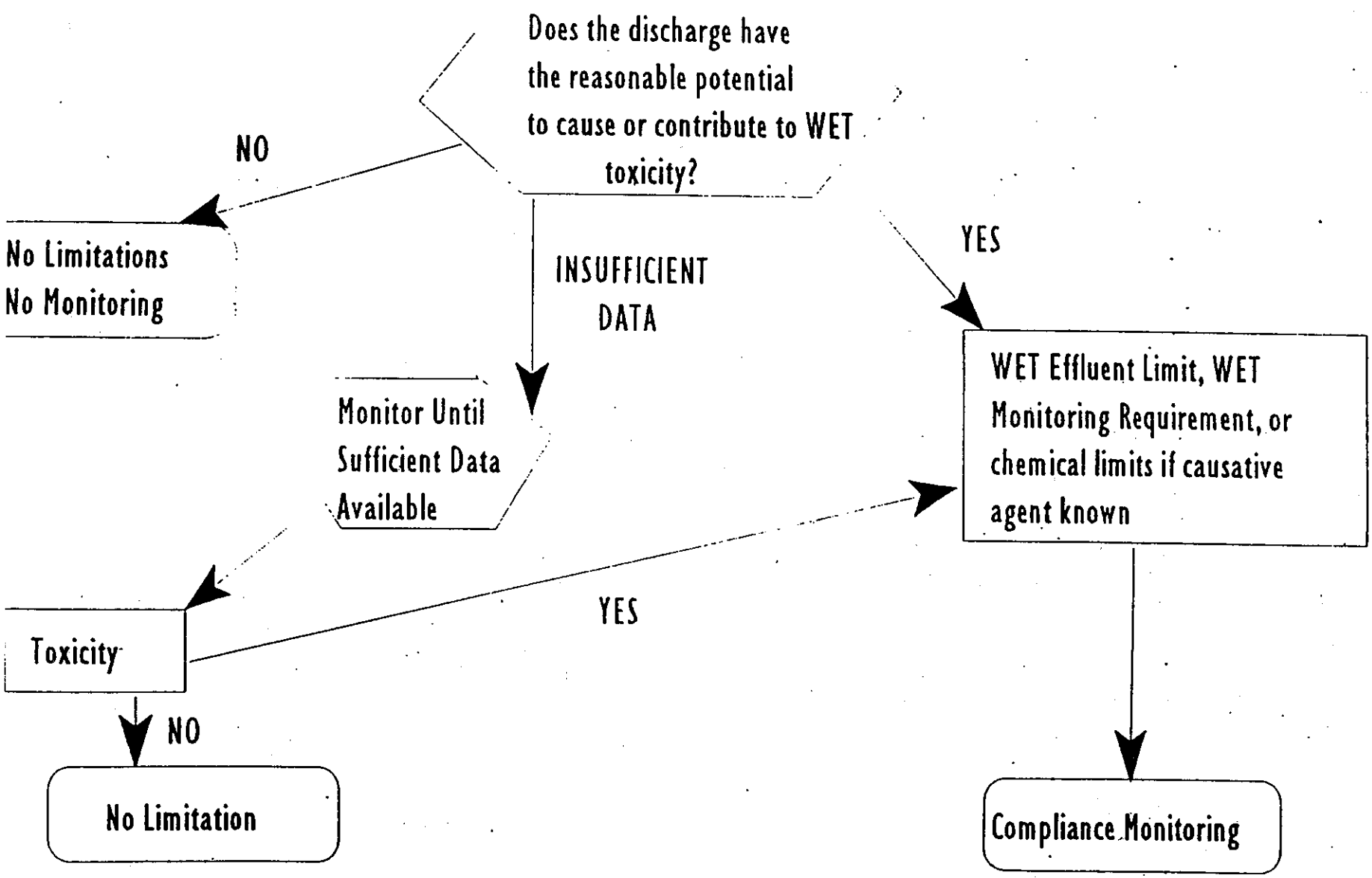
Despite low, and ever-declining, funding, toxicity testing of surface waters has proved to be powerful water quality screening tool. Given the relative short time this tool has been used, it has an exceptional record for indicating water quality problems. Specifically, toxicity testing with subsequent TIEs and chemical analyses have an excellent record in locating the geographic source, land use practices, and chemical causes of water quality problems.

Surface water quality toxicity testing studies plus TIE results also have evoked several Department of Fish and Game hazard assessments for specific pesticides. These assessments include the development of water quality criteria for the pesticides. In the last ten years, ambient water toxicity testing in association with TIE and analytical chemistry results have yielded the potential for several changes in land/water use practices.

APPENDIX J

ESTABLISHING REASONABLE POTENTIAL

ESTABLISHING WET PERMIT LIMITS OR MONITORING REQUIREMENTS



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re 1 Flowchart for establishing WET permit limits or conditions

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BASIS FOR ESTABLISHING REASONABLE POTENTIAL

NPDES regulations at 40 CFR 122.44(d) require the permit writer to establish effluent limitations for pollutants, including whole effluent toxicity (WET), which are discharged in amounts that cause, have the reasonable potential to cause, or contribute to an excursion above State water quality standards, including State narrative objectives for water quality.

As required under 40 CFR 122.44(d)(1)(ii), the permit writer must consider a number of factors in establishing reasonable potential including existing controls on point and nonpoint sources of pollution, pollutant variability in the effluent, sensitivity of toxicity test species, and dilution in the receiving water. The following discussions outline the tiered methodology followed when conducting a reasonable potential evaluation. Regulations supporting reasonable potential determinations are discussed in the TSD (see Chapter 3).

Justification for imposing water quality-based effluent limitations based on reasonable potential is required in the statement of basis, or fact sheet [see 40 CFR 122.44(d)(vi)(C)].

ESTABLISHING REASONABLE POTENTIAL WITH FACILITY-SPECIFIC DATA

Where facility-specific effluent data are available, reasonable potential is evaluated in a sequential (i.e., tiered) process. The first-tier analysis may be performed by using a simple steady-state mass balance equation. The mass balance equation relates the mass of pollutants upstream of a point source discharge, to the mass of pollutants downstream after mixing of the discharge in the receiving water is complete. The general mass balance equation for the recommended steady-state model (see Training Manual for NPDES Permit Writers, EPA 833-B-93-003, March 1993, pp 6-10) is:

$$QdCd + QsCs = QrCr, \text{ where}$$

Qd = waste discharge flow in million gallons per day (MGD), or cubic feet per second (cfs)

Cd = waste discharge pollutant concentration in toxic units for WET (TUa or TUc)

Qs = background in-stream flow in MGD or cfs above point of discharge during critical flow conditions

%Qs= percent of upstream flow allowed by mixing zone standard

Cs = background in-stream pollutant concentration in toxic units for WET (TUa or TUc)

Qr = resultant in-stream flow after discharge in MGD or cfs: %Qs + Qd

Cr = resultant in-stream pollutant concentration in toxic units for WET (TUa or TUc) in the stream reach (after complete mixing).

For reasonable potential determinations, this equation is rearranged to solve for the resultant in-stream concentration (Cr) at the edge of the mixing zone:

$$Cr = \frac{(Qd)(Cd) + (Qs)(Cs)}{Qr}$$

Using the mass balance equation, Cr should be calculated using conservative (i.e., critical) assumptions for background in-stream receiving water flow (Qs), background in-stream receiving water pollutant concentration (Cs), waste discharge flow (Qd) and waste discharge pollutant concentration (Cd). Critical waste discharge conditions should be represented by the highest observed pollutant concentration and waste discharge flow. Critical background in-stream receiving water flows are: 1) the 1Q10 flow (1-day low flow over a 10-year recurrence interval) for calculating acute effects and 2) the 7Q10 flow (consecutive 7-day low flow over a 10-year recurrence interval) for calculating chronic effects. The State of Alaska uses 30Q2 (consecutive 30-day low flow over a 2-year recurrence interval). Where possible, background in-stream pollutant concentrations should correlate with critical background in-stream flows, as critical pollutant concentrations occur during low flows, or are associated with stormwater. For WET, Regions 9 and 10 recommend that background be assumed to be zero, unless data are available. Ambient low flow data, developed by the U.S. Geological Survey, are available through STORET.

Once the projected maximum in-stream pollutant concentration (Cr) is calculated, this value can be compared to the appropriate water quality criterion (WQC). Where Cr is greater than the WQC, reasonable potential is established for that pollutant at the specified effect level (i.e., acute or chronic). When reasonable potential is demonstrated, water quality-based effluent limitations must then be developed for WET.

If the projected maximum resultant in-stream pollutant concentration (Cr) is less than the WQC, the permit writer must then exercise judgement to determine whether reasonable potential exists. This judgement depends on how large the difference is between Cr and the applicable WQC, the uncertainty of maximum effluent concentrations, type of discharger, and the sensitivity of the receiving water. To assist in making this judgement, a second-tier assessment may be performed that statistically addresses the uncertainty of maximum effluent concentrations for individual pollutants. The second-tier analysis is a six step process (see TSD, Box 3-2, p. 53) and is conducted for an effluent pollutant data set as follows:

1. Calculate the coefficient of variation (CV), where the CV is the standard deviation over the mean (σ/μ) (see TSD, Appendix E). For sample sizes less than 10 ($k < 10$) a default CV of 0.6 can be used (see TSD, Box 3-2, p. 53).

2. Choose uncertainty multiplier from Table 3-1 or 3-2 (see TSD, p. 54) using k and the CV. The 99% confidence level and 99% probability basis (Table 3-1) is recommended.

3. Calculate the adjusted maximum effluent concentration by multiplying the uncertainty multiplier times the highest observed effluent concentration (Cd).

4. Re-calculate the maximum resultant in-stream pollutant concentration (Cr) using the adjusted maximum effluent concentration (Cd) and the mass balance equation.

5. Compare Cr with the applicable criterion. Reasonable potential is established when Cr exceeds the criterion.

When reasonable potential is established by either first- and/or second-tier analyses, a water quality-based effluent limitation must be included in the permit for WET. A case example is presented at the end of this appendix.

ESTABLISHING REASONABLE POTENTIAL WITHOUT FACILITY-SPECIFIC EFFLUENT DATA

Where facility-specific effluent data are lacking, the permit writer may still conduct a reasonable potential evaluation. Establishing reasonable potential under such circumstances requires a systematic consideration of all applicable factors in 40 CFR 122.44(d)(1)(ii) (see TSD, pp. 50-51 and Box 3-1, p. 49) including:

- Existing ambient water quality data;
- Available dilution in the receiving water;
- Type of receiving water and designated uses;
- Industry/POTW type and nature of the discharges;
- Compliance history and historical toxic impacts; and
- Information from permit application or DMRs.

If a review of ambient monitoring data shows in-stream exceedances or near exceedances of a criterion for toxicity and WET is present in the discharge, reasonable potential is clearly established and effluent limitations for WET should be included in the permit. The in-stream exceedance of the toxicity criterion indicates that the receiving water body cannot assimilate any additional load of toxicity. Consequently, compliance with the criterion for toxicity must be met at the end-of-pipe (i.e., no dilution).

FINDING NO REASONABLE POTENTIAL

Where existing effluent monitoring data show no reasonable potential for excursions above ambient applicable criteria, the permit need not contain water quality-based effluent limitations. However, the permit writer may include monitoring requirements in the permit to continue to re-affirm initial reasonable potential determinations and to monitor for effluent changes (see TSD, pp. 59, 64).

CASE EXAMPLE

Facility Description

A regional wastewater treatment plant (Regional Plant) discharges to a river. The Regional Plant treatment train consists of coarse screening, aerated grit chambers, primary sedimentation, pure oxygen activated sludge, secondary clarification, and disinfection using chlorination/dechlorination systems. The river in the vicinity of the discharge is influenced by tides and slack flows and flow reversals may occur. In order to insure rapid mixing in the receiving water and prevent a breakdown in jet diffusion, the secondary effluent is diverted to an on-site emergency storage basin. Once the river flow is sufficient for adequate mixing of the effluent, the discharge is resumed. Design effluent flow is 180 MGD.

Data

Based on information provided, the 7Q10 is estimated to be 7500 cfs. Using the design flow of 180 MGD, this would correspond to an instream dilution of 26:1. Based on the analysis provided for the diversion of the effluent during low flow periods, a minimum dilution of 14:1 would occur infrequently, as a result of extreme high tides and low flow conditions, is a short-duration event (less than 1-hour in duration), and is used to assess for the exceedance of the CMC (i.e., acute effects).

The following table is a summary of the results of 20 chronic tests conducted by the facility. Based on those results, the value for k is 20, the highest effluent concentration of WET observed was 16 TUc, and the CV is 0.9. The uncertainty multiplier from Table 3-1 is 3.2.

Toxicity test results, TUc	2,2,2,4,4,4,2,2,2,2,2,4,4,4,8,2,>16,16,2,2,8
ACR	10
Chronic dilution	26:1
Acute dilution	14:1
CV	0.9
k	20
Uncertainty multiplier (RPF)	3.2

In order to evaluate reasonable potential for the acute criterion, the chronic results need to be converted to TUa, i.e., 16/ACR = 1.6 TUa.

Acute: (1.6 TUa)(3.2)/14 = 0.4 TUa
0.4 TUa > 0.3 TUa (acute criterion)

Chronic: (16 TUc)(3.2)/26 = 1.9 TUc
1.9 TUc > 1.0 TUc (chronic criterion)

Based on these results, both acute and chronic criteria for toxicity have demonstrated a reasonable potential to be exceeded. Permit limits for toxicity must be developed this discharge.

APPENDIX K

CALCULATING WATER QUALITY-BASED EFFLUENT LIMITATIONS

OVERVIEW

Water quality-based effluent limitations (WQBELs) are based on maintaining effluent quality at a level that will comply with appropriate criteria, even during critical conditions in the receiving water. These effluent limitations are based on the allowable effluent loading concentration, or waste load allocation[1] (WLA). Pollutant WLAs can be adjusted for effluent variability using statistics calculated from historical effluent data; these adjusted WLAs define the desired levels of performance, or targeted long-term average discharge conditions (LTAs) for specific applicable criterion effect levels (i.e., acute or chronic). Permit limits are calculated using statistics derived from historical effluent data and the most limiting target LTA for a specific applicable criterion.

The coefficient of variation (CV) is the critical statistic calculated for each pollutant using historical effluent data. Where historical data are insufficient (i.e., $k < 10$), the CV may be estimated by 0.6 (see TSD, Appendix E, p. E-3). Statistical derivation procedures for the average monthly limit (AML) for whole effluent toxicity (WET) should assume that at least one sample (n) will be taken per month.

The WLA required to protect against both acute and chronic effects under critical conditions may be calculated using either steady-state or dynamic models. For chronic WET and other cases, a WLA for a WET is not apportioned under a total maximum daily load (TMDL) for the receiving water. In such cases, the allowable effluent loading concentration (Cd) based on steady-state assumptions may be substituted for the more rigorously determined WLA. The steady-state model is the mass balance formula, $QdCd + QsCs = QrCr$, used in reasonable potential evaluations. However, the equation is rearranged to solve for the effluent concentration (Cd), or WLA, necessary to achieve the appropriate applicable criterion. For compliance purposes, the criterion for toxicity is set equal to Cr, where Cr is the applicable criterion:

$$WLA = Cd = \frac{[Cr\ (Qd + \%Qs)] - [(Cs)(\%Qs)]}{Qd}\ , \text{ where}$$

Qd = waste discharge flow in million gallons per day (MGD), or cubic feet per second (cfs)

Cd = waste discharge pollutant concentration in toxic units for WET (TUa or TUc)

Qs = background in-stream flow in MGD or cfs above point of discharge

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[1] "Wasteload allocation" is the portion of a receiving water's total maximum daily load that is allocated to one of its existing or future point sources of pollution.

Cs = background in-stream pollutant concentration in toxic units for WET (TUa or TUc); setting Cs = 0 is recommended for WET

%Qs = percent of upstream flow allowed by mixing zone standard

Qr = resultant in-stream flow after discharge in MGD or cfs: %Qs + Qd

Cr = applicable toxicity criterion = resultant in-stream pollutant concentration in toxic units for WET (TUa or TUc), in the stream reach (after complete mixing)

In most cases, this steady-state model should be used to calculate the WLA (i.e., allowable effluent concentration) that will meet acute and chronic water quality criteria for the protection of aquatic life at 1Q10 and 7Q10 design flows, respectively (see TSD, p. 68). Ambient low flow data from the U.S. Geological Survey are available on STORET.

When calculating the WLA, it should be noted that if State water quality standards and plans do not explicitly allow the application of mixing zones, the appropriate applicable criterion must be met at the end-of-pipe (i.e., applicable criterion = Cr = Cd = WLA). Where mixing zones are allowed, appropriate State procedures should be applied.

If adequate receiving water flow and effluent concentration data are available to estimate frequency distributions, dynamic modeling techniques can be used to calculate allowable effluent loadings that will more precisely maintain water quality standards (see TSD, p. 97). However, the steady-state mass balance equation, when coupled with the recommended conservative assumptions, should be adequately protective of receiving water beneficial uses.

WLAs calculated using State water quality criteria for WET can have both acute and chronic requirements, whereas WLAs determined using some other State water quality criteria for WET may have only chronic requirements. For permit implementation, acute and chronic WLAs need to be converted to maximum daily limits (MDLs) and average monthly limits (AMLs). For effluent-dominated waters (EDWs) and other low flow situations, MDLs and monthly medians should be used (see Chapter 2). The following methodology (see TSD, Box 5-2, p. 100; Figure 5-4, p. 101; and Tables 5-1, 5-2 and 5-3, pp. 102-103, 106) is designed to derive permit limits for specific pollutants and WET to achieve calculated WLAs at the 99% confidence level for MDLs and the 95% confidence level for AMLs.

1. Using the mass balance equation to solve for the allowable effluent concentration (Cd), or WLA, for WET:

   a. Set Cr equal to acute, chronic criteria.

b. Background receiving water (Qs), discharge (Qd) flows, and background pollutant concentration (Cs) should represent critical conditions.

c. Solve for acute (WLAa) and chronic (WLAc) waste load allocations.

2. Convert the acute WLA to chronic toxic units (WLAa,c).

$$WLAa,c \text{ (in TUc)} = WLAa \text{ (in TUa)} \cdot ACR$$

3. To calculate the coefficient of variation (CV):

a. Use effluent data set of 'k' observations (k is ≥ 10) to calculate the mean (μ) and standard deviation (σ) (see TSD, Appendix E).

b. Calculate the coefficient of variation (CV), where $CV = \sigma/\mu$.

c. Where the effluent data set is small ($k < 10$), the conservative value of 0.6 is recommended to estimate the CV (see TSD, Appendix E, p. E-3).

4. To determine long-term averaged discharge conditions (LTAs):

Use the following equations to calculate acute and chronic long-term average discharge conditions (LTAa,c and LTAc) that will satisfy the acute and chronic waste load allocation (WLAa,c and WLAc). The CV calculated above is used to estimate both acute and chronic WLA multipliers (see TSD, Table 5-1, p. 102).

$$LTAa,c = WLAa,c \cdot e^{[0.5\sigma^2 - z\sigma]}$$

$$LTAc = WLAc \cdot e^{[0.5\sigma_4^2 - z\sigma_4]} \text{ , where}$$

$e^{[0.5\sigma^2 - z\sigma]}$ = acute WLA multiplier
$e^{[0.5\sigma_4^2 - z\sigma_4]}$ = chronic WLA multiplier
$z =$ 2.326 for the 99th percentile occurrence probability for the LTA is recommended

5. Determine the lower (more limiting) long-term average discharge condition (LTA).

$$LTA = \text{minimum (LTAa,c or LTAc)}$$

6. Calculate the maximum daily permit limit (MDL) and average monthly permit limit (AML) using the lower (more limiting) long-term average discharge condition.

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

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

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

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

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

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

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

n = number of samples/month

Following these procedures, the maximum daily limit (MDL) and average monthly limit (AML) may be then incorporated into the permit as justifiable water quality-based effluent limitations.

EXAMPLES

No Dilution Available

This first example is a publicly owned treatment works (POTW) discharging to an effluent-dominated stream. The example shows the steps that a permitting authority would take to establish a water quality-based effluent limit for WET. Examples showing how it was determined that this POTW discharge needs a limit for WET are contained in Appendix J, Establishing Reasonable Potential.

General site description and information. This facility discharges up to 5.8 MGD. Based on the available information, the acute to chronic ratio (ACR) is 10. The CV, based on available data, is 0.7, the water quality criterion for chronic toxicity is 1.0 TUc, and the acute criterion for acute toxicity used is 0.3 TUa. The State water quality standards allow an assumption of complete mix.

Determine wasteload allocations. The WLA is used to determine the level of effluent concentration that will comply with water quality standards in receiving waters. Using the information available on dilution, WLAs were calculated for WET using the complete mix equation:

$$WLA\ (Cd) = ([Cr(Qd+Qs)] - [(Cs)(Qs)])/Qd$$

Since this is an effluent-dominated situation, and background concentration Cs is set to zero, the equation simplifies to

$$WLA = Cr[(Qd+Qs)/Qd]$$

$$WLAa = 0.3 \cdot 1 = 0.3\ TUa$$

$$WLAa,c = WLAa \cdot ACR = 0.3 \cdot 10 = 3.0\ TUa$$

Calculate long term averages (LTAs). The process for calculating LTAs for toxicity is the same as for chemical-specific pollutants except for the additional step of needing to express the WLA for acute toxicity in equivalent chronic toxic units by multiplying by the ACR of 10.

$$LTAa,c = WLAa,c \cdot e^{[0.5\sigma^2 - z\sigma]}$$

LTAa,c = 3 x .281, where

.281 is the acute WLA multiplier for CV = 0.7 at the 99th percentile (from Table 5-1, p. 102 of the TSD)

LTAa,c = .843 TUc

$$LTAc = WLAc \cdot e^{[0.5\sigma_4^2 - z\sigma_4]}$$

LTAc = 1 x .481, where

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

LTAc = 0.481 TUc

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

Calculate the maximum daily limit (MDL). Using the equations given above in step 5, the MDL is calculated as:

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

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

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

MDL = .481 x 3.56 (from the LTA multiplier in Table 5-2, on p. 102 of the TSD)

MDL = 1.7 TUc

Calculate the average monthly limit (AML). Using the equations in step 5, the 95th percentile and monthly sampling, the AML is calculated as:

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

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

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

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

AML = .481 x 1.65, where

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

AML = 0.8 TUc

APPENDIX L

QUALITY ASSURANCE PROGRAMS

STATUTORY AND REGULATORY CONSIDERATIONS

Discharge Monitoring Report-Quality Assurance (DMR-QA)

The DMR-QA program evaluates the ability of a major NPDES permittee to analyze and report data. This program is intended to improve overall analytical performance for self-monitoring data. Authority for requiring participation is granted under section 308(a) of the Clean Water Act. In the DMR-QA program, major permittees who have effluent toxicity limits or effluent toxicity self-monitoring requirements are required to analyze "blind" reference toxicant samples with the type of toxicity test required in their NPDES permit. The permittees' results are compared to the true value and an evaluation of the reported data is sent to the permittees. Permittees are expected to use the same personnel and methods employed for reporting NPDES data to analyze the samples. Permittees are required to follow the instructions for reporting results and include a signed certification statement in accordance with 40 CFR Part 122.22.

Toxicity samples, unlike the chemistry samples, are shipped directly to the laboratory performing the tests, either an in-house laboratory or a commercial laboratory. The list of toxicity support laboratories is generated from the information received from the announcement letters sent to the permittees. It is the permittee's responsibility to notify the laboratory that they will be receiving the toxicity samples. The laboratories are only required to perform the type of tests required in the permit, not all of the tests available.

Both the *permittee* and the *support laboratory* are responsible for submitting the toxicity test results by the designated due date. Support laboratories must submit results to the permittee and the EPA contractor coordinating the DMR-QA study. Permittees that perform their own toxicity tests are required to submit their data twice, once on the toxicity data report form and once on the permittees data report form. Instruction packages received by both the permittee and laboratory contain the data report forms and further instructions on reporting requirements.

WET testing DMR-QA results are compiled annually by the EPA contractor coordinating the study. Permittees, EPA Regional Offices, and State coordinators receive performance evaluation reports on the DMR-QA study results approximately 5 months after the data is reported. Regulatory agencies (states and EPA) can conduct follow-up investigations to address poor or incomplete DMR-QA results, failure to participate, or late submittal of DMR-QA results.

Permittees (or contract support laboratories) that receive reports evaluating their results as "not acceptable" or "unusable" must submit a written response explaining the reason(s) for these results. This letter should be submitted to the state and/or regional DMR-QA coordinator.

The general schedule for the DMR-QA study is outlined below. Tasks in italics indicate those tasks to be conducted by the EPA contractor coordinating the DMR-QA study; those in normal format are those tasks required by the permittee. Since the study schedule spans two fiscal years, years one and two are labeled as FY 1 and FY 2.

TABLE L-1. DMR-QA STUDY MILESTONES

ACTION ITEM:	DUE DATE:
Study announcement letter sent to participants	*November FY 1*
Name & address of toxicity laboratory performing tests submitted to EPA contractor	Late December FY 1
Samples shipped to participants	*January - February FY 2*
Analyses performed	Approximately 7 weeks
Results from participants due to EPA contractor	March - April FY 2
Report mailed to participants and DMR-QA Coordinators	*August - September FY 2*
Corrective action letters (written response) due to study coordinator	October FY 2

Contacts. Technical assistance with toxicity test conditions, data reporting, and instructions assistance should be addressed to John Helm, the EPA Headquarters contact for the toxicity testing DMR-QA program, at (202) 564-4144 (EST).

The EPA contractor coordinating the DMR-QA study from September 29, 1994 to September 30, 1999 is ManTech Environmental Technology, Inc. The contractor should be contacted for study schedule, sample shipment, and the availability of additional reference toxicants. The ManTech contact for regional and state coordinators is Terry Bundy at (919) 818-5743 (EST). The ManTech contact for permittees is Stewart Nicholson at (919) 406-2164 (EST).

The Regional coordinator or state coordinator should be contacted for the study schedule, corrections in permittee information, and technical assistance. The state and EPA Region contacts are listed below.

TABLE L-2. DMR REGION 9 STATE COORDINATORS

Region 9 Carolyn Tambwekar, USEPA Laboratory 1337 S. 46th St., Bldg. 201 Richmond, CA 94804-4698 (510) 412-2383	Hawaii Randy Chow State Laboratories Division Department of Health P.O. Box 3378 Honolulu, HI 96801 (808) 586-4501
American Samoa Executive Secretary Environmental Quality Commission American Samoa Government Pago, Pago, AS 96799 (684) 633-2304	Nevada Wendall McCurry Division of Environmental Protection Department of Health 201 South Fall Street Carson City, NV 89701 (702) 687-4670
Arizona Wynand Nimmo Division of State Lab Services 1520 West Adams Street Phoenix, AZ 85007 (602) 542-1188	Northern Islands Russell Mechem Division of Environmental Quality Mariana Island P.O. Box 1304 Saipan, CM 96950 (670) 234-1003
California Bill Ray State Water Resources Control Board P.O. Box 944213 Sacramento, CA 94244-2130 (916) 657-1123	

TABLE L-3. DMR REGION 10 STATE COORDINATORS

Alaska, Idaho Lisa Macchio Regional Coordinator US EPA - WD 135 1200 Sixth Street Seattle, WA 98101 (206) 553-1834	Washington Stewart Lombard Washington State Department of Ecology Quality Assurance Section P.O. Box 488-2350 Colchester Manchester, WA 98353 (206) 895-4649
Oregon Renato Dulay (Industrial) Judy Johndohl (Municipal) Department of Environmental Quality Executive Building 811 SW Sixth Avenue Portland, OR 97204 (503) 229-6896	

Arizona Program

The Arizona Department of Health Services, Office of Environmental Laboratory Licensure and Certification shall license laboratories to perform aquatic toxicity tests on wastewater samples. The licensing is mandated by law in Arizona Revised Statutes, Title 36, Public Health and Safety, Chapter 4.3, Article 1, Section 36-495 to 36-495.16. Certification of methods for acute and chronic toxicity testing of effluent water will be dependent upon which manual is referenced in the facilities' NPDES permit(s). Laboratories requesting licensure for acute and chronic wastewater tests must conclude the application process and pass an on-site survey.

The initial on-site survey is immediately scheduled after concluding the application process. The survey will include a review of data (historical and present), standard operating procedures, EPA DMR-QA studies and quality assurance procedures. To maintain licensure, on-site surveys are performed annually.

A report containing statements of deficiencies and recommendations listing areas in which the laboratory was deficient during the on-site survey would be sent within 30 working days. The laboratory would then be licensed if there are no deficiencies, or after the deficiencies have been resolved. Interested parties should conduct Arizona Department of Health Services at (602) 255-3454 for additional information or to obtain an application.

California Program

The California Department of Health Services, Environmental Laboratory Accreditation Program (ELAP) shall certify laboratories to perform aquatic toxicity tests on wastewater samples. Certification of laboratories is mandated by law in Title 22, CCR, Division 4, Chapter 19, Sections 64801-64827. Certification is available for all types of acute and chronic wastewater tests that are currently (1/12/95) required by the State Water Resources Control Board.

Three steps must normally be completed to obtain ELAP registration to conduct aquatic toxicity tests. The process includes application submission, site inspection and data review, and resolution of deficiencies.

Following acceptance of a laboratory's application to the program, a site inspection is performed. As part of the audit, historical test data, written laboratory procedures and reference toxicant control charts are reviewed. Also, if performance evaluation results are available from a study such as the EPA DMR/QA, they will also be reviewed for the determination of competence to conduct aquatic toxicity tests.

A report evaluating any deficiencies found during the entire audit will be sent to the participating laboratory. Following resolution of all significant deficiencies, a laboratory will be registered by ELAP for aquatic toxicity testing.

Laboratories are reviewed biennially to maintain registration by ELAP. Interested parties should conduct ELAP at (510) 540-2800 or (916) 323-4769 for additional information or to obtain an application.

Washington Program

The Washington State Department of Ecology Environmental Laboratory Accreditation Program was authorized by the Revised Code of Washington (RCW) 4321A.230 in 1987. Subsequently, Washington Administrative Code (WAC) 173-50 established the accreditation program primarily for environmental laboratories that submit data to the Department of Ecology. The program is administered by the QA Section.

The requirements for use of accredited laboratories for reporting discharge monitoring data are in other WACs that regulate the state and NPDES permit programs. The Department of Ecology also has a policy of requiring managers responsible for ordering laboratory services to use accredited laboratories whenever possible.

The program currently covers waters and water-related (e.g., sludge and sediments) tests. Accreditation is by specific method in the categories of general chemistry, trace metals, organics,

radiochemistry, microbiology and bioassay. Fees are charged by method and parameter, with a maximum fee for each category. In some cases, other avenues of accreditation may be available at reduced fees. Third-party accreditation can be recognized, such as by the American Association for Laboratory Accreditation and the Army Corps of Engineers. In addition, certification by the Washington Department of Health can be recognized for specific methods, and reciprocity agreements have been established with three other states. No laboratories have yet been accredited for toxicity tests by third-party agreement or reciprocity.

The QA section assists all laboratories participating in the accreditation program to the extent resources allow. The process begins with submission of an application and payment of fees. Out-of-state laboratories will also be required to pay actual travel costs. The application describes personnel, equipment, facilities, and other aspects of laboratory capabilities. An acceptable quality assurance document should also be submitted at this time, and laboratories must have acceptable performance evaluation sample results if such samples are available. The final step is the on-site audit of the laboratory. Emphasis in the audit is on documentation. In particular, auditors examine documentation in the laboratory to verify procedures specified in the quality assurance manual for sample handling, analysis, and data handling are being carried out. The accuracy of information provided in the application is also verified. Generally, on-site audits address: personnel, facility and equipment, sample management, data management, quality assurance and quality control, and methods being used.

A narrative audit is subsequently sent to the laboratory. Problems are identified and specific recommendations for resolution made. The narrative audit identifies actions which must be completed before accreditation can be granted. If accreditation is warranted, the laboratory is issued a certificate and scope of accreditation listing the methods for which it is certified. Accreditation is by parameter and method, so a laboratory may be accredited for some requested methods, but not for others.

Accreditation is normally for a period of one year. To maintain its accreditation, a laboratory must: continue to successfully analyze performance evaluation samples twice yearly; report significant changes in personnel status; submit any updates of the QA document; and submit a new application and renewal fees yearly. Laboratory re-audits are conducted at a normal frequency of once every three years.

APPENDIX M

SUGGESTED GENERIC TRE WORKPLANS

GENERIC TOXICITY REDUCTION EVALUATION WORKPLAN (TRE)
INDUSTRIAL

1. Information and Data Acquisition
   a. Regulatory information
      i. NPDES permit limits
      ii. Trigger
   b. Facility monitoring data
      i. NPDES monitoring data
      ii. In-house monitoring data
      iii. State agency monitoring data
   c. Plant and Process Description
      i. Process and treatment plant description
         (1) numbers and types of streams
         (2) their size
         (3) scheduled changes or events in process stream operation
         (4) types and configurations of equipment
         (5) flow equalization facilities
         (6) records of treatment plant upsets
      ii. Physical/chemical monitoring data
         (1) chemical analyses of process streams
         (2) physical/chemical analyses of treatment streams

2. Housekeeping
   a. Initiation of housekeeping study
      i. Identify areas which may contribute to toxicity
      ii. Reduce these contributions through best management practices (BMPs), administrative, and procedural controls
   b. Evaluation of housekeeping practices
      i. Review of plant policies
      ii. "Walk-through" inspection
   c. Identification of potential problem areas
      i. Probability of release of toxic material
      ii. Type and frequency of release which may occur
      iii. Quantity of toxic substances involved
      iv. Toxicity of substances released
      v. Potential downstream impact of the substances released
      vi. Effect of release on final effluent
   d. Identification of corrective measures
      i. Area cleanup
      ii. Process or operational changes
      iii. Material loss collection and recovery
      iv. Chemical and biological testing of contained waters prior to release from diked storage areas
      v. Increased storage capacity for contained waters
      vi. Equipment modifications or changes
   e. Selection of corrective measures
   f. Implementation of corrective measures

3. Treatment Plant Optimization
   a. Evaluation of influent wastestreams
      i. Raw chemicals or materials used in the process
      ii. Byproducts or reaction products produced during the process
      iii. Reaction vessels, valves, piping systems, overflow points, and other mechanical aspects of the system
      iv. Wastestreams produced, volumes, and routing paths

v. Non-point sources

b. Description and evaluation of the treatment system
   i. Design basis for each constituent, including variability in flow conditions and concentrations
   ii. Treatment sequence
   iii. Performance projections by constituents
   iv. Operational flexibility of each process
   v. Treatment objectives and projected effluent standards

c. Analysis of treatment system operation
   i. Flow loading
   ii. Mass loading
   iii. Frequency and impact of shock loadings
      (1) normal cleaning and maintenance
      (2) spills and upsets
   iv. Changes in operating procedures

4. Chemical optimization

a. Information gathering
   i. Examination of wastestreams produced by specific production processes
   ii. Chemicals and raw materials and their contaminants and by-products used in the process
   iii. Chemicals used in treatment
   iv. Chemicals and material use rates
   v. Percentage of chemical in final product
   vi. Chemical reuse and waste recycling activities

b. Process chemical review
   i. List all chemicals used
   ii. List all quantities
   iii. Determine pounds per product
   iv. Determine pounds per gallon of wastewater discharged

c. MSDS information review
   i. Obtain MSDS for all process chemicals discharged
   ii. Highlight MSDS sections on aquatic toxicity
   iii. Examine Hazardous Ingredient section and note "hazardous substances" listed
   iv. Categorize all chemicals by hazard and irritation potential and use standard references to obtain aquatic toxicity information, if possible

d. Chemical composition screen of incoming raw materials

e. Outcome of chemical optimization phase
   i. List of all chemicals used in processing and manufacturing the product
   ii. MSDS and literature reviews will be on file when needed
   iii. List of all chemicals and raw material purchased on a monthly basis and a record of production volumes during the same time period

GENERIC TOXICITY REDUCTION EVALUATION (TRE) WORKPLAN
POTW

1. Information and Data Acquisition
   a. Operations and performance review
      i. NPDES permit requirements
         (1) Effluent limitations
         (2) Special Conditions
         (3) Monitoring data and compliance history
      ii. POTW design criteria
         (1) Hydraulic loading capacities
         (2) Pollutant loading capacities
         (3) Biodegradation kinetics calculations/assumptions
      iii. Influent and effluent conventional pollutant data
         (1) Biochemical oxygen demand (BOD_5)
         (2) Chemical oxygen demand (COD)
         (3) Suspended solids (SS)
         (4) Ammonia
         (5) Residual chlorine
         (6) pH
      iv. Process control data
         (1) Primary sedimentation-hydraulic loading capacity and BOD ans SS removal
         (2) Activated sludge-Food -to-microorganism (F/M) ratio, mean cell residence time (MCRT), mixed liquor suspended solids (MLSS), sludge yield, and BOD and COD removal
         (3) Secondary clarification-hydraulic and solids loading capacity, sludge volume index and sludge blanket depth
      v. Operations information
         (1) Operating logs
         (2) Standard operating procedures
         (3) Operations and maintenance practices
      vi. Process sidestream characterization data
         (1) Sludge processing sidestreams
         (2) Tertiary filter backwash
         (3) Cooling water
      vii. Combined sewer overflow (CSO) bypass data
         (1) Frequency
         (2) Volume
      viii. Chemical coagulant usage for wastewater treatment and sludge processing
         (1) Polymer
         (2) Ferric chloride
         (3) Alum

b. POTW influent and effluent characterization data
   i. Toxicity
   ii. Priority pollutants
   iii. Hazardous pollutants
   iv. SARA 313 pollutants
   v. Other chemical-specific monitoring results
c. Sewage residuals (raw, digested, thickened and dewatered sludge and incinerator ash) characterization data
   i. EP toxicity
   ii. Toxicity Characteristic Leaching Procedure (TCLP)
   iii. Chemical analysis
d. Industrial waste survey (IWS)
   i. Information on IUs with categorical standards or local limits and other significant non-categorical IUs
   ii. Number of IUs
   iii. Discharge flow
   iv. Standard Industrial Classification (SIC) code
   v. Wastewater flow
      (1) Types and concentrations of pollutants in the discharge
      (2) Products manufactured
   vi. Description of pretreatment facilities and operating practices
   vii. Annual pretreatment report
   viii. Schematic of sewer collection system
   ix. POTW monitoring data
      (1) Discharge characterization data
      (2) Spill prevention and control procedures
      (3) Hazardous waste generation
   x. IU self-monitoring data
      (1) Description of operations
      (2) Flow measurements
      (3) Discharge characterization data
      (4) Notice of slug loading
      (5) Compliance schedule (if out of compliance)
   xi. Technically based local limits compliance reports
   xii. Waste hauler monitoring data and manifests
   xiii. Evidence of POTW treatment interferences (i.e., biological process inhibition)

APPENDIX N

SAMPLE FACT SHEET LANGUAGE

OPTION 1

Under 40 CFR 122.44(d), permits must contain limits on whole effluent toxicity when a discharge has reasonable potential to cause or contribute to an exceedance of the water quality standard ("reasonable potential"). Toxicity testing requirements and limits as contained in Item ____ on Page ___ and in Part ___ have been included to ensure that the effluent from Outfall(s) ___ conform(s) with appropriate State water quality standards and/or regulations, and/or Regional guidance as contained in the document, "Regions 9 and 10 Guidance for Implementing Whole Effluent Toxicity Testing Programs", dated May 31, 1996, as appropriate. Due to the intermittent nature of the discharges from this facility, acute whole effluent toxicity (WET) conditions have been included. Because acute WET limits were in the previous NPDES permit, §402(o)(1) of the CWA is applicable for Outfall 001. Because no acute WET data are available for Outfall 002, monitoring only will be required. Since it is possible that a discharge may last more than 4 days, chronic WET monitoring provisions have also been included for both outfalls. For Outfall 001, if reasonable potential to exceed appropriate State water quality standards and/or regulations is found to exist, the permit may be reopened to include a chronic WET limit. For Outfall 002, if reasonable potential to exceed appropriate State water quality standards and/or regulations is found to exist, the permit may be reopened to include an acute and/or chronic WET limit, as appropriate. EPA notes that the State has not granted a mixing zone for chronic WET to this facility. Until such time as a mixing zone is granted for this parameter, EPA will evaluate the chronic WET monitoring results and base reasonable potential on 100% effluent, at the end of the pipe. The inclusion of a chronic whole effluent toxicity limit in the permit is authorized and required by 40 CFR §122.44(d)(1)(v). The inclusion of an acute whole effluent toxicity limit in the permit is authorized and required by 40 CFR §122.44(d)(1)(iv).

For chronic testing, the permittee is required to perform the following tests: *Pimephales promelas* (fathead minnow), larval survival and growth test and *Ceriodaphnia dubia*, three-brood, 7-day survival and reproduction test. Either static renewal or flow-through testing may be used. For acute testing, the permit requires a 96-hour LC_{50} test using *Onchorynchus kisutch* (coho salmon).

The permit allows for a reduction in monitoring frequency to a specified frequency if no individual test result is greater than the target (or limit) divided by the reasonable potential factor (based on number of samples required for monitoring). The target is equal to the criterion times the dilution allowed (in this case, the target equals 1.0 TUc times 100, or 100 TUc). Since quarterly monitoring is required for the first year, the reasonable potential factor from Table 3-1 in the TSD is 4.7 (at a CV of 0.6 and 4 samples). If no test results are greater than the value specified above (21.3 TUc), it would be reasonable to assume that the discharge has low probability of causing an impact to receiving waters. If there are no significant changes to the facility, a reduced frequency would be appropriate. The TSD recommends that if no reasonable potential exists, that monitoring be conducted once before permit reissuance. If there is a limit for WET, the minimum monitoring frequency allowed is annual.