STATE WATER RESOURCES CONTROL BOARD
RESOLUTION NO. 91-82

APPROVAL OF CALIFORNIA REGIONAL WATER QUALITY CONTROL BOARD, SAN FRANCISCO BAY REGION MODIFIED GUIDELINES FOR THE EFFLUENT TOXICITY CHARACTERIZATION PROGRAM

WHEREAS:

1. On December 17, 1986, the California Regional Water Quality Control Board, San Francisco Bay Region (San Francisco Bay Regional Board) adopted Resolution No. 86-14 establishing an Effluent Toxicity Control Program (Control Program) in the Water Quality Control Plan for San Francisco Bay Basin, and the State Water Resources Control Board (State Board) approved the Control Program on May 21, 1987 (Resolution No. 87-49).

2. The first element of the Control Program is the Effluent Toxicity Characterization Program (Characterization Program) which requires that toxicity of selected effluent discharges be characterized.

3. The San Francisco Bay Regional Board adopted Characterization Program Guidelines on August 19, 1987 through Resolution No. 87-107 to implement the Characterization Program.

4. On April 21, 1988, through Resolution No. 88-50, the State Board approved the Characterization Program Guidelines with the condition that the Control Program maintain consistency with future statewide toxicity control programs.

5. Recent Characterization Program results, participant input, and the need to maintain consistency with the California Ocean Plan, the California Inland Surface Waters Plan, and the California Enclosed Bays and Estuaries Plan required that the Characterization Program Guidelines be modified.

6. The Modified Guidelines will result in a more cost-effective Characterization Program and will be more responsive to the biomonitoring needs of the San Francisco Bay Region.

7. On May 15, 1991, the San Francisco Bay Regional Board adopted the Modified Guidelines (Attachment 1) under Resolution 91-083 (Attachment 2) to ensure consistency with adopted statewide toxicity control programs as required by the State Board.

8. Subsequent to adoption of Resolution 91-083, the Bay Area Dischargers Association expressed specific concerns regarding Toxicity Identification Evaluation and Toxicity Reduction Evaluation (TIE/TRE) protocols for Publicly-Owned Treatment Works (POTWs); therefore, by memorandum of August 19, 1991 (Attachment 3), the Executive Officer of the San Francisco Bay Regional Board requested the State Board to approve the

Modified Guidelines with the exception of the provisions of concern in Section C-3.b.15 (Dilution Assessments) and Section D [Criteria for Requiring a Toxicity Reduction Evaluation (TRE)].

9. This project involves guidelines for conducting toxicity characterization studies and, as such, is exempt from the provisions of the California Environmental Quality Act in accordance with Title 14, California Code of Regulations, Chapter 3, Section 15306.

10. San Francisco Bay Regional Board guidelines require State Board approval pursuant to Section 13245.5 of the Porter-Cologne Water Quality Control Act.

THEREFORE BE IT RESOLVED:

That the State Board:

1. Approves the San Francisco Bay Regional Board's Modified Guidelines for the Effluent Toxicity Characterization Program as adopted by the San Francisco Bay Regional Board through Resolution 91-083 on May 15, 1991 with the exception of Section C-3.b.15 (Dilution Assessments) and Section D [Criteria for Requiring a Toxicity Reduction Evaluation (TRE)].

2. Understands that the San Francisco Bay Regional Board will reconsider the provisions of the Modified Guidelines regarding Dilution Assessments and Toxicity Reduction Evaluations during the forthcoming Basin Plan review process.

CERTIFICATION

The undersigned, Administrative Assistant to the Board, does hereby certify that the foregoing is a full, true, and correct copy of a policy duly and regularly adopted at a meeting of the State Water Resources Control Board held on September 5, 1991.

Maureen Marché
Administrative Assistant to the Board

CALIFORNIA REGIONAL WATER QUALITY CONTROL BOARD
SAN FRANCISCO BAY REGION

RESOLUTION 91-083

Modified Guidelines for the Effluent Toxicity Characterization Program

I. Whereas, on December 17, 1986, the Regional Board adopted Basin Plan Amendments including an Effluent Toxicity Characterization Program; and, on May 21, 1987, the State Board also adopted the Program; and,

II. Whereas, the Regional Board adopted Guidelines for the Effluent Toxicity Characterization Program on August 19, 1987, to implement its approach to using biomonitoring procedures for evaluating effluent toxicity; and, on April 23, 1988, the State Board conditionally approved the Guidelines; and,

III. Whereas, Program results to date and discussions with past and prospective Program participants indicate a need to refine the Program's requirements to make them more cost-effective and responsive to the Region's biomonitoring needs, and,

IV. Whereas, this Regional Board has determined that there are no State mandated local costs under Section 2231 of the Revenue and Taxation Code as a result of the foregoing regulation because such regulation is not an executive regulation by virtue of Section 2209 of the Revenue and Taxation Code, now

V. THEREFORE, BE IT RESOLVED that this Regional Board adopts the modified guidance set forth in the attached document "Modified Guidelines for the Effluent Toxicity Characterization Program", and directs the Executive Officer to transmit the guidelines to the State Board for approval.

I, Steven R. Ritchie, Executive Officer, do hereby certify the foregoing is a full, true, and correct copy of a resolution adopted by the California Regional Water Quality Control Board, San Francisco Bay Region, on May 15, 1991.

Steven R. Ritchie,
Executive Officer

ATTACHMENT 2

AUG 26 1991

MODIFIED GUIDELINES

EFFLUENT TOXICITY CHARACTERIZATION PROGRAM

DRAFT

APRIL, 1991

CALIFORNIA REGIONAL WATER QUALITY CONTROL BOARD

SAN FRANCISCO BAY REGION

COPIES AVAILABLE UPON REQUEST FROM
THE SAN FRANCISCO BAY REGIONAL BOARD

MODIFIED GUIDELINES

EFFLUENT TOXICITY CHARACTERIZATION PROGRAM

APRIL, 1991

CALIFORNIA REGIONAL WATER QUALITY CONTROL BOARD

SAN FRANCISCO BAY REGION

MODIFIED GUIDELINES *

for the

EFFLUENT TOXICITY CHARACTERIZATION PROGRAM

CALIFORNIA REGIONAL WATER QUALITY CONTROL BOARD
SAN FRANCISCO BAY REGION

Steven R. Ritchie,
Executive Officer

DRAFT
April, 1991

Prepared by:

Lynn Suer, Ph.D.
Program Coordinator

* Modified from S.L. Anderson et al. July 17, 1987. Proposed Guidelines. Effluent Toxicity Characterization Program. Final Draft.

ERRATA

MODIFIED GUIDELINES
for the
EFFLUENT TOXICITY CHARACTERIZATION PROGRAM

San Francisco Bay Regional Water Quality Control Board

DRAFT, April 1991

1. Appendix B. Page 4. Add the following reference to the "Protocol" column in the rows for Strongylocentrotus purpuratus and Dendraster excentricus: Dinnel et al. (1987), as adapted by Anderson, Appendix C, herein.

2. Appendix B. Page 6. Replace (16) Red Abalone . . . 20% with the following:

   (16) Red Abalone.
   a. Average larval abnormality should not exceed 20% in the reference toxicant or effluent tests.
   b. Brine control results must not be significantly different from dilution water contol results in the effluent test, using a t-test and an alpha level of 0.05.
   c. The response from the 56 micrograms/liter zinc treatment must be significantly different from the control response.
   d. The between-replicate variability must be low enough that the ANOVA Error Mean Square (MS) does not exceed 100.00 in the reference toxicant test (using arcsine transformed percentage abnormality data in degrees).

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EXECUTIVE SUMMARY

The Effluent Toxicity Characterization Program is one component of the Toxicity Control Program adopted in the 1986 Basin Plan. Dischargers in the San Francisco Bay Region that participate in the Effluent Toxicity Characterization Program, perform toxicity studies based on guidance from the Technical Support Document for Water Quality Based Toxics Control (EPA, 1991). Currently, the Regional Board requires participation in this Program under Section 13267 of the California Water Code.

The purpose of these investigations is to characterize the acute and chronic toxicity of effluents and to characterize ambient toxicity adjacent to discharge sites. There are several potential regulatory outcomes of this study-based approach to effluent toxicity. Results will be used to identify locations at which Toxicity Reduction Evaluations are warranted. Results will also be used to establish permit limits and biomonitoring requirements for NPDES permits. Toxicity limits are required in NPDES permits, according to Statewide Water Quality Control Plans (Ocean Plan (1990), and Water Quality Control Plans for Inland Surface Waters and Enclosed Bays and Estuaries (1991)).

Twenty dischargers have either completed or are currently involved in this Program, and have followed Guidelines previously adopted by the Regional Water Quality Control Board and approved by the State Board (Anderson et al., 1987). In July 1991, sixteen additional dischargers will be required to participate in this Program, and will follow the modified guidance contained herein.

The major elements and overall technical rationale for the Program remain unchanged, and are discussed is Sections II and III of this document, as well as the 1987 Guidelines. These major elements include:

1. A quality assurance/quality control testing round, for currently non-eligible laboratories, to demonstrate proficiency in toxicity testing,

2. Effluent screening to determine the three most sensitive species, and

3. Repetitive testing of the most sensitive species to evaluate the variability of the effluent.

EPA guidance (Technical Support Document, 1985) was adapted in developing the original (1987) Guidelines for this Program. Some of the adaptations have since been incorporated into the more recent (1991) version of this document. The only remaining deviation of this Program's requirements from EPA guidance is an increase in the number of species used to screen effluents discharged into the highly variable and complex, estuarine waters of San Francisco Bay.

Modifications of the 1987 Effluent Toxicity Characterization Program Guidelines, described herein, take into account information provided by interim and final

reports submitted by dischargers, as well as discussions with Program participants and interested persons. The modifications and rationale are described in Section III. Briefly, the modifications are:

1. Elimination of 96-hour acute tests from the Screening Phase.

2. Additional guidance for testing effluent in which the potential for ammonia toxicity exists.

3. Revisions of the list of recommended species for testing.

4. Reduced Variability Phase testing requirements for seasonal, non-contact cooling water, and groundwater discharges.

5. Additional guidance for study plan preparation, data review, and reporting.

The cost of performing effluent characterization studies will vary greatly among sites, as estimates range from $15,000 for a non-contact cooling water discharge conducting a Partial Study to $150,000 for a non-seasonal discharger conducting a Full Study. This cost is distributed over a period of two years.

Program quality assurance will continue to include: use of test protocols specified by EPA, ASTM and the State Water Quality Control Plans; a preliminary QA/QC round for currently non-eligible laboratories; review of study plans by Regional Board staff; the use of reference toxicants in the Variability Phase; concurrent testing of ambient toxicity by dischargers or Regional Board staff; surveillance by Regional Board staff, which may include testing of split samples; public access to data.

The criteria for requiring a TRE, established previously by the Regional Board (Anderson, 1989) will remain unchanged. These criteria include: 1) No Observed Effect Concentrations (calculated from toxicity test results) that are less than Instream Waste Concentrations (estimated from dilution studies), 2) Acute toxicity (i.e., lethality within 96 hours) exceeding Basin Plan limits, using the critical life stage tests recommended for this program, and 3) Ambient toxicity that can be linked to a specific discharge.

I. INTRODUCTION

The Effluent Toxicity Characterization Program is one component of the 1986 Basin Plan Toxicity Control Program: The four major components of the Toxicity Control Program are: 1) effluent characterization (the toxicity-based approach), 2) development of water quality objectives for specific pollutants (the chemical-specific approach), 3) system modelling and wasteload allocation, and 4) effluent limit derivation. The Program is based on the Technical Support Document for Water Quality-Based Toxics Control (EPA, 1991; subsequently referred to as the TSD). The TSD provides guidance on implementation of a national biomonitoring policy (Federal Register 49 (46): 9016-9019). EPA requires that States implement this policy, but they encourage states to adapt their guidelines for regional implementation.

The purpose of the Effluent Toxicity Characterization Program is to collect definitive data in order to predict the potential for receiving water impacts due to toxicity. Predictions are based on measurements of whole effluent toxicity and assessments of dilution. More specifically, the statistical results of laboratory toxicity tests, expessed as No Observed Effect Concentrations (NOEC's), are compared with estimates of In-stream Waste Concentrations (IWC's), based on estimates of dilution. If an NOEC value is less than the predicted IWC (NOEC < IWC), then it is concluded that an impact to receiving waters is likely. The Screening and Variability Phases of this Program are designed to reduce uncertainties with respect to species sensitivity and effluent variability, so that safety margins need not be applied in assessing risks of impacts due to toxicity.

There are several potential regulatory outcomes of this program. First, Toxicity Reduction Evaluations may be required if data indicate significant toxicity outside the zone of initial dilution (NOEC< IWC). Second, results of this program may be used to refine existing NPDES biomonitoring requirements for acute toxicity. Third, data generated by this Program will be used to derive chronic toxicity limitations and biomonitoring requirements for NPDES permits, as mandated by Statewide Water Quality Control Plans applicable to dischargers in this Region (Ocean Plan (1990) and State Water Quality Control Plans for Inland Surface Waters and Enclosed Bays and Estuaries (1991)). Mixing zone policy is currently being reviewed for application to toxicity limits, as a basis for future permit limit derivation.

Program costs will vary greatly, ranging from $15,000 for discharges of non-contact cooling water to $150,000 for non-seasonal discharges of treated effluent. These estimates are based on an average unit cost of $2,400 per chronic toxicity test with parallel reference toxicant test. The 1986 Basin Plan review process provided an opportunity for over one year of comment on the reasonableness of the Program approach and costs, and there was almost unanimous support for the Program. Some dischargers currently conduct hourly monitoring to establish compliance with a single water-quality based permit level (0.0 total residual chlorine). Failure to adopt new methods to assess and control other toxic effects of discharges that are known to harm aquatic life would constitute a failure to protect beneficial uses, such as

valuable sport and commercial fisheries. As millions of dollars have been invested in treatment plants in this region, the Effluent Toxicity Characterization Program is a reasonable requirement to evaluate the efficacy of these facilities.

The TSD (1991) provides guidance on determining the "reasonable potential" of a discharge to cause an excursion above a water quality standard. In the absence of existing toxicity data for making this determination, EPA recommends that dischargers be required to collect data 12-18 months in advance of permit development to narrow the uncertainties in determining "reasonable potential." Furthermore, the EPA recommends that the requirement to generate chronic toxicity data should not be removed, based on considerations of dilution alone (TSD, pp. 85-6, 92). The goals and requirements of this program are consistent with this guidance.

Twenty major dischargers were initially required to participate in this Program (1986 Basin Plan, Table IV-2). Program Guidelines were adopted in 1987, and testing began in 1988. Nine of these dischargers have submitted final Program reports, as of April, 1991. Results of the Program to date, as well as results of the closely linked Ambient Toxicity Characterization Program are summarized in Anderson et al. (1990).

Sixteen more dischargers will be required to participate, beginning July 1, 1991, and will follow modifications of the 1987 Guidelines, as described herein. Dischargers are included in the second group if one of four criteria are met: 1) flows exceed 10 million gallons per day, 2) discharge could impact a marsh, 3) less than 10:1 dilution, or 4) discharge contains significant industrial inputs, as indicated by a pre-treatment program, if the treatment facility is a sewage treatment plant.

In developing the 1987 Program Guidelines, Regional Board staff critically evaluated and adapted guidance provided by EPA's TSD for regional application. The application of EPA guidance is more difficult in a complex estuary than in a river or open-ocean site. Sources of added uncertainty that occur in an estuary include the greater complexity of hydrodynamic modelling, highly variable receiving water chemistry, and the paucity of toxicity test methods using estuarine species. EPA Region IX has supported the Regional Board's adaptations of the TSD in stating that "the specific adaptations made to the approach in the TSD are appropriate given the uniqueness and complexity of the Bay-Delta estuary" (EPA Comments on Proposed Guidelines, June 15, 1987. Available at Regional Board.) In addition, the 1987 Guidelines were subject to extensive public review.

The modifications of the 1987 Guidelines, described herein, incorporate knowledge gained from Program results to date. The rationale for these modifications is presented in Section III. As more data become available and are reviewed, it is possible that further modifications will be considered. Further modifications will not increase the cost of the Program, as the current Program requirements are sufficient for meeting the Program goals. Regional Board staff are cognizant, however, of the need to reduce requirements that do not produce worthwhile information, while maintaining the integrity of the Program. Regional Board staff will coordinate the Program with all participants, and will make every effort to share technical information and obtain outside review of the Program as it progresses.

II. PROGRAM FOR DEFINITIVE DATA GENERATION

The purpose of this section is to explain the required work for this Program. The technical rationale for the requirements is discussed in Section III.

A. Site definitions: A similar program will be followed at all sites, but specific Screening and Variability Phase requirements will differ for five cases:

Case 1. OCEAN DISCHARGES to locations on the outer coast.

Case 2. MARINE DISCHARGES into San Francisco Bay (receiving water salinities greater than 20 ppt approximately 75% of the time).

Case 3. BRACKISH DISCHARGES into San Francisco Bay (receiving water salinities ranging between 5 and 20 ppt approximately 75% of the time).

Case 4. GROUNDWATER DISCHARGES into surface waters.

Case 5. NON-CONTACT COOLING WATER DISCHARGES

B. General program requirements

1. QA/QC Round.

If a discharger chooses to conduct the toxicity testing for this Program in-house, or selects a contract laboratory that is not currently on the list of eligible laboratories (available from Regional Board), then the discharger or discharger representative must participate in a quality assurance test round.

2. Effluent Screening.

A Study Plan for screening the effluent will be submitted to Regional Board staff for their review. After approval of the plan, the discharger will conduct toxicity tests as a preliminary evaluation of the degree of toxicity and to determine the three most sensitive species. After testing is complete, a Screening Phase Report, summarizing the results, will be prepared and submitted.

3. Variability Testing. A Variability Study Plan for repeated testing of the effluent, using the three most sensitive species determined from Screening Phase results, will be submitted to Regional Board staff for their review. After approval of the plan by the Regional Board Executive Officer, the discharger will initiate testing. At a minimum, one interim

report and one final Variability Phase Report, summarizing the results, will be submitted.

C. Specific Requirements

1. QA/QC Round

a. Schedule (Table 1)

Participation in a QA/QC round will only be required if a discharger chooses to conduct toxicity tests in-house or with a contractor not presently listed as eligible to participate in this Program. This round will be conducted only once, in **July, 1991**, for two ranks of dischargers.

Dischargers, their contract laboratories, or any other commercial laboratory wishing to participate in a QA/QC Round must contact Regional Board staff in writing by **June 1, 1991.**

b. Procedures

The QA/QC round will involve synchronous testing, using three chronic toxicity tests specified by Regional Board staff. The discharger or representative will be given an "unknown" toxicant with which to conduct the tests. Criteria for acceptable test performance will be provided in advance of testing. If the tests are not completed successfully, then the discharger must contract with a laboratory that has successfully completed the QA/QC round.

2. Effluent Screening

a. Schedule (Table 1).

The Screening and Variabaility Phase Program requirements for the two ranks of dischargers are staggered by four months. Dischargers will submit a Screening Phase Study Plan for review by Regional Board staff according to the schedule in Table 1.

Approximately two months will be allowed for review and revision of the Study Plan before actual testing begins. Effluent screening will be conducted once and will require successful completion of a battery of standard chronic or critical-lifestage toxicity tests.

A Screening Phase report, summarizing results of the toxicity tests, must be submitted at the same time that the Variability Phase Study

Table 1. Proposed schedule for Effluent Toxicity Characterization Program 1991-1993

(Some flexibility may be allowed on a case-by-case basis)

PROGRAM REQUIREMENTS	DISCHARGER RANK	
	Rank 1	Rank 2
QA/QC Round		
Notify Regional Board of intent to participate	6/1/91	6/1/91
Initiate Testing	7/1/91	7/1/91
Submit Results	8/1/91	8/1/91
Screening Phase		
Submit Study Plan	8/1/91	12/1/91
Initiate Testing	10/1/91	2/1/92
Submit Final Report	1/1/92	4/1/92
Variability Phase		
Submit Study Plan	1/1/92	4/1/92
Initiate Testing	3/1/92	6/1/92
Submit Interim Report	9/1/92	12/1/92
Submit Final Report	6/1/93	10/1/93

Plan (Table 1) is submitted. The Screening report is due three months after initiation of Screening tests.

Flexibility in the screening phase schedule may be allowed on a case-by-case basis to allow for seasonal availability of organisms, pre-screening tests or test repetition. The technical rationale for delaying a series of test must be documented. Pre-screening tests may be necessary to refine laboratory procedures (e.g., in the case of potential ammonia effects).

b. Procedures.

2.b.1 Test species. A battery of "chronic" (primarily short-term critical life stage tests), selected from Table 2, will be conducted during the Screening Phase. In all cases, a fish, an invertebrate, and a plant must be included among the tests. The number of fresh, brackish, and salt water species that must be screened (Table 3) will depend upon receiving water salinity, as specified below, except when effluent salinities are routinely above 5 to 10 parts per thousand. In the latter case, only marine and brackish water species should be used.

OCEAN DISCHARGE: Four marine species will be tested.

MARINE DISCHARGE TO SF BAY: Four marine or brackish species and two freshwater species will be tested.

BRACKISH DISCHARGE TO SF BAY: The *Menidia beryllina* (Atlantic Silverside minnow) growth test must be conducted at all brackish sites, as it is the only standard chronic test that spans a wide range of salinities. In addition, two marine and three freshwater tests will be tested.

DISCHARGE TO FRESHWATER TRIBUTARY: Of the dischargers currently required to participate in this Program, only groundwater dischargers fit this case. These dischargers will screen their effluent using three freshwater tests: *Ceriodaphnia dubia* (water flea), *Pimephales promelas* (fathead minnow), and *Selenastrum capricornutum*. (algae).

Table 2. Species and test protocols recommended for critical lifestage toxicity testing

SPECIES	BIOLOGICAL EFFECTS EVALUATED	CALIFORNIA RESIDENT +/-	LAB-REARED VS. WILD STOCK	REFERENCE
FRESHWATER				
Ceriodaphnia sp. (Crustacean)	survival, reproduction	-	Lab cultured	EPA, 1989
Pimephales promelas (Fathead minnow)	survival, growth	+	Lab cultured	EPA, 1989
Selenastrum capricornutum (unicellular alga)	cell division rate	-	Lab cultured	EPA, 1989
MARINE				
Mysidopsis bahia (Crustacean)	survival, growth, fecundity	-	Lab cultured	EPA, 1987
Molluscs (Mytilus edulis - mussel) (Crassostrea gigas - oyster) (Haliotis rufescens - abalone)	embryo development, survival	+	Wild stock or Field cultured	ASTM, 1989 ASTM, 1989 B.S. Anderson et al., 1990
Echinoderms (Strongylocentrotus purpuratus, S. franciscanus - urchins) (Dendraster excentricus - sand dollar)	fertilization success	+	Wild stock	Dinnel et al., 1989, as adapted by Anderson (Appendix C, herein)
Diatom Plants (Skeletonema costatum) (Thalassiosira pseudonana)	cell division rate	+	Lab cultured	ASTM, 1990
Macrocystis pyrifera (giant kelp)	percent germination, germ tube length	+	Wild stock	B.S. Anderson et al., 1990
Champia parvula (red algae)	number of cystocarps	-	Lab cultured	EPA, 1987
MARINE/BRACKISH				
Menidia beryllina	survival, larval growth	+	Lab cultured	EPA, 1987

Table 3. Short-term critical life stage toxicity test requirements for Screening Phase of Effluent Toxicity Characterization Program

REQUIREMENTS	RECEIVING WATER CHARACTERISTICS			
	DISCHARGE TO COAST	DISCHARGE TO SAN FRANCISCO BAY		
	Ocean	Marine	Brackish	Freshwater
Taxonomic Diversity	1 plant 1 invertebrate 1 fish	1 plant 1 invertebrate 1 fish	1 plant 1 invertebrate 1 fish	1 plant 1 invertebrate 1 fish
Number of tests of each salinity type				
Freshwater	0	2	3	3
Brackish	0	0-1	1	0
Marine	4	3-4	2	0
Total number of tests	4	6	6	3

2.b.2. Ammonia screening. All dischargers will be required to include, in their Screening Study Plan, an evaluation of the potential for toxicity due to ammonia in receiving waters and under toxicity testing conditions.

The evaluation of impacts of ammonia on receiving waters will be based on predictions of unionized ammonia concentrations and comparisons with concentrations that are known to be toxic. Unionized ammonia concentrations will be determined from measurements of total ammonia concentrations in effluents, pH, salinity, temperature and dilution under worst case conditions. A table for calculating unionized ammonia concentrations from total ammonia is attached as Appendix A.

The evaluation of the potential for ammonia toxicity during testing will use a similar approach. Concentrations of unionized ammonia in test containers will be based on effluent ammonia concentrations and likely laboratory test conditions.

If it can be shown that ammonia **does not deleteriously impact receiving waters,** yet could exert toxic effects under toxicity test conditions, then procedures may be used to minimize these potential effects. If it is determined that ammonia could deleteriously impact receiving waters, then a Toxicity Reduction Evaluation may be warranted. Several procedures for minimizing ammonia toxicity during testing are:

- Deep water dischargers (receiving $\geq$ 10:1 dilution) may dilute their effluent prior to testing, such that the highest concentration tested is less than 100%, but no less than 60%. This will reduce ammonia concentrations and associated toxicity. In addition, procedures to minimize sample aeration and/or to control pH should be used. However, several test protocols (e.g., urchin test) have strict pH requirements and may not, therefore, be amenable to pH adjustment. Pre-screening tests should be performed to establish the most effective procedures.

- Shallow water dischargers (< 10:1 dilution) may not dilute their effluent prior to testing, but should establish procedures to minimize sample aeration and/or control pH.

Pre-screening tests should be performed to establish the most effective procedures.

- Removal of ammonia, by air-stripping or exposure to zeolite minerals, is the least preferred method for reducing ammonia toxicity during toxicity testing, as other potentially toxic pollutants of concern may also be removed. Application of these methods may be considered on a case-by-case basis.

2.b.3. Test Synchrony. All screening tests must be performed concurrently.

2.b.4. Sample Collection. Tests will be conducted using 24-hour composites of effluent. Composite samples must be collected each day for tests requiring renewals. Common composite samples should be split among all the screening tests to provide uniformity.

2.b.5. Dilution Waters. Standard dilution waters (see Section 3.b.6) will be used during effluent screening. Ambient dilution waters will only be used during Variability Phase testing.

2.b.6. Dilution Series. During the Screening Phase, the highest concentration tested must be either 100% effluent, or the highest concentration possible, given the sensitivities of the test organisms to commercial salts. Certain tests require the addition of natural brine, rather than commercial salts, because the latter exerts a toxic effect. In these cases, the highest effluent concentrations that can be conducted will be 60-70% effluent, depending upon the concentration of the natural brine.

Shallow water dischargers (with no dilution) should avoid using tests that cannot be conducted at 100% effluent, as potential impacts at In-stream Waste Concentrations (100%) could not, then, be evaluated.

Individual test protocols often specify the dilution factor that should be followed, once the maximum concentration has been determined. For example, if the test recommends a factor of 0.5, then the dilutions would be 100%, 50%, 25%, 12.5%, 6.25% and 0% (control), if 100% is the maximum effluent concentration tested.

2.b.7. Controls. For a standard dilution water test, one standard dilution water control is required. A salt control is also required, when the salinity of the test solutions is adjusted with natural seawater brine or commercial salts. Control salinity should be the same as that used in the effluent test treatments.

All controls must meet the acceptability criteria for a given test (Appendix B).

2.b.8. Salt Addition. Natural, seawater brine or commercial salts may be used to adjust salinities. Commercial salts (depending on brand, stock) can sometimes be toxic. A salt control is required for all tests using either natural brine or commercial salts.

2.b.9. Water Chemistry Measurements. Water chemistry measurements are specified by individual test protocols. However, daily ammonia and chlorine compliance measurements during the test period must also be reported by all dischargers, as appropriate.

2.b.10 Additional Protocol Refinements.. Dischargers and their contract laboratories should carefully review refinements of existing protocols that are required in conducting toxicity tests for this program (Appendix C). In particular, note this Region's required procedures for conducting the echinoderm fertilization test. Modifications or additional refinements may occur during the implementation of this Program, as additional information becomes available.

2.b.11. Reference Toxicants. Parallel reference toxicant tests are not required, but are recommended during the Screening Phase. These will be specified by the Regional Board prior to the initiation of testing.

2.b.12. Required Level of Effort for Obtaining Wild Stock.

As specified by the March 21, 1990 Status Report to the Regional Board, a <u>minimum</u> effort to obtain spawning organisms consists of ordering 50 (oysters and urchins) or 100 (mussels) specimens from two suppliers. Documentation could consist of order forms or verification of order placed by phone (signed and dated entries in a bound notebook).

2.b.13. Data Review. Dischargers are responsible for reviewing all toxicity test results, including raw data, to assure acceptability of test results and accurate interpretation and reporting. Criteria for evaluating data are presented in Appendix C. The discharger must attach to each data submittal a signed statement that data have been thoroughly reviewed.

2.b.14. Test Repeats. Any test failures during the Screening Phase will warrant a test repeat.

2.b.15. Reporting. Toxicity test results must be submitted according to the requirements in Appendix D.

3. Variability Testing

a. Schedule (Table 1)

Dischargers will submit a Variability Phase Study Plan according to the schedule in Table 1. Approximately two months will be allowed for review and approval of the Variability Study Plan before testing is initiated.

b. Procedures

3.b.1. Species Selection. Based on the results of the Screening Phase, the three most sensitive species will comprise a test battery for repeated testing during the Variability Phase. In making the species selection, test batteries should include the most sensitive species that can be tested with ambient dilution water, the most sensitive species overall, and a species to provide phylogenetic diversity. For example, if two fish were selected for the first two categories, then the third species should be either a plant or an invertebrate. If two species are equally sensitive, then species that are available year round are preferred.

In addition, shallow-water dischargers should, if possible, select sensitive tests that can be conducted at 100% effluent. Groundwater dischargers will use the same three freshwater species used to screen their effluent (see 2.b.1).

3.b.2. Number of Test Batteries (Table 4). The number of test batteries conducted during the Variability Phase will depend upon the **degree of toxicity, discharge case type,** and the **seasonality of the discharge.**

Table 4. The number of test batteries required for Full and Partial Variability Phase studies.

RECEIVING WATER TYPE	DISCHARGE AND STUDY TYPES			
	EFFLUENT		COOLING WATER	TREATED GROUNDWATER
	Continuous Full, Partial	Seasonal Full, Partial	Full, Partial	Full, Partial
OCEAN	18, 6	NA	NA	NA
SF BAY or TRIBUTARY Marine	18, 6	# mos* + 3, (# mos +3)/3	6**, 1	NA
Brackish	18, 6	# mos + 3, (# mos + 3)/3	6**, 1	NA
Freshwater	NA	NA	NA	6, 3

* # mos = the number of months of actual discharge during the Variability Phase of this program.

** If a Full Variability Study is warranted, then the influent and effluent streams will be tested concurrently, using identical three species batteries on three separate occasions to equal six batteries.

NA = not applicable, as none of the dischargers beginning the Program in July 1991 fall into that category.

Degree of toxicity

Dischargers will perform a **Full Variability Study**, when the effluent shows significant toxicity during screening, and a **Partial Variability Study**, when there is no significant toxicity. A **Full Study** is warranted if statistically significant toxicity relative to controls occurs in any of the screening tests at 100% effluent (i.e. the NOEC < 100% effluent) or at the highest concentration that can be conducted (See 2.b.6). Statistical tests and endpoints of significance are specified in the test protocols (also see Appendix B).

If the degree of toxicity exhibited during the Screening or Variability Phases is sufficient to warrant a **Toxicity Reduction Evaluation (see 3.D)**, then Variability Phase testing will be discontinued. If the discharger completes a TRE and corrects the toxicity problem, a limited program to confirm the elimination of toxicity or to develop a water quality-based effluent limit may be initiated.

The exact number of test batteries required for **Full and Partial Studies** depends upon the Discharge Case type and Seasonality, as explained further below.

Discharge Case Type (assuming continuous discharge):

Ocean and Marine/Brackish Discharges to San Francisco Bay: Full = 18 batteries; Partial = 6 batteries.

Non-Contact Cooling Water Discharges: Full= 6 batteries; Partial = 1 battery. The full study will involve concurrent testing of influent and effluent streams, using identical, three species batteries. This concurrent testing will be conducted three times (2 batteries X 3 test periods = 6 batteries)

Groundwater Discharges: Full = 6 batteries; Partial = 3 batteries.

Seasonal Discharges:

The number of test batteries required for Full and Partial studies will be adjusted to account for the proportion of the year that the effluent is actually discharged during the Variability Phase. The total number of tests for a Full Study will be equal to the number of months of discharge during the Variability Phase plus three. For a Partial Study, the required number will be equal to one-third the number required for a Full Study. For example, the number of required tests for a nine month discharger would be 9 + 3 = 12 for a Full Study and 12 / 3 = 4 for a Partial Study.

3.b.3. The Timing of Variability Test Batteries. Test batteries should be scheduled after considering all factors that could contribute to effluent variability. These factors are more fully described in Appendix E.

3.b.4. Test Synchrony. All tests within a test battery must be performed concurrently as static renewals, using composite samples split among the three effluent tests. Short-term tests ($\leq$ 96 hours) must be initiated within the test period of the longer term tests.

3.b.5. Sample Collection. Tests will be conducted using 24-hour composites. Samples must be collected each day for tests requiring renewals, unless there are technical reasons for less frequent sampling. In no case may sample holding times exceed 48 hours.

3.b.6. Dilution Water. Standard dilution waters and salts must be used for two of the three effluent tests in a battery (Standard dilution waters are <u>always</u> required for reference toxicant tests). Standard dilution waters may be either synthetically prepared or natural waters that are documented as non-toxic. Bodega Bay seawater is recommended as a standard dilution water for marine/brackish tests. See individual test protocols for standard dilution waters for freshwater testing.

One test in a battery must be conducted with <u>ambient dilution water</u> to monitor the receiving water contribution to the toxicity of an effluent. For discharges into receiving waters with unidirectional flow, collection of ambient

dilution waters should be conducted upstream of the discharge site. For estuarine sites with tidal flow, ambient dilution water collection should be focused on periods of low tidal exchange and on slack tides, at a station that is not in the influence of the discharge. Samples should be collected approximately one meter below the surface.

It is recommended that receiving water samples be collected every day for tests requiring daily renewals. However, in cases where the collection of samples is strategically difficult, ambient samples may be collected every other day, then stored for use on the following day.

3.b.7. Salt Addition. See Section 2.b.8.

3.b.8. Controls. For a standard dilution water test, one standard dilution water control is required. A salt control is also required, when the salinity of the test solutions is adjusted with natural seawater brine or commercial salts. Control salinity should be the same as that used in the effluent test treatments.

In addition, for an ambient dilution water test, both a standard dilution water control and ambient dilution water control must be included, so that the presence of ambient toxicity can be determined. The standard dilution water control requirement is generally met by the standard water control used in the parallel reference toxicant test.

All controls must meet the acceptability criteria for a given test (Appendix C), except for ambient water controls. Ambient controls may be toxic relative to standard dilution water controls.

3.b.8. Reference Toxicant Testing. Parallel reference toxicant tests, using toxicants specified by Regional Board staff, must accompany each effluent test. Reference toxicant tests provide valuable information with which to evaluate relative sensitivies of test organisms, as well as laboratory performance. The reference toxicants will be specified prior to Screening Phase testing, based on further review of existing Program data.

Reference toxicant and effluent tests should be identical with respect to the number of replicates and test organisms.

3.b.9. Water Chemistry Measurements. See Section 2.b.9.

3.b.10 Additional Protocol Refinements. See Section 2.b.10.

3.b.11. Required Level of Effort for Obtaining Wild Stock. See Section 2.b.12.

3.b.12.. Data Review. See Section 2.b.13.

3.b.13 Test Repeats. If more than one test fails in a battery, then the entire battery must be repeated. If, however, only one test fails in a battery, then a repeat of that single test may be required. Two single test failures will be allowed over the course of Variability Phase testing without requiring repeats. After that, failure of one test in a battery for any reason will warrant a test repeat.

3.b.14. Reporting. Interim and Final Variability Phase Reports must be submitted according to the schedule in Table 1. Reporting requirements are attached as Appendix D.

3.b.15. Dilution Assessments. Dilution assessments (plume modelling, dye studies) may be required on a case-by-case basis. Regional Board staff will work with individual dischargers to design dilution assessments appropriate to an estuary. Plume modelling and estimation of initial dilution could be used to evaluate the potential for toxic impact outside the zone of initial dilution, or to distinguish the contributions of multiple discharges to observed ambient toxicity. In most cases, tracer studies should be implemented to verify modelling predictions.

D. Criteria for Requiring a Toxicity Reduction Evaluation (TRE). The criteria for triggering a TRE, specified in Anderson et al. (1989) remain unchanged. Dischargers are responsible for reporting results that indicate the need for a Toxicity Reduction Evaluation within **two months** of completion of tests indicating that need.

1. First Half of the Variability Phase (prior to submitting interim report).

a. A TRE will be required if any 6 data points indicate that the concentration of effluent outside the zone of initial dilution (i.e., IWC) is greater than the No Observed Effect Concentration (IWC > NOEC). This requirement applies to any 6 data points, whether they are obtained using different species or at different times.

b. A TRE will be required if any 3 data points indicate that the IWC is more than twice the NOEC (IWC > 2 NOEC).

2. Second Half of the Variability Phase (after submitting interim report).

During the second half of the Variability Phase (after submitting the interim report), TRE criteria are more stringent:

a. Any 3 data points for which the IWC equals or exceeds the NOEC will trigger some level of TRE.

b. TRE's will be required when the Basin Plan acute toxicity limit is exceeded, based on survival data from short-term chronic tests.

III. TECHNICAL RATIONALE FOR PROGRAM REQUIREMENTS

The technical rationale for the overall design of this Program remains unchanged and is thoroughly described in the 1987 Guidelines (Anderson et al., 1987, Section III). Briefly, the 1987 Guidelines explain the relationship between this Program's requirements and guidance provided by EPA's Technical Support Document. It emphasizes how the Program is designed to narrow margins of uncertainty in assessing risks due to toxicity. It also describes deviations from EPA guidance intended to improve risk assessments in a highly variable estuarine environment, such as San Francisco Bay. Specific topics covered in the rationale include: the inclusion of both acute and chronic tests, as well as both freshwater and marine tests in effluent screening; the use of ambient dilution waters during variability testing; methods for testing receiving waters to verify predictions based on effluent tests; and species recommended for acute, chronic and critical-lifestage toxicity testing, using static-renewal exposure methods.

This section will not repeat topics covered by the 1987 Guidelines. Instead, it will provide the rationale for modifications in Program requirements, as follows:

A. Elimination of 96-hour acute tests from the Screening Phase.

Valuable information has been obtained from acute tests used to screen eight municipal and ten industrial effluents to date (Anderson et al., 1989, Tables 1-4). More than 50% of the effluents were acutely toxic, despite the fact that dischargers were meeting NPDES acute toxicity requirements, based on 96-hour flow-through tests with two fish species. Acute toxicity was observed using diatoms (3 species), mysid shrimp (*Neomysis mercedes* and *Mysidopsis bahia*), sanddabs (*Citharichthys stigmaeus*), rainbow trout (*Onchorhyncus mykiss*), and microtox bacteria (*Photobacterium phosphoreum*).

Elimination of the acutes tests from the Screening Phase will not, however, impair this Region's ability to evaluate potential impacts of acute toxicity. This conclusion is based on the following rationale:

- The diatom tests are categorized herein as chronic tests, and remain on the list of recommended test species.

- *Neomysis mercedes* (resident mysid shrimp) is no longer on the recommended list. However, the test using *Mysidopsis bahia* (East Coast mysid shrimp), which has both survival and growth endpoints, is still included. *Neomysis* was never one of the three most sensitive species during the Screening Phase. Furthermore, it is seasonally available, whereas *Mysidopsis* is available year round from laboratory stocks.

- Rainbow trout are no longer on the recommended list of test species. However, all refineries in the San Francisco Bay Region are required to

conduct flow-through compliance biomonitoring using rainbow trout, and rainbow trout is one of three fish species that may be selected for NPDES biomonitoring by all other dischargers.

- Acute toxicity was observed using microtox bacteria in two refinery effluents. However, this species was never one of the three most sensitive species during the Screening Phase, and subsequent testing during the Variability Phase indicates that this species is relatively insensitive to discharges in this Region.

- The sanddab test is, therefore, the only sensitive acute test that is no longer on the recommended list and has no substitute. Sanddabs were among the three most sensitive tests for four of eight municipal dischargers. The rationale for removing this species is its heightened sensitivity to ammonia, as explained below.

B. Additional guidance for testing effluent with the potential for toxicity due to ammonia.

Three of the four dischargers exhibiting acute toxicity using sanddabs conducted Toxicity Identification Evaluations. In all cases, ammonia was determined to be a primary cause of acute toxicity, although other toxicants could also have exerted an effect. Two of these dischargers (EBMUD and San Francisco) demonstrated that the toxicity was associated with aeration that occurred when salts were mixed with the effluent to achieve the appropriate test salinity. One discharger (San Francisco) estimated that acute sanddab toxicity occurred at unionized ammonia concentrations as low as 0.1 mg/L.

The potential for deleterious impacts to receiving waters due to ammonia was evaluated for all four discharges. Concentrations of unionized ammonia were predicted for receiving waters under worst case conditions of pH, temperature, salinity and dilution. These concentrations were then compared with the lowest toxic concentrations cited in EPA's ammonia criteria document. In all cases, it was concluded that the potential for deleterious impacts to receiving waters was negligible.

Therefore, Regional Board staff have concluded that ammonia can, in some cases, produce toxicity test results that are not predictive of potential receiving water impacts. In these cases, the ability to characterize effects of other, persistent pollutants may be compromised by the acutely toxic effects of ammonia, expressed under certain test conditions. Since there is little evidence to suggest that the sanddab acute test is more sensitive to non-ammonia pollutants than the critical-lifestage tests recommended for testing, it has been removed from the list.

However, all dischargers participating in this Program must fully evaluate the potential for ammonia toxicity in receiving waters and under toxicity test conditions prior to effluent screening. If this evaluation indicates that ammonia is not toxic in receiving waters, but could be toxic under test conditions, then procedures may be used to minimize potential ammonia effects, as described in Section 2.b.2.

The dischargers' evaluations must be based on calculations using worst case ammonia and receiving water conditions, rather than field measurements of ammonia. The rationale for this is that worst case conditions are relatively rare, and are unlikely to occur within the timeframe allowed for Study Plan preparation.

C. Removal of *Lemna minor* (duckweed), *Laminaria saccharina* (alga), and *Lytechinus anamesus* (urchin), and addition of the algae *Macrocystis pyrifera* (giant kelp) and *Champia parvula* (red alga) and the urchin *Strongylocentrotus franciscanus* to the list of species recommended for critical life-stage testing.

These changes provide consistency with the California Ocean Plan (1990), and the California Water Quality Control Plans for Inland Surface Waters and Enclosed Bays and Estuaries (1991). The critical lifestage test, which measures germination and germ tube length of *Macrocystis pyrifera* has been recently developed by the Marine Bioassay Project and is considered ready for routine use. Although the red alga *Champia parvula* is an east coast species, it is readily available in laboratory culture and is, therefore, a practical indicator species.

D. Reduced Variability Phase testing requirements for seasonal discharges, and non-contact cooling water discharges, and groundwater discharges.

1. Seasonal Discharges

The 1987 Guidelines require municipal and industrial dischargers to conduct eighteen test batteries over a one year period for a Full Study (significant toxicity observed during effluent screening) and six test batteries for a Partial Study (no toxicity during effluent screening). This frequency of testing was based on the need to document both short-term and long-term effluent variability. One battery per month (on average) was considered sufficient to evaluate long term variability, and six batteries were added to enable characterization of short-term variability with more frequent (e.g. weekly) testing.

Seasonal dischargers will be required to test with the same testing frequency as non-seasonal dischargers so that the goal of characterizing both long and short-term variability is satisfied. For a Full Study, the required number of batteries will, therefore, be equal to the number of months of discharge (for long-term evaluation) plus three (for short-term

evaluation). As an example, a Full Study for a 9 month discharge would involve 9 + 3 = 12 test batteries; a Partial Study would involve 12/3 = 4 batteries. Dischargers may not be able to exactly predict the period of discharge during the Variability Phase of this Program. However, dischargers should plan their budgets to allow for a "typical" discharge period.

2. Non-contact cooling water discharges

The primary source of variability of cooling water discharges should be the influent water, in the absence of process wastes or chemical additions. The cooling system could contribute to toxicity through the leaching of metals. However, this source of toxicity is expected to be relatively invariable, and should require relatively little repeated testing to characterize.

Therefore, dischargers of non-contact cooling water will be required to screen their discharge for the presence of toxicity. If toxicity is observed, then six additional test batteries will be required, using the most sensitive three species. Influent and effluent streams will be tested concurrently, using identical test batteries, to determine the relative contributions of source water vs. the cooling system to toxicity. This concurrent, parallel testing will be repeated three times. If toxicity is not observed during the Screening Phase, dischargers of non-contact cooling water will conduct only one follow-up test battery, using the three most sensitive species.

3. Treated groundwater discharges.

Treated groundwater discharges should be less variable than treated municipal and industrial wastes, since the influent stream (extracted groundwater) is relatively invariable. However, treatment processes could significantly increase variability. Therefore, Variability Phase testing requirements for groundwater dischargers should be intermediate between those for non-contact cooling water discharges and treated wastewater discharges.

The 1987 Guidelines and subsequent correspondence established reduced Program requirements for groundwater discharges. These involved quarterly testing, using a battery of three freshwater chronic tests. Additional tests would be required, if significant toxicity was observed. The testing requirements for groundwater dischargers, described herein (Section II), are similar to the earlier requirements, except that the first test battery has been designated as a screening battery, which will determine the number of subsequent test batteries.

E. Additional specifications regarding data review.

Each discharger must designate, in their study plans, one or more persons responsible for reviewing test data. Each submitted report will include a signed statement that the toxicity test data, including raw data, have been thoroughly reviewed to determine if Program requirements have been met, and toxicity results are valid according to test protocols. Criteria for acceptable tests are summarized in Appendix B.

Regional Board staff have found that although the quality of testing and reporting has been generally high, errors and misinterpretations of test results are not uncommon. In some cases, these can affect decisions regarding the need for a Toxicity Reduction Evaluation.

F. Additional specifications regarding data reporting.

Previous reporting requirements were specified in Anderson (1989) and Anderson et al. (1990a). These included 1) summary tables, 2) summary graphs, 3) hard copies of raw test data (laboratory benchsheets), and 4) raw data entered electronically onto computer disks in Toxstat format.

These requirements remain unchanged with one exception: Summary information for each toxicity test must be entered, in tabular form, onto a spreadsheet which has specific headings and instructions for data entry (Appendix D). At a minimum, dischargers must submit hardcopies of this spreadsheet with all information correctly entered. Dischargers are strongly encouraged to enter the information electronically onto 3.5" double-sided, double-density floppy disks, using Excel 2.2 software for the MacIntosh computer or 5 1/4 inch DOS formatted DS/DD 360 K diskettes, using Lotus software for an IBM compatible computer.

This spreadsheet takes the place of the tabular format (1 above) previously required, and is urgently needed to provide a consistent framework for reporting Program information. Previous submittals have often contained incomplete information and reporting formats have been extremely variable. It is important to correct this situation in order to enhance the timeliness of data review, facilitate in-depth analyses of Program results, and increase public access to Program information.

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V. LIST OF APPENDICES

Appendix A. Table for calculating the unionized fraction of ammonia (NH_3) from total ammonia concentrations.

Appendix B. Criteria for acceptable test results

Appendix C. Required and recommended technical refinements of toxicity test protocols.

Appendix D. Toxicity test data reporting requirements.

Appendix E. Information on Variability Phase Study Plans submittals

APPENDIX A

APPENDIX A

Instructions for calculating the unionized fraction of ammonia from total ammonia concentrations.

From: San Francisco Bay Regional Water Quality Control Plan (1982)

The water quality objective for un-ionized ammonia (NH_3) was established for the un-ionized fraction rather than total ammonia ($NH_3 + NH_4^+$) because it is far more toxic than the ionized fraction (NH_4^+). Unfortunately, it is impossible to directly measure the un-ionized fraction of ammonia in the relevant concentration range. However, it can be calculated from easily measured quantities: total ammonia, pH, TDS or salinity, and temperature. The basic equation is:

$$\text{un-ionized ammonia} = NH_3\text{-}N = \frac{\text{Total Ammonia}}{1 + 10^X}$$

where $X = pKa - pH$

Table gives pKa values for different temperature and TDS (or salinity) conditions.

TABLE OF AMMONIA PKa AS A FUNCTION OF TEMPERATURE AND TDS OR SALINITY

TEMP	TOTAL DISSOLVED SOLIDS, TDS MG/L								SALINITY, G/KG						
C	0	250	500	750	1000	1500	2000	3000	5	10	15	20	25	30	35
0	10.091	10.118	10.131	10.141	10.149	10.162	10.172	10.159	10.192	10.212	10.229	10.240	10.253	10.261	10.263
1	10.049	10.081	10.095	10.105	10.113	10.126	10.136	10.152	10.156	10.175	10.193	10.207	10.217	10.224	10.227
2	10.009	10.045	10.059	10.069	10.077	10.090	10.100	10.116	10.119	10.139	10.156	10.170	10.181	10.187	10.190
3	9.973	10.010	10.023	10.033	10.041	10.054	10.065	10.081	10.083	10.103	10.120	10.134	10.145	10.151	10.154
4	9.938	9.975	9.988	9.998	10.006	10.019	10.029	10.045	10.047	10.067	10.084	10.096	10.109	10.116	10.118
5	9.903	9.940	9.953	9.963	9.971	9.984	9.994	10.010	10.012	10.032	10.049	10.053	10.073	10.080	10.083
6	9.868	9.904	9.915	9.928	9.936	9.949	9.959	9.975	9.976	9.998	10.013	10.027	10.038	10.044	10.047
7	9.833	9.869	9.880	9.893	9.901	9.914	9.924	9.940	9.941	9.961	9.978	9.992	10.002	10.009	10.012
8	9.796	9.835	9.848	9.858	9.866	9.879	9.890	9.906	9.906	9.926	9.943	9.957	9.966	9.974	9.977
9	9.764	9.801	9.814	9.824	9.832	9.845	9.855	9.871	9.871	9.891	9.908	9.922	9.933	9.939	9.942
10	9.730	9.767	9.780	9.790	9.798	9.811	9.821	9.838	9.837	9.857	9.874	9.888	9.895	9.905	9.908
11	9.696	9.733	9.746	9.756	9.764	9.777	9.788	9.804	9.804	9.822	9.839	9.853	9.864	9.871	9.873
12	9.662	9.699	9.713	9.723	9.731	9.744	9.754	9.770	9.768	9.788	9.805	9.819	9.830	9.837	9.839
13	9.629	9.666	9.680	9.690	9.698	9.711	9.721	9.737	9.735	9.755	9.772	9.786	9.796	9.803	9.806
14	9.597	9.633	9.647	9.657	9.665	9.678	9.688	9.704	9.701	9.721	9.738	9.752	9.763	9.770	9.772
15	9.564	9.601	9.610	9.624	9.632	9.645	9.655	9.671	9.668	9.688	9.705	9.719	9.730	9.737	9.739
16	9.531	9.567	9.581	9.591	9.599	9.612	9.622	9.638	9.635	9.654	9.672	9.686	9.696	9.703	9.706
17	9.498	9.535	9.546	9.558	9.566	9.579	9.589	9.605	9.604	9.621	9.638	9.652	9.663	9.669	9.672
18	9.465	9.502	9.516	9.526	9.534	9.546	9.557	9.573	9.568	9.588	9.604	9.619	9.630	9.636	9.639
19	9.433	9.470	9.483	9.493	9.501	9.514	9.524	9.540	9.535	9.555	9.572	9.586	9.597	9.604	9.606
20	9.401	9.438	9.451	9.461	9.469	9.482	9.492	9.508	9.503	9.523	9.540	9.554	9.564	9.571	9.574
21	9.369	9.406	9.420	9.430	9.438	9.451	9.461	9.477	9.471	9.491	9.508	9.522	9.532	9.539	9.542
22	9.338	9.375	9.388	9.398	9.406	9.419	9.424	9.446	9.439	9.459	9.476	9.490	9.501	9.507	9.510
23	9.307	9.344	9.352	9.367	9.375	9.368	9.398	9.415	9.408	9.426	9.445	9.459	9.469	9.476	9.479
24	9.276	9.313	9.324	9.337	9.345	9.352	9.365	9.384	9.377	9.396	9.414	9.425	9.439	9.445	9.446
25	9.246	9.283	9.296	9.306	9.314	9.327	9.337	9.353	9.346	9.366	9.383	9.397	9.407	9.414	9.417
26	9.215	9.251	9.265	9.275	9.283	9.296	9.306	9.322	9.314	9.334	9.351	9.365	9.376	9.382	9.385
27	9.184	9.221	9.234	9.244	9.252	9.265	9.275	9.291	9.283	9.303	9.320	9.334	9.344	9.351	9.354
28	9.153	9.190	9.204	9.214	9.222	9.234	9.245	9.261	9.252	9.272	9.286	9.303	9.313	9.320	9.323
29	9.123	9.160	9.173	9.183	9.191	9.204	9.214	9.231	9.221	9.241	9.252	9.272	9.282	9.289	9.292
30	9.093	9.130	9.143	9.153	9.161	9.174	9.184	9.201	9.190	9.210	9.227	9.241	9.252	9.259	9.261

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APPENDIX B

APPENDIX B

Criteria for Evaluating Toxicity Data for the
San Francisco Bay Regional Water Quality Control Board
Effluent Toxicity Characterization Program

Prepared by Lynn Suer and Erika Hoffman

Toxicity test data should be evaluated by the discharger for acceptability according to the following criteria. If any criteria are not met, notify Regional Board staff.

I. Synchronous Testing (Variability Phase)

A. Tests for all three species in a battery should be conducted synchronously. Note: Short-term chronic tests (40 min to 96 hrs) may be run at any time within the test period of the longer-term tests.

B. Parallel reference toxicant tests must be conducted synchronously with effluent tests.

II. Sample Handling/Holding Times

A. Storage time for all samples should be less than 48 hours, as specified in the EPA chronic manual (EPA/600/4-87/028, p. 20).

B. Storage temperature for all samples should be 4 degrees C., as specified in the EPA chronic manual (EPA/600/4-87/028, p. 20).

III. Controls

A. Required controls

1. Dilution water controls

a. For a standard dilution water test, one standard water control is required.

b. For an ambient dilution water test, two controls are required:

b.1. Standard water control
b.2. Ambient water control

Note: Standard water may be either synthetically prepared or natural water that is documented as non-toxic (e.g., Bodega Bay seawater).

2. Salt controls

A salt control is also required, when the salinity of the test solutions is adjusted with natural seawater brine or commercial salts.

B. Control salinity should be the same as that used in the effluent test treatments.

C. All controls must meet the acceptability criteria for a given test (See Protocol Test Summary Table), except for ambient water controls. Ambient controls may be toxic relative to standard dilution water controls.

IV. Dose-response

A. If a response occurs in effluent tests, then the response should increase with effluent concentration. An erratic patern may indicate that certain variables (e.g., water quality, organism weight) were not adequately controlled or randomized.

B. Reference toxicant tests should show an increasing response with increasing concentration over the entire dose range. If the dose range is "missed", then the organisms may be relatively insensitive or overly sensitive.

V. Check for consistency with attached Test Protocol Summary.

	Species	Common Name	Test Type (1)	Duration	Endpoint	Temp. (C)	Salinity (ppt)
Freshwater							
Fish	*Pimephales promelas*	Fathead minnow	S/R	7 d	surv./growth	25	0
Invertebrate	*Ceriodaphnia dubia*	Water flea	S/R	7 d or 3 broods	surv./growth	25	0
Plant	*Selenastrum capricornutum (5)*	algae	S	96 h	growth rate	25	0
Marine/Brackish							
Fish	*Menidia beryllina*	Inland silverside	S/R	7 d	surv./growth	25	8 to 35
Invertebrates	*Mysidopsis bahia*	Mysid shrimp	S/R	7 d	surv./growth/repro.	26-27	20 to 30
	Strongylocentrotus purpuratus	Purple sea urchin	S	20 min sperm (9)	fertilization	12 or 15	30-35
	Dendraster excentricus	Sand dollar	S	20 min sperm (9)	fertilization	12 or 15	30-35
	Mytilus edulis	Bay mussel	S	48 h (13)	larval develop./surv.	16	28-35
	Crassostrea gigas	oyster	S	48 h (13)	larval develop./surv.	20	28-35
	Haliotis rufescens	red abalone	S	48 h (13)	larval development	15	32-35
Plants	*Skeletonema costatum (5)*	algae	S	96 h	growth rate	20	24-35
	Thalassiosira pseudonana	algae	S	96 h	growth rate	20	24-35
	Macrocystis pyrifera	giant kelp	S	48 h	germination/germ tube length	15	34
	Champia parvula	red algae	S	48 H (20)	cystocarp formation	22-24	30

APPENDIX B - EFFLUENT TOXICITY CHARACTERIZATION PROGRAM TOXICITY TEST PROTOCOLS

Species	Age of test organism	# organisms/ replicate	# reps./conc.	Statistics (2)	Accepta-bility	Protocol
Pimephales promelas	<48 hr	10 to 15	3 or 4	LC50,EC50,NOEC	(3)	EPA/600/4-89/001
Ceriodaphnia dubia	<24 h, within 8 hr	1	10	LC50,EC50, NOEC	(4)	EPA/600/4-89/001
Selenastrum capricomutum (5)	4-7 d culture	10e4 cells/ml	at least 3	EC50	(6)	EPA/600/4-89/001
Menidia beryllina	7-9 d	10 to 15	3 or 4	LC50,EC50,NOEC	(7)	EPA 600/4-87/028
Mysidopsis bahia	7 d	5	8	LC50,EC50,NOEC	(8)	EPA 600/4-87/028
Strongylocentrotus purpuratus	(10)	(11)	at least 3	EC50,NOEC	(12)	
Dendraster excentricus	(10)	(11)	at least 3	EC50, NOEC	(12)	
Mytilus edulis	(14)	15-30 embryos/ml	at least 3	LC50, EC50, NOEC	(15)	ASTM E724-89
Crassostrea gigas	(14)	15-30 embryos/ml	at least 3	LC50,EC50, NOEC	(15)	ASTM E724-89
Haliotis rufescens	(14)	5 embryos/ml	at least 3	LC50, EC50,NOEC	(16)	SWRCB 90-10WQ
Skeletonema costatum (5)	4-7 d culture	2 e4	at least 3	EC50,NOEC	(17)	ASTM E 1218-90
Thalassiosira pseudonana	4-7 d culture	2 e4	At least 3	EC50,NOEC	(17)	ASTM E 1218-90
Macrocystis pyrifera	(18)	7,500 zoospores/ml	5	EC50,NOEC	(19)	SWRCB 90-10 WQ
Champia parvula	(21)	(22)	at least 3	EC50, NOEC	(23)	EPA 600/4-87/028

Attached Notes to Toxicity Test Protocol Summary

(1) F: Flow; S: Static; S/R: Static renewal

(2) 95% confidence limits should be calculated for all LC50 and EC50 values.

(3) Fathead minnow: 80% average survival and 0.25 mg/larvae (average dry weight) in the control.

(4) Water flea: 80% average survival and 15 neonates/female in the control

(5) Test using *Skeletonema* and *Selenastrum* algae species may be considered as either acute or chronic tests because of the duration of exposure (96 hr = acute) and the endpoint measured (growth rate = chronic). For this Program, they are considered to be chronic.

(6) For *Selenastrum*:: Control density should be greater than 2e5 cells/ml and control variability among replicates should not exceed 20%.

(7) Silverside minnow: 80% average survival and 0.5 mg/larvae (average dry weight) in the control

(8) Mysid shrimp: 80% average survival and 0.20 mg/mysid (average dry weight) in the control.

(9) Purple sea urchin and sand dollar: 40 minute total test time (20 minute sperm exposure time + 20 minute fertilization time).

(10) Purple sea urchin and sand dollar: Eggs and sperm should be used within 5 hours of collection assuming that they are kept at 4C during this period.

(11) Purple sea urchin and sand dollar: Test should be performed using a sperm/egg ratio no greater than 1500:1. The optimum ratio should be determined by pre-tests (see Appendix D, p.3).

(12) Purple sea urchin and sand dollar: 75-95% average control fertilization.

(13) Bay mussel and oyster: Test should be run for 48 hours or until controls show greater than 70% "normal" development ("normal" meaning development to the D-shaped veliger stage).

(14) All mollusc species: Exposure to embryos should begin no more than 1 hour after fertilization.

(15) Bay mussel and oyster: Greater than 70% average survival and greater than 70% average normal development in the control.

(16) Red abalone: Average control larval abnormality should not exceed 20%.

(17) *Skeletonema* and *Thalassiosira* : Average control density should be greater than 1e5 cells/ml and control variability among replicates should not exceed 20%.

(18) Release of zoospores should not exceed two hours.

(19) a. Mean germination must be at least 70% in both the reference toxicant control and the brine control of the effluent test.
b. Mean germination-tube length must be at least 10 micrometers in both the reference toxicant control and the brine control for the effluent test.
c. Brine control results must not be significantly different from dilution water control results.
d. The germination NOEC in the copper reference toxicant test must be below 110 micrograms per liter; the germination-tube growth NOEC must be below 35 micrograms per liter (chemically verified copper concentrations).
e. The between-replicate variability for the germination data must be low enough that the ANOVA Mean Square (MS) does not exceed 70.00 in the reference toxicant test (using arcsine transformed percentage germination data in degrees). This corresponds to a Dunnett's Standard Error (SE) of 5.29 (with n = 5 replicates).
f. The between-replicate variability for germ-tube growth data must be low enough that the ANOVA Mean Square (MS) does not exceed 12.00 in the reference toxicant test (using untransformed length data). This corresponds to a Dunnett's Standard Error (SE) of 2.19 (with n = 5 replicates).

(20) Sexually mature male and female branches of *Champia* are exposed to effluent for two days. However, this is followed for a 5 to 7 day recovery period in control medium, so test duration is 7 to 9 days.

(21) Cultured plants must be sexually mature.

(22) Each test chamber must have 5 female branch tips and 1 male plant.

(23) *Champia parvula* : Control mortality may not exceed 20%. Plants should not fragment in the controls or lower exposure concentrations (this indicates stress). Control plants should average 10 or more cystocarps.

APPENDIX C

APPENDIX C

REQUIRED AND RECOMMENDED PROTOCOL REFINEMENTS

Reference: S.L. Anderson. September 1, 1989 mailing to Effluent Toxicity Characterization Mailing List.

MENIDIA

1. Minimum control weights of 0.5mg/fish are attainable in greater than 80% of the tests run in our laboratory.

Comment: *None of the commenters reported difficulties in attaining minimum control weights*

2. Menidia should be fed twice a day, at a minimum.

Recommendation: *It is advised that feeding occur at least twice a day. However, laboratories may use their own discretion as to whether this should occur twice per day, three times per day or once per day with supplemental feeding ad libitum.*

3. The age of larvae at the beginning of the test should not be greater than 7-9 days. Although 10-11 days are also allowable in the EPA protocol, there is no compelling reason to extend the timeframe. A narrower timeframe will ensure greater consistency between laboratories.

Requirement: *Most commenters felt that 7-8 days would be too restrictive. However, data submitted to the Regional Board indicate that tests initiated with 8-d old fish provide results that consistently meet the 0.5 mg/fish control weight. As such, Regional Board staff will require that tests be initiated on fish that are not older than 9-d. Early on, there will be no penalty to laboratories that cannot meet this criterion if they inform Regional Board staff in writing of the difficulties that they may be having and propose a schedule to resolve the difficulties.*

4. Salinity adjustments using a variety of commercial sea salts results in acceptable control performance. Please list the salts used in your laboratory below.

Comment: *Commenters reported successful results using 40 Fathoms and Instant Ocean.*

5. Salinity of effluent tests does not have to be raised above 8ppt. It is probably best to minimize sample alterations by not utilizing unnecessary salt adjustments. Note: This statement does not apply to effluents run with ambient dilution waters, and any salinity below 6ppt is too low.

Requirement: *The salinity of the effluent test should be adjusted to the approximate receiving water salinity for the site. However, the salinity should never be less than 8 ppt.*

6. Control salinities should be adjusted to match the salinity used for the effluent series (or that of the ambient water).

Requirement: *See above*

7. The laboratories should have flexibility in determining whether the reference toxicant series should be run at the same salinity as the effluent series.

Requirement: *The salinity of the reference toxicant series should be adjusted to the receiving water salinity unless the laboratory is grouping the reference toxicant work for more than one client. In the latter case, the salinity of the reference toxicant series does not have to match that of the receiving water, but the laboratory must document in their reports the fact that the test was being run for multiple clients, the name of the clients and the dates of the test. If the salinity is adjusted to that of the receiving water and multiple clients happen to have similar salinities, the above does not have to be reported.*

8. The appropriate control for most effluent tests will be salts added to distilled or mineral water. Seawater controls are also required for effluent tests using ambient dilution waters. In those cases, laboratories should run seawater controls and dilute them with distilled or mineral water if necessary.

Requirement: *For tests in which 100% effluent is one of the dilutions studied, artificial salts should be used to adjust salinity. As such, the appropriate control for this test would be artificial salts in mineral water (Forty Fathoms and Arrowhead water have been used successfully). If the effluent test is for lower dilutions, natural seawater may be used for salt adjustment, and the appropriate control would be natural seawater diluted with mineral water.*

9. Approximately 24h of salinity acclimation may be required before a test is initiated. Salinity acclimation should be accomplished by making adjustments of approximately 5 ppt per day.

Recommendation: *Regional Board staff suggest that salinity acclimation be conducted at a rate of 5ppt per day. One commenter suggested 3ppt every second day, but this does not appear to be necessary as successful tests are routinely conducted with more abrupt acclimations. One commenter believed standardization would be valuable. If you receive your 7-d old larvae at approximately 20 ppt, fish can be acclimated to any salinity between 10 and 30 ppt in 2 days. This means that tests can be started by the 9-day age limit.*

10. Minor changes in water chemistry measurements might be useful. It seems logical to measure salinity and pH at the beginning of the water renewal rather than at the end.

Comment: *Salinity, pH and DO can be measured before the renewals are conducted rather than at the end of the 24-h renewal period.* DO should also be meaured at the end of the 24-h period.

ECHINODERM FERTILIZATION

1. "Dry" sperm collection techniques should be used, and sperm should be stored on ice.

Requirement: *All reviewers commented that dry collection techniques would be preferrable. As such, this will be a project requirement.*

2. Eggs should be held on ice after being spawned into chilled seawater.

Recommendation: *A diversity of opinion by reviewers was noted. Staff recommend that eggs be maintained at any temperature between 4-12°C.*

3. The laboratories should be allowed to decide whether they prefer to run 60 min or 20 min exposure times.

Requirement: *Laboratories involved in this program will be required to run 20 min. exposures. Any laboratory that has difficulty with this requirement should notify their clients in writing and copy their comments to Susan Anderson of the Regional Board staff. No penalty to the discharger will be incurred, as long as the laboratory documents their concerns and a schedule for resolving them. There was nearly unanimous agreement among commenters that 60-min exposures can compromise the viability of sperm. There was also concurrence that the 10-min exposure time used by researchers at the Bodega Marine laboratory was scientifically valid but logistically difficult for laboratories conducting varying volumes of tests under varying conditions. As such, the 20-min exposure time achieves uniform sperm viability in a more practical timeframe.*

4. The appropriate sperm:egg ratio for a given test should be determined each time the test is run. Note: this can be accomplished if dry sperm collection techniques are used. Four sperm:egg ratios (1500:1, 1000:1, 500:1, and 100:1) are tested while the gametes are on ice. Either control performance or response to a reference toxicant test is evaluated.

Requirement: *The appropriate sperm:egg ratio must be determined each time the test is run. The lab should test sperm:egg ratios of 1000:1, 500:1 and 300:1 using natural seawater and the brine control. The test should be terminated after a 20-min exposure and counted (only one replicate per ratio for this pre-test). The lowest sperm:egg ratio giving fertilization in excess of 90% should be used for the test.*

5. Sample pH should be adjusted to 8.0.

Requirement: *At a minimum, pH must be measured, reported, and adjusted to 8.0 + 0.5. It is highly recommended that pH be adjusted to 8.0 + 0.1. This specification applies to the water into which the eggs are spawned, the test water, and the dilution water for the reference toxicant series.*

6. Brine prepared from natural seawater provides the best control performance in the echinoderm tests. If you do not agree, describe the brine you use including the names of the salts and the type of diluent water.

Recommendation: *Brine prepared from natural, 0.45um-filtered seawater should be used whenever possible. For tests in which the toxicity of 100% effluent is evaluated, commercial salts should be added directly to the effluent. If it is not significant to test 100% effluent at a given site, restrict the tests to 67% or less and use natural brine whenever possible. Most investigators find that 2X-3X brine is reliable. Never use a brine for which the salts have begun to precipitate out.*

7. Brine controls and natural seawater controls should be run for every test. For sites at which 100% effluent is being tested, an additional brine plus salt control is also needed.

Requirement: *For tests in which 100% effluent must be evaluated, a commercial sea salt control is a requirement. See additional comments above.*

8. The eggs and sperm of one male and one female can be used (rather than pooling four of each) because the parallel reference toxicant data will provide an estimate of between-animal variability. It should be up to the laboratories to decide whether they want to pool gametes from separate individuals.

Recommendation: *Most reviewers believed that it would be valuable, but not essential, for spawners to be pooled. Consequently, we recommend that spawners be pooled when possible, but in the event that only one spawner of either sex is available, pooling is not essential.*

9. Scoring criteria are adequately defined in the protocols. Please report below whether you report "blebbed" eggs as fertilized or unfertilized.

Requirement: *Apparently, blebbed eggs are being reported inconsistently. It is not known whether this could have an impact on the NOEC derived in any given test. Consequently, staff recommend that blebbed eggs, asymmetric eggs, and eggs with low elevation of the fertilization membrane be recorded separately. Statistics should be run two ways. Once in which the embryos are reported as normal and once in which the embryos are reported as abnormal.*

10. Filtration of ambient dilution waters through 37 um filters improves control performance.

Comment: *Most laboratories have had little experience with ambient samples. Regional Board staff recommend the use of 37-um filtration for all tests except the algae tests. The latter tests employ 0.45-um filtration. Tests using filtration with lower pore sizes than 37 um (except algae) will not be accepted.*

MOLLUSC DEVELOPMENT

1. Both the ASTM 1980 and 1987 protocols are acceptable. There is no need to restrict laboratories to use of only the 1987 protocol.

Comment: *One of the two commenters believed that greater standardization is desirable. In the absence of strong opinion on this topic, staff recommend that the existing protocol flexibility be maintained.*

2. Initial embryo densities for survivorship calculations should be determined by making embryo counts on three subsamples taken at the intiation of the test (or fixed subsamples that may be counted at a later date).

Requirement: *The above describes the most commonly accepted approach to determining intial densities. However, commenters have indicated that three samples are the bare minimum and 4-5 are adviseable. Therefore, laboratories should follow the approach described above and use 3 or more subsamples.*

3. Laboratories should have flexibility in selecting water temperatures to be used to stimulate spawning. However, gametes should not be exposed to temperatures exceeding those listed in the ASTM protocol.

Requirement: *There was no disagreement on this topic. Laboratories should excercise care in following the guidelines specified.*

4. If small culture vessels (25 ml or less) are used for the test, copper sulfate cannot be used as the reference toxicant because the large surface area:volume ratio causes a significant proportion of the copper to bind to the glass.

Comment: *Apparently, little information is available on this topic. Please share your observations with Regional Board staff as data become available.*

5. The eggs and sperm of one male and one female can be used (rather than pooling three of each) because the parallel reference toxicant data will provide an estimate of between-animal variability. It should be up to the laboratories to decide whether they want to pool gametes from separate individuals.

Recommendation: *See comment #8 for echinoderm test.*

6. Scoring criteria are adequately defined. Please report below whether you record abnormally shaped D-hinges as normal or abnormal.

Requirement: *Abnormally-shaped D-hinge larvae should be reported as abnormal.*

7. Brine prepared from natural seawater provides acceptable control performance. Please specify below any other acceptable salt formulations you have used.

Recommendation: *Natural brine is the most reliable option for salt adjustment.*

8. Both seawater and brine controls are necessary for each test.

Requirement: *Both types of controls are now considered a program requirement. Please refer to comments #6 and #7 on echinoderm.*

9. Survival is never a more sensitive endpoint than abnormality.
Please elaborate if you have data to the contrary.

Comment: *No substantial data was submitted on this topic. As such, investigators in this program must continue to report both surviviorship and abnormalities.*

FATHEAD MINNOW

1. This test should be run with <24-hr old fish. On occasion, fish may exhibit stress from transport, and 24- to 48-hr old fish can be used. This extended observation period ensures the investigator that the test has not been initiated on weak fish.

Requirement: *These tests can now be conducted with 48-hr old fish. However, staff caution that the fish must be received at 24-hr old, and tests must be initiated no later than 48-hr. The age specification is being relaxed because many investigators believe this will aid them in identifying fish stressed during shipment.*

2. Minimum weights of 0.25 mg per fish are acheivable in more than 80% of the tests run in our laboratory.

Comment: *No difficulties were reported.*

3. Fish should be weighed by determining the weight of all 10 fish pooled, not by taking individual weights.

Requirement: *Fish must be pooled for weighing. Tests data using individual fish weights will not be accepted.*

CERIODAPHNIA

1. Control reproduction is acheivable in greater than 80% of the tests run in our laboratory. Please specify below the control water you use.

Comment: *Control reproduction is apparently achievable in all laboratories. The most commonly used dilution water is 10% Perrier in EPA moderately hard water, spring water or laboratory water. Other reported dilution waters were: "aged" culture water, a lab blend water bubbled for 7d with YCT, EPA moderately hard without the Perrier, and bottled spring water combined with Nanopure grade water to a hardness of 80-100 mg/l.*

2. The basic YCT plus algae feed is acceptable.

Recommendation: *This feed formulation appears to work well for the majority of investigators, and it is recommended for future testing. However, it should be noted that one group reported that trout chow batch qualities vary substantially and that batches can become rapidly "soured".*

SKELETONEMA/SELANASTRUM

1. The reference toxicant for the diatom tests must be selected. Please record your suggestions below.

Comment: *Both copper sulfate and potassium dichromate were recommended. Use of either of these is acceptable until further information is available.*

2. EDTA should be added to the culture medium for Selenastrum.

Comment: *Laboratories may use best professional judgement on this topic. The only two reviewers who commented on this topic found the use of EDTA adviseable.*

3. Thalassiosira is a good substitute for Skeletonema.

Comment: *Thalassiosira can be used as a substitute for Skeletonema in this program. However, laboratories cannot switch species in the middle of a study.*

4. Initial algal densities should be 10,000 per ml.

Requirement: *While slight variations will be observed, initial algal densities should be approximately 10,000 cells/mL*

5. Salinity adjustment for Skeletonema tests can be accomplished with either commercial seasalts or natural brine. Please specify the methods you use below.

Comment: *Little information was reported on this topic. One commenter reported that natural brine provided optimal control performance and another reported that 40 fathoms povided good results as well.*

6. For Skeletonema, all tests should include a seawater control and a brine control.

Requirement: *The two types of controls described above are now required.*

DATA ANALYSIS AND STATISTICS

1. Statistics on acute tests run during the Variability Phase should include reporting of LC50 and LOEC/NOEC.

Requirement: *This topic requires a policy-based decision rather than a strictly technical decision. It is now a program requirement that both LC50 and LOEC/NOEC be reported for acute tests run during the Variability Phase. It is intended that the LOEC/NOEC be calculated using only survivorship data. For acute tests, observable sublethal effects should be reported, as appropriate, but not included in statistical evaluations.*

2. For the chronic tests, NOEC and LOEC should be determined and reported for each endpoint evaluated (e.g. growth and survival) in each test. Other useful determinations include ChV, AEC etc.

Comment: *No disagreement was noted. One investigator commented that LC50/EC50 should also be reported. Staff agree that this is adviseable.*

AMBIENT WATER SAMPLING

1. Sites for ambient water sampling should be identified on a site-specific basis. However, general guidelines for sampling should include recording tide condition, initial salinity and time of collection. Samples should be collected approximately 0.5-1.0 m below the surface using clean, biocompatible containers and/or pumps.

Requirement: *Use the above general guidelines for ambient water sampling.*

2. Ambient water samples should be filtered to 37um and salinity adjusted before each test.

Requirement: *With the exception of the algal bioassays, tests using pore sizes smaller than 37 um for filtration will be considered unacceptable.*

3. For tests requiring 7-day renewals, ambient waters should be renewed daily using samples collected daily.

Requirement: *THE DAILY RENEWALS FOR 7-DAY TEST SERIES CAN BE CONDUCTED WITH 3 DIFFERENT AMBIENT SAMPLES RATHER THAN DAILY AMBIENT SAMPLES. Effluent must be collected daily.*

OTHER

1. The time between effluent sample collection and test initiation should not exceed 48 h.

Requirement: *The time between the completion of effluent sampling and test initiation should*

not exceed 48h.

2. The reference toxicant for Mysidopsis and for the acute tests has not been specified. Please record your suggestions below.

Comment: *Cadmium chloride and potassium chromate have been suggested for Mysidopsis. Either may be used until further information is available. Potassium chromate has been suggested as a reference toxicant for acute tests.*

APPENDIX D

APPENDIX D

Reporting Requirements for the
Effluent Toxicity Characterization Program

Interim and Final Variability Reports must contain the following:

- Tabulated information according to the attached spreadsheet format (pp. 2-10 of this Appendix). At a minimum, dischargers must submit hardcopies of the completed spreadsheet. Dischargers with access to Excel 2.2 software for the MacIntosh computer are strongly encouraged to enter the information electronically onto a 3.5 inch, double-sided, double-density floppy disk.

- Graphical summaries according to the attached format (p.11 of this Appendix).

- Hardcopies of raw data (laboratory bench sheets).

- Raw data entered electronically onto 5 1/4 inch DOS formatted DS/DD 360 K diskettes. Submit datasets in TOXSTAT format in ASCII, using one file for each analysis. Each file should be structured as follows:

TITLE OF ANALYSIS
NUMBER OF GROUPS
REPS GROUP 1
REPS GROUP 2
.
. (# REPS FOR EACH GROUP)
.
REPS LAST GROUP
ID FOR GROUP 1 (CONTROL GROUP)
DATUM GROUP 1, REP 1
DATUM GROUP 1, REP 2
.
. (GROUP 1 REPS)
.

APPENDIX D. NOTES TO SPREADSHEET

Cell: A1
Note: Discharger: Name the NPDES discharger whose effluent has been tested

Cell: B1
Note: Laboratory: Enter the name of the commercial lab or enter in-house, if tests were conducted at the discharger's facility.

Cell: C1
Note: Bat: Enter the number of the test battery. A test battery consists of 6 tests: 3 effluent tests using different species plus 3 parallel reference toxicant tests. The total number of batteries will vary among dischargers, but should not be greater than 18.

Cell: D1
Note: Species: Enter the scientific, generic name of the species used in the test.

Cell: E1
Note: Sample Date: Enter the date that the 24 hour composite sample was taken.

Cell: F1
Note: Enter the date that the sample of receiving water was collected for the ambient test.

Cell: G1
Note: Test Date: Enter the month/date/ year when the specific test referred to in column D was started.

Cell: H1
Note: Salinity: Enter the salinity range of the test solutions (parts per thousand) without entering the units.

Cell: I1
Note: [NH3]Max: Enter the maximum concentration of ammonia nitrogen achieved during any of the test days, at the maximum effluent concentration.

Cell: J1
Note: C1, Diluent ID: Describe the solution used to dilute the effluent. This is control solution 1, or C1.

Cell: K1
Note: C2, Ambient ID: Name the location where ambient SF Bay water was collected. This is control solution 2, or C2, for toxicity tests using ambient dilution waters.

Cell: L1
Note: C3, Salt ID: Identify the salts added to the test solutions to achieve the desired salinity. If commercial salts were used, identify the commercial name, such as Forty Fathoms, Instant Ocean, etc. If a natural brine was used, specify the location of the ambient waters used to prepare the brine. For example, enter: Bodega brine. The salt control is C3.

Cell: M1
Note: Control ID: Identify the controls used in the toxicity tests as C1, C2 and C3

Cell: N1
Note: [Eff]: Enter the Effluent Concentrations as a decimal fraction. For example, enter 0.125 for 12.5% and 1 for 100%. Enter 0 for controls.

Cell: O1
Note: # Reps: Enter the number of replicates for each effluent concentration.

Cell: P1
Note: Ave Survival: Enter average survival data. Define the survival units, such as no. of embyros per ml., or percent surviving, as a note in the first row entry for a specific test (if you are using Excel). See Notes P9 and P17 as examples. Add a footnote to your table, if you are submitting hard copy only.

Cell: Q1
Note: *Ac,C1: Indicate the statistical significance of the average acute value in relation to C1 by entering an *. If the value is not significantly different than C1, then leave the space blank.

Cell: R1
Note: *Ac,CX: Indicate the statistical significance of the average acute value relative to CX by entering an *. If the value is not significantly different than CX, then leave the space blank. Define CX as a note in the first row entry for the specific test (or as a footnote, if only a hardcopy is submitted). See Note R9 as an example.

Cell: S1
Note: <, >: The < or > sign entered here and all such signs hereafter apply to the values in the following column.

Cell: T1
Note: Enter the LC50 value that is obtained when C1 is used as the basis for statistical comparison.

Cell: U1
Note: 95% CL: Enter the 95% confidence limits for the LC50,C1 value entered in Column 18. If the limits have not been calculated, then enter NC. Hereafter, all 95% CL will refer to the value in the preceeding column.

Cell: W1
Note: LC50,CX: Enter the LC50 value that is obtained when C2 or C3 is used as the basis for statistical comparison. Define CX as a note in the first row entry for the specific test. See Note W9 as an example. Enter NA if statistical analysis is based on only one control value.

Cell: Z1
Note: AcNOEC,C1: Enter the NOEC obtained from statistical evaluation of the survival data. This is the Acute NOEC or AcNOEC. In this column, enter the NOEC, when C1 is used as the control value in the statistical evaluation.

Cell: AB1
Note: AcNOEC,CX: Enter the Acute NOEC value, when CX is used as the control value in the statistical analysis. Define CX in the first row entry for the specific test.

Cell: AC1
Note: [Eff]: Enter 0 for control concentrations and enter other concentrations as decimal fractions. These should be same numbers as in column N.

Cell: AD1
Note: Ave C: Enter the average values for the chronic endpoint. Define the units as a note in the first row entry of the specific test. See Notes AD9 and AD17 as examples.

Cell: AE1
Note: *ChC1: Indicate the statistical significance of the average chronic value in relation to C1 by entering an a *. If the value is not significantly different than C1, then leave the space blank.

Cell: AF1

Note: *ChCX: Indicate the statistical significance of the average chronic value relative to CX by entering an *. If the value is not significantly different than CX, then leave the space blank. Define CX as a note in the first row entry for the specific test. See Note AF12 as an example.

Cell: AH1
Note: EC50, C1: Enter the EC50 value, based on the C1 control value, if this has been calculated. Enter NA, if statistical analysis is not based on this control.

Cell: AK1
Note: EC50,CX: Enter the EC 50 value that is obtained when CX is used as the basis for statistical comparison. Define CX as a note in the first row entry for the specific test. For example CX=C2. Enter NA if statistical analysis is based on only one control value.

Cell: AN1
Note: ChNOEC,C1: Enter the NOEC value determined from chronic values, based on C1 as the control.

Cell: AP1
Note: ChNOEC,CX: Enter the NOEC value determined from chronic values, based on CX as a control Define CX as a note in the first row entry for the specific test. For example CX=C2.

Cell: AQ1
Note: Ref Tox ID: Identify the chemical used as reference toxicant and the units of concentration used. Be specific, and use a note, if additional space is needed. For example: If $CuSO_4 \cdot 6H_2O$ is the stock compound, state whether the concentration units refer to the compound or to elemental copper.

Cell: AR1
Note: Reps RT: Enter the number of replicates in reference toxicant tests.

Cell: AS1
Note: [RT]: Enter the reference toxicant concentrations.

Cell: AT1
Note: Ave S RT: Enter the average survival data for the reference toxicant tests. Define the units as a note in the first row of the specific test. See Note AT2 as an example.

Cell: AU1
Note: *Ac,RT: Indicate if the average survival data for a specific concentrations is significantly different than C1 by entering an *. Leave the space blank, if there is no significant difference.

Cell: AW1
Note: LC50 RT: Enter the LC50 value based on acute data.

Cell: AZ1
Note: AcNOEC,RT: Enter the acute NOEC value for the reference toxicant test.

Cell: BB1
Note: [RT]: Enter the concentrations used in reference toxicant test, starting with 0 for the control.

Cell: BC1
Note: Enter the average values for chronic endpoint of each of the reference toxicant concentrations. Define the chronic units as a note in the first row entry for the test.

Cell: BD1

Note: *Ch,RT: Indicate average chronic values that are statistically different than the control with an *. Leave blank if there is no significant difference.

Cell: BF1
Note: EC50 RT: Enter the EC50 value calculated from chronic values

Cell: BI1
Note: ChNOEC,RT: Enter the chronic NOEC value

Cell: AT2
Note: Percent surviving

Cell: P9
Note: larvae/ml

Cell: R9
Note: CX=C2

Cell: W9
Note: CX=C2

Cell: AD9
Note: percent abnormal

Cell: BC9
Note: percent abnormality

Cell: AF12
Note: CX=C2

Cell: P17
Note: Mean Proportion Surviving

Cell: AD17
Note: Mean weight (mg)

	A	B	C	D	E	F	G	H	I	J
1	Discharger	Laboratory	Bat	Species	Eff. Sample Date	Amb. Sample I	Test Date	Sal	[NH3]Max	Cl,Diluent ID
2	Timbuctu SD	Tests R Us	1	Ceriodaphnia	10/7/89		10/8/89	0	10	Mineral Water
3					10/8/89					
4					10/9/89					
5					10/10/89					
6					10/11/89					
7					10/12/89					
8					10/13/89					
9	Timbuctu SD		1	Crassostrea	10/9/89	10/8/89	10/10/89	28-30	10	Bodega Water
10										
11										
12										
13										
14										
15										
16										
17	Timbuctu SD		1	Menidia	10/7/89		10/8/89	32-34	10	Bodega Water
18					10/8/89					
19					10/9/89					
20					10/10/89					
21					10/11/89					
22					10/12/89					
23					10/13/89					

APPENDIX D. SPREADSHEET FORMAT FOR SUBMITTING PROGRAM INFORMATION

	K	L	M	N	O	P	Q	R	S	T	U
1	C2,Ambient ID	C3,Salt ID	Cont. ID	[Eff]	# Reps	Ave Sur	*Ac,C1	*Ac,CX	<, >	LC50,C1	95%CL
2	NA	NA	C1	0	15	0.00		NA		0.555	0-.75
3				0.13	15	73.3	*				
4				0.25	15	73.3	*				
5				0.5	15	73.3	*				
6				0.75	15	0	*				
7				1	15	0	*				
8											
9	Dumbarton Br.	Bodega brine	C1	0	3	15.5				0.043	.020-.063
10			C2	0	3	10.7	*				
11			C3	0	3	12.9	*				
12				0.044	3	8.3	*	*			
13				0.088	3	6.1	*	*			
14				0.175	3	4.9	*	*			
15				0.35	3	0.5	*	*			
16				0.7	3	0.3	*	*			
17	NA	40 Fathoms	C1	0	3	0.975			>	1	NC
18			C3	0	3	0.95					
19				0.13	3	0.925					
20				0.25	3	0.975					
21				0.5	3	0.95					
22				0.75	3	0.925					
23				1	3	0.575	*	*			

	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI
1	<, >	LC50,CX	95% CL	<, >	AcNOEC,C1	<, >	AcNOEC,CX	[Eff]	Ave C	*ChC1	*ChCX	<, >	EC50,C1	95% CL
2		NA		<	0.13		NA	0	15.5		NA		0.87	0.5-1
3								0.13	17.4					
4								0.25	18.8					
5								0.5	15.9					
6								0.75	5	*				
7								1	NA					
8														
9		0.115	.101-.130	<	0.044		0.044	0	0.077			>	0.175	NC
10								0	0.113					
11								0	0.036					
12								0.044	0.272	*	*			
13								0.088	0.228	*	*			
14								0.175	0.219	*	*			
15								0.35	0					
16								0.7	0.5					
17	>	1	NC		0.75		0.75	0	0.864			>	0.75	NC
18								0	0.639					
19								0.13	0.646					
20								0.25	0.575					
21								0.5	0.587					
22								0.75	0.49	*				
23								1	0.556	*				

APPENDIX D. SPREADSHEET FORMAT FOR SUBMITTING PROGRAM INFORMATION

	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX
1	<, >	EC50,CX	95% CL	<, >	ChNOEC,CI	<, >	ChNOEC,CX	Ref Tox ID	Reps RT	[RT]	Ave S RT	*Ac,RT	<, >	LC50 RT	95% CL
2		NA			0.5		NA	NaCl g/L	15	0	100			1.09	0-2
3									15	0.13	73.7				
4									15	0.25	80				
5									15	0.5	80				
6									15	1	60	*			
7									15	2	0	*			
8															
9	>	0.175	NC	<	0.044	<	0.044	CuS04 ug/L	3	0	15.5			0.14	0.11-.18
10									3	0.09	10.3	*			
11									3	0.18	6.3	*			
12									3	0.35	4.5	*			
13									3	0.7	2.5	*			
14									3	1.4	2	*			
15															
16															
17	>	1	NC		0.5		1	CuSO4 (ug/L	3	0	0.975			216	158-286
18									3	25	100				
19									3	100	0.9				
20									3	250	0.325	*			
21									3	500	0.175	*			
22									3	1000	0.15	*			
23															

	AY	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI
1	<, >	AcNOEC,RT	<, >	[RT]	Ave C RT	*Ch,RT	<, >	EC50 RT	95% CL	<, >	ChNOEC,RT
2		0.5		0	15.5			0.87	0.5-1		0.5
3				0.13	17.4						
4				0.25	18.8						
5				0.5	15.9						
6				1	5	*					
7				2	ND						
8											
9	<	0.088		0	0.077		>	1.4	NC		0.35
10				0.09	0.097						
11				0.18	0.2						
12				0.35	0.294						
13				0.7	0.474	*					
14				1.4	0.467	*					
15											
16											
17		100		0	0.864			444	382-571		250
18				25	0.661						
19				100	0.771						
20				250	0.657						
21				500	0.384	*					
22				1000	0.491	NA					
23											

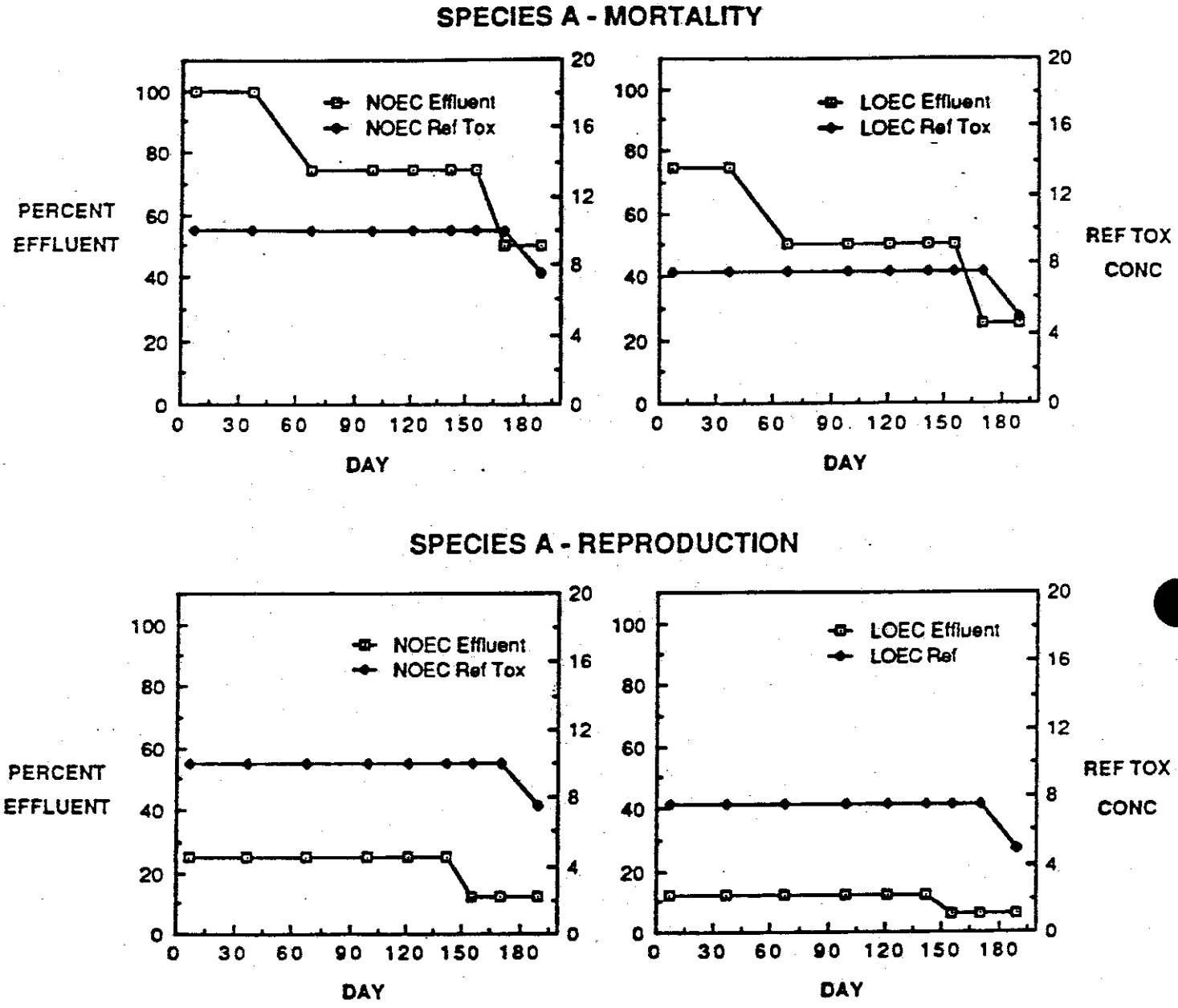

Figure 1. Graphical format for toxicity test with 2 endpoints (mortality and reproduction) and reference toxicants.

APPENDIX E

APPENDIX E

Information on Variability Phase Submittals

From: S.L. Anderson. July 13, 1988 mailing to Effluent Toxicity Characterization Program Mailing List.

Variability phase submittals [study plans] should contain a discussion of expected effluent variability with all proposals and conclusions supported with quantitative data. Rationale for a long-term, short-term, or mixed or unknown variability case should be provided wherever possible. It is acknowledged that few cases exist for which significant short term variability can be documented. Conventional effluent monitoring data will not be considered sufficient. Data should be presented on conventional parameters such as BOD, suspended solids and flows as well as toxic contaminants and past bioassay data. Data should be presented in the most specific units possible (e.g., daily or weekly averages are preferred over monthly averages and standard deviations should be provided). Data should also be provided on the retention times of your facility, influent quality and variation, treatment unit removal efficiencies, and industrial load quality and variation. Other factors that should be considered include: the effect of seasonal variations of flow and ambient temperature on the wastewater treatment system, particularly during transition periods; maintenance, start-up, and shut-down of treatment units; and bypassing of treatment units.

Municipal dischargers should submit pretreatment data and wastewater treatment plant influent data with analysis and discussion of seasonality in treatment plant inputs and removal efficiencies. Data on one recent wet weather year and one recent dry weather year are sufficient.

Industrial dischargers should submit a detailed description of processes which generate wastewater and the expected variability of that wastewater along with wastewater treatment plant influent data. For example, discussion of a refinery operation could include: changes in type, quality, and quantity of crude processes; start-up, operation, and shut down of process units.

The aspects of effluent variability mentioned above should be used to formulate a proposed sampling schedule for the variability phase of your program. One logical approach to consider is to conduct 8-10 series of three tests on a random basis throughout the year and then to schedule 8-10 series of three tests keyed to specific treatment plant changes.