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WATER QUALITY CONTROL PLAN FOR
INLAND SURFACE WATERS, ENCLOSED BAYS, AND ESTUARIES OF CALIFORNIA

OCTOBER 19, 2018

DIVISION OF WATER QUALITY

STATE WATER RESOURCES CONTROL BOARD

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

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I. INTRODUCTION

This Water Quality Control Plan for Inland Surface Waters, Enclosed Bays, and Estuaries of California (Plan) was adopted by the State Water Resources Control Board (State Water Board) under authority provided by Water Code sections 13140 and 13170. Except as otherwise indicated, this Plan establishes provisions for water quality and sediment quality that apply to all INLAND SURFACE WATERS, ENCLOSED BAYS, and ESTUARIES AND COASTAL LAGOONS of the state, including both waters of the United States and surface waters of the state. These provisions do not apply to OCEAN WATERS, including Monterey Bay and Santa Monica Bay. In accordance with Water Code section 13170, except where otherwise noted, the provisions contained within this Plan supersede any Regional Water Quality Control Plans (Basin Plans) for the same waters to the extent of any conflict. All terms in capital letters are defined in Appendix A.

II. BENEFICIAL USES

Water body-specific beneficial use designations contained in the Basin Plans and other statewide plans, including future amendments to those plans, are incorporated by reference into this Plan.

III. WATER QUALITY OBJECTIVES

A. *[Reserved]*

B. Aquatic Toxicity

Aquatic toxicity is the adverse response of aquatic organisms from exposure to chemical or physical agents, and/or their synergistic effects in effluent or receiving water. Acute aquatic toxicity refers to adverse response (typically lethality) from a short-term exposure. Chronic aquatic toxicity generally refers to a sub-lethal adverse response.

1. Applicable Beneficial Uses

The following water quality objectives for chronic and acute toxicity establish minimum requirements to protect AQUATIC LIFE beneficial uses including, but not limited to, warm freshwater habitat (WARM), cold freshwater habitat (COLD), wildlife habitat (WILD), estuarine habitat (EST), preservation of rare, threatened, or endangered species (RARE), migration of aquatic organisms (MIGR), spawning reproduction and/or early development (SPWN), marine habitat (MAR), inland saline water habitat (SAL), and wetland habitat (WET).

2. Aquatic Toxicity Water Quality Objectives

a. Numeric Chronic Aquatic Toxicity Objective

The chronic aquatic toxicity water quality objective is expressed as a NULL HYPOTHESIS and an ALTERNATIVE HYPOTHESIS with a REGULATORY MANAGEMENT DECISION (RMD) of 0.75, where the following NULL HYPOTHESIS shall be used:

H_o: Mean RESPONSE (ambient receiving water) ≤ 0.75 • mean RESPONSE (control)

In general terms, the NULL HYPOTHESIS is the following statement: the ambient receiving water is toxic because the test organism RESPONSE (e.g., survival, reproduction, growth) in the ambient receiving water sample is less than or equal to 75 percent of the test organism RESPONSE in the control water sample.

And where the following ALTERNATIVE HYPOTHESIS shall be used:

H_a: Mean RESPONSE (ambient receiving water) > 0.75 • mean RESPONSE (control)

In general terms, the ALTERNATIVE HYPOTHESIS is the following statement: the ambient receiving water is not toxic because the test organism RESPONSE (e.g., survival, reproduction, growth) in the ambient receiving water sample is greater than 75 percent of the test organism RESPONSE in the control water sample.

Attainment of the water quality objective is demonstrated by conducting CHRONIC TOXICITY TESTING as described in Section IV.B.1.b and rejecting this NULL HYPOTHESIS in accordance with the TEST OF SIGNIFICANT TOXICITY (TST) statistical approach described in Section IV.B.1.c. When the NULL HYPOTHESIS is rejected, the ALTERNATIVE HYPOTHESIS is accepted in its place, and there is no exceedance of the chronic toxicity water quality objective. Failing to reject the NULL HYPOTHESIS (referred to as a "fail") is equivalent to an exceedance of the chronic toxicity water quality objective.

b. Numeric Acute Aquatic Toxicity Objective

The acute aquatic toxicity water quality objective is expressed as a NULL HYPOTHESIS and ALTERNATIVE HYPOTHESIS with an RMD of 0.80, where the following NULL HYPOTHESIS shall be used:

H_o: Mean RESPONSE (ambient receiving water) ≤ 0.80 • mean RESPONSE (control)

In general terms, the NULL HYPOTHESIS is the following statement: the ambient receiving water is toxic because the test organism RESPONSE (e.g., survival) in the ambient receiving water sample is less than or equal to 80 percent of the test organism RESPONSE in the control water sample.

And where the following ALTERNATIVE HYPOTHESIS shall be used:

H_a: Mean RESPONSE (ambient receiving water) > 0.80 • mean RESPONSE (control)

In general terms, the ALTERNATIVE HYPOTHESIS is the following statement: the ambient receiving water is not toxic because the test organism RESPONSE (e.g., survival) in the ambient receiving water sample is greater than 80 percent of the test organism RESPONSE in the control water sample.

Attainment of the water quality objective is demonstrated by conducting ACUTE TOXICITY TESTING as described in Section IV.B.1.b and rejecting this NULL HYPOTHESIS in accordance with the TST statistical approach described in Section IV.B.1.c. When the NULL HYPOTHESIS is rejected, the ALTERNATIVE HYPOTHESIS is accepted in its place, and there is no exceedance of the acute toxicity water quality objective. Failing to reject the NULL HYPOTHESIS (referred to as a "fail") is equivalent to an exceedance of the acute toxicity water quality objective.

3. Interaction of Toxicity Provisions with Basin Plans and the SIP

In accordance with Water Code section 13170, except where otherwise noted, the TOXICITY PROVISIONS supersede any Regional Water Quality Control Plans (Basin Plans) for the same waters to the extent of any conflict. The TOXICITY PROVISIONS supersede section 4 of the Policy for Implementation of Toxics Standards for Inland Surface Waters, Enclosed Bays, and Estuaries of California (SIP).

The TOXICITY PROVISIONS in Section III.B.2 and Section IV.B, except as defined in this section, supersede Basin Plan toxicity provisions to the extent that:

(A) The Basin Plan provisions specify methods of assessing compliance with any numeric or narrative water quality objectives for acute and chronic aquatic toxicity; and
(B) The Basin Plan provisions regard aquatic toxicity testing and/or interpretation of aquatic toxicity testing results; and
(C) The Basin Plan provisions are in conflict with the TOXICITY PROVISIONS.

The TOXICITY PROVISIONS in Section III.B.2 and Section IV.B, notwithstanding the above, do not supersede:

(D) The narrative toxicity water quality objectives (e.g., 'no toxic POLLUTANTS in toxic amounts'); and
(E) Any Basin Plan provisions regarding the application of narrative toxicity water quality objectives to derive chemical specific limits, targets, and other thresholds; and
(F) Any site-specific toxicity water quality objective established in a Basin Plan. In addition, the TOXICITY PROVISIONS in Section III.B.2 and Section IV.B do not apply to that water body.

Any total maximum daily loads (TMDLs), including their implementation provisions, adopted by a Regional Water Board prior to the effective date of these TOXICITY PROVISIONS, remain in effect, and do not require reconsideration (for purposes of compliance with the TOXICITY PROVISIONS). Nothing in this section limits the Regional Water Board's authority to reconsider a TMDL and its implementation provisions.

4. Interaction of Toxicity Provisions with Narrative and Numeric Toxicity Water Quality Objectives

Section IV.B. includes a program of implementation for toxicity that shall be used to assess whether ambient receiving water meets the numeric aquatic toxicity water quality objectives, whether a PERMITTING AUTHORITY shall require aquatic toxicity effluent limitations for non-storm water National Pollutant Discharge Elimination System (NPDES) dischargers, and whether dischargers' effluent complies with applicable permit terms.

Compliance with narrative toxicity water quality objectives is determined by use of indicator species, analysis of species diversity, pollution density, toxicity tests or other appropriate method as specified by the PERMITTING AUTHORITY. The PERMITTING AUTHORITY may also consider all material and relevant information submitted by the discharger and other interested parties and numerical criteria and guidelines for toxic substances developed by the State Water Board, the California Office of Environmental Health Hazard Assessment, the California Department of Health Services, the U.S. Food and Drug Administration, the National Academy of Sciences, the U.S. EPA, and other appropriate organizations, to evaluate compliance with narrative toxicity water quality objectives.

The PERMITTING AUTHORITY shall have discretion regarding the application of narrative toxicity water quality objectives to derive chemical specific effluent limitations, receiving water limitations, targets, and other thresholds.

In addition to implementing the requirements of Section IV.B. using a species and endpoint identified in Table 1 of Section IV.B.1.b., the PERMITTING AUTHORITY shall have discretion regarding the application of narrative toxicity water quality objectives to derive effluent limitations for aquatic toxicity endpoints not addressed by any of the acute and chronic aquatic toxicity test methods identified in Table 1 of Section IV.B.1.b (e.g., endocrine disruption).

The PERMITTING AUTHORITY shall have discretion regarding the application of narrative or numeric toxicity water quality objectives to derive narrative effluent or receiving water limitations.

The PERMITTING AUTHORITY shall not include numeric effluent limitations for aquatic toxicity endpoints addressed by any of the acute and chronic toxicity test methods identified in Table 1 of Section IV.B.1.b to implement either the toxicity narrative or numeric water quality objectives except as indicated in section IV.B.2.e.

IV. PROGRAMS OF IMPLEMENTATION

A. *[Reserved]*

B. Aquatic Toxicity

The following sections shall be used to assess whether ambient receiving water meets the numeric aquatic toxicity water quality objectives, whether a PERMITTING AUTHORITY shall require aquatic toxicity effluent limitations for non-storm water National Pollutant Discharge Elimination System (NPDES) dischargers, and whether dischargers' effluent complies with applicable permit terms. Specific requirements for NON-STORM WATER NPDES DISCHARGERS, STORM WATER DISCHARGERS, and NONPOINT SOURCE dischargers are described, respectively, in Section IV.B.2, IV.B.3, and IV.B.4.

1. Required Toxicity Testing Methods and Analyses

a. Toxicity Testing Sample and Location

To determine if ambient water meets the numeric aquatic water quality objective (non-specific to a discharger), the ambient water sample shall be a representative sample of the waterbody.

For compliance with a receiving water limitation for a specific discharger, the ambient water sample shall be from a location specified by the PERMITTING AUTHORITY.

For compliance with an effluent limitation for a specific discharger, effluent samples shall be from a location specified by the PERMITTING AUTHORITY. Dilution and control waters should be obtained from an area unaffected by the discharge in the receiving waters. For rivers and streams, dilution water should be obtained immediately upstream of the wastewater outfall. Standard dilution water, as defined by the test methods, can be used if the above sources exhibit toxicity or if approved by the PERMITTING AUTHORITY.

b. Toxicity Test Methods

CHRONIC TOXICITY TESTS shall be conducted using one or more of the test species in Table 1 selected by the PERMITTING AUTHORITY in accordance with the TOXICITY PROVISIONS, and shall follow methods identified in the Code of Federal Regulations, title 40, part 136 or included in the following United States Environmental Protection Agency (U.S. EPA) method manuals: Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition (EPA-821-R-02-013); Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, Third Edition (EPA-821-R-02-014); and Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms, First Edition (EPA-600-R-95-136).

Table 1. Bioequivalence Values (b), Test Species Tier Classification, and False Negative Rate (α error) for toxicity test methods.

EPA Toxicity Test Method	Bioequivalence Value (b)	Tier	False Negative (α Error)
Chronic Freshwater Methods			
Ceriodaphnia dubia (water flea) Survival and reproduction	0.75	I	0.20
Pimephales promelas (fathead minnow) Survival and growth	0.75	I	0.25
Selenastrum capricornutum (green alga) Growth	0.75	I	0.25
Chronic West Coast Marine Methods			
Atherinops affinis (topsmelt) Survival and growth	0.75	I	0.25
Dendraster excentricus (sand dollar); *Strongylocentrotus purpuratus* (purple urchin) Fertilization	0.75	I	0.05
Dendraster excentricus (sand dollar); Strongylocentrotus purpuratus (purple urchin) Larval development	0.75	I	0.05
Haliotis rufescens (red abalone) Larval development	0.75	I	0.05
Mytilus sp. (mussels); *Crassostrea gigas* (oyster) Larval development	0.75	I	0.05
Macrocystis pyrifera (giant kelp) Germination and germ-tube length	0.75	I	0.05
Chronic East Coast Marine Methods			
Menidia beryllina (inland silverside) Survival and growth	0.75	II	0.25
Americamysis bahia (mysid) Survival and growth	0.75	II	0.15
Acute Freshwater Methods			
Ceriodaphnia dubia (water flea); *Daphnia magna* (water flea); *Daphnia pulex* (water flea); *Hyalella azteca* (amphipod) Survival	0.80	I	0.10
Pimephales promelas (fathead minnow); *Oncorhynchus mykiss* (rainbow trout); *Salvelinus fontinalis* (brook trout) Survival	0.80	I	0.10
Acute Marine Methods			
Atherinops affinis (topsmelt) Survival	0.80	I	0.10
Americamysis bahia (mysid) Survival	0.80	II	0.10
Menidia beryllina (inland silverside) Survival	0.80	II	0.10

Notes: The false positive rate (β error) is set at 0.05 for all toxicity test methods. The bioequivalence value (b) is equivalent to the RMD.

ACUTE TOXICITY TESTS shall be conducted using one or more of the test species in Table 1 selected by the PERMITTING AUTHORITY in accordance with the TOXICITY PROVISIONS and shall follow methods established in Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition (EPA-821-R-02-012).

These methods specify a minimum number of REPLICATES. However, additional test REPLICATES may be conducted to increase test sensitivity and confidence in the results.

Test method selection is determined by salinity and tier classification (refer to Table 1 in this Section). Freshwater test methods shall be used for receiving waters in which salinity is less than 1,000 mg/L at least 95 percent of the time, and marine test methods shall be used for receiving waters in which salinity is equal to or greater than 1,000 mg/L at least 95 percent of the time. In all other instances, the PERMITTING AUTHORITY has discretion to choose either freshwater test or marine test methods for receiving waters. The PERMITTING AUTHORITY shall specify in the permit or monitoring requirements whether freshwater or marine test methods shall be used. The PERMITTING AUTHORITY may require use of freshwater test methods for dischargers that discharge freshwater effluent to marine waters. Tier I test species shall be used unless Tier I species are not readily available, in which case the PERMITTING AUTHORITY may allow the use of Tier II test species.

Test results shall be analyzed using the TEST OF SIGNIFICANT TOXICITY (TST) as described in Section IV.B.1.c. To the extent that U.S. EPA-approved methods require that observations should be made of organism RESPONSES in multiple concentrations of effluent or receiving water, the INSTREAM WASTE CONCENTRATION (IWC) shall be included as one of the selected concentrations, and the TST shall be conducted using the IWC and control as described in Section IV.B.1.c.

c. Test of Significant Toxicity

Aquatic toxicity test data shall be analyzed using the TEST OF SIGNIFICANT TOXICITY (TST) as described below in Steps 1 through 7. For any chronic toxicity test method with both lethal and sub-lethal endpoints, the sub-lethal endpoint data shall be used in Steps 1 through 7. For any chronic toxicity test method with more than one sub-lethal endpoint (giant kelp), the data for each sub-lethal endpoint shall be independently analyzed using Steps 1 through 7. The TST is applicable for a data analysis of an IWC compared to a control. For assessing whether receiving waters meet the water quality objectives, the undiluted ambient water shall be used as the IWC.

Step 1: Conduct the aquatic toxicity test according to procedures in the appropriate test method manual, as described in Section IV.B.1.b.

Step 2: Determine if there is no variance in the ENDPOINT (i.e., determine if all REPLICATES in each concentration have the same exact RESPONSE).

If there is no variance in the ENDPOINT in both concentrations being compared, compute the PERCENT EFFECT, as described in Section IV.B.1.d.

If the PERCENT EFFECT at the IWC is ≥ the RMD, the sample is declared toxic and the test result is "fail." If the PERCENT EFFECT at the IWC is < the RMD, the sample is declared non-toxic and the test result is "*Pass.*" Skip steps 3-7.

If there is variance in the ENDPOINT in both concentrations being compared, follow Steps 3-7.

Step 3: Use the data to calculate the mean RESPONSE for the control and IWC. If the data consists of proportions from a binary response (e.g., for survival, germination, and fertilization) transform the data using the arcsine square root transformation before calculating the mean RESPONSE for the control and IWC.

The arcsine square root transformation is used for such data to stabilize the variance and satisfy the normality requirement. To conduct the arcsine square root transformation, the response proportion (RP) for each REPLICATE (e.g., percent survival, percent fertilization), expressed as a decimal fraction (where 1.00 = 100 percent) for each treatment, is first calculated:

$$RP = \frac{\text{Number of Organisms with Response}}{\text{Number of Organisms Exposed}}$$

The square root value of the response proportion is then arcsine transformed before calculating the mean RESPONSE and analysis in Step 4. Note: Excel and most statistical software packages can calculate arcsine square root values.

If $0 < RP < 1$,

then the angle (in radians) = $\arcsin(\sqrt{(RP)})$.

If $RP = 0$,

then the angle (in radians) = $\arcsin(\sqrt{1/4n})$,
Where n = number of ORGANISMS used for each REPLICATE.

If $RP = 1$

then the angle (in radians) = $\arcsin(\sqrt{1-(1/4n)})$,
Where n = number of ORGANISMS used for each REPLICATE.

Use the transformed data in the following steps.

Step 4: Conduct Welch's t-test (Zar 1996) using the following equation to obtain the calculated *t* value:

$$t = \frac{\overline{Y}_t - b \bullet \overline{Y}_c}{\sqrt{\frac{S_t^{\ 2}}{n_t} + \frac{b^2 S_c^{\ 2}}{n_c}}}$$

Where:

$\overline{Y}_c$ = Mean RESPONSE for the control

$\overline{Y}_t$ = Mean RESPONSE for the IWC

S_c^2 = Estimate of the variance for the control

S_t^2 = Estimate of the variance for the IWC

n_c = Number of REPLICATES for the control

n_t = Number of REPLICATES for the IWC

b = 0.75 for chronic tests; 0.80 for acute tests
(Note: b is equivalent to the RMD)

Note on the use of Welch's t-test: Welch's t-test is appropriate to use when there are an unequal number of REPLICATES between control and the IWC. When sample sizes of the control and treatment are the same (i.e., $n_t = n_c$), Welch's t-test is equivalent to the Student's t-test (Zar 1996).

Step 5: Adjust the degrees of freedom using the following equation:

$$v = \frac{\left(\frac{S_t^{\ 2}}{n_t} + \frac{b^2 S_c^{\ 2}}{n_c}\right)^2}{\frac{\left(\frac{S_t^{\ 2}}{n_t}\right)^2}{n_t - 1} + \frac{\left(\frac{b^2 S_c^{\ 2}}{n_c}\right)^2}{n_c - 1}}$$

Using Welch's t-test, the degrees of freedom is the value obtained for *v* in the equation above. When *v* is a non-integer, round *v* to the next smallest integer, and that number is used as the degrees of freedom.

Step 6: Compare the calculated *t* value from Step 4 with the critical *t* value in Table 2 using the test method-specific alpha values shown in Table 1 of Section IV.B.1.b. To obtain the critical *t* value, look across the table for the alpha value that corresponds to the toxicity test method and then look down the table for the appropriate degrees of freedom.

Step 7: If the calculated *t* value is less than the critical *t* value, the NULL HYPOTHESIS is not rejected, and the test result is "fail." If the calculated *t* value is greater than the critical *t* value, the NULL HYPOTHESIS is rejected, and the test result is "pass".

d. Percent Effect

The PERCENT EFFECT at the IWC shall be calculated for each ENDPOINT in an aquatic toxicity test. Calculate the PERCENT EFFECT at the IWC using untransformed data and the following equation:

$$\text{Percent Effect at the IWC} = \frac{\text{Mean Control Response} - \text{Mean IWC Response}}{\text{Mean Control Response}} \bullet 100$$

e. Reporting

Results obtained from toxicity tests shall be reported to the PERMITTING AUTHORITY as either a "pass" or a "fail," and the PERCENT EFFECT at the IWC for each endpoint. The results and any required supporting data shall be submitted in the format specified by the PERMITTING AUTHORITY.

Table 2. Critical values of the t-distribution; one-tailed probability is assumed.

α Error					
Degrees of Freedom (v)	**0.25**	**0.20**	**0.15**	**0.10**	**0.05**
1	1	1.3764	1.9626	3.0777	6.3138
2	0.8165	1.0607	1.3862	1.8856	2.92
3	0.7649	0.9785	1.2498	1.6377	2.3534
4	0.7407	0.941	1.1896	1.5332	2.1318
5	0.7267	0.9195	1.1558	1.4759	2.015
6	0.7176	0.9057	1.1342	1.4398	1.9432
7	0.7111	0.896	1.1192	1.4149	1.8946
8	0.7064	0.8889	1.1081	1.3968	1.8595
9	0.7027	0.8834	1.0997	1.383	1.8331
10	0.6998	0.8791	1.0931	1.3722	1.8125
11	0.6974	0.8755	1.0877	1.3634	1.7959
12	0.6955	0.8726	1.0832	1.3562	1.7823
13	0.6938	0.8702	1.0795	1.3502	1.7709
14	0.6924	0.8681	1.0763	1.345	1.7613
15	0.6912	0.8662	1.0735	1.3406	1.7531
16	0.6901	0.8647	1.0711	1.3368	1.7459
17	0.6892	0.8633	1.069	1.3334	1.7396
18	0.6884	0.862	1.0672	1.3304	1.7341
19	0.6876	0.861	1.0655	1.3277	1.7291
20	0.687	0.86	1.064	1.3253	1.7247
21	0.6864	0.8591	1.0627	1.3232	1.7207
22	0.6858	0.8583	1.0614	1.3212	1.7171
23	0.6853	0.8575	1.0603	1.3195	1.7139
24	0.6849	0.8569	1.0593	1.3178	1.7109
25	0.6844	0.8562	1.0584	1.3163	1.7081
26	0.684	0.8557	1.0575	1.315	1.7056
27	0.6837	0.8551	1.0567	1.3137	1.7033
28	0.6834	0.8546	1.056	1.3125	1.7011
29	0.683	0.8542	1.0553	1.3114	1.6991
30	0.6828	0.8538	1.0547	1.3104	1.6973
inf	0.6745	0.8416	1.0364	1.2816	1.6449

2. Implementation for Non-Storm Water NPDES Dischargers

The PERMITTING AUTHORITY shall include the requirements specified in this Section (Section IV.B.2) for NPDES permits issued, reissued, renewed, or reopened after the effective date of these provisions for NON-STORM WATER NPDES DISCHARGERS.

a. Species Sensitivity Screening

i. Non-Storm Water NPDES Dischargers Required to Conduct Species Sensitivity Screening for Chronic Toxicity

All NON-STORM WATER NPDES DISCHARGERS shall conduct a SPECIES SENSITIVITY SCREENING for chronic toxicity either prior to, or within 18 months after the first issuance, reissuance, renewal, or reopening (to address toxicity requirements) of the permit after the effective date of these TOXICITY PROVISIONS. The PERMITTING AUTHORITY may require a SPECIES SENSITIVITY SCREENING for chronic toxicity prior to every subsequent issuance, reissuance, renewal, or reopening (to address toxicity requirements) of the permit. At a minimum, a SPECIES SENSITIVITY SCREENING shall be conducted no less than once every ten years unless the discharger is participating in a regional monitoring program approved by the PERMITTING AUTHORITY and the PERMITTING AUTHORITY determines that 1) the discharger has conducted a valid species sensitivity screening using test methods and statistical analysis required by these provisions and 2) the nature of the effluent has not changed since the last species sensitivity screening.

ii. Non-Storm Water NPDES Dischargers Required to Conduct Species Sensitivity Screening for Acute Toxicity.

Except for PUBLICLY OWNED TREATMENT WORKS (POTW) dischargers, all NON-STORM WATER NPDES DISCHARGERS shall conduct a SPECIES SENSITIVITY SCREENING for acute toxicity, either prior to, or within 18 months after the first issuance, reissuance, renewal, or reopening (to address toxicity requirements) of the permit after the effective date of these TOXICITY PROVISIONS. The PERMITTING AUTHORITY may require a SPECIES SENSITIVITY SCREENING for acute toxicity prior to every subsequent issuance, reissuance, renewal, or reopening (to address toxicity requirements) of the permit. At a minimum, a SPECIES SENSITIVITY SCREENING shall be conducted no less than once every ten years.

For POTW dischargers, the PERMITTING AUTHORITY may, in its discretion, require a SPECIES SENSITIVITY SCREENING for acute toxicity. This determination must be documented in the NPDES fact sheet (or equivalent document).

iii. Type and Frequency of Testing in a Species Sensitivity Screening

A SPECIES SENSITIVITY SCREENING for chronic toxicity includes four sets of testing conducted within one year, each set of testing consisting of, at a minimum, one vertebrate, one invertebrate, and one aquatic plant/algae from

Table 1 of Section IV.B.1.b. For CONTINUOUS DISCHARGERS, the four sets of testing shall be conducted over four consecutive quarters. For NON-CONTINUOUS DISCHARGERS, the four sets of testing shall be evenly distributed across the CALENDAR YEAR to the extent feasible.

A SPECIES SENSITIVITY SCREENING for acute toxicity includes four sets of testing conducted within one year, each set of testing consisting of, at a minimum, one vertebrate and one invertebrate from Table 1 of Section IV.B.1.b. For CONTINUOUS DISCHARGERS, the four sets of testing shall be conducted over four consecutive quarters. For NON-CONTINUOUS DISCHARGERS, the four sets of testing shall be evenly distributed across the CALENDAR YEAR to the extent feasible.

For dischargers granted a dilution credit or a MIXING ZONE for toxicity, the PERMITTING AUTHORITY may direct that a higher concentration of effluent than the IWC be used for SPECIES SENSITIVITY SCREENING to increase the likelihood that potential effects might be observed.

For seasonal and intermittent dischargers, testing in a specific SPECIES SENSITIVITY SCREENING can be conducted using effluent that is not discharged into surface waters (e.g., effluent discharged onto land because of summer prohibition on discharges into surface waters, etc.) as long as the effluent is representative of the effluent that will be discharged to surface waters.

iv. Determination of the Most Sensitive Species

The PERMITTING AUTHORITY has the discretion to choose how the MOST SENSITIVE SPECIES is selected from the SPECIES SENSITIVITY SCREENING. The PERMITTING AUTHORITY should generally select the species in the SPECIES SENSITIVITY SCREENING exhibiting the highest PERCENT EFFECT at the IWC as the MOST SENSITIVE SPECIES. The PERMITTING AUTHORITY shall indicate how the MOST SENSITIVE SPECIES is selected from the SPECIES SENSITIVITY SCREENING (e.g., species exhibiting highest percent effect, species with most number of “fails” etc.) in the NPDES permit.

The PERMITTING AUTHORITY shall specify the MOST SENSITIVE SPECIES and IWC in the NPDES permit. When the selected species cannot be used, including for example when the discharger encounters unresolvable test interference or cannot secure a reliable supply of test organisms, the PERMITTING AUTHORITY may specify a different species as the MOST SENSITIVE SPECIES. In such cases, the next applicable species shall be selected by the PERMITTING AUTHORITY as the MOST SENSITIVE SPECIES. The selection of the MOST SENSITIVE SPECIES must be documented in the NPDES fact sheet (or equivalent document).

When the SPECIES SENSITIVITY SCREENING is conducted within 18 months of the issuance, reissuance, renewal, or reopening (to address toxicity requirements) of the permit after the effective date of these TOXICITY PROVISIONS, then the PERMITTING AUTHORITY shall specify in the NPDES

permit a species as the MOST SENSITIVE SPECIES until the SPECIES SENSITIVITY SCREENING is conducted. The NPDES permit shall indicate the method of determining the MOST SENSITIVE SPECIES from the SPECIES SENSITIVITY SCREENING, and a provision indicating that the Executive Director or Executive Officer may select and document the species determined to be the MOST SENSITIVE SPECIES from the SPECIES SENSITIVITY SCREENING test. When that species cannot be used, such as when discharger encounters unresolvable test interference or cannot secure a reliable supply of test organisms, the Executive Director or Executive Officer may specify the next applicable species as the MOST SENSITIVE SPECIES and document that determination.

b. Reasonable Potential

If a REASONABLE POTENTIAL analysis is required pursuant to this Section, a REASONABLE POTENTIAL analysis shall be conducted prior to every permit issuance, reissuance, renewal, or reopening (to address toxicity requirements).

i. Non-Storm water NPDES Dischargers Required to Conduct Reasonable Potential Analysis for Chronic Toxicity.

Except for POTW dischargers authorized to discharge at a rate equal to or greater than 5.0 MGD, all NON-STORM WATER NPDES DISCHARGERS shall conduct a REASONABLE POTENTIAL analysis for chronic toxicity, pursuant to the procedures specified in Section IV.B.2.b.iii, for review and approval by the PERMITTING AUTHORITY. A REASONABLE POTENTIAL analysis for chronic toxicity is not required for POTW dischargers authorized to discharge at a rate equal to or greater than 5.0 MGD, because the PERMITTING AUTHORITY shall include an effluent limitation for these dischargers pursuant to Section IV.B.2.e.

ii. Non-Storm Water NPDES Dischargers Required to Conduct Reasonable Potential Analysis for Acute Toxicity.

Except for POTW dischargers, all NON-STORM WATER NPDES DISCHARGERS shall conduct a REASONABLE POTENTIAL analysis for acute toxicity, pursuant to the procedures in Section IV.B.2.b.iii, for review and approval by the PERMITTING AUTHORITY. The PERMITTING AUTHORITY may require POTW dischargers to conduct a REASONABLE POTENTIAL analysis for acute toxicity, pursuant to the procedures in Section IV.B.2.b.iii, for review and approval by the PERMITTING AUTHORITY. The PERMITTING AUTHORITY shall document the decision whether to conduct a REASONABLE POTENTIAL analysis for acute toxicity in the NPDES fact sheet (or equivalent document).

iii. Reasonable Potential Analysis

All toxicity test data generated within five years prior to permit issuance, reissuance, renewal, or reopening (to address toxicity requirements) that is representative of effluent quality during discharge conditions shall be evaluated in determining REASONABLE POTENTIAL. Data generated within those five years from a minimum of four tests using species specified by the PERMITTING

AUTHORITY and selected from Table 1 of Section IV.B.1.b must be conducted at the IWC and be analyzed using the TST. If this minimum data is unavailable and there is representative effluent, the PERMITTING AUTHORITY shall require the discharger to conduct additional toxicity tests at the IWC, using a species selected by the PERMITTING AUTHORITY from Table 1 of Section IV.B.1.b, and to analyze the results using the TST. The PERMITTING AUTHORITY may also evaluate older toxicity test data to determine REASONABLE POTENTIAL.

A discharge has REASONABLE POTENTIAL to cause or contribute to an excursion above the chronic toxicity water quality objectives specified in Section III.B.2.a, if any of the CHRONIC TOXICITY TESTS result in a "fail" at the IWC, or if any of the CHRONIC TOXICITY TESTS have a PERCENT EFFECT at the IWC greater than 10 percent.

A discharge has REASONABLE POTENTIAL to cause or contribute to an excursion above the acute toxicity water quality objectives specified in Section III.B.2.b, if any of the ACUTE TOXICITY TESTS result in a "fail" at the IWC, or if any of the ACUTE TOXICITY TESTS have a PERCENT EFFECT at the IWC greater than 10 percent.

Furthermore, other information or data, including, but not limited to, fish die off observation, lack of available dilution, or existing data on toxic POLLUTANTS, may be used by the PERMITTING AUTHORITY to determine if there is REASONABLE POTENTIAL to cause or contribute to an excursion above the toxicity water quality objectives specified in Section III.B.2.

For Non-Storm Water NPDES Dischargers that do not have an effluent discharge prior to permit issuance, reissuance, renewal or reopening (to address toxicity requirements) that is representative of the quality of the proposed discharge, the PERMITTING AUTHORITY may use non-facility specific monitoring data and other information to determine reasonable potential, consistent with 40 CFR 122.44(d)(1)(ii).

The PERMITTING AUTHORITY'S determination that there is or is no REASONABLE POTENTIAL must be documented in the NPDES fact sheet (or equivalent document).

If a REASONABLE POTENTIAL analysis indicates no REASONABLE POTENTIAL for either chronic or acute toxicity, the PERMITTING AUTHORITY may include a reopener clause in the permit authorizing the PERMITTING AUTHORITY to reopen the permit, reevaluate REASONABLE POTENTIAL, and add MAXIMUM DAILY EFFLUENT LIMITATIONS (MDEL) and MEDIAN MONTHLY EFFLUENT LIMITATIONS (MMEL), if warranted, after the evaluation of new data and information.

If a REASONABLE POTENTIAL analysis indicates there is REASONABLE POTENTIAL for the discharge to cause or contribute to an exceedance of either the chronic or the acute toxicity water quality objective, then the PERMITTING AUTHORITY shall include the corresponding MDEL and MMEL in the NPDES permit.

c. MDEL and MMEL Compliance Monitoring

All NON-STORM WATER NPDES DISCHARGERS that demonstrate REASONABLE POTENTIAL for chronic toxicity and all POTW dischargers that are authorized to discharge at a rate equal to or greater than 5.0 MGD shall conduct monitoring for compliance with the chronic toxicity MDEL and MMEL. All NON-STORM WATER NPDES DISCHARGERS that demonstrate REASONABLE POTENTIAL for acute toxicity shall conduct monitoring for compliance with the acute toxicity MDEL and MMEL. The compliance monitoring for the MDEL and MMEL includes ROUTINE MONITORING and MMEL COMPLIANCE TESTS.

Toxicity tests of the MOST SENSITIVE SPECIES conducted at the IWC and analyzed using the TST shall be used to determine compliance with the MDEL and MMEL. The PERMITTING AUTHORITY shall specify in the permit the specific type of testing (e.g. the MOST SENSITIVE SPECIES and the concentration of the IWC) that will be used to determine compliance with the chronic toxicity MDEL and MMEL and acute toxicity MDEL and MMEL, as applicable. The toxicity test in ROUTINE MONITORING and MMEL COMPLIANCE TESTS shall be the MOST SENSITIVE SPECIES toxicity test and shall be analyzed using the TST at the IWC.

The PERMITTING AUTHORITY shall specify the day of the month that corresponds to the start of a CALENDAR MONTH, and the day of the month and the month(s) that correspond to the start of the CALENDAR QUARTER, AND CALENDAR YEAR in an NPDES permit or Water Code section 13383 Order.

For dischargers that conduct ROUTINE MONITORING at a less than monthly frequency, the CALENDAR MONTH begins from the initiation of the ROUTINE MONITORING test.

ROUTINE MONITORING and MMEL COMPLIANCE TESTS shall be conducted in accordance with this section. ROUTINE MONITORING and MMEL COMPLIANCE TESTS continue during any required TOXICITY REDUCTION EVALUATION (TRE). When there is no effluent available to initiate a ROUTINE MONITORING test or MMEL COMPLIANCE TEST(s), the test is not required and ROUTINE MONITORING continues in the frequency specified in the permit.

i. Routine Monitoring for Chronic Toxicity

(A) Routine Monitoring Schedule for Chronic Toxicity

For NON-STORM WATER NPDES DISCHARGERS authorized to discharge, at a rate equal to or greater than 5.0 MGD, the frequency of ROUTINE MONITORING shall be specified in the NPDES permit as follows:

"The discharger shall conduct at least one CHRONIC TOXICITY TEST every CALENDAR MONTH during which there is expected to be at least 15 days of discharge. A sample for the ROUTINE MONITORING test shall be taken at a time that would allow corresponding MMEL COMPLIANCE TESTS to be initiated within the same CALENDAR MONTH as the ROUTINE MONITORING test."

For NON-STORM WATER NPDES DISCHARGERS authorized to discharge at a rate less than 5.0 MGD, the frequency of ROUTINE MONITORING shall be specified in the NPDES permit as follows:

"The discharger shall conduct at least one CHRONIC TOXICITY TEST each CALENDAR QUARTER during which there is expected to be at least 15 days of discharge. A sample for the ROUTINE MONITORING test shall be taken at a time that would allow corresponding MMEL COMPLIANCE TESTS to be initiated within the same CALENDAR MONTH as the ROUTINE MONITORING test."

The PERMITTING AUTHORITY shall have the discretion to require NON-STORM WATER NPDES DISCHARGERS with an MDEL and an MMEL in their permit to conduct more frequent chronic toxicity ROUTINE MONITORING than that which is prescribed in this subsection. The PERMITTING AUTHORITY may approve a reduction in the frequency of ROUTINE MONITORING in accordance with the requirements in Section IV.B.2.c.i.(B). At a minimum, a chronic toxicity ROUTINE MONITORING test shall be conducted at least once per CALENDAR YEAR. The rationale for requiring more frequent or reduced ROUTINE MONITORING must be documented in the NPDES fact sheet (or equivalent document) or Water Code section 13383 Order.

Consistent with the required frequency, the PERMITTING AUTHORITY has discretion to or not to specify the exact dates or time period in which a sample for ROUTINE MONITORING shall be taken within the defined ROUTINE MONITORING period (e.g., a requirement to initiate test within five days of the start of the CALENDAR QUARTER, a requirement to sample between the 10^{th} and the 15^{th} of each month, etc.). To the extent feasible, ROUTINE MONITORING test shall be evenly distributed across the CALENDAR YEAR or period of seasonal or intermittent discharge.

(B) Reduced Routine Monitoring Schedule for Chronic Toxicity

The PERMITTING AUTHORITY may approve a reduction in the frequency of the ROUTINE MONITORING specified in Section IV.B.2.c.i.(A) for dischargers upon reissuance, renewal, or reopening (to address toxicity requirements) of an NPDES permit when during the prior five consecutive years the following conditions have been met:

1) The MDEL and MMEL as specified in Section IV.B.2.e have not been exceeded;
2) The toxicity provisions in the applicable NPDES permit(s) have been followed.

The PERMITTING AUTHORITY may approve a reduced frequency ROUTINE MONITORING schedule from one CHRONIC TOXICITY TEST per CALENDAR MONTH, as required in Section IV.B.2.c.i.(A) to one per CALENDAR QUARTER. The PERMITTING AUTHORITY may approve a reduced frequency ROUTINE MONITORING schedule from one CHRONIC

TOXICITY TEST per CALENDAR QUARTER, as required in Section IV.B.2.c.i.(A), to two CHRONIC TOXICITY TESTS per CALENDAR YEAR. In addition, the PERMITTING AUTHORITY may approve a reduced frequency of one CHRONIC TOXICITY TEST per Calendar year when the following conditions have been met: (1) the discharger has an initial dilution of at least 10:1, and (2) for dischargers authorized to discharge, at a rate equal to or greater than 5.0 MGD, the PERMITTING AUTHORITY requires additional monitoring in accordance with Section IV.B.1.

The PERMITTING AUTHORITY shall require dischargers on an approved reduced frequency ROUTINE MONITORING schedule to return to a ROUTINE MONITORING schedule, as described in Section IV.B.2.c.i.(A), if the requirements listed above cease to be met. The PERMITTING AUTHORITY may also require dischargers on an approved reduced frequency ROUTINE MONITORING schedule to return to a ROUTINE MONITORING schedule, as described in Section IV.B.2.c.i.(A), for other reasons including major changes to the treatment facility or changes to the quality of the influent. Upon returning to a ROUTINE MONITORING schedule described in Section IV.B.2.c.i.(A), dischargers will need to, once again, meet the two conditions listed in this section for at least a period of five years to be granted another discretionary chronic toxicity ROUTINE MONITORING reduction.

The PERMITTING AUTHORITY may also approve a temporary reduction in the frequency of the ROUTINE MONITORING specified in Section IV.B.2.c.i.(A) for dischargers conducting a TRE. When a discharger is conducting a TRE, the PERMITTING AUTHORITY may temporarily reduce the ROUTINE MONITORING frequency to two CHRONIC TOXICITY TESTS per CALENDAR YEAR. The PERMITTING AUTHORITY shall require dischargers under a temporary reduced frequency to return to a ROUTINE MONITORING schedule, as described in Section IV.B.2.c.i.(A), either at the conclusion of the TRE or one year after the initiation of the TRE, whichever occurs sooner. Upon returning to a ROUTINE MONITORING schedule described in Section IV.B.2.c.i.(A), dischargers will need to meet the conditions 1-2 listed in this section to be granted a discretionary monitoring reduction.

ii. Routine Monitoring for Acute Toxicity

If REASONABLE POTENTIAL is demonstrated for acute toxicity, in accordance with the provisions specified in Section IV.B.2.b, the discharger shall conduct acute toxicity ROUTINE MONITORING in addition to any other required chronic toxicity ROUTINE MONITORING.

The monitoring period shall be specified in the NPDES permit and be at a frequency determined by the PERMITTING AUTHORITY but no less than once per CALENDAR YEAR. A ROUTINE MONITORING test shall be initiated at a time that would allow corresponding MMEL COMPLIANCE TESTS to be initiated within the same CALENDAR MONTH as the ROUTINE MONITORING test. The PERMITTING AUTHORITY has discretion to or not to specify the exact dates or

time period in which a sample for ROUTINE MONITORING shall be taken (e.g., a requirement to initiate test within five days of the start of the CALENDAR QUARTER, a requirement to sample between the 10th and the 15th of each month, etc.). To the extent feasible, ROUTINE MONITORING tests shall be evenly distributed across the CALENDAR YEAR or period of seasonal or intermittent discharge.

iii. Additional Routine Monitoring Tests for TRE Determination and Compliance

For NON-STORM WATER NPDES DISCHARGERS with a ROUTINE MONITORING frequency of less than monthly, an additional ROUTINE MONITORING test shall be required when there is one violation of the MDEL or MMEL, but not two violations in a single CALENDAR MONTH. This additional ROUTINE MONITORING test is not required if the discharger is already conducting a TRE, or if the discharger is required to conduct ROUTINE MONITORING at or more frequent than a monthly frequency.

This additional ROUTINE MONITORING test is used to determine if a TRE is necessary. This additional ROUTINE MONITORING test is also used for compliance purposes, and could require MMEL COMPLIANCES TESTS.

This additional ROUTINE MONITORING test shall be conducted in the successive CALENDAR MONTH after the CALENDAR MONTH in which the MMEL or MDEL violation occurred.

When there is no effluent available to initiate this additional ROUTINE MONITORING test, this additional ROUTINE MONITORING test shall not be required, ROUTINE MONITORING continues in the frequency specified in the permit, and the PERMITTING AUTHORITY shall have discretion to require a TRE.

iv. MMEL Compliance Tests

If an acute or chronic toxicity ROUTINE MONITORING test results in a “fail” at the IWC, then NON-STORM WATER NPDES DISCHARGERS shall conduct a maximum of two MMEL COMPLIANCE TESTS. The MMEL COMPLIANCE TESTS shall be initiated within the same CALENDAR MONTH that the first ROUTINE MONITORING test was initiated that resulted in the “fail” at the IWC. If the first chronic MMEL COMPLIANCE TEST results in a “fail” at the IWC, then the second MMEL COMPLIANCE TEST is waived. For the purposes of MMEL COMPLIANCE TEST, for dischargers that conduct ROUTINE MONITORING at a less than monthly frequency, the CALENDAR MONTH begins from the initiation of the ROUTINE MONITORING test.

When there is no effluent available to initiate an MMEL COMPLIANCE TEST, the MMEL COMPLIANCE TEST shall not be required, and ROUTINE MONITORING continues in the frequency specified in the permit.

d. Mixing Zones and Dilution Credits

The PERMITTING AUTHORITY may grant MIXING ZONES and DILUTION CREDITS to dischargers in accordance with the provisions of this section. The allowance of MIXING ZONES for chronic aquatic toxicity is discretionary and shall be determined on a discharge-by-discharge basis. A PERMITTING AUTHORITY may consider allowing MIXING ZONES and DILUTION CREDITS for chronic aquatic toxicity only for discharges with a physically identifiable point of discharge that are regulated through an NPDES permit issued by the PERMITTING AUTHORITY. The following conditions must be met in allowing a MIXING ZONE:

A MIXING ZONE shall not:

1) compromise the integrity of the entire water body;
2) cause acutely toxic conditions to AQUATIC LIFE passing through the MIXING ZONE;
3) adversely impact biologically sensitive or critical habitats, including, but not limited to, habitat of species listed under federal or state endangered species laws; or
4) overlap a MIXING ZONE from different outfalls.

If a PERMITTING AUTHORITY allows a MIXING ZONE and DILUTION CREDIT, the permit shall specify the method by which the MIXING ZONE was derived, the DILUTION RATIO calculated, the IWC granted, and the point(s) in the receiving water where the applicable objectives must be met. The application for the permit shall include, to the extent feasible, the information needed by the PERMITTING AUTHORITY to make a determination on allowing a MIXING ZONE, including the calculations for deriving the appropriate receiving water and effluent flows, and/or the results of a MIXING ZONE study. MIXING ZONE studies may include, but are not limited to, tracer studies, dye studies, modelling studies, and monitoring upstream and downstream of the discharge that characterize the extent of actual dilution.

When a MIXING ZONE and DILUTION CREDIT is granted by the PERMITTING AUTHORITY, the IWC is the concentration of effluent in the receiving water after mixing as determined by the PERMITTING AUTHORITY. When a mixing zone is granted, the IWC is the inverse of 1 plus the DILUTION CREDIT or IWC = 1/(1+D), where D = DILUTION CREDIT. The PERMITTING AUTHORITY may set the IWC at a concentration of effluent greater than the inverse of 1 plus the DILUTION CREDIT in order to protect beneficial uses, or because of site-specific conditions. For the purpose of toxicity tests, in no case shall the Permitting Authority set the IWC at less than the inverse of 1 plus the DILUTION RATIO. For completely mixed discharges the dilution credit may be equivalent to the dilution ratio. If no DILUTION CREDIT is granted for toxicity, then the undiluted effluent shall be used as the IWC.

The DILUTION RATIO shall be determined using the parameters specified in Table 3.

Table 3: Parameters for Calculating a Dilution Ratio

In Calculating A DILUTION RATIO For:	Use the Critical Low Flow Of The Upstream Receiving Water Of:	Use the Discharge Effluent Flow Of:
Acute Toxicity Objective	Lowest flow that occurs for one day with a statistical frequency of once every 10 years	Maximum daily flow (i.e., the maximum flow sample of all samples collected in a calendar day) during period of discharge.
Chronic Toxicity Objective	The average low flow that occurs for seven consecutive days with a statistical frequency of once every 10 years.	Four-day average of daily maximum flows (i.e., the average of daily maximums taken from the data set in four-day intervals.) during period of discharge.

e. Effluent Limitation Provisions

i. Chronic Toxicity Effluent Limitations

(A) Chronic Toxicity MDEL

Except when the MOST SENSITIVE SPECIES does not include the survival ENDPOINT the PERMITTING AUTHORITY shall include the following MDEL in the NPDES permit if REASONABLE POTENTIAL is demonstrated for chronic toxicity in accordance with the provisions specified in Section IV.B.2.b, or if a POTW is authorized to discharge at a rate equal to or greater than 5.0 MGD:

"No {MOST SENSITIVE SPECIES} CHRONIC TOXICITY TEST shall result in a "fail" at the IWC for the sub-lethal ENDPOINT measured in the test and a PERCENT EFFECT for the survival ENDPOINT greater than or equal to 50 percent."

If the MOST SENSITIVE SPECIES CHRONIC TOXICITY TEST does not include the survival ENDPOINT, then the PERMITTING AUTHORITY shall include the following MDEL:

"No {MOST SENSITIVE SPECIES} CHRONIC TOXICITY TEST shall result in a "fail" at the IWC for any sub-lethal ENDPOINT measured in the test and a PERCENT EFFECT for that sub-lethal ENDPOINT greater than or equal to 50 percent."

The PERMITTING AUTHORITY shall specify the MOST SENSITIVE SPECIES and the IWC in the NPDES permit. A MDEL violation may require

the implementation of a TRE in accordance with the provisions of Section IV.B.2.f.

(B) Chronic Toxicity MMEL

The PERMITTING AUTHORITY shall include the following MMEL in the NPDES permit if REASONABLE POTENTIAL is demonstrated for chronic toxicity in accordance with the provisions specified in Section IV.B.2.b, or if a POTW is authorized to discharge at a rate equal to or greater than 5.0 MGD:

"No more than one {MOST SENSITIVE SPECIES} CHRONIC TOXICITY TEST initiated in a CALENDAR MONTH may result in a "fail" at the IWC for any ENDPOINT."

The PERMITTING AUTHORITY shall specify the MOST SENSITIVE SPECIES and the IWC in the NPDES permit. A MMEL violation may require the implementation of a TRE, in accordance with the provisions of Section IV.B.2.f.

ii. Acute Toxicity Effluent Limitations

(A) Acute Toxicity MDEL

THE PERMITTING AUTHORITY shall include the following MDEL in the NPDES permit if REASONABLE POTENTIAL is demonstrated for acute toxicity:

"No {MOST SENSITIVE SPECIES} ACUTE TOXICITY TEST may result in a "fail" at the IWC for the survival ENDPOINT and a PERCENT EFFECT for the survival ENDPOINT greater than or equal to 50 percent."

The PERMITTING AUTHORITY shall specify the MOST SENSITIVE SPECIES and the IWC in the NPDES permit. A MDEL violation may require the implementation of a TRE in accordance with the provisions of Section IV.B.2.f.

(C) Acute Toxicity MMEL

THE PERMITTING AUTHORITY shall include the following MMEL in the NPDES permit if REASONABLE POTENTIAL is demonstrated for acute toxicity in accordance with the provisions specified in Section IV.B.2.b: "No more than one {MOST SENSITIVE SPECIES} ACUTE TOXICITY TEST initiated in a CALENDAR MONTH may result in a "fail" at the IWC for the survival ENDPOINT."

The PERMITTING AUTHORITY shall specify the MOST SENSITIVE SPECIES and the IWC in the NPDES permit. An MMEL violation may require the implementation of a TRE, in accordance with the provisions of Section IV.B.2.f.

f. Toxicity Reduction Evaluation

A TRE is required when a NON-STORM WATER NPDES DISCHARGER has any combination of two or more MDEL or MMEL violations within a single CALENDAR MONTH or within two successive CALENDAR MONTHS. In addition, if other information indicates toxicity (e.g., results of additional monitoring, fish kills, or intermittent recurring toxicity, etc.), then the PERMITTING AUTHORITY shall have discretion to require a TRE.

The discharger shall conduct a TRE in accordance with a TRE Work Plan as approved by the PERMITTING AUTHORITY. When TREs are required of multiple dischargers, the dischargers may coordinate the TREs with the approval of the PERMITTING AUTHORITY. ROUTINE MONITORING, as specified in Section IV.B.2.c, shall continue during a TRE.

g. Flow-Through Acute Toxicity Testing Systems

The PERMITTING AUTHORITY may require additional toxicity compliance provisions in the NPDES permit specific to FLOW-THROUGH ACUTE TOXICITY TESTING SYSTEMS, including but not limited to additional effluent limitations or additional monitoring requirements. For existing flow through systems that are not amenable to use of the TST, the PERMITTING AUTHORITY shall specify the statistical analysis and ENDPOINT (e.g., fail/pass, no observed effect concentration (NOEC), etc.). These additional requirements do not substitute toxicity provisions in Section IV.B.2.

If the PERMITTING AUTHORITY requires monitoring with FLOW-THROUGH ACUTE TOXICITY TESTING SYSTEMS constructed after the effective date of these TOXICITY PROVISIONS, those FLOW-THROUGH ACUTE TOXICITY TESTING SYSTEMS shall be designed to facilitate analysis of results using the TST, and the PERMITTING AUTHORITY shall require analysis of results to be conducted using the TST.

h. Additional Monitoring

In addition to effluent limitation compliance monitoring and monitoring specific to FLOW-THROUGH ACUTE TOXICITY TESTING SYSTEMS, the PERMITTING AUTHORITY has the discretion to require dischargers to conduct additional toxicity testing. This testing can include, but is not limited to the following, special studies, additional test species, testing with additional dilutions or higher concentrations of effluent than the IWC, or using test species not included in Table 1 of Section IV.B.1.b The PERMITTING AUTHORITY can require this testing in an NPDES permit or a Water Code section 13383 Order. The rationale for requiring additional monitoring must be documented in the NPDES fact sheet (or equivalent document) or Water Code section 13383 Order.

The PERMITTING AUTHORITY shall specify in the permit the specific type of testing (e.g. the MOST SENSITIVE SPECIES and the concentration of the IWC) that will be used to determine compliance with the MDEL and MMEL. To the extent any of the additional monitoring described above requires the use of receiving water, different

species, different effluent concentrations than the IWC, or different test methods, that monitoring cannot be used to determine compliance with the toxicity effluent limitations specified in Section IV.B.2.e.

i. Violation Reporting

All toxicity tests of the MOST SENSITIVE SPECIES at the IWC shall be used for determining compliance with any toxicity MDEL or MMEL contained in the discharger's permit. NON-STORM WATER NPDES DISCHARGERS shall notify the PERMITTING AUTHORITY of a violation of a toxicity MDEL or MMEL as soon as the discharger learns of the violation but no later than 24 hours of the discharger receiving the monitoring results.

j. Exceptions

i. Small Disadvantaged Communities

The PERMITTING AUTHORITY is authorized to exempt POTWs only serving SMALL DISADVANTAGED COMMUNITIES from some or all of the provisions of Section IV.B.2 if the PERMITTING AUTHORITY makes a finding that the discharge will have no REASONABLE POTENTIAL to cause or contribute to an exceedance of the toxicity water quality objectives. The REASONABLE POTENTIAL conclusion necessary to exempt SMALL DISADVANTAGED COMMUNITIES need not be based on the REASONABLE POTENTIAL analysis methods set forth in Section IV.B.2.b. For POTWs only serving SMALL DISADVANTAGED COMMUNITIES that do not have an effluent discharge prior to permit issuance, reissuance, renewal, or reopening (to address toxicity requirements) that is representative of the quality of the proposed discharge, the PERMITTING AUTHORITY is authorized to make this determination and exempt the POTW only after the first year of effluent discharge.

If exempt, the PERMITTING AUTHORITY shall include the water quality objectives in Section III.B.2 as a receiving water limitation in the NPDES permit and the PERMITTING AUTHORITY shall have the discretion to assign ROUTINE MONITORING as necessary. ROUTINE MONITORING schedules for POTWs only serving SMALL DISADVANTAGED COMMUNITIES shall not exceed the applicable frequency specified in Section IV.B.2.c for the discharger's authorized rate of discharge.

ii. Insignificant Discharges

The PERMITTING AUTHORITY is authorized to exempt certain NON-STORM WATER NPDES DISCHARGERS from some or all of the provisions of Section IV.B.2 if the PERMITTING AUTHORITY makes a finding that the discharge will have no REASONABLE POTENTIAL to cause or contribute to an exceedance of the toxicity water quality objectives. The REASONABLE POTENTIAL conclusion necessary to exempt INSIGNIFICANT DISCHARGES need not be based on the REASONABLE POTENTIAL analysis methods set forth in Section IV.B.2.b.

If exempt, the PERMITTING AUTHORITY shall include the water quality objectives in Section III.B.2 as a receiving water limitation in the NPDES permit and the PERMITTING AUTHORITY shall have the discretion to assign ROUTINE MONITORING as necessary. ROUTINE MONITORING schedules for INSIGNIFICANT DISCHARGES shall not exceed the applicable frequency specified in Section IV.B.2.c for the discharger's authorized rate of discharge.

3. Implementation for Storm Water Dischargers Regulated Pursuant to NPDES Permits

The PERMITTING AUTHORITY shall have discretion to require toxicity monitoring using any test method. For all STORM WATER dischargers with existing chronic or acute toxicity monitoring requirements with test methods described in Section IV.B.1.b, the PERMITTING AUTHORITY shall issue Water Code section 13267 or 13383 Orders within one year of the effective date of these TOXICITY PROVISIONS that requires the statistical approach, percent effect, and reporting to be conducted in accordance with Section IV.B.1.c, IV.B.1.d, & IV.B.1.e commencing within one year from the date of the Order.

If after the effective date of these TOXICITY PROVISIONS, the PERMITTING AUTHORITY issues new or reissued chronic or acute toxicity monitoring requirements with test methods described in Section IV.B.1.b, then the PERMITTING AUTHORITY shall require the statistical approach, percent effect, and reporting to be conducted in accordance with Section IV.B.1.c, IV.B.1.d, and IV.B.1.e.

The PERMITTING AUTHORITY shall have discretion to require test methods not described in Section IV.B.1.b, except as required by federal law. This determination must be documented in the NPDES fact sheet (or equivalent document) or Water Code section 13267 or 13383 Order. Multi-concentration testing is not required except to the extent required by federal law or specified by the PERMITTING AUTHORITY.

4. Implementation for Nonpoint Source and Other Non-NPDES Dischargers

The PERMITTING AUTHORITY shall have discretion to require toxicity monitoring using any test method. For all NONPOINT SOURCE and other non-NPDES dischargers with existing chronic or acute toxicity monitoring requirements with test methods described in Section IV.B.1.b, the PERMITTING AUTHORITY shall issue a Water Code section 13267 Order within one year of the effective date of these TOXICITY PROVISIONS that requires the statistical approach, percent effect, and reporting to be conducted in accordance with Section IV.B.1.c, IV.B.1.d, and IV.B.1.e, commencing within one year from the date of the Order.

If after the effective date of these TOXICITY PROVISIONS, the PERMITTING AUTHORITY issues new or renewed chronic or acute toxicity monitoring requirements with test methods described in Section IV.B.1.b, then the PERMITTING AUTHORITY shall require the statistical approach, percent effect, and reporting to be conducted in accordance with Section IV.B.1.c, IV.B.1.d, & IV.B.1.e.

The PERMITTING AUTHORITY shall have discretion to require test methods not described in Section IV. B.1.b, except as required by federal law. This determination must be documented in the WDR (or equivalent document) or Water Code section 13267 Order. Multi-concentration testing is not required except to the extent required by federal law or specified by the PERMITTING AUTHORITY.

5. Variances and Exceptions to the Toxicity Water Quality Objectives

a. Waters of the U.S.

The PERMITTING AUTHORITY may, in compliance with CEQA, and subsequent to a public hearing, grant a variance to the numeric and narrative water quality objectives for toxicity. Water quality standard variances are subject to review and approval of the U.S. EPA, in accordance with Code of Federal Regulations, Title 40, section 131.14. {Note: This paragraph or similar provision may be added as part of an earlier amendment to the ISWEBE.}

b. Waters of the State That are Not Also Waters of the U.S.

The PERMITTING AUTHORITY may, after compliance with CEQA, allow short-term or seasonal exceptions from meeting numeric and narrative water quality objectives for toxicity if determined to be necessary to implement control measures for resource or pest management (e.g., vector or weed control, pest eradication, or fishery management) conducted by public entities.

The discharger shall notify potentially affected members of the public and governmental agencies. Also, the discharger shall submit to the PERMITTING AUTHORITY all of the following:

1) A detailed description of the proposed action, including the proposed method of completing the action;
2) A time schedule;
3) A discharge and receiving water quality monitoring plan (before project initiation, during the project, and after project completion, with the appropriate quality assurance and quality control procedures);
4) CEQA documentation;
5) Contingency plans;
6) Identification of alternate water supply (if needed); and
7) Residual waste disposal plans.

Additionally, upon completion of the project, the discharger shall provide certification by a qualified biologist that the receiving water beneficial uses have been restored. A qualified biologist is a biologist who has the knowledge and experience in the ecosystem where the resource or pest management control measure is implemented so that he or she can adequately evaluate whether the beneficial uses of the receiving waters have been protected and/or restored upon completion of the project.

APPENDIX A: Glossary

ACUTE TOXICITY TEST: A test to determine an adverse effect (usually lethality) on a group of test organisms during a short-term exposure (e.g. 24, 48, or 96 hours).

ALTERNATIVE HYPOTHESIS: A statement used to propose a statistically significant relationship in a set of given observations. Under the TST approach, when the NULL HYPOTHESIS is rejected, the ALTERNATIVE HYPOTHESIS is accepted in its place, indicating a relationship between variables and an acceptable level of toxicity.

AQUATIC LIFE: Aquatic life refers to aquatic organisms.

CALENDAR MONTH(S): A period of time from a day of one month to the day before the corresponding day of the next month if the corresponding day exists, or if not to the last day of the next month (e.g., from January 1 to January 31, from June 15 to July 14, or from January 31 to February 28).

CALENDAR QUARTER: A period of time defined as three consecutive CALENDAR MONTHS.

CALENDAR YEAR: A period of time defined as twelve consecutive CALENDAR MONTHS.

CHRONIC TOXICITY TEST: A test to determine an adverse effect (sub-lethal or lethal) on a group of test organisms during an exposure of duration long enough to assess sub-lethal effects.

CONTINUOUS DISCHARGERS: Facilities that discharge without interruption throughout its operating hours, except for infrequent shutdowns for maintenance, process changes, or other similar activities, and that discharge throughout the CALENDAR YEAR.

DILUTION CREDIT: The amount of dilution granted to a discharge in the calculation of a water quality-based effluent limitation, based on the allowance of a specified MIXING ZONE. It is calculated from the DILUTION RATIO or determined through conducting a MIXING ZONE study or modeling of the discharge and the receiving water.

DILUTION RATIO: The critical low flow of the upstream receiving water divided by the flow of the effluent discharged.

ENCLOSED BAYS: Indentations along the coast that enclose an area of oceanic water within distinct headlands or harbor works. ENCLOSED BAYS include all bays where the narrowest distance between headlands or outermost harbor works is less than 75 percent of the greatest dimension of the enclosed portion of the bay. This definition includes, but is not limited to: Humboldt Bay, Bodega Harbor, Tomales Bay, Drakes Estero, San Francisco Bay, Morro Bay, Los Angeles Harbor, Upper and Lower Newport Bay, Mission Bay, and San Diego Bay.

ENDPOINT: A measured RESPONSE of a receptor to a stressor. An endpoint can be measured in a toxicity test or field survey.

ESTUARIES and COASTAL LAGOONS: Waters at the mouths of streams where fresh and OCEAN WATERS mix during a portion of the year. Mouths of streams that are temporarily

separated from the ocean by sandbars shall be considered as estuaries. Estuarine waters will generally be considered to extend from a bay or the open ocean to the upstream limit of tidal action, but it may be considered to extend seaward if significant mixing of fresh and salt water occurs in the open coastal waters. The waters described by this definition include, but are not limited to, the Sacramento-San Joaquin Delta as defined by Water Code section 12220, Suisun Bay, Carquinez Strait downstream to Carquinez Bridge, and appropriate areas of the Smith, Klamath, Mad, Eel, Noyo, and Russian Rivers.

FLOW-THROUGH ACUTE TOXICITY TESTING SYSTEMS: A toxicity testing system where an effluent sample is either pumped continuously from the sampling point directly to a dilutor system, or collected and placed in a tank adjacent to the test laboratory and pumped continuously from the tank to a dilutor system.

INLAND SURFACE WATERS: All surface waters of the state (including waters of the United States) that do not include the ocean, ENCLOSED BAYS, or ESTUARIES AND COASTAL LAGOONS.

INSIGNIFICANT DISCHARGES: NPDES discharges that are determined to be a very low threat to water quality by the PERMITTING AUTHORITY.

INSTREAM WASTE CONCENTRATION (IWC): The concentration of effluent in the receiving water after mixing as determined by the PERMITTING AUTHORITY. For purposes of aquatic toxicity, when a MIXING ZONE and DILUTION CREDIT are granted for a NON-STORMWATER NPDES DISCHARGER, the IWC shall be determined as indicated in Section IV.B.2.d. For a NON-STORMWATER NPDES DISCHARGER, if no MIXING ZONE is allocated, then the undiluted effluent (100 percent) shall be used as the IWC. For assessing whether receiving waters meet the numeric water quality objectives, the undiluted ambient water shall be used as the IWC in the TEST OF SIGNIFICANT TOXICITY (TST) as indicated in Section IV.B.1.c.

MAXIMUM DAILY EFFLUENT LIMITATION (MDEL): For the purposes of chronic and acute aquatic toxicity, an MDEL is an effluent limitation based on the outcome of the TEST OF SIGNIFICANT TOXICITY (TST) approach and the resulting PERCENT EFFECT at the IWC, as described in Section IV.B.2.e.

MEDIAN MONTHLY EFFLUENT LIMITATION (MMEL): For the purposes of chronic and acute aquatic toxicity, an MMEL is an effluent limitation based on a maximum of three independent toxicity tests, analyzed using the TST, as described in Section IV.B.2.e.

MMEL COMPLIANCE TESTS: For the purposes of chronic and acute aquatic toxicity, MMEL COMPLIANCE TESTS are a maximum of two tests that are used in addition to the ROUTINE MONITORING test to determine compliance with the chronic and acute toxicity MMEL.

MIXING ZONE: A limited zone within a receiving water that is allocated for mixing with a wastewater discharge where a water quality objective can be exceeded without causing adverse effects to the overall water body.

MOST SENSITIVE SPECIES: The single species selected from an array of test species to be used in a single species laboratory test series to determine toxic effects of effluent or ambient water.

NON-CONTINUOUS DISCHARGERS: Facilities that do not discharge in a continuous manner or do not discharge throughout the CALENDAR YEAR (e.g. intermittent and seasonal dischargers).

NON-STORM WATER NPDES DISCHARGERS: Dischargers that are regulated pursuant to one or more NPDES permit(s) but excluding any discharges subject to the United States Code title 33 section 1342(p). This includes dischargers that discharge a combination of treated municipal or industrial waste water and storm water.

NONPOINT SOURCES: Sources that do not meet the definition of a POINT SOURCE, as defined below.

NULL HYPOTHESIS: A statement used in statistical testing that has been put forward either because it is believed to be true or because it is to be used as a basis for argument, but has not been proved.

OCEAN WATERS: The territorial marine waters of the state, as defined by California law, to the extent these waters are outside of ENCLOSED BAYS, ESTUARIES, and COASTAL LAGOONS. Discharges to OCEAN WATERS are regulated in accordance with the State Water Board's California Ocean Plan.

PERCENT EFFECT: The value that denotes the difference in RESPONSE between the test concentration and the control, divided by the mean control RESPONSE, and multiplied by 100.

PERMITTING AUTHORITY: The State Water Board or a regional water board that issues a permit, waste discharge requirements, water quality certification, or other authorization for the discharge or proposed discharge of waste. To the extent that the action is delegable, the term "Permitting Authority" can include the Executive Officer or Executive Director.

POINT SOURCE: Any discernible, confined and discrete conveyance including, but not limited to any pipe, ditch, channel, tunnel, conduit, well, discrete fissure, container, rolling stock, concentrated animal feeding operation, or vessel or other floating craft, from which POLLUTANTS are or may be discharged. This term does not include agricultural storm water discharges and return flows from irrigated agriculture.

POLLUTANT: Defined in section 502(6) of the CWA as "dredged spoil, solid waste, incinerator residue, filter backwash, sewage, garbage, sewage sludge, munitions, chemical wastes, biological materials, radioactive materials, heat, wrecked or discarded equipment, rock, sand, cellar dirt and industrial, municipal, and agricultural waste discharged into water."

PUBLICLY OWNED TREATMENT WORKS (POTW): Facilities owned by a state or municipality that store, treat, recycle, and reclaim municipal sewage or industrial wastes of a liquid nature. Similar facilities that are privately, instead of publicly owned, are included in this definition for purposes of Section IV.B.

REASONABLE POTENTIAL: A designation used for a waste discharge that is projected or calculated to cause or contribute to an excursion above a water quality standard.

REGULATORY MANAGEMENT DECISION (RMD): The decision that represents the maximum allowable error rates and thresholds for toxicity and non-toxicity that would result in an acceptable risk to AQUATIC LIFE.

REPLICATES: Two or more independent organism exposures of the same treatment (i.e. effluent concentration) within a toxicity test. REPLICATES are typically conducted with separate test chambers and test organisms, each having the same effluent concentration.

RESPONSE: A measured biological effect (e.g., survival, reproduction, growth) as a result of exposure to a stimulus.

ROUTINE MONITORING: Required monitoring that occurs during a permit term. For purposes of Section IV.B.2, ROUTINE MONITORING refers to the required toxicity testing described in Section IV.B.2.c, and is used to determine violations of the MDEL, and is used with MMEL COMPLIANCE TESTS to determine violations of the MMEL.

SMALL DISADVANTAGED COMMUNITIES: Municipalities with populations of 20,000 persons or less, or a reasonably isolated and divisible segment of a larger municipality encompassing 20,000 persons or less, with an annual median household income that is less than 80 percent of the statewide annual median household income.

SPECIES SENSITIVITY SCREENING: An analysis to determine the single MOST SENSITIVE SPECIES from an array of test species to be used in a single species laboratory test series.

STORM WATER: Same meaning set forth in 40 Code of Federal Regulations section 122.26(b)(13) (Nov. 16, 1990).

TEST OF SIGNIFICANT TOXICITY (TST): A statistical approach used to analyze aquatic toxicity test data, as described in Section IV.B.1.c.

TOXICITY IDENTIFICATION EVALUATIONS (TIEs): Techniques used to identity the unexplained cause(s) of toxic event. TIE involves selectively removing classes of chemicals through a series of sample manipulations, effectively reducing complex mixtures of chemicals in natural waters to simple components for analysis. Following each manipulation, the toxicity sample is assessed to see whether the toxicant class removed was responsible for the toxicity.

TOXICITY PROVISIONS: Refers to Section III.B and Section IV.B of the Water Quality Control Plan for Inland Surface Waters, Enclosed Bays, and Estuaries of California (Plan)

TOXICITY REDUCTION EVALUATION (TRE): A study conducted in a step-wise process designed to identify the causative agents of effluent or ambient toxicity, isolate the sources of toxicity, evaluate the effectiveness of toxicity control options, and then confirm the reduction in toxicity. A TIE may be required as part of the TRE, if appropriate.

APPENDIX B: Examples of Compliance Determination for Toxicity Effluent Limitations

Chronic *Ceriodaphnia dubia* test, example 1.

Step 1: Conduct the aquatic toxicity test according to the procedures in the appropriate test method manual, as described in Section IV. B.1.b of the Provisions. The corresponding results are reported below and used for the following example calculations**.**

Replicate/Statistic	Control Reproduction	Control Survival	IWC Reproduction	IWC Survival
1	29	1	31	1
2	38	1	28	1
3	31	1	25	1
4	34	1	28	1
5	36	1	22	1
6	35	1	21	1
7	30	1	27	1
8	31	1	26	1
9	36	1	29	1
10	34	1	30	1
Mean	33.4	1	26.7	1
Standard Deviation	2.989	0	3.268	0
# of REPLICATES (n)	10	10	10	10

Step 2: Determine if there is no variance in the ENDPOINT for each concentration. If there is no variance in both concentrations being compared, compute the PRECENT EFFECT as described in Section IV.B.1.d of the Provisions.

If there is variance in the ENDPOINT in both concentrations, then proceed with Steps 3-7.

For this example, the reproduction ENDPOINT would be used in the TST calculation. Both the Control and the IWC reproduction data have a standard deviation greater than 0 (i.e., both concentrations do have variance), so step 2 is not relevant and proceed to step 3.

Step 3: Calculate the mean RESPONSE for both concentrations and determine if an arcsine square root transformation in necessary.

Because reproduction data are not proportions of a binary response, this step is not necessary. Proceed to step 4.

Step 4: Conduct Welch's t-test, in this case for reproduction

$$t = \frac{\overline{Y_t} - b \times \overline{Y_c}}{\sqrt{\frac{{S_t}^2}{n_t} + \frac{b^2 {S_c}^2}{n_c}}} = \frac{26.7 - (0.75 \times 33.4)}{\sqrt{\frac{10.68}{10} + \frac{(0.75)^2 (8.93)}{10}}} = 1.32$$

Step 5: Adjust the degrees of freedom.

$$v = \frac{\left(\frac{{S_t}^2}{n_t} + \frac{b^2 {S_c}^2}{n_c}\right)^2}{\frac{\left(\frac{{S_t}^2}{n_t}\right)^2}{n_t - 1} + \frac{\left(\frac{b^2 {S_c}^2}{n_c}\right)^2}{n_c - 1}} = \frac{\left(\frac{10.68}{10} + \frac{(0.75)^2 (8.93)}{10}\right)^2}{\frac{\left(\frac{10.68}{10}\right)^2}{10 - 1} + \rightleftarrows \frac{\left(\frac{(0.75)^2 (8.93)}{10}\right)^2}{10 - 1}} = 15$$

Step 6: Compare the calculated t-value with the critical t-value:

Given 15 degrees of freedom and an alpha level set at 0.20, the critical t-value = 0.87 (obtained from Table 2 in the Provisions). The calculated t-value from step 4 = 1.32, which is greater than the critical t-value of 0.87.

Step 7: 1.32 > 0.87 = pass

The calculated t-value (1.32) is greater than the critical t-value (0.87), so the NULL HYPOTHESIS is rejected, and the test result is a "pass".

Conclusion: The test in example 1 indicates compliance with both the MDEL and the MMEL.

Reporting: Calculate the PERCENT EFFECT for all endpoints and report as required by Section IV.B.1.d of the Provisions.

$$\text{Reproduction \% Effect at IWC} = \frac{33.4 - 26.7}{33.4} \bullet 100 = 20.1\%$$

$$\text{Survival \% Effect at IWC} = \frac{1 - 1}{1} \bullet 100 = 0\%$$

Chronic *Ceriodaphnia dubia* test, example 2.

Step 1: Conduct the aquatic toxicity test according to the procedures in the appropriate test method manual, as described in Section IV. B.1.b of the Provisions. The corresponding results are reported below and used for the following example calculations.

Replicate/Statistic	Control Reproduction	Control Survival	IWC Reproduction	IWC Survival
1	29	1	19	1
2	38	1	18	0
3	31	1	6	0
4	34	1	11	0
5	36	1	20	1
6	35	1	10	0
7	30	1	18	1
8	31	1	32	1
9	36	1	25	1
10	34	1	18	0
Mean	33.4	1	17.70	0.5
Standard Deviation	2.989	0	7.499	0.5
# of REPLICATES (n)	10	10	10	10

Step 2: Determine if there is no variance in the ENDPOINT for each concentration. If there is no variance in both concentrations being compared, compute the PRECENT EFFECT as described in Section IV.B.1.d of the Provisions.

If there is variance in the ENDPOINT in both concentrations, then proceed with Steps 3-7.

For this example, the reproduction ENDPOINT would be used in the TST calculation. Both the Control and the IWC reproduction data have a standard deviation greater than 0 (i.e., both concentrations do have variance), so step 2 is not relevant and proceed to step 3.

Step 3: Calculate the mean RESPONSE for both concentrations and determine if an arcsine square root transformation is necessary.

Because reproduction data are not proportions of a binary response, this step is not necessary. Proceed to step 4.

Step 4: Conduct Welch's t-test.

$$t = \frac{\overline{Y_t} - b \times \overline{Y_c}}{\sqrt{\frac{{S_t}^2}{n_t} + \frac{b^2 {S_c}^2}{n_c}}} = \frac{17.70 - (0.75 \times 7.499)}{\sqrt{\frac{56.24}{10} + \frac{(0.75)^2 (8.93)}{10}}} = -2.9696$$

Step 5: Adjust the degrees of freedom.

$$v = \frac{\left(\frac{{S_t}^2}{n_t} + \frac{b^2{S_c}^2}{n_c}\right)^2}{\frac{\left(\frac{{S_t}^2}{n_t}\right)^2}{n_t - 1} + \frac{\left(\frac{b^2{S_c}^2}{n_c}\right)^2}{n_c - 1}} = \frac{\left(\frac{56.24}{10} + \frac{(0.75)^2(8.93)}{10}\right)^2}{\frac{\left(\frac{56.24}{10}\right)^2}{10-1} + \frac{\left(\frac{(0.75)^2\ (8.93)}{10}\right)^2}{10-1}} = 10$$

Step 6: Compare the calculated t-value with the critical t-value:

Given 10 degrees of freedom and an alpha level set at 0.20, the critical t-value = 0.8791 (obtained from Table 2 in these Provisions). The calculated t-value from step 4 = -2.9696, which is less than the critical t-value of 0.8791.

Step 7: -2.9696 < 0.8791 = fail

The calculated t-value (-2.9696) is less than the critical t-value (0.8791), so the NULL HYPOTHESIS is not rejected, and the test result is a "fail".

Conclusion: Because the test in example 2 resulted in a "fail", up to 2 more MMEL compliance tests would need to be conducted to determine compliance with the MMEL. In addition, because the *Ceriodaphnia dubia* test does include a survival ENDPOINT, the percent effect for the survival ENDPOINT must be calculated to determine compliance with the MDEL (see Reporting section below).

Reporting: Calculate the PERCENT EFFECT for all endpoints and report as required by Section IV.B.1.d of the Provisions.

$$\text{Reproduction \% Effect at IWC} = \frac{33.4 - 17.70}{33.4} \bullet 100 = 47.0\%$$

$$\text{Survival \% Effect at IWC} = \frac{1 - 0.5}{1} \bullet 100 = 50\%$$

Conclusion: Because the percent effect at the IWC for the survival ENDPOINT is greater than 50% and the test result was a "fail", the test in example 2 indicates a violation of the MDEL.

Acute fish survival test

Step 1: Conduct the aquatic toxicity test according to the procedures in the appropriate test method manual, as described in Section IV. B.1.b of the Provisions. The corresponding results are reported below and used for the following example calculations.

Replicate/Statistic	Control	IWC
1	10	7
2	10	8
3	10	8
4	10	9
Mean	10	8
Standard Deviation	0.000	0.816
# of REPLICATES (n)	4	4

Step 2: Determine if there is no variance in the ENDPOINT for each concentration. If there is no variance in both concentrations being compared, compute the PRECENT EFFECT as described in Section IV.B.1.d of the Provisions.

If there is variance in the ENDPOINT in both concentrations, then proceed with Steps 3-7.

In this example, the survival ENDPOINT would be used in the TST calculation. The IWC data has variance (i.e., standard deviation greater than zerio), so step 2 is not relevant and proceed to step 3.

Step 3: Calculate the mean RESPONSE for both concentrations and determine if an arcsine square root transformation is necessary.

For this example, survival data are a proportion of a binary response variable, so the data must be transformed using the arcsine square root transformation before calculating the mean RESPONSE for the control and the IWC.

Arcsine square root transformed data

Replicate/Statistic	Control	Treatment
1	1.412	0.991
2	1.412	1.107
3	1.412	1.107
4	1.412	1.249
Mean	1.412	1.11
Standard Deviation	0.000	0.106
# of REPLICATES (n)	4	4

Use the transformed data in the table above for the calculations in steps 4-7.

Step 4: Conduct Welch's t-test.

$$t = \frac{\overline{Y_t} - b \times \overline{Y_c}}{\sqrt{\frac{{S_t}^2}{n_t} + \frac{b^2 {S_c}^2}{n_c}}} = \frac{1.111 - (0.80 \times 1.412)}{\sqrt{\frac{0.027}{4} + \frac{(0.80)^2\ (0.00)}{4}}} = -0.03$$

Step 5: Adjust the degrees of freedom.

$$v = \frac{\left(\frac{{S_t}^2}{n_t} + \frac{b^2 {S_c}^2}{n_c}\right)^2}{\frac{\left(\frac{{S_t}^2}{n_t}\right)^2}{n_t - 1} + \frac{\left(\frac{b^2 {S_c}^2}{n_c}\right)^2}{n_c - 1}} = \frac{\left(\frac{0.027}{4} + \frac{(0.80)^2 (0.00)}{4}\right)^2}{\frac{\left(\frac{0.027}{4}\right)^2}{4 - 1} + \rightleftarrows \frac{\left(\frac{(0.80)^2\ (0.00)}{4}\right)^2}{4 - 1}} = 3$$

Step 6: Compare the calculated t-value with the critical t-value:

Given 3 degrees of freedom and an alpha level set at 0.10, the critical t-value = 1.64 (obtained from Table 2 in these Provisions). The calculated t-value from step 4 = -0.03, which is less than the critical t-value of 1.64.

Step 7: -0.03 < 1.64 = fail.

The calculated t-value -0.03) is less than the critical t-value (1.64), so the NULL HYPOTHESIS is not rejected, and the test result is a "fail".

Conclusion: Because the test in example 3 resulted in a "fail", up to 2 more MMEL compliance tests would need to be conducted to determine compliance with the MMEL. In addition, because the acute fish survival test does include a survival ENDPOINT, the percent effect for the survival ENDPOINT must be calculated to determine compliance with the MDEL (see Reporting section below).

Reporting: Calculate the PERCENT EFFECT for all endpoints and report as required by Section IV.B.1.d of the Provisions

$$\%\ \text{Effect at IWC} = \frac{10 - 8}{10} \bullet 100 = 20\%$$

Conclusion: Because the percent effect at the IWC for the survival ENDPOINT is less than 50%, the test in example 3 indicates compliance with the MDEL.