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State Policy for Water Quality Control: Toxicity Provisions

[Also for inclusion in the Water Quality Control Plan for Inland Surface Waters, Enclosed Bays, and Estuaries of California for waters of the United States]



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I. INTRODUCTION

This State Policy for Water Quality Control: Toxicity Provisions (TOXICITY PROVISIONS) was adopted by the State Water Resources Control Board (State Water Board) under authority provided by Water Code sections 13140 and 13170.¹ Except as otherwise indicated, the TOXICITY PROVISIONS establishes provisions for water 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 TOXICITY PROVISIONS do not apply to OCEAN WATERS, including Monterey Bay and Santa Monica Bay. All terms that are defined in Appendix A are reflected in capital letters.

II. AQUATIC TOXICITY WATER QUALITY OBJECTIVES

II.A. Aquatic Toxicity

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

As used in Section II, 'ambient water' refers to a sample taken from the water body of concern that may or may not be influenced by a discharge.

II.B. Applicable Beneficial Uses

The following water quality objectives for chronic and acute aquatic 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, or early development (SPWN); marine habitat (MAR); inland saline water habitat (SAL); and wetland habitat (WET).

¹ NOTE: The portions of the TOXICITY PROVISIONS that apply to waters for which water quality standards are required by the Federal Water Pollution Control Act and acts amendatory thereof or supplementary thereto (i.e., waters of the United States) will be incorporated into the Water Quality Control Plan for Inland Surface Waters, Enclosed Bays, and Estuaries of California. Future incorporation of those portions of the TOXICITY PROVISIONS, as adopted, into the water quality control plan will be considered non-substantive amendments. At that time, formatting and other organizational edits necessary for incorporation into the water quality control plan will be addressed.

II.C. Aquatic Toxicity Water Quality Objectives

II.C.1. 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_0 : Mean RESPONSE (ambient water) $\leq 0.75 \cdot$ mean RESPONSE (control)

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

And where the following ALTERNATIVE HYPOTHESIS shall be used:

H_a : Mean RESPONSE (ambient water) $> 0.75 \cdot$ mean RESPONSE (control)

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

Attainment of the water quality objective is demonstrated by conducting CHRONIC AQUATIC TOXICITY TESTING as described in Section III.B.2 and rejecting this NULL HYPOTHESIS in accordance with the TEST OF SIGNIFICANT TOXICITY (TST) statistical approach described in Section III.B.3. When the NULL HYPOTHESIS is rejected, the ALTERNATIVE HYPOTHESIS is accepted in its place, and there is no exceedance of the chronic aquatic toxicity water quality objective. Failing to reject the NULL HYPOTHESIS (referred to as a "fail") is equivalent to an exceedance of the chronic aquatic toxicity water quality objective.

II.C.2. 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_0 : Mean RESPONSE (ambient water) $\leq 0.80 \cdot$ mean RESPONSE (control)

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

And where the following ALTERNATIVE HYPOTHESIS shall be used:

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

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

Attainment of the water quality objective is demonstrated by conducting ACUTE AQUATIC TOXICITY TESTING as described in Section III.B.2 and rejecting this NULL HYPOTHESIS in accordance with the TST statistical approach described in Section III.B.3. When the NULL HYPOTHESIS is rejected, the ALTERNATIVE HYPOTHESIS is accepted in its place, and there is no exceedance of the acute aquatic toxicity water quality objective. Failing to reject the NULL HYPOTHESIS (referred to as a "fail") is equivalent to an exceedance of the acute aquatic toxicity water quality objective.

II.D. Interaction of Toxicity Provisions with Basin Plans and the State Implementation Policy

In accordance with Water Code section 13170, except where otherwise noted, the TOXICITY PROVISIONS automatically supersede any Regional Water Quality Control Plans (Basin Plans) for waters of the United States to the extent of any conflict. Consistent with its authority in Water Code sections 13140 and 13142, the State Water Board has also determined that the TOXICITY PROVISIONS shall supersede any Regional Water Quality Control Plans (Basin Plans) for all waters of the state 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, also known as the State Implementation Policy or the SIP.

The TOXICITY PROVISIONS 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 or chronic aquatic toxicity; or
- (B) The Basin Plan provisions regard aquatic toxicity testing or interpretation of aquatic toxicity testing results; or
- (C) The Basin Plan provision is a numeric aquatic toxicity water quality objective that is not a site-specific water quality objective; or
- (D) The Basin Plan provisions are in conflict with the TOXICITY PROVISIONS.

The TOXICITY PROVISIONS, notwithstanding the above, do not supersede the following Basin Plan provisions:

- (A) The narrative toxicity water quality objectives (e.g., 'no toxic POLLUTANTS in toxic amounts'); or

-
- (B) Any Basin Plan provisions regarding the application of narrative toxicity water quality objectives to derive chemical-specific limits, targets, and other thresholds; or
 - (C) Any site-specific toxicity water quality objective or site-specific toxicity implementation provisions established in a Basin Plan. In addition, the TOXICITY PROVISIONS do not apply to segments of the water body in which the site-specific toxicity water quality objective apply; or
 - (D) Any total maximum daily loads (TMDLs) related to aquatic toxicity, including their implementation provisions, established prior to the effective date of these TOXICITY PROVISIONS. Section III also applies to all dischargers subject to TMDL requirements except to the extent the PERMITTING AUTHORITY determines that any specific aquatic toxicity TMDL requirements are more protective than any comparable requirements of Section III in which case those specific TMDL requirements will apply in lieu of the comparable requirements of Section III. Nothing in this section limits the Regional Water Board's authority to reconsider a TMDL and its implementation provisions that were established prior to the effective date of these TOXICITY PROVISIONS.

II.E. Interaction of Toxicity Provisions with Narrative and Numeric Aquatic Toxicity Water Quality Objectives

Compliance with narrative toxicity water quality objectives may be determined by use of indicator species, analysis of species diversity, population 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 United States Environmental Protection Agency (U.S. EPA), and other appropriate organizations, to evaluate compliance with narrative toxicity water quality objectives.

The PERMITTING AUTHORITY may apply narrative toxicity water quality objective(s) to derive chemical-specific effluent limitations, chemical-specific receiving water limitations, targets, and other thresholds.

The PERMITTING AUTHORITY may apply narrative toxicity water quality objective(s) to derive narrative receiving water limitations (e.g., "the discharge must not cause or contribute to toxics in toxic amounts in the receiving water"), numeric receiving water limitations, numeric effluent limitations, narrative effluent limitations, or a combination of limitations.

The PERMITTING AUTHORITY may rely solely on the numeric aquatic toxicity water quality objectives in Section II.C to address non-chemical specific aquatic toxicity unless there is information to suggest that the numeric aquatic toxicity water quality objective would not protect all aquatic species in the relevant water body.

If the PERMITTING AUTHORITY includes a numeric aquatic toxicity receiving water limitation in a National Pollutant Discharge Elimination System (NPDES) permit using any of the acute or chronic aquatic toxicity test methods identified in Table 1 of Section III.B.2, then the receiving water limitation shall be derived from the applicable numeric water quality objective(s) specified in Section II, except as provided in Section II.D for more protective TMDL-based requirements.

If the PERMITTING AUTHORITY includes a numeric aquatic toxicity effluent limitation in an NPDES permit using any of the acute or chronic aquatic toxicity test methods identified in Table 1 of Section III.B.2, then the effluent limitation shall be derived from the applicable numeric water quality objective(s) specified in Section II, except as provided in Section II.D for more protective TMDL-based requirements.

For NON-STORM WATER NPDES DISCHARGERS, the PERMITTING AUTHORITY shall assess whether to require numeric aquatic toxicity effluent limitations in accordance with Section III.C, and, if required, shall include in an NPDES permit the applicable numeric effluent limitation(s) specified in Section III.C.5 and Section III.C.6, except as provided in Section II.D for more protective TMDL-based requirements. For NON-STORM WATER NPDES DISCHARGERS, if the PERMITTING AUTHORITY includes in an NPDES permit the applicable numeric effluent limitation(s) specified in Section III.C.5 or Section III.C.6, or both, it shall not include any other numeric effluent limitations using test methods identified in Table 1 of Section III.B.2.

III. AQUATIC TOXICITY PROGRAM OF IMPLEMENTATION

III.A. Introduction

The following sections shall be used to assess whether ambient water meets the numeric aquatic toxicity water quality objectives, whether a PERMITTING AUTHORITY shall require aquatic toxicity effluent limitations for non-storm water NPDES dischargers, and whether dischargers' effluent complies with applicable permit terms related to aquatic toxicity. Specific requirements for NON-STORM WATER NPDES DISCHARGERS, STORM WATER DISCHARGERS, and NONPOINT SOURCE dischargers are described, respectively, in Section III.C, III.D, and III.E.

As used in section III, 'ambient water' refers to a sample taken from the water body of concern that may or may not be influenced by a discharge.

III.B. Required Toxicity Testing Methods and Analyses

III.B.1. Toxicity Testing Sample and Location

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

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, the effluent sample shall be from a location specified by the PERMITTING AUTHORITY. Dilution water and control water shall be prepared and used as specified by the test methods.

III.B.2. Toxicity Test Methods

CHRONIC AQUATIC 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 other U.S. EPA-approved methods, or included in the following 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).

ACUTE AQUATIC 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 other U.S. EPA-approved methods, or included 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 statistical power and confidence in the results.

Table 1. Toxicity Test Methods, Regulatory Management Decision (RMD), β Error, and α Error

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

U.S. EPA Toxicity Test Method	Tier	RMD (b)	β Error	α Error
<i>Americamysis bahia</i> (mysid) Survival	II	0.80	0.05	0.10
<i>Menidia berylina</i> (inland silverside) Survival	II	0.80	0.05	0.10

Table 1 Notes: The bioequivalence value (b) is equivalent to the RMD.
 The β error is the probability of declaring a sample toxic when it is not toxic.
 The α error is the probability of declaring a sample non-toxic when it is toxic.

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 may choose either freshwater test methods 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 or inland saline 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 TST as described in Section III.B.3. To the extent that U.S. EPA-approved methods require that observations be made of organisms' RESPONSE 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 III.B.3.

III.B.3. 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 aquatic 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 aquatic 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 ambient water meets the water quality objectives, the undiluted ambient water shall be used as the IWC for purposes of the data analysis as described below.

Step 1: Conduct the aquatic toxicity test according to procedures in the appropriate test method manual, as described in Section III.B.2.

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 III.B.4.

If the PERCENT EFFECT at the IWC is \geq 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 either concentration 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:

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

Where:

\bar{Y}_c = Mean RESPONSE for the control

\bar{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 III.B.2. 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.”

III.B.4. 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:

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

III.B.5. 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.

Degrees of Freedom (v)	α Error				
	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

III.C. Implementation for Non-Storm Water NPDES Dischargers

The PERMITTING AUTHORITY shall include the requirements specified in this Section (Section III.C) for NPDES permits issued, reissued, renewed, or reopened after the effective date of these provisions for NON-STORM WATER NPDES DISCHARGERS. For discharges from combined sewer systems, the acute and chronic aquatic toxicity water quality objectives will be implemented in accordance with the Combined Sewer Overflow (CSO) Control Policy.

III.C.1. Instream Waste Concentration

The PERMITTING AUTHORITY may grant MIXING ZONES and DILUTION CREDITS for the numeric aquatic toxicity objectives to dischargers in accordance with Section 1.4.2 of the Policy for Implementation of Toxics Standards for Inland Surface Water, Enclosed Bays, and Estuaries of California (2005).

The INSTREAM WASTE CONCENTRATION (IWC) is the concentration of effluent in the receiving water after mixing as determined by the PERMITTING AUTHORITY. The PERMITTING AUTHORITY shall determine the IWC prior to every permit issuance, reissuance, renewal, or reopening (if the permit reopening is to address toxicity requirements), prior to conducting a SPECIES SENSITIVITY SCREENING, and prior to determining REASONABLE POTENTIAL.

When a MIXING ZONE and DILUTION CREDIT is granted by the PERMITTING AUTHORITY, the IWC is the inverse of 1 plus the DILUTION CREDIT multiplied by 100 percent ($IWC = 100\% \times [1/(1+D)]$ where D = DILUTION CREDIT) unless the PERMITTING AUTHORITY selects a higher concentration of effluent as the IWC in order to protect beneficial uses, or because of site-specific conditions, or both. The PERMITTING AUTHORITY shall document the basis for the selection of a higher concentration of effluent as the IWC, if applicable, in the NPDES fact sheet (or equivalent document). If no DILUTION CREDIT is granted for toxicity, then the undiluted effluent shall be used as the IWC.

The PERMITTING AUTHORITY shall specify the IWC to be used in monitoring required in Section III.C.4 and used for compliance with the MAXIMUM DAILY EFFLUENT LIMITATION (MDEL) and MEDIAN MONTHLY EFFLUENT LIMITATION (MMEL) in the NPDES permit.

III.C.2. Species Sensitivity Screening

III.C.2.a. Non-Storm Water NPDES Dischargers Required to Conduct Species Sensitivity Screening for Chronic Aquatic Toxicity

The PERMITTING AUTHORITY shall require NON-STORM WATER NPDES DISCHARGERS to conduct a SPECIES SENSITIVITY SCREENING for chronic

aquatic toxicity as part of a report of waste discharge (ROWD), as a permit condition, or both.

III.C.2.a.i. Initial Species Sensitivity Screening

All NON-STORM WATER NPDES DISCHARGERS shall conduct a SPECIES SENSITIVITY SCREENING for chronic aquatic toxicity either prior to, or within 18 months after, the first issuance, reissuance, renewal, or reopening (if the permit reopening is to address toxicity requirements) of the permit after the effective date of these TOXICITY PROVISIONS. The PERMITTING AUTHORITY may allow use of aquatic toxicity test data in the SPECIES SENSITIVITY SCREENING generated within ten years prior to the first issuance, reissuance, renewal, or reopening (if the permit reopening is to address toxicity requirements) of the permit after the effective date of these TOXICITY PROVISIONS when the data are representative of the effluent and either (1) the SPECIES SENSITIVITY SCREENING is conducted in accordance with Section III.C.2.c or (2) the PERMITTING AUTHORITY accepts the use of the data in the SPECIES SENSITIVITY SCREENING, the data are analyzed using the TST, and the data are from chronic aquatic toxicity testing of, at minimum, one vertebrate, one invertebrate, and one aquatic plant/algae from Table 1 of Section III.B.2.

III.C.2.a.ii. Subsequent Species Sensitivity Screening

Following the first issuance, reissuance, renewal, or reopening (if the permit reopening is to address toxicity requirements) of the permit after the effective date of these TOXICITY PROVISIONS, the PERMITTING AUTHORITY shall require the discharger to conduct a SPECIES SENSITIVITY SCREENING prior to any subsequent issuance, reissuance, renewal, or reopening (if the permit reopening is to address toxicity requirements) of the permit if (1) the discharger has not conducted a SPECIES SENSITIVITY SCREENING in accordance with Section III.C.2.c within the previous 15 years or (2) if the effluent used in the last SPECIES SENSITIVITY SCREENING is no longer representative of the effluent.

The PERMITTING AUTHORITY may require a SPECIES SENSITIVITY SCREENING for chronic aquatic toxicity prior to every issuance, reissuance, renewal, or reopening (if the permit reopening is to address toxicity requirements) of the permit.

III.C.2.b. Non-Storm Water NPDES Dischargers Required to Conduct Species Sensitivity Screening for Acute Aquatic Toxicity.

The PERMITTING AUTHORITY may require NON-STORM WATER NPDES DISCHARGERS to conduct a SPECIES SENSITIVITY SCREENING for acute aquatic toxicity. The basis for requiring a SPECIES SENSITIVITY SCREENING for acute aquatic toxicity shall be documented in the NPDES fact sheet (or equivalent document).

If the PERMITTING AUTHORITY requires NON-STORM WATER NPDES DISCHARGERS to conduct a SPECIES SENSITIVITY SCREENING for acute aquatic toxicity, the PERMITTING AUTHORITY shall require NON-STORM WATER NPDES DISCHARGERS to conduct a SPECIES SENSITIVITY SCREENING as part of a ROWD, as a Water Code section 13383 Order, as a permit condition, or combination of these options.

III.C.2.c. Type and Number of Tests Required for a Species Sensitivity Screening

For chronic aquatic toxicity, each set of testing in the SPECIES SENSITIVITY SCREENING shall consist of, at a minimum, one vertebrate, one invertebrate, and one aquatic plant/algae from Table 1 of Section III.B.2. For acute aquatic toxicity, each set of testing in the SPECIES SENSITIVITY SCREENING shall consist of, at a minimum, one vertebrate and one invertebrate from Table 1 of Section III.B.2. The testing in the SPECIES SENSITIVITY SCREENING shall be analyzed in accordance with the statistical approach and PERCENT EFFECT specified in Section III.B.3 and III.B.4 and reported in accordance with Section III.B.5.

For CONTINUOUS DISCHARGERS, a SPECIES SENSITIVITY SCREENING includes four sets of testing, with a set of testing conducted in each quarter of a year.

For NON-CONTINUOUS DISCHARGERS, a set of testing shall be conducted in each quarter in which there is expected to be at least 15 days of discharge. For NON-CONTINUOUS DISCHARGERS that discharge in only one quarter of the year in which there is expected to be at least 15 days of discharge, two sets of testing shall be conducted within the same quarter. For NON-CONTINUOUS DISCHARGERS that do not discharge in any quarter in which there is expected to be at least 15 days of discharge, the PERMITTING AUTHORITY shall indicate if a SPECIES SENSITIVITY SCREENING is required and the number of sets of testing to be conducted in that SPECIES SENSITIVITY SCREENING. If a SPECIES SENSITIVITY SCREENING is not required, the PERMITTING AUTHORITY shall specify the MOST SENSITIVE SPECIES.

When there is no effluent available to complete tests in one of the sets in a SPECIES SENSITIVITY SCREENING, that set of testing shall not be required.

For dischargers granted a DILUTION CREDIT and MIXING ZONE for aquatic 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 NON-CONTINUOUS 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.

III.C.2.d. Determination of the Most Sensitive Species

The PERMITTING AUTHORITY has the discretion to choose the approach for selecting the MOST SENSITIVE SPECIES from the SPECIES SENSITIVITY SCREENING (e.g., species exhibiting highest PERCENT EFFECT, species with the most number of “fails” etc.). However, the PERMITTING AUTHORITY shall select the species in the SPECIES SENSITIVITY SCREENING exhibiting the highest PERCENT EFFECT at the IWC as the approach for selecting the MOST SENSITIVE SPECIES, unless the PERMITTING AUTHORITY identifies the basis for selecting a different approach in the NPDES fact sheet (or equivalent document).

When the SPECIES SENSITIVITY SCREENING is conducted within 18 months after the issuance, reissuance, renewal, or reopening (if the permit reopening is to address toxicity requirements) of the permit after the effective date of these TOXICITY PROVISIONS, then the NPDES permit shall identify the species that will serve as the MOST SENSITIVE SPECIES until the SPECIES SENSITIVITY SCREENING is conducted, and shall include a reopener clause requiring the PERMITTING AUTHORITY to reopen the permit to specify the MOST SENSITIVE SPECIES once the SPECIES SENSITIVITY SCREENING is completed. The reopener clause shall include a statement that reopening is not required if the SPECIES SENSITIVITY SCREENING indicates the MOST SENSITIVE SPECIES is the same as the species as the MOST SENSITIVE SPECIES already identified in the NPDES permit.

The PERMITTING AUTHORITY shall specify the MOST SENSITIVE SPECIES and IWC in the NPDES permit. In the NPDES permit, the PERMITTING AUTHORITY may also delegate to the Executive Officer or Executive Director, as applicable, the authority to allow the temporary use of the next appropriate species as the MOST SENSITIVE SPECIES when the discharger submits documentation and the Executive Officer or Executive Director, as applicable, determines that the discharger has encountered unresolvable test interference or cannot secure a reliable supply of test organisms. The “next appropriate species” is a species in Table 1 in the same test method classification (e.g., chronic aquatic toxicity test methods, acute aquatic toxicity test method), in the same salinity classification (e.g., freshwater or marine), and in the same taxon as the MOST SENSITIVE SPECIES. When there are no other species in Table 1 in the same taxon as the MOST SENSITIVE SPECIES, the “next appropriate species” is the species exhibiting the highest PERCENT EFFECT at the IWC tested in the SPECIES SENSITIVITY SCREENING other than the MOST SENSITIVE SPECIES.

III.C.3. 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 (if the permit reopening is to address toxicity requirements).

III.C.3.a. Non-Storm Water NPDES Dischargers Required to Conduct Reasonable Potential Analysis for Chronic Aquatic Toxicity

Except for POTW dischargers that are authorized to discharge at a rate equal to or greater than 5.0 million gallons per day (MGD) and are required to have a pretreatment program by the terms of 40 CFR § 403.8(a) (effective January 1, 2020), all NON-STORM WATER NPDES DISCHARGERS shall conduct a REASONABLE POTENTIAL analysis for chronic aquatic toxicity, pursuant to the procedures specified in Section III.C.3.c, for review and approval by the PERMITTING AUTHORITY. A REASONABLE POTENTIAL analysis for chronic aquatic toxicity is not required for POTW dischargers that are authorized to discharge at a rate equal to or greater than 5.0 MGD and are required to have a pretreatment program by the terms of 40 CFR § 403.8(a) (effective January 1, 2020), because the PERMITTING AUTHORITY shall include an effluent limitation for these dischargers pursuant to Section III.C.5.

III.C.3.b. Non-Storm Water NPDES Dischargers Required to Conduct Reasonable Potential Analysis for Acute Aquatic Toxicity

The PERMITTING AUTHORITY may require NON-STORM WATER NPDES DISCHARGERS to conduct a REASONABLE POTENTIAL analysis for acute aquatic toxicity, pursuant to the procedures in Section III.C.3.c, for review and approval by the PERMITTING AUTHORITY. A chronic aquatic toxicity test is generally protective of both chronic and acute aquatic toxicity. The situations that may warrant a reasonable potential analysis for acute aquatic toxicity include, but are not limited to, discharges to waterbodies inhabited by threatened or endangered species if a chronic aquatic toxicity test surrogate is not available, discharges with high dilution rates as high dilutions may mask chronic effects, or a situation in which the chronic aquatic toxicity test is not adequately protective of aquatic life beneficial uses. The PERMITTING AUTHORITY shall document the basis for the decision whether to conduct a REASONABLE POTENTIAL analysis for acute aquatic toxicity in the NPDES fact sheet (or equivalent document).

III.C.3.c. Reasonable Potential Analysis

Prior to conducting a REASONABLE POTENTIAL analysis, the PERMITTING AUTHORITY shall evaluate data as described in section III.C.3.c.i. The PERMITTING AUTHORITY shall then determine REASONABLE POTENTIAL in accordance with section III.C.3.c.ii.

III.C.3.c.i. Data to be Evaluated in Reasonable Potential Analysis

All aquatic toxicity test data generated within five years prior to permit issuance, reissuance, renewal, or reopening (if the permit reopening is to address toxicity requirements) that is representative of effluent quality during discharge conditions shall be evaluated in determining REASONABLE POTENTIAL.

If any of the evaluated toxicity test data conducted at the IWC were not analyzed using the TST, the PERMITTING AUTHORITY shall require the discharger to reanalyze the toxicity test data conducted at the IWC using the TST.

When the data from the evaluated aquatic toxicity tests were not conducted at the IWC, then the PERMITTING AUTHORITY shall either require the discharger to reanalyze the toxicity test data conducted at a higher concentration of effluent than the IWC using the TST, if that higher concentration was tested, or require the discharger to conduct a minimum of four aquatic toxicity tests at the IWC using a species from Table 1 of Section III.B.2 and analyzed using the TST.

When the evaluated data does not include data from a minimum of four aquatic toxicity tests, then the PERMITTING AUTHORITY shall require the discharger to conduct a minimum of four aquatic toxicity tests at the IWC using a species from Table 1 of Section III.B.2 and analyzed using the TST.

The PERMITTING AUTHORITY may also evaluate older toxicity test data that is representative of effluent quality to determine REASONABLE POTENTIAL. The PERMITTING AUTHORITY may also require the discharger to conduct additional aquatic toxicity tests using a species from Section III.B.2 and analyzed using the TST to determine REASONABLE POTENTIAL.

III.C.3.c.ii. Reasonable Potential Determination

A discharge has REASONABLE POTENTIAL to cause or contribute to an excursion above the chronic aquatic toxicity water quality objectives specified in Section II.C.1, if any of the CHRONIC AQUATIC TOXICITY TESTS result in a “fail” at the IWC, or if any of the CHRONIC AQUATIC 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 aquatic toxicity water quality objectives specified in Section II.C.2, if any of the ACUTE AQUATIC TOXICITY TESTS result in a “fail” at the IWC, or if any of the ACUTE AQUATIC 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, data using a different concentration than the IWC, lack of available dilution, water quality and beneficial uses of the receiving water, the presence of

endangered or threatened species or critical habitat, 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 aquatic toxicity water quality objectives specified in Section II.C.

For NON-STORM WATER NPDES DISCHARGERS that do not have an effluent discharge prior to permit issuance, reissuance, renewal or reopening (if the permit reopening is 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 shall be documented in the NPDES fact sheet (or equivalent document).

If a REASONABLE POTENTIAL analysis indicates no REASONABLE POTENTIAL for either chronic or acute aquatic toxicity, the PERMITTING AUTHORITY shall 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 aquatic toxicity water quality objective, then the PERMITTING AUTHORITY shall include the corresponding MDEL and MMEL in the NPDES permit.

III.C.4. Aquatic Toxicity Monitoring

The PERMITTING AUTHORITY shall include aquatic toxicity monitoring requirements in an NPDES permit or Water Code Section 13383 Order for all NON-STORM WATER NPDES DISCHARGERS as specified in this section. The required aquatic toxicity monitoring includes ROUTINE MONITORING, and when applicable, either MEDIAN MONTHLY EFFLUENT TARGET (MMET) TESTS or MMEL COMPLIANCE TESTS. ROUTINE MONITORING, MMET TESTS, and MMEL COMPLIANCE TESTS shall be conducted at the IWC using the MOST SENSITIVE SPECIES identified by the PERMITTING AUTHORITY and shall be analyzed using the TST.

When there is no effluent available to complete a ROUTINE MONITORING test, MMET TEST, or MMEL COMPLIANCE TEST, the test shall not be required, and ROUTINE MONITORING continues at the frequency specified in the permit.

The PERMITTING AUTHORITY may also require dischargers to conduct additional toxicity testing. This testing can include, but is not limited to, special studies, additional test species, testing with additional dilutions or higher concentrations of effluent than the IWC, testing using test species not included in Table 1 of Section III.B.2, or monitoring specific to FLOW-THROUGH ACUTE TOXICITY TESTING SYSTEMS. The PERMITTING AUTHORITY can require this testing in an NPDES permit, Water Code section 13383 Order, or both. The rationale for requiring additional monitoring shall be documented in the NPDES fact sheet (or equivalent document), Water Code section 13383 Order, or both.

For purposes of this section, initiation of a test means when the sample is collected. For purposes of this section, completion of a test means when the test has been terminated at the prescribed time (e.g., 96 hours, 7 days) and test acceptability criteria have been met.

III.C.4.a. Defining the Start of the Calendar Month, Calendar Quarter, and Calendar Year

Several portions of the TOXICITY PROVISIONS require the PERMITTING AUTHORITY to include requirements in the NPDES permit, and any applicable section 13383 order, that rely upon the use of a CALENDAR MONTH, CALENDAR QUARTER, and CALENDAR YEAR (e.g., targets, effluent limitations, monitoring) regardless of the discharger's required monitoring frequency. For all purposes, the start of the CALENDAR MONTH, CALENDAR QUARTER, and CALENDAR YEAR for each discharger shall be as specified in this section.

The PERMITTING AUTHORITY shall specify the day of the month that corresponds to the start of a CALENDAR MONTH in the NPDES permit and any applicable Water Code section 13383 Order, for dischargers that are required to conduct ROUTINE MONITORING at a monthly or greater than monthly frequency. For dischargers that are required to conduct ROUTINE MONITORING at a less than monthly frequency, the CALENDAR MONTH begins from the initiation of the ROUTINE MONITORING test.

The PERMITTING AUTHORITY shall specify the day and the month that correspond to the start of each CALENDAR QUARTER and the start of the CALENDAR YEAR in the NPDES permit and any applicable Water Code section 13383 Order.

In setting the start of the CALENDAR MONTH, CALENDAR QUARTER, and CALENDAR YEAR, the PERMITTING AUTHORITY shall consider relevant scheduling constraints identified by the discharger and applicable laboratories.

The PERMITTING AUTHORITY may specify the exact dates or time period in which any required aquatic toxicity test shall be initiated within an applicable monitoring period (e.g., a requirement to initiate a test within five days of the start of the

CALENDAR QUARTER, a requirement to initiate a test between the 10th and the 15th of each CALENDAR MONTH, etc.).

III.C.4.b. Toxicity Monitoring for Dischargers Required to Comply with Numeric Aquatic Toxicity Effluent Limitations

The PERMITTING AUTHORITY shall require chronic aquatic toxicity monitoring pursuant to this subsection for all NON-STORM WATER NPDES DISCHARGERS that demonstrate REASONABLE POTENTIAL for chronic aquatic toxicity and all POTW dischargers that are authorized to discharge at a rate equal to or greater than 5.0 MGD and are required to have a pretreatment program by the terms of 40 CFR § 403.8(a) (effective January 1, 2020). The PERMITTING AUTHORITY shall require acute aquatic toxicity monitoring pursuant to this subsection for all NON-STORM WATER NPDES DISCHARGERS that demonstrate REASONABLE POTENTIAL for acute aquatic toxicity.

ROUTINE MONITORING and MMEL COMPLIANCE TESTS continue during any required TOXICITY REDUCTION EVALUATION (TRE).

III.C.4.b.i. Routine Monitoring for Chronic Aquatic Toxicity

III.C.4.b.i(A). Routine Monitoring Schedule for Chronic Aquatic 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 AQUATIC TOXICITY TEST every CALENDAR MONTH during which there is expected to be at least 15 days of discharge. Initiation of the ROUTINE MONITORING test shall be at a time that would allow any required MMEL COMPLIANCE TESTS to be initiated within the same CALENDAR MONTH as the ROUTINE MONITORING test.”

Except for POTW dischargers authorized to discharge at a rate of less than or equal to 1.0 MGD, 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 AQUATIC TOXICITY TEST each CALENDAR QUARTER during which there is expected to be at least 15 days of discharge.”

For POTW dischargers authorized to discharge at a rate of less than or equal to 1.0 MGD, the frequency of ROUTINE MONITORING shall be specified in the NPDES permit as follows:

“The discharger shall conduct at least two CHRONIC AQUATIC TOXICITY TESTS each CALENDAR YEAR during which there is expected to be at least 15 days of discharge in at least one CALENDAR QUARTER.”

The PERMITTING AUTHORITY may require NON-STORM WATER NPDES DISCHARGERS to conduct more frequent chronic aquatic 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 III.C.4.b.i(B). The PERMITTING AUTHORITY shall require the discharger to conduct a chronic aquatic toxicity ROUTINE MONITORING test at a minimum frequency of two chronic toxicity tests per CALENDAR YEAR during any CALENDAR YEAR in which there is expected to be at least 15 days of discharge in at least one CALENDAR QUARTER.

The rationale for requiring more frequent or reduced ROUTINE MONITORING shall be documented in the NPDES fact sheet (or equivalent document), Water Code section 13383 Order, or both.

To the extent feasible, ROUTINE MONITORING tests shall be evenly distributed across the CALENDAR YEAR or period of seasonal or intermittent discharge.

III.C.4.b.i(B). Reduced Routine Monitoring Schedule for Chronic Aquatic Toxicity

If an NPDES permit includes the MDEL and MMEL as specified in Section III.C.5, the PERMITTING AUTHORITY may approve a reduction in the frequency of the ROUTINE MONITORING specified in Section III.C.4.b.i(A) upon reissuance, renewal, or reopening (if the permit reopening is to address toxicity requirements) of the NPDES permit when during the prior five consecutive years the following conditions have been met:

- 1) The MDEL and MMEL as specified in Section III.C.5 have not been violated;
- 2) The toxicity requirements in the applicable NPDES permit(s) have been followed.

If an NPDES permit does not include the MDEL and MMEL as specified in Section III.C.5, the PERMITTING AUTHORITY may approve a reduction in the frequency of the ROUTINE MONITORING specified in Section III.C.4.b.i(A) for dischargers upon reissuance, renewal, or reopening (if the permit reopening is to address toxicity requirements) of the NPDES permit when during the prior five consecutive years the following conditions have been met:

-
- 1) The discharger has complied with the toxicity requirements in the applicable NPDES permit(s); and
 - 2) A minimum of ten chronic aquatic toxicity tests have been conducted at the IWC or at a concentration of effluent higher than the IWC; and
 - 3) All chronic aquatic toxicity test data are analyzed or reanalyzed using the TST; and
 - 4) No chronic aquatic toxicity test resulted in a “fail” at the IWC or, if the aquatic toxicity test was not conducted at the IWC, at a concentration of effluent higher than the IWC.

If the conditions are met and the PERMITTING AUTHORITY approves a reduced ROUTINE MONITORING schedule, the frequency of ROUTINE MONITORING may be reduced from one CHRONIC AQUATIC TOXICITY TEST per CALENDAR MONTH, as required in Section III.C.4.b.i(A), to one per CALENDAR QUARTER. The frequency of ROUTINE MONITORING may be reduced from once per CALENDAR MONTH to two per CALENDAR YEAR when the discharger has an initial dilution of at least 10:1 and the PERMITTING AUTHORITY requires a minimum of two additional monitoring tests conducted using the MOST SENSITIVE SPECIES at a concentration of effluent that is at least double the IWC.

If the conditions are met and the PERMITTING AUTHORITY approves a reduced ROUTINE MONITORING schedule, the frequency of ROUTINE MONITORING may be reduced from one CHRONIC AQUATIC TOXICITY TEST per CALENDAR QUARTER, as required in Section III.C.4.b.i(A), to two CHRONIC AQUATIC TOXICITY TESTS per CALENDAR YEAR.

The PERMITTING AUTHORITY shall specify in the NPDES permit that dischargers on an approved reduced frequency ROUTINE MONITORING schedule shall return to a ROUTINE MONITORING schedule, as described in Section III.C.4.b.i(A), if the conditions 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 III.C.4.b.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 III.C.4.b.i(A), dischargers will need to, once again, meet the conditions listed in this section for at least a period of five years to be granted another discretionary reduction in the frequency of chronic aquatic toxicity ROUTINE MONITORING.

The PERMITTING AUTHORITY may include a provision in the NPDES permit authorizing the Executive Officer or Executive Director to temporarily reduce the frequency of ROUTINE MONITORING from the frequency described in Section III.C.4.b.i(A) to a minimum of two CHRONIC AQUATIC TOXICITY

TESTS per CALENDAR YEAR when the discharger is conducting aquatic toxicity testing as part of the TRE during that CALENDAR YEAR.

The PERMITTING AUTHORITY shall require dischargers under a temporary reduced frequency to return to a ROUTINE MONITORING schedule, as described in Section III.C.4.b.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 III.C.4.b.i(A), dischargers will need to meet the conditions listed in this section to be granted a discretionary reduction in the frequency of monitoring.

III.C.4.b.ii. Routine Monitoring for Acute Aquatic Toxicity

If REASONABLE POTENTIAL is demonstrated for acute aquatic toxicity, in accordance with the provisions specified in Section III.C.3, the discharger shall conduct acute aquatic toxicity ROUTINE MONITORING in addition to any other required chronic aquatic 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. To the extent feasible, ROUTINE MONITORING tests shall be evenly distributed across the CALENDAR YEAR or period of seasonal or intermittent discharge.

III.C.4.b.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 frequently than a monthly frequency.

This additional ROUTINE MONITORING test shall be initiated within two weeks after the CALENDAR MONTH in which the MMEL or MDEL violation occurred. The CALENDAR MONTH of the violation and the CALENDAR MONTH of the additional ROUTINE MONITORING shall be considered “successive calendar months” for purposes of determining whether a TRE is required under section III.C.8. 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 result in the need to conduct MMEL COMPLIANCE TESTS.

III.C.4.b.iv. MMEL Compliance Tests

If an acute or chronic aquatic toxicity ROUTINE MONITORING test results in a “fail” at the IWC, then NON-STORM WATER NPDES DISCHARGERS shall complete 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 because the first chronic MMEL COMPLIANCE TEST that results in a “fail” constitutes a violation and so the second MMEL COMPLIANCE TEST is not required.

III.C.4.c. Chronic Toxicity Monitoring For Dischargers Not Required to Comply with the Numeric Chronic Aquatic Toxicity Effluent Limitations

The PERMITTING AUTHORITY shall include monitoring requirements pursuant to this subsection for NON-STORM WATER NPDES DISCHARGERS that are not required to comply with the chronic toxicity effluent limitations indicated in Section III.C.5. The monitoring includes chronic aquatic toxicity ROUTINE MONITORING and MMET TESTS. This “target” monitoring shall be used with the MAXIMUM DAILY EFFLUENT TARGET (MDET) and MEDIAN MONTHLY EFFLUENT TARGET (MMET) indicated in Section III.C.7 to determine whether a TRE is needed.

III.C.4.c.i. Chronic Aquatic Toxicity Routine Monitoring

The PERMITTING AUTHORITY shall require the discharger to complete ROUTINE MONITORING at a minimum frequency of two chronic toxicity tests per CALENDAR YEAR during any CALENDAR YEAR in which there is expected to be at least 15 days of discharge in at least one CALENDAR QUARTER. Initiation of a ROUTINE MONITORING test shall be at a time that would allow corresponding MMET TESTS to be initiated within the same CALENDAR MONTH as the ROUTINE MONITORING test.

To the extent feasible, ROUTINE MONITORING tests shall be evenly distributed across the CALENDAR YEAR or period of seasonal or intermittent discharge.

III.C.4.c.i(A). Additional Routine Monitoring Tests for TRE Determination

For NON-STORM WATER NPDES DISCHARGERS with a ROUTINE MONITORING frequency of less than monthly, an additional ROUTINE MONITORING test shall be required when one MDET or MMET is not met, but not two, 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 frequently than a monthly frequency.

This additional ROUTINE MONITORING test shall be initiated within two weeks after the CALENDAR MONTH in which the MMET or MDET was not met. The CALENDAR MONTH in which the MMET or MDET was not met and the CALENDAR MONTH of the additional ROUTINE MONITORING shall be considered “successive calendar months” for purposes of determining whether a TRE is required under section III.C.8. This additional ROUTINE MONITORING test is used to determine if a TRE is necessary. This additional ROUTINE MONITORING test could result in the need to conduct MMET TESTS.

III.C.4.c.ii. MMET Tests

If a chronic aquatic toxicity ROUTINE MONITORING test results in a “fail” at the IWC, then NON-STORM WATER NPDES DISCHARGERS shall complete a maximum of two MMET TESTS. The MMET 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 MMET TEST results in a “fail” at the IWC, then the second MMET TEST is waived.

III.C.4.d. Replacement Tests for Routine Monitoring, MMET Tests, or MMEL Compliance Tests

When a required toxicity test for ROUTINE MONITORING, MMET TESTS, or MMEL COMPLIANCE TESTS is not completed, a new toxicity test to replace the toxicity test that was not completed shall be initiated as soon as possible. The new toxicity test shall replace the ROUTINE MONITORING, MMET TESTS, or MMEL COMPLIANCE TESTS, as applicable, for the CALENDAR MONTH in which the toxicity test that was not completed was required to be initiated, even if the new toxicity test is initiated in a subsequent month. The new toxicity test for ROUTINE MONITORING, MMET TESTS, or MMEL COMPLIANCE TESTS, as applicable, and any MMET TESTS or MMEL COMPLIANCE TESTS required to be conducted due to the results of the new toxicity test shall be used to determine compliance with the effluent limitations or to determine if the MMET and the MDET are met for the CALENDAR MONTH in which the toxicity test that was not completed was required to be initiated. The new toxicity test and any MMET TESTS or MMEL COMPLIANCE TESTS required to be conducted due to the results of the new toxicity test shall not be used to substitute for any other required toxicity tests.

The PERMITTING AUTHORITY shall include a statement in the NPDES permit, Water Code section 13383 Order, or both that any specific monitoring event is not required to be initiated in the required time period when the PERMITTING AUTHORITY determines that the test was not initiated in the required time period due to circumstances outside of the discharger’s control that were not preventable with the reasonable exercise of care, and the discharger promptly initiates, and ultimately completes, a replacement test.

III.C.5. Chronic Aquatic Toxicity Effluent Limitations

The PERMITTING AUTHORITY shall include the chronic toxicity effluent limitations according to this section if REASONABLE POTENTIAL is demonstrated for chronic aquatic toxicity in accordance with the provisions specified in Section III.C.3, or if a POTW is authorized to discharge at a rate equal to or greater than 5.0 MGD and is required to have a pretreatment program by the terms of 40 CFR § 403.8(a) (effective January 1, 2020).

The PERMITTING AUTHORITY shall specify in the NPDES 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 aquatic toxicity effluent limitations. All toxicity tests of the MOST SENSITIVE SPECIES conducted at the IWC and analyzed using the TST shall be used to determine compliance with the chronic toxicity effluent limitations. To the extent any monitoring 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 chronic aquatic toxicity effluent limitations specified in this section.

III.C.5.a. For permit issuance, reissuance, renewal, or reopening that occurs after the effective date of the Provisions and prior to January 1, 2024

The requirement to include the MMEL indicated in Section III.C.5.d using *Ceriodaphnia dubia* as the MOST SENSITIVE SPECIES shall, to the extent consistent with federal law, take effect on a statewide basis starting January 1, 2024. The PERMITTING AUTHORITY shall apply one of the following four scenarios if the PERMITTING AUTHORITY issues, reissues, renews, or reopens (if the permit reopening is to address toxicity requirements) the NPDES permit after the effective date of the Provisions and prior to January 1, 2024:

Scenario 1: For NON-STORMWATER NPDES DISCHARGERS with no numeric chronic aquatic toxicity effluent limitations in their current permit and when *Ceriodaphnia dubia* is identified as the MOST SENSITIVE SPECIES according to Section III.C.2, the PERMITTING AUTHORITY shall include the MDEL indicated in Section III.C.5.c and the MMET indicated in Section III.C.7.b using *Ceriodaphnia dubia* as the MOST SENSITIVE SPECIES if the PERMITTING AUTHORITY determines that an MMEL using *Ceriodaphnia dubia* is not required by federal law. The PERMITTING AUTHORITY shall document the basis for this determination in the NPDES fact sheet (or equivalent document). Whenever the PERMITTING AUTHORITY determines that an MMEL using *Ceriodaphnia dubia* must be included in the NPDES permit in order to comply with federal law, the MMEL indicated in Section III.C.5.d using *Ceriodaphnia dubia* as the MOST SENSITIVE SPECIES shall be required instead of the MMET indicated in Section III.C.7.b.

If the PERMITTING AUTHORITY includes the MMET using *Ceriodaphnia dubia*, the PERMITTING AUTHORITY shall indicate that the MMET using *Ceriodaphnia dubia*

shall be in effect only through December 31, 2023, and that starting January 1, 2024, the discharger must comply with the MDEL indicated in Section III.C.5.c using *Ceriodaphnia dubia* and the MMEL indicated in Section III.C.5.d using *Ceriodaphnia dubia* as the MOST SENSITIVE SPECIES.

Scenario 2: For NON-STORMWATER NPDES DISCHARGERS with no numeric chronic aquatic toxicity effluent limitations in their current permit and when the MOST SENSITIVE SPECIES identified by the PERMITTING AUTHORITY is not *Ceriodaphnia dubia*, the PERMITTING AUTHORITY shall include the MDEL indicated in Section III.C.5.c and MMEL indicated in Section III.C.5.d using the MOST SENSITIVE SPECIES.

Scenario 3: For NON-STORMWATER NPDES DISCHARGERS with numeric chronic aquatic toxicity effluent limitations in their current permit and when the MOST SENSITIVE SPECIES identified by the PERMITTING AUTHORITY is *Ceriodaphnia dubia*, the PERMITTING AUTHORITY shall include in the NPDES permit one of the two following options: (Option A) the MDEL indicated in Section III.C.5.c and the MMEL indicated in Section III.C.5.d using *Ceriodaphnia dubia* as the MOST SENSITIVE SPECIES, or (Option B) the MDEL indicated in Section III.C.5.c using *Ceriodaphnia dubia* as the MOST SENSITIVE SPECIES, the MMEL indicated in Section III.C.5.d using *Pimephales promelas* (fathead minnow) or *Selenastrum capricornutum* (green alga) as the MOST SENSITIVE SPECIES, and the MMET indicated in Section III.C.7.b using *Ceriodaphnia dubia* as the MOST SENSITIVE SPECIES. The PERMITTING AUTHORITY may choose to include Option B in the NPDES permit if it determines that an MMEL using *Ceriodaphnia dubia* is not required by federal law. The PERMITTING AUTHORITY shall document the basis for this determination in the NPDES fact sheet (or equivalent document). Whenever the PERMITTING AUTHORITY determines an MMEL using *Ceriodaphnia dubia* must be included in the NPDES permit in order to comply with federal law, the PERMITTING AUTHORITY shall include Option A in the NPDES permit.

If the PERMITTING AUTHORITY chooses to include Option B in the NPDES permit, the PERMITTING AUTHORITY shall indicate that Option B is in effect only through December 31, 2023, and that starting January 1, 2024, the discharger must comply with the MDEL indicated in Section III.C.5.c using *Ceriodaphnia dubia* and the MMEL indicated in Section III.C.5.d using *Ceriodaphnia dubia* as the MOST SENSITIVE SPECIES.

Scenario 4: For NON-STORMWATER NPDES DISCHARGERS with numeric chronic aquatic toxicity effluent limitations in their current permit and when the MOST SENSITIVE SPECIES identified by the PERMITTING AUTHORITY is not *Ceriodaphnia dubia*, the PERMITTING AUTHORITY shall include the MDEL indicated in Section III.C.5.c and the MMEL indicated in Section III.C.5.d using the MOST SENSITIVE SPECIES.

III.C.5.b. For permit issuance, reissuance, renewal, or reopening that occurs on or after January 1, 2024

If the PERMITTING AUTHORITY issues, reissues, renews, or reopens (if the permit reopening is to address toxicity requirements) the NPDES permit on or after January 1, 2024, the PERMITTING AUTHORITY shall include the MDEL indicated in Section III.C.5.c and the MMEL indicated in Section III.C.5.d.

III.C.5.c. Chronic Aquatic 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 aquatic toxicity in accordance with the provisions specified in Section III.C.3, or if a POTW is authorized to discharge at a rate equal to or greater than 5.0 MGD and is required to have a pretreatment program by the terms of 40 CFR § 403.8(a) (effective January 1, 2020):

“No {MOST SENSITIVE SPECIES} CHRONIC AQUATIC 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 AQUATIC TOXICITY TEST does not include the survival ENDPOINT, then the PERMITTING AUTHORITY shall include the following MDEL:

“No {MOST SENSITIVE SPECIES} CHRONIC AQUATIC 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, the IWC, and require the use of the TST to analyze the test results in the NPDES permit. A violation of the MDEL may require the implementation of a TRE in accordance with the provisions of Section III.C.8.

III.C.5.d. Chronic Aquatic Toxicity MMEL

The PERMITTING AUTHORITY shall include the following MMEL in the NPDES permit if REASONABLE POTENTIAL is demonstrated for chronic aquatic toxicity in accordance with the provisions specified in Section III.C.3, or if a POTW is authorized to discharge at a rate equal to or greater than 5.0 MGD and is required to have a pretreatment program by the terms of 40 CFR § 403.8(a) (effective January 1, 2020):

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

The PERMITTING AUTHORITY shall specify the MOST SENSITIVE SPECIES, the IWC, and require the use of the TST to analyze the test results in the NPDES permit. A violation of the MMEL may require the implementation of a TRE, in accordance with the provisions of Section III.C.8.

III.C.6. Acute Aquatic Toxicity Effluent Limitations

The PERMITTING AUTHORITY shall include the acute aquatic toxicity effluent limitations according to this section if REASONABLE POTENTIAL is required and demonstrated for acute aquatic toxicity in accordance with the provisions specified in Section III.C.3.

The PERMITTING AUTHORITY shall specify in the NPDES 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 acute toxicity effluent limitations. All toxicity tests of the MOST SENSITIVE SPECIES conducted at the IWC and analyzed using the TST shall be used to determine compliance with the acute toxicity effluent limitations. To the extent any monitoring 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 acute aquatic toxicity effluent limitations specified in this section.

III.C.6.a. Acute Aquatic Toxicity MDEL

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

“No {MOST SENSITIVE SPECIES} ACUTE AQUATIC TOXICITY TEST shall 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, the IWC, and require the use of the TST to analyze the test results in the NPDES permit. A violation of the MDEL may require the implementation of a TRE in accordance with the provisions of Section III.C.8.

III.C.6.b. Acute Aquatic Toxicity MMEL

THE PERMITTING AUTHORITY shall include the following MMEL in the NPDES permit if REASONABLE POTENTIAL is demonstrated for acute aquatic toxicity in accordance with the provisions specified in Section III.C.3:

“No more than one {MOST SENSITIVE SPECIES} ACUTE AQUATIC TOXICITY TEST initiated in a CALENDAR MONTH shall result in a “fail” at the IWC for the survival ENDPOINT.”

The PERMITTING AUTHORITY shall specify the MOST SENSITIVE SPECIES, the IWC, and require the use of the TST to analyze the test results in the NPDES permit. A violation of the MMEL may require the implementation of a TRE, in accordance with the provisions of Section III.C.8.

III.C.7. Targets for a Toxicity Reduction Evaluation

The PERMITTING AUTHORITY shall include the following chronic aquatic toxicity MDET and chronic aquatic toxicity MMET in the NPDES permit for NON-STORM WATER NPDES DISCHARGERS that are not required to comply with the chronic toxicity effluent limitations indicated in Section III.C.5. When MDET and MMET are not met, an obligation to conduct a TRE may be triggered, as specified in Section III.C.8.

III.C.7.a. Chronic Aquatic Toxicity MDET

When required by the TOXICITY PROVISIONS, the PERMITTING AUTHORITY shall include the following MDET in the NPDES permit if the MOST SENSITIVE SPECIES includes the survival ENDPOINT:

“No {MOST SENSITIVE SPECIES} CHRONIC AQUATIC 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 AQUATIC TOXICITY TEST does not include the survival ENDPOINT, then the PERMITTING AUTHORITY shall include the following MDET:

“No {MOST SENSITIVE SPECIES} CHRONIC AQUATIC 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, the IWC, and require the use of the TST to analyze the test results in the NPDES permit. Not meeting the MDET may require the implementation of a TRE in accordance with the provisions of Section III.C.8. Not meeting the MDET is not a violation of an effluent limitation.

III.C.7.b. Chronic Aquatic Toxicity MMET

When required by these Provisions, the PERMITTING AUTHORITY shall include the following MMET in the NPDES permit:

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

The PERMITTING AUTHORITY shall specify the MOST SENSITIVE SPECIES, the IWC, and require the use of the TST to analyze the test results in the NPDES permit. Not meeting the MMET may require the implementation of a TRE, in accordance with the provisions of Section III.C.8. Not meeting the MMET is not a violation of an effluent limitation.

III.C.8. 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. A TRE is also required when a NON-STORM WATER NPDES DISCHARGER does not meet any combination of two or more MDET or MMET within a single CALENDAR MONTH or within two successive CALENDAR MONTHS. In addition, if other information indicates toxicity (e.g., results of additional monitoring, results of monitoring at a higher concentration than the IWC, fish kills, intermittent recurring toxicity), then the PERMITTING AUTHORITY may require a TRE. A TRE may also be required when there is no effluent available to complete a ROUTINE MONITORING test, MMET TEST, or MMEL COMPLIANCE TEST.

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 shall continue during a TRE.

III.C.9. Flow-Through Acute Toxicity Testing Systems

The PERMITTING AUTHORITY may include requirements in the NPDES permit specific to FLOW-THROUGH ACUTE TOXICITY TESTING SYSTEMS, including but not limited to additional effluent limitations or additional monitoring requirements. If the PERMITTING AUTHORITY includes requirements specific to existing flow-through systems, which generate data that cannot be analyzed using the TST, the PERMITTING AUTHORITY shall specify the statistical approach or the calculation to be used to analyze acute toxicity results (e.g., t-test, percent survival). These additional requirements do not substitute for the requirements in Section III.C.

If the PERMITTING AUTHORITY requires the construction of a FLOW-THROUGH ACUTE TOXICITY TESTING SYSTEM after the effective date of these TOXICITY PROVISIONS, that FLOW-THROUGH ACUTE TOXICITY TESTING SYSTEM shall be designed to be amenable to using the TST to analyze the acute toxicity results, and the PERMITTING AUTHORITY shall require analysis of the acute toxicity results to be conducted using the TST.

III.C.10. Violation Reporting and Target 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.

NON-STORM WATER NPDES DISCHARGERS shall notify the PERMITTING AUTHORITY of not meeting any MDET or MMET as soon as the discharger learns of it, but no later than 24 hours of the discharger receiving the monitoring results.

III.C.11. Exemptions

III.C.11.a. Insignificant Discharges

The PERMITTING AUTHORITY is authorized to exempt certain NON-STORM WATER NPDES DISCHARGERS from some or all of the provisions of Section III.C if the PERMITTING AUTHORITY makes a finding that the discharge will have no REASONABLE POTENTIAL to cause or contribute to an exceedance of the numeric aquatic 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 III.C.3.

If exempt, the PERMITTING AUTHORITY shall include the water quality objectives in Section II.C as receiving water limitations in the NPDES permit and the PERMITTING AUTHORITY may assign ROUTINE MONITORING as necessary. ROUTINE MONITORING schedules for INSIGNIFICANT DISCHARGES shall not be more frequent than the applicable frequency specified in Section III.C.4 for the discharger's authorized rate of discharge.

III.C.11.b. Biological Pesticide and Residual Pesticide Discharges

The PERMITTING AUTHORITY is authorized to exempt biological pesticide or residual pesticide discharges¹ regulated by an NPDES permit from some or all of the provisions of Section III.C if the PERMITTING AUTHORITY makes a finding pursuant to the Code of Federal Regulations, title 40, part 122.44(k)(3) that it is infeasible to establish numeric effluent limitations for the biological pesticide or residual pesticide discharges. If exempt, the PERMITTING AUTHORITY shall include the water quality objectives in Section II.C as receiving water limitations in

¹ The term 'pesticide', as used in this section, includes, but is not limited to, pesticide, adulticide, larvicide, algaecide, and herbicide.

the NPDES permit. The determination for the exemption shall be documented in the NPDES fact sheet (or equivalent document).

III.C.11.c. Drinking Water System Discharges

The PERMITTING AUTHORITY is authorized to exempt drinking water system discharges regulated by an NPDES permit from some or all of the provisions of Section III.C if the PERMITTING AUTHORITY makes a finding that the discharges will have no REASONABLE POTENTIAL to cause or contribute to an exceedance of the numeric aquatic toxicity water quality objectives, or that reasonable potential exists only due to discharges of chlorine and chlorine effluent limitations are included in the NPDES permit. The REASONABLE POTENTIAL conclusion necessary to exempt drinking water system discharges need not be based on the REASONABLE POTENTIAL analysis methods set forth in Section III.C.3. If exempt, the PERMITTING AUTHORITY shall include the water quality objectives in Section II.C as receiving water limitations in the NPDES permit.

III.C.11.d. Natural Gas Facilities Discharges

The PERMITTING AUTHORITY is authorized to exempt discharges from hydrostatic testing of natural gas facilities and discharges from site dewatering related to excavation, construction, testing, maintenance, or repair of natural gas facilities regulated by an NPDES permit from some or all of the provisions of Section III.C if the PERMITTING AUTHORITY makes a finding that the discharges will have no REASONABLE POTENTIAL to cause or contribute to an exceedance of the numeric aquatic toxicity water quality objectives, or that reasonable potential exists only due to discharges of chlorine and chlorine effluent limitations are included in the NPDES permit. The REASONABLE POTENTIAL conclusion necessary to exempt natural gas facilities discharges need not be based on the REASONABLE POTENTIAL analysis methods set forth in Section III.C.3. If exempt, the PERMITTING AUTHORITY shall include the water quality objectives in Section II.C as a receiving water limitation in the NPDES permit.

III.D. Implementation for Storm Water Dischargers Regulated Pursuant to NPDES Permits

The PERMITTING AUTHORITY may require toxicity monitoring using any test method. For all STORM WATER dischargers with existing chronic or acute aquatic toxicity monitoring requirements with test methods described in Section III.B.2, the PERMITTING AUTHORITY shall issue Water Code section 13267 or 13383 Order(s) 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 III.B.3, III.B.4, and III.B.5, 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 aquatic toxicity monitoring requirements with test methods described in Section III.B.2, then the PERMITTING AUTHORITY shall require the statistical approach, PERCENT EFFECT, and reporting to be conducted in accordance with Section III.B.3, III.B.4, and III.B.5.

The PERMITTING AUTHORITY may require test methods not described in Section III.B.2, except as required by federal law. This determination must be documented in the NPDES fact sheet (or equivalent document), Water Code section 13267 or 13383 Order(s), or both. Multi-concentration testing is not required except to the extent required by federal law or specified by the PERMITTING AUTHORITY.

III.E. Implementation for Nonpoint Source and Other Non-NPDES Dischargers

The PERMITTING AUTHORITY may require toxicity monitoring using any test method. For all NONPOINT SOURCE and other NON-NPDES DISCHARGERS with existing chronic or acute aquatic toxicity monitoring requirements with test methods described in Section III.B.2, the PERMITTING AUTHORITY shall issue a Water Code section 13267 Order(s) 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 III.B.3, III.B.4, and III.B.5, 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 aquatic toxicity monitoring requirements with test methods described in Section III.B.2, then the PERMITTING AUTHORITY shall require the statistical approach, PERCENT EFFECT, and reporting to be conducted in accordance with Section III.B.3, III.B.4, and III.B.5.

The PERMITTING AUTHORITY may require test methods not described in Section III.B.2, except as required by federal law. This determination shall be documented in the waste discharge requirement (or equivalent document), Water Code section 13267 Order(s), or both. Multi-concentration testing is not required except to the extent required by federal law or specified by the PERMITTING AUTHORITY.

III.F. Variances and Exceptions to the Numeric Aquatic Toxicity Water Quality Objectives

III.F.1. Waters of the U.S.

The PERMITTING AUTHORITY may, after compliance with California Environmental Quality Act (CEQA) if applicable, grant a variance to the numeric or narrative water quality objectives for aquatic toxicity in accordance with Water Quality Standards Variances provisions adopted by State Water Board Resolution No. 2018-0038.

III.F.2. 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 or narrative water quality objectives for aquatic 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 private or 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 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 to adequately evaluate whether the beneficial uses of the receiving waters have been protected or restored upon completion of the project.

APPENDIX A: Glossary

ACUTE AQUATIC TOXICITY TEST: A test to determine an adverse effect (usually lethality) on a group of aquatic 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 AQUATIC TOXICITY TEST: A test to determine an adverse effect (sub-lethal or lethal) on a group of aquatic 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 testing for NON-STORM WATER NPDES DISCHARGERS, the IWC shall be as described in Section III.C.1. 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 III.B.3.

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 Sections III.C.5 and III.C.6.

MAXIMUM DAILY EFFLUENT TARGET (MDET): For the purposes of chronic and acute aquatic toxicity, an MDET is a target used to determine whether a TRE should be conducted. Not meeting the MDET is not a violation of an effluent limitation.

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 Sections III.C.5 and III.C.6.

MEDIAN MONTHLY EFFLUENT TARGET (MMET): For the purposes of chronic and acute aquatic toxicity, an MMET is a target based on a maximum of three independent toxicity tests used to determine whether a TRE should be conducted. Not meeting the MMET is not a violation of an effluent limitation.

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 aquatic toxicity MMEL and MDEL.

MMET TESTS: For the purposes of chronic and acute aquatic toxicity, for dischargers not required to comply with numeric chronic toxicity effluent limitations, MMET TESTS are a maximum of two tests that are used in addition to the ROUTINE MONITORING test to determine whether a TRE should be conducted.

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: Dischargers that do not discharge in a continuous manner or do not discharge throughout the CALENDAR YEAR (e.g., intermittent and seasonal dischargers).

NON-NPDES DISCHARGERS: Dischargers of waste that could affect the quality of waters of the state that are not regulated by the NPDES program.

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 SOURCE: A source that does 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 III.

REASONABLE POTENTIAL: A designation used for a waste discharge that is projected or calculated to cause or contribute to an instream 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.

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) which states, ‘Storm water means storm water runoff, snow melt runoff, and surface runoff and drainage.

TEST OF SIGNIFICANT TOXICITY (TST): A statistical approach used to analyze aquatic toxicity test data, as described in Section III.B.3.

TOXICITY IDENTIFICATION EVALUATIONS (TIEs): Techniques used to identify the unexplained cause(s) of toxic event. A 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 the State Policy for Water Quality Control: Toxicity Provisions.

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 Determinations 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 III.B.2 of the TOXICITY 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 PERCENT EFFECT as described in Section III.B.4 of the TOXICITY 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, in this case for reproduction

$$t = \frac{\bar{Y}_t - b \times \bar{Y}_c}{\sqrt{\frac{S_t^2}{n_t} + \frac{b^2 S_c^2}{n_c}}} = \frac{26.7 - (0.75 \times 33.4)}{\sqrt{\frac{10.68}{10} + \frac{(0.75)^2 (8.93)}{10}}} = 1.32$$

Step 5: Adjust the degrees of freedom.

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

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 TOXICITY 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 III.B.4 of the TOXICITY PROVISIONS.

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

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

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 III.B.2 of the TOXICITY 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 PERCENT EFFECT as described in Section III.B.4 of the TOXICITY 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.

$$t = \frac{\bar{Y}_t - b \times \bar{Y}_c}{\sqrt{\frac{S_t^2}{n_t} + \frac{b^2 S_c^2}{n_c}}} = \frac{17.70 - (0.75 \times 33.4)}{\sqrt{\frac{56.24}{10} + \frac{(0.75)^2 (8.93)}{10}}} = -2.9696$$

Step 5: Adjust the degrees of freedom.

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

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 TOXICITY PROVISIONS). The calculated t-value from step 4 = -2.9696, which is less than the critical t-value of 0.8791.

Step 7: $-2.9696 < 0.8791 = \text{fail}$

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 III.B.4 of the TOXICITY PROVISIONS.

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

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

Conclusion: Because the PERCENT EFFECT at the IWC for the survival ENDPOINT is 50% and the test result was a “fail,” the test in example 2 indicates a violation of the MDEL.

Acute fish survival test, example 3.

Step 1: Conduct the aquatic toxicity test according to the procedures in the appropriate test method manual, as described in Section III.B.2 of the TOXICITY 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 PERCENT EFFECT as described in Section III.B.4 of the TOXICITY 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 zero), 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.

$$t = \frac{\bar{Y}_t - b \times \bar{Y}_c}{\sqrt{\frac{S_t^2}{n_t} + \frac{b^2 S_c^2}{n_c}}} = \frac{1.111 - (0.80 \times 1.412)}{\sqrt{\frac{0.027}{4} + \frac{(0.80)^2 (0.00)}{4}}} = -0.03$$

Step 5: Adjust the degrees of freedom.

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

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 TOXICITY 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 III.B.4 of the TOXICITY PROVISIONS

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

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.