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California Association of Sanitation Agencies
California Water Environment Association

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Via Electronic Mail
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SUBJECT: COMMENTS ON THE PRELIMINARY DRAFT POLICY FOR WHOLE EFFLUENT TOXICITY ASSESSMENT AND CONTROL

Dear Mr. Hann:

Tri-TAC and the California Association of Sanitation Agencies (CASA) appreciate the opportunity to provide written comments on the State Water Resources Control Board's (State Water Board's) Preliminary Draft Policy for Whole Effluent Toxicity Assessment and Control (Policy). Our associations represent public wastewater agencies providing sewer collection, wastewater treatment and water recycling services to millions of Californians. Tri-TAC and CASA are fully committed to the effective and appropriate implementation of the Whole Effluent Toxicity (WET) program and strongly support the use of WET testing as a tool to address uncertainties associated with chemical specific monitoring and biological assessment. The following comments are respectfully submitted with this premise in mind, and with the intent to improve the implementation of toxicity test provisions designed to assess the water quality of surface waters, enclosed bays, and estuaries within the State of California.

Tri-TAC and CASA have a number of serious concerns regarding the Policy, as detailed below.[1] The Policy is based upon the United States Environmental Protection Agency's (USEPA) recently released Test of Significant Toxicity (TST). The overarching concern is that use of the TST will lead to numerous "false positive" results, where non-toxic discharges and receiving waters are incorrectly identified as toxic. This, in turn, will lead to the wasting of significant State and Regional Water Board and publicly owned treatment works' (POTWs') resources to respond to non-toxic, false positive indications of toxicity. Taken to its logical conclusion, the Policy could also ultimately lead to inappropriate use of public funds to provide unnecessary treatment plant upgrades based on non-existent biological community impacts. Tri-TAC and CASA believe that numeric limits for low levels of chronic toxicity are inappropriate, as low-level chronic toxicity has not been linked to instream biological impacts, and that there are significant technical problems with the TST methodology.

Numeric Limits for Chronic Toxicity are Unnecessary and Problematic

We acknowledge that the discharge of wastewater that is toxic may represent a significant threat to beneficial uses and support State Water Board efforts to reduce and eliminate toxic discharges in all waters of the state. However, numeric limits for chronic toxicity are inappropriate and unnecessary to protect water quality within the State, for the reasons detailed below. We strongly support the use of narrative limits with accelerated monitoring and toxicity reduction evaluation (TRE) triggers, an approach that has been effectively implemented in California for several years. This step-wise approach is consistent with guidance from the USEPA, both at the national[2] and regional[3] levels, supported by a diverse national expert advisory panel[4] formed by SETAC and funded by the USEPA to provide guidance on WET issues, and by the State Water Board Toxicity Task Force[5] specifically assembled to provide guidance on the regulatory use of toxicity test within the State.

Biological Systems are Inherently Variable

WET tests are not chemical measurements; they are instead measures of how certain organisms respond to a particular water sample. As such, the measurements are influenced by a number of factors that may be wholly unconnected to toxicity including

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[1] Tri-TAC and CASA also have significant legal and policy concerns regarding the preliminary draft policy. Given the informal nature of this review, we have limited our comments to technical issues but fully intend to raise these additional concerns during the formal review process.
[2] Technical Support Document for Water Quality-Based Toxics Control, EPA Office of Water, March 1991, EPA/505/2-90-001, p. 62, Section 3.3.7.
[3] EPA Regions 9 and 10 Guidance for Implementing Whole Effluent Toxicity Testing Programs, EPA, May 31, 1996, pp. 2-1, 4-1, and 5-2.
[4] Society of Environmental Toxicology and Chemistry (SETAC) WET Expert Advisory Panels, http://www.setac.org/wettre.html, Sections 1 and 4.
[5] Memo to Members of the State Water Resources Control Board from the Toxicity Task Force, September 27, 1995. Recommendations 2, 5, 9, and 10.

ionic changes in water chemistry, presence/absence of trace elements in the water, seasonality, light levels, and temperature. While WET test procedures attempt to minimize variability, they cannot eliminate it altogether. USEPA guidance warns:

> *The interpretation of the results of the analysis of data from any of the toxicity tests described in this manual can become problematic because of the inherent variability and sometimes unavoidable anomalies in biological data.*[6]

> *The allowable frequency for criteria excursions should refer to true excursions of the criteria, not to spurious excursions caused by analytical variability or error.*[7]

Chronic Toxicity Tests Are Poor Predictors of Instream Impacts

Although there is a common perception that the results of WET tests are relatively good predictors of instream biological impacts, scientific research has not shown this to be true. The most definitive study conducted on the subject indicates that chronic WET tests are generally poor predictors in instream impacts even when using the more robust EC/IC25 statistical analyses.[8] Several quoted findings of this study include:

> *In general, poor agreement was observed between WET results and instream biological condition, contrary to results previously reported by EPA and other research entities.*

> *Results from acute and chronic Ceriodaphnia tests were not significantly related to measures of instream impairment – even after accounting for habitat factors (p>0.05).*

A subsequent follow-up WERF study published in 2007 described nearly identical findings,[9] even though it focused on effluent-dominated streams where effluent WET tests would be expected to be more predictive of in stream effects. Therefore, the use of a sensitive and likely over-protective final effluent WET objective will not result in greater protection of receiving biological conditions than a more reasonable WET objective.

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[6] EPA. Short-Term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Water to Freshwater Organisms, Fourth Ed., EPA-821-R-02-013. October 2002. Section 9.4.1.1, p. 39.
[7] Technical Support Document for Water Quality-Based Toxics Control, EPA Office of Water, March 1991, EPA/505/2-90-001. See Appendix entitled "Technical Support Document for Water Quality Based Toxics Control – Responsiveness Summary," p. 11.
[8] Evaluating Whole Effluent Toxicity Testing as an Indicator of Instream Biological Condition. Water Environment Research Foundation (WERF) Project Report 95-HHE-1. 1999.
[9] Evaluation of WET Testing as an Indicator of Aquatic Health in Effluent-Dominated Streams. Water Environment Research Foundation Project Report 03-ECO-2T. 2007.

Chronic Toxicity Triggers Provide an Effective Means of Addressing Toxicity

For the reasons described above, Tri-TAC and CASA continue to strongly support the use of narrative limits with prescriptive and numeric accelerated monitoring and TRE triggers. This has been the State's practice for over 10 years, one that has been demonstrated to be effective at controlling and eliminating toxicity. Although it is not possible for POTWs to predict when "toxicity" is entering the plant, as it can derive from diffuse and intermittent or even one-time sources, it is appropriate and reasonable to expect dischargers to aggressively seek the source(s) of the toxicity and, once identified, take immediate action to reduce the source(s). Failure on the part of a discharger to adequately implement this process in response to toxicity would constitute a violation of the narrative toxicity limitation and open the discharger to the imposition of administrative liability and other enforcement actions.

False Positive Rates Under the Policy Would Be Unacceptably High

Tri-TAC and CASA are tremendously concerned that the Policy will lead to an excessive frequency of false positives (i.e., incorrectly identifying a non-toxic effluent as toxic). The recently released USEPA guidance on the TST[10] (TST Guidance) contends that a 5% false positive error rate for individual tests is incorporated into the TST analysis. While even a 5% false positive rate is unacceptably high from a discharger point of view, it is important to note that the actual false positive rate would be even higher. The explicit 5% false positive error rate built into the TST is actually a regulatory management decision made by USEPA to identify no more than 5% of the tests with a 10% effect or less as "toxic." This means that the 5% false positive error rate only applies to tests with a 10% effect or less. The false positive error rate among samples with an effect of 10% to 25% will be significantly higher.

The State Water Board staff have apparently attempted to address the excessive rate of false positives on individual tests through the proposed implementation procedures, these implementation procedures would not reduce the number of false violations identified to an acceptable level. However, though the language of the Policy is ambiguous, it appears that the intent of the State Water Board staff is that a test result of "fail" during routine monitoring (using two concentrations – a control and the Instream Waste Concentration (IWC)) would not be considered a violation but rather an "excursion." A discharger having such an excursion would initiate an accelerated monitoring schedule consisting of six additional tests (using five concentrations). A violation would only occur if one or more of the accelerated tests failed at the IWC. While such an approach is a step in the right direction, it does not go far enough in reducing the incidence of false violations. Moreover, as many permits contain language prohibiting "excursions above water quality standards," this approach to differentiate a

[10] National Pollutant Discharge Elimination (NPDES) System Test of Significant Toxicity. EPA 833-R-10-003, June 2010.

trigger exceedance from a violation may actually create a different avenue for incurring violations.

In order to determine the rate at which false positive results and false violations would occur, Tri-TAC examined several different data sets as well as the false positive error rate built inherently into the TST. The results are detailed below.

USEPA Non-Toxic Blank Samples Are Identified as Toxic 15% of the Time Using the TST

USEPA conducted an extensive inter-laboratory WET study[11] in 2000, in support of WET method development. As part of this study, USEPA prepared and distributed a number of non-toxic "blank" samples and sent them out to laboratories for WET analysis. The raw results from this study were obtained for the blanks that were analyzed for *Ceriodaphnia dubia* chronic toxicity and evaluated using the TST. Since all these samples were known non-toxic blank samples, any identification of toxicity would be a false positive error. **An absolutely unacceptable 15% of the USEPA blank, non-toxic samples tested with *Ceriodaphnia dubia* would have been incorrectly identified as toxic using the TST.**

Using this 15% false positive error rate for single tests, calculations were performed to determine the probability that a false violation would occur, assuming that an excursion in a single test had to be confirmed during one of six follow-up tests. This follows the implementation procedure proposed in the Policy. **The probability that a false violation would occur (i.e., that the proposed numeric limit would be exceeded) in discharge that had zero toxicity would be 9.4%**.[12] This means that if a discharger had an effluent that was fully non-toxic, with a monthly monitoring requirement for chronic toxicity, the discharger would be expected to experience, on average, one false violation each year, or six false positive violations over the 5-year course of an NPDES permit cycle. Dischargers could incur significant liability under the Clean Water Act and state law for these false violations.

Non-Toxic Reference Samples Are Identified as Toxic 12% of the Time Using the TST

Another set of data was analyzed to determine the potential rate of false positive results and false violations. This data set consisted of data from fathead minnow survival and growth chronic bioassays conducted repeatedly at a single laboratory during the

[11] U.S. EPA. Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods-Vol. 1 & 2; EPA-821-B-01-004; September, 2001

[12] False positive violation rate calculated as the single test false positive error rate multiplied by the binomial test probability of exceeding at least one of the six accelerated tests assuming the same single test false positive error rate.

course of one year on a reference toxicant concentration that had a mean, median, and 70th percentile effect of less that 10% (n = 49). This means that overall, the expected effect on fathead minnows from the reference toxicant solution should be less than 10%, so the solution is officially "non-toxic." When the data were analyzed using the TST, fully 12% of the individual tests would have been declared "toxic."

At a 12% false positive error rate on individual samples, the probability of having a false violation (showing a violation where the "true" or actual effect is less than 10%) using the implementation procedure in the Policy would be 6.4%.[13] At this false positive violation rate, an average discharger would be expected to experience almost one false violation per year, or four over the course of a 5-year permit cycle.

High False Positive Error Rates Would Cause Undue Impairment Listings

In addition to the issues associated with final effluent numeric limit compliance determination, the false positive error rates associated with the proposed chronic toxicity objective are also likely to result in nearly every waterbody being ultimately included on the 303(d) list due to toxicity related impairments. This would potentially give a grossly inaccurate portrayal of the condition of California's water bodies and result in hundreds of unnecessary Total Maximum Daily Loads (TMDLs) being required in waters that are in reality meeting all aquatic life beneficial uses. Table 3.1 of California's 303(d) listing policy[14] specifies that if two or more of 24 measurements in a waterbody exceed the water quality objective, the waterbody will be listed as impaired. At a 15% false positive error rate, the probability of listing a non-toxic water body (i.e., of observing at least two TST exceedances in 24 samples) is 89%. Even at a 12.5% error rate, the probability of listing a non-toxic waterbody is an unacceptably high 82%.

High False Positive Error Rates Would Waste State and Local Resources

The false positive error rate associated with the TST combined with the implementation procedures that include single test numeric chronic WET limits being proposed by the State Water Board is troublesome for a number of reasons. For regulators, false violations divert enforcement resources away from real water quality violations. False determinations of impairment ultimately consume regulatory resources through unnecessary 303(d) listings and development of associated TMDLs. For dischargers, false violations subject POTWs to unnecessary and unwarranted enforcement action and citizen lawsuits. Dischargers are also put in the untenable position of being required to solve a problem that does not exist or to expend resources

[13] False positive violation rate calculated as the single test false positive error rate multiplied by the binomial test probability of exceeding at least one of the six accelerated tests assuming the same single test false positive error rate.
[14] Water Quality Control Policy for Developing California's Clean Water Act Section 303(d) List, State Water Resources Control Board. Adopted September 2004.

attempting to identify sources of toxicity that do not exist. In the most extreme cases, dischargers could be forced to perform treatment upgrades that are aimed at the wrong target and thus, will not improve biological communities. Given that the State is not required to implement the TST, these new costs represent a new unfunded state mandate for some categories of dischargers.

The TST Should Only Be Used in Conjunction With a EC/IC25

While issuance of the USEPA TST Guidance means that there is now an allowable statistical approach to determine toxicity in addition to the existing No Observable Effect Concentration (NOEC) and EC/IC25 methods, the **only** statistical analysis specifically recommended for NPDES compliance purposes in the promulgated toxicity methods remains the point estimate technique (i.e., EC/IC25).[15] Furthermore, both the TST Guidance and the USEPA Technical Support Document for Water Quality-based Toxics Control[16] concur that the appropriate effect level threshold for defining unacceptable chronic toxicity is 25% or an EC/IC25.

Due to the high false positive rate under the TST, use of the TST alone to determine toxicity is not appropriate. If the State Water Board staff believe that it is necessary to use the TST in order to address the issue of false negatives caused by high variability within a laboratory, the State Water Board should combine the TST with a minimum observed effect of 25% as established by the point estimate test. In order for a sample to be considered toxic, it would have to fail the TST AND demonstrate a minimum observed effect of 25%. In instances that the TST indicates an exceedance but a minimum effect of 25% is not observed, the results would be considered inconclusive and the test repeated. Such a dual metric approach would ensure that all samples having an observed effect level over 25% would be considered toxic. It would also ensure that laboratories conduct precise tests with good test control replication, because otherwise the TST will provide inconclusive results. It would further address the concerns of dischargers relating to excessive false positive results and the potential for results with very low effects being considered toxic. A dual metric approach provides certainty that a toxic event occurred, and that the event was both statistically and biologically significant.

Although Tri-TAC and CASA prefer the use of a narrative approach, if numeric limitations are proposed, compliance with those limitations should be determined through the use of two separate statistical methods to be used to determine toxicity is provided for in the promulgated method for measuring chronic toxicity. The method allows the use of

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[15] Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, 2002, EPA-821-R-02-013, pg. 41, section 9.5.1. "**Note: For the NPDES permit program, the point estimation techniques are the preferred statistical methods in calculating endpoints for effluent toxicity tests**."

[16] Technical Support Document for Water Quality-based Toxics Control, U.S. EPA Office of Water, March 1991, EPA/505/2-90-001

a NOEC and/or an EC/IC25 to measure toxicity. The State of Colorado recently adopted a policy on regulating WET in discharges that requires both the NOEC and the IC25 to be exceeded to establish toxicity.[17] Similar dual metric definitions for chronic toxicity have been implemented in Region 2 and 8 of California, North Carolina, and South Carolina.

Clarification Needed on Interpretation of Violation Occurrences

Section 7.b of the Policy appears to indicate that a result of "fail" during routine monthly testing at two concentrations (control and IWC) is not a numeric limit violation but rather represents a trigger to conduct accelerated confirmatory monitoring. A "fail" result during the confirmatory accelerated monitoring would constitute a violation. However, in the second sentence of Section 7 on page 10, the Policy states that accelerated testing would be initiated "no later than fourteen days from the date of the *violation*." (Emphasis added.) This implies that an initial "fail" result during routine monthly testing may be considered a violation. The term "violation" in this sentence should be replaced with "trigger exceedance" to more clearly indicate the intent of the policy. The first sentence of the next paragraph is also confusing as to when a numeric limit violation occurs. This apparent discrepancy can be addressed if a period is added after "(TRE)" and then a new sentence added that indicates that a TRE will be conducted if one accelerated test fails at the IWC.

Stringency Considerations

In the "Applicability of Policy" section of the Policy, it is stated that the Policy only establishes minimum requirements; Regional Water Quality Control Boards may impose more stringent requirements. Tri-TAC and CASA strongly disagree with this provision of the Policy. One impetus for the Policy is a perceived need for statewide consistency to replace the varying approaches in regional Basin Plans. Allowing regional boards to deviate from the Policy in one direction is inconsistent with this goal and with the very specialized nature of establishing toxicity limitations. There are many technical and statistical considerations with respect to establishing numeric toxic limits. Such limits should be set only after a thorough analysis of the technical and environmental impacts associated with setting such limits. They should not be done on a permit-by-permit basis, as would currently be allowed under the Policy.

Reasonable Potential Calculations

It is not clear why, in contrast to chemical-specific objectives, the draft Policy assumes that major POTWs discharges exhibit Reasonable Potential (RP) for chronic toxicity. Additionally, the RP procedures contained in the Policy use a different criterion

[17] Implementation of the Narrative Standard for Toxicity in Discharge Permits Using Whole Effluent Toxicity (WET) Testing, Colorado Water Quality Control Division, Policy WQP-28, effective September 30, 2010.

(an observed effect of 10% in one or more tests) than the objective. There does not appear to be a single example in California where chemical-specific objectives in NPDES permits were implemented using different criteria to determine permit limits versus the criteria used to determine RP for the same constituent. The RP criterion of a 10% reduction in the chronic response at the IWC compared to the control appears to be unnecessarily stringent. The Policy does not explain how this criterion was determined or developed and it appears to be arbitrary.

Technical Comments on the TST

Tri-TAC and CASA comment on several technical concerns regarding the USEPA TST method as described in the referenced USEPA guidance. The Code of Federal Regulations (CFR) Part 136-approved chronic toxicity methods allow for the use of the NOEC and/or point estimates (i.e., EC/IC25) exclusively. Furthermore, as previously discussed, these promulgated methods specifically recommend use of point estimates for NPDES compliance determination. Neither the TST method nor USEPA's TST Guidance as referenced in the Policy have been formally peer reviewed and the analytical procedures have not been promulgated into 40 CFR Part 136. Since WET is a method-dependent parameter, a change in data analysis essentially changes the water quality criteria. This is most obviously demonstrated by the fact that the TST does not always produce the same toxicity determination as the promulgated NOEC or EC/IC25. Peer review of the proposed Policy is strongly recommended since no peer review, formal public comment, or promulgation was incorporated into the development of the USEPA TST Guidance.

The other significant technical comment is related to the apparent bias associated with the arc-sin square root transformation of binomial data without a similar transformation or correction applied to the "b" or bioequivalency factor. Like percentage data, the "b" or bioequivalency factor is also a binomial parameter. Failure to account for this when transforming binomial data results in significant increases in transformed variance and effect. Figure 1 graphically displays this inherent bias in transformed response at observed effects greater than 0% and less than 25% in fathead minnow chronic toxicity results (survival endpoint) conducted by a single laboratory. This ultimately results in increasing the likelihood of identifying such tests as toxic using the TST introducing an unwanted positive bias.

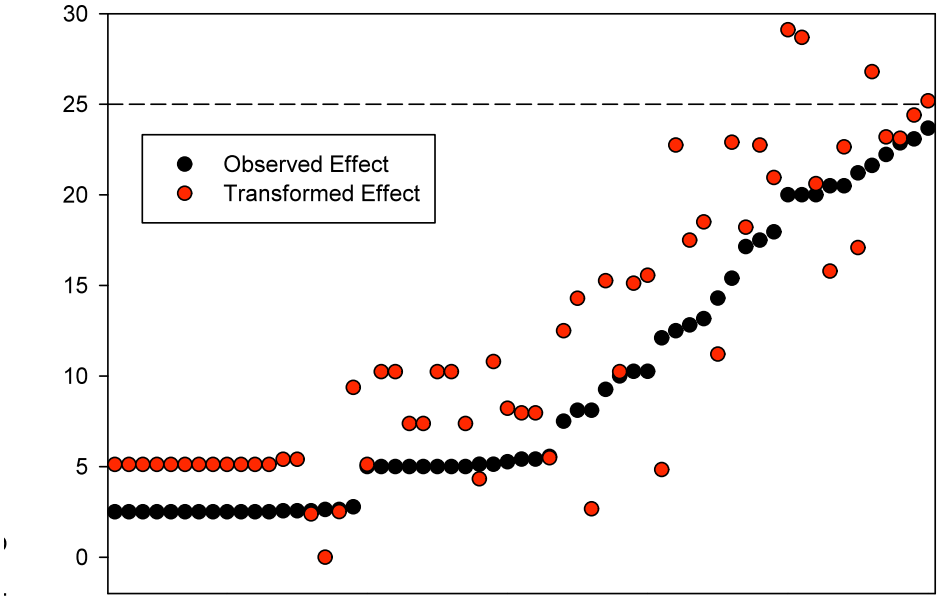

Figure 1. Fathead Minnow survival effects between 0% and 25%. All tests conducted in 2009 by a single laboratory.
Proportionality Constants (b) Used in the TST Results in an Under-Estimation of the False Positive Error Rate

The proportionality constant (fixed fraction), b, values assigned in the proposed TST method are not appropriate and consequently the statistical power of the test to reject the hypothesis that treatment and control are not bioequivalent is unacceptably low; i.e. it results in false positives more than the nominal 5% of the time. By assigning appropriate, b, values based upon the USEPA developed 75th percentile value of CVs would improve the regulatory application of proposed TST method and will be consistent with the current State Implementation Policy regarding chemical measurements. Details of this concern are contained in Appendix 1.

Tri-TAC and CASA again thank the State Water Board for this opportunity to provide early input into the Policy. We look forward to working with the State Water Board as it continues to develop Policy.

If you have any questions about these comments or require additional information, please contact Bobbi Larson at (916) 469-3887 or blarson@somachlaw.com.

Sincerely,

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Tri-TAC

Roberta L. Larson
Director of Legal & Regulatory Affairs
CASA

cc: Brian Ogg, State Water Board
Darrin Polhemus, State Water Board
Rik Rasmussen, State Water Board

Appendix A

Abstract: The proportionality constant (fixed fraction), b, values assigned in the proposed TST method are not appropriate and consequently the statistical power of the test to reject the hypothesis that treatment and control are not bioequivalent is unacceptably low i.e. it results in false positives more than the nominal 5 percent of the time. By assigning appropriate, b, values based upon the USEPA developed 75th percentile value of CVs would improve the regulatory application of proposed TST method and will be consistent with the current SIP policy regarding chemical measurements.

Discussion: The USEPA's TST document [(1)] (page 2, paragraph 2) states " . . . the rate of false negatives in the NPDES WET program has not been controlled. Put another way, the statistical power of these tests, the ability to correctly classify the IWC as toxic . . . has not been controlled." The TST program is designed to control both false negatives and false positives.

In the proposed TST program the statistical power of the test is expressed as 1-β, where β is associated with the false positives. The β is set at 0.05. Thus the nominal statistical power of the test is 0.95 (1 – 0.05) i.e. no more than 5 percent of the time, a truly acceptable effluent (≤ 10 percent mean effect at IWC) will be declared toxic.

In order to obtain the stated (nominal) statistical power of test, the proportionality constant, b, is set at 0.75 for chronic and 0.8 for acute WET methods (see the TST recommended statistical method equation on page 6 of SWRCB proposal [(2)] and page A-2 of the USEPA TST document[(1)] or attachments 1, 2, and 6).

The proportionality constant, b, value of 0.75 for chronic and 0.8 for acute is developed by the USEPA based upon the USEPA's analysis of the α levels for various WET methods for a desired β value of 0.05 (statistical power of 0.95).

Our review of the TST recommended statistical method reveals that the actual statistical power of the test is much lower than nominal of 0.95. In other words, the value of the proportionality constant, b, is artificially higher than actually required for an appropriate CV and number of replicates for a given WET test. Consequently, the TST recommended statistical method would declare effluent toxic, when indeed it is not, more than the stated 5 percent of time (the nominal false positive error, β = 0.05). The reason this happens is that an artificially higher value of the proportionality constant, b, would result in not rejecting the hypothesis that the treatment and control are not bioequivalent more than the stated 5 percent of the time. We illustrate this problem using the USEPA's TST document[(1)] case examples.

In case examples 1 and 2 (see attachment #1 and #2) the mean *Ceriodaphnia dubia* reproduction (26.7) in treatment (IWC) in both cases is identical. So is true for the controls (33.4). The effluent passes the acceptable toxicity threshold (RMD) i.e. the mean effect in the treatment (IWC), 26.7 is ≥ 0.75 of the mean effect in the control, 25.0 (33.4 X 0.75). However the TST statistical method declares the effluent toxic in case example 1 and non-toxic in case example 2 (note that the effluent sample is the same and the toxicity effects are identical in both tests or between Labs).

The reason the effluent is declared toxic in case example 1 is that the calculated 't' value of 0.82 is lower than the critical 't' of 0.87 for a given degree of freedom and 'α' value. Hence the hypothesis that treatment and control are not bioequivalent is not rejected. Thus the effluent is declared "toxic" when indeed it is not as shown by case example 2 and the RMD threshold. Note that the proportionality constant, b, in equation (step 4 and 5) is set at 0.75 (see attachment # 1 and # 2). This value appears to be based upon a CV of 0.20 (see table 9 from Erickson & McDonald [(3)], attachment # 3, value, b, required for CV = 0.20, k=2, n=10). The value of 0.75 renders the test a stated power of about 0.90 (α = 0.05). Turns out that the value of 0.75 is artificially high because it is based upon a CV of 0.2 which is artificially low or represents a less than 50th percentile value of CVs routinely obtained within and between laboratories (see USEPA [(4)] Table 3.2 and 3.4, attachment # 4). Use of a proportionality constant, b, corresponding to less than the 50th percentile value of CVs imparts the test an unacceptably low statistical power or unacceptably high false positive rates. Although the 95th percentile value would be desirable for accurate regulatory decision, the 75th percentile value is reasonable because it is consistent with the current SWRCB SIP policy regarding chemical measurements. The SIP uses 80th percentile value of ML for chemical regulatory decisions. Therefore, an appropriate CV for *Ceriodaphnia dubia* reproduction test is 0.45 which represents a 75th percentile value of CVs (see attachment # 4). The corresponding value of, b, is 0.51 (see attachment # 3 for value, b, required for k=2, n=10 and CV=0.45).

By substituting 0.51 for, b, in equation (step 4 and 5 in attachment # 1) the calculated 't' is 9, which is greater than the critical 't' at α = 0.05. Thus the hypothesis that the treatment and control are not bioequivalent is rejected and the effluent is correctly declared "non toxic."

Case example 3 (see attachment # 5) suffers from the same problem as illustrated using case example 1 and 2 above. The appropriate CV for Fish (Fathead minnow) Growth Test is 0.38 (see attachment # 4) and the corresponding proportionality constant, b, value is 0.38 (k=2, n=4, CV=0.35, see attachment # 3). By substituting 0.38 for, b, in equation (step 4 and 5 in attachment # 3) the calculated 't' is greater than the critical 't.' Thus the hypothesis that the treatment and contact are not bioequivalent is rejected and the effluent is correctly declared "non toxic."

Like case example 1 and 3, in case example 4 the effluent (see attachment # 6) is declared toxic even though the effluent clearly meets the acceptable toxicity threshold (RMD) i.e. the mean percent survival in treatment (IWC) 87.5 percent is ≥ 0.80 of the mean survival in the control, 80 percent (0.80 x 100). According to the TST procedure, the value, b, in equation (step 4 and 5) is set at 0.8. The appropriate CV for Fish Acute Toxicity Test (Fathead minnow survive) is 0.32 (see attachment # 4). The corresponding value of, b, is 0.43 (k=2, n=4, and CV=0.30, see attachment # 3). By substituting 0.43 for, b, in equation (step 4 and 5) the calculated 't,' 3.5 is greater than the critical 't' at $\alpha = 0.05$ and thus the hypothesis is rejected and effluent is correctly declared "non toxic."

References:

1. USEPA: National Pollutant Discharge Elimination System, Test of Significant Toxicity, Implementation Document, September 4, 2009.
2. SWRCB: Policy for Whole Effluent Toxicity Assessments and Control, 2010
3. William Erickson and L.L. McDonald: Test for Bioequivalence of Control Media and Test Media in Studies of Toxicity. Environ. Toxicol., & Chem. Vol. 14 No. 7 PP 1247-1256 (1995).
4. USEPA: Understanding and Accounting for Method Variability in Whole Effluent Toxicity Application under the National Pollutant Discharge Elimination System (June 30, 2000).