

# CITY OF SAN BERNARDINO MUNICIPAL WATER DEPARTMENT

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August 21, 2012

Charles R. Hoppin, Chairman and Members  
State Water Resources Control Board  
1001 I Street  
Sacramento, CA 95814

c/o Jeanine Townsend, Clerk to the Board  
[commentletters@waterboards.ca.gov](mailto:commentletters@waterboards.ca.gov)

**SUBJECT: Comment Letter: Draft Policy for Toxicity Assessment and Control**

Dear Chairman Hoppin and Members:

The City of San Bernardino Water Department discharges highly-treated recycled water to the Santa Ana River in accordance with NPDES Permit No. CA8000304. We perform routine chronic toxicity testing every month and have demonstrated an outstanding record of consistent compliance for more than 16 years. Since 1996, when our tertiary treatment facility went on-line, San Bernardino's effluent has rarely failed a toxicity test and has never failed two consecutive tests. Thus, the following comments are submitted from our perspective as a permittee that is already producing a non-toxic effluent. We are deeply concerned that proposed procedures in the Draft Policy for Toxicity Assessment and Control will more frequently characterize San Bernardino's discharge as toxic when, in fact, it is not.

- 1) The proposed policy's null hypothesis-based numeric water quality objective initially presumes any effluent sample water is toxic, i.e., that organisms exposed to any given effluent sample will have less than 75% of the growth or reproduction of similar organisms exposed to a non-toxic control sample, until the WET test results prove otherwise. Such an assumption is completely inappropriate where a discharger has

years of historical data indicating that its effluent is not toxic and that effluent-exposed organisms perform as well or better than the control. Such is the case in San Bernardino. This is precisely this pattern of consistent compliance that has enabled the Santa Ana Regional Board to conclude that San Bernardino had no reasonable potential for chronic toxicity and to issue San Bernardino a permit without an effluent limit for chronic toxicity. Therefore, we strongly object to any policy or test procedure that presumes San Bernardino's effluent is toxic until proven otherwise because such an assumption is inconsistent with the historical record and is contrary to EPA's guidance on how to properly assess reasonable potential.

- 2) Since San Bernardino's current permit was issued in December of 2006, San Bernardino has performed more than 70 chronic toxicity tests and observed only 3 failures. This failure rate is BELOW what one would expect to occur by random chance (<5% of all tests). We reanalyzed all of this data using EPA's TST procedure and found that the number of apparent test "failures" more than doubles under the SWRCB's proposed policy (see Appendix A). All of these additional "failures" would have been deemed to have passed both of the statistical endpoints (NOEC & IC25) currently authorized under EPA's regulations at 40 CFR Part 136. In each instance, reproduction among the effluent-exposed organisms was actually greater than 75% of that exhibited by the control group; nevertheless, under the TST procedure, San Bernardino's effluent would be declared toxic despite clear data indicating to the contrary.

Similar side-by-side comparisons performed by other NPDES permittees in the Santa Ana watershed identified similar problems. Inland Empire Utilities Agency and Yucaipa Valley Water District both found that the number of reported test failures increased using the proposed TST procedure compared to the existing NOEC or IC25 methods (see e.g., Appendix B). Results derived from the TST procedure are clearly not consistent with or comparable to those produced using the previously promulgated WET test methods.

- 3) The proposed TST method has not been approved under 40 CFR Part 136. Federal regulations require dischargers to perform tests using the standard methods promulgated under 40 CFR Part 136 when assessing compliance with effluent limitations in an NPDES permit.<sup>1</sup> Because the proposed TST procedure is not a promulgated method, it cannot be used in lieu of the currently recommended NOEC or IC25 endpoints.<sup>2</sup> In addition, the TST procedure specifies that the toxicity test be performed using only two test concentrations: a control group and an effluent-exposed group. However, EPA's official promulgated WET method requires that tests used to determine compliance with an NPDES effluent limit consist of a control group and "a minimum of

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<sup>1</sup> See, for example, 40 CFR Part 136.1(b) and U.S. EPA. 64 FR 149, 42464 (Aug. 4, 1999)

<sup>2</sup> U.S. EPA. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms - 4<sup>th</sup> Ed. October, 2002. EPA-821-R-02-013. See §1.9 @ pg. 2.

five effluent concentrations" in order to evaluate the validity of the dose-response relationship.<sup>3</sup> EPA explains that:

"The agency is concerned that single concentration, pass/fail, toxicity tests do not provide sufficient concentration-response information on effluent toxicity to determine compliance. It is the Agency's policy that all effluent toxicity tests include a minimum of five effluent concentrations and a control."<sup>4</sup>

Despite this admonition, the SWRCB is proposing that a single effluent concentration be used as a pass/fail test to determine compliance. According to EPA, additional concentrations are essential in order to reduce the number of false positives:

"In today's action, EPA proposes to require the review of concentration-response relationships generated for all multi-concentration WET tests reported under the NPDES program. EPA proposes to modify section 10 of the two chronic method manuals and section 12 of the acute method manual to incorporate this required test review procedure...Use of the concentration-response review procedures would ensure that a valid concentration-response relationship is demonstrated prior to the determination of toxicity...the use of these review procedures reduced the rate of reported false positives in the WET Variability Study from 11.1% to 3.7% in the *Ceriodaphnia dubia* Survival and Reproduction Test and from 12.5% to 4.35% in the Fathead minnow Larval Survival and Growth Test."<sup>5</sup>

Since the TST procedure does not use multiple different effluent concentrations, the TST procedure produces insufficient data to evaluate the validity of the dose-response relationship. Without this important tool to identify anomalous results that frequently lead to false indications of toxicity, it is not surprising that the TST reports twice as many test failures as are observed when using the promulgated method. According to the two EPA scientists most directly responsible for developing the current WET test methods:

"A predictable dose-response curve is one of the mandatory requirements for a valid toxicity test. We would never accept analytical results from an instrument producing an abnormal standard curve. The

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<sup>3</sup> U.S. EPA. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms - 4<sup>th</sup> Ed. October, 2002. EPA-821-R-02-013. See Table 1 @ pg. 76 and Table 3 @ pg. 165.

<sup>4</sup> U.S. EPA. Whole Effluent Toxicity: Guidelines Establishing Test Procedures for the Analysis of Pollutants - Supplementary Information Document (SID) Oct. 2, 1995; pg. 28.

<sup>5</sup> U.S. EPA. 66 FR 189, 49799-49800 (Sept. 28, 2001); For final rule confirming requirement to review concentration response relationship see U.S. EPA. 67 FR 223, 69962 (Nov. 19, 2002)

predictable dose-response curve, that is increasing toxicity with increasing concentration, is the analogue of the analytical standard curve and is of equal importance in toxicity testing."<sup>6</sup> (emphasis added)

"The dose response curve is the basis for the validity of a toxicity test. The control serves as the starting point from which the dose response is evaluated. If a dose response is not obtained, then toxicity cannot be inferred."<sup>7</sup> (emphasis added)

The TST procedure fails to provide the necessary dose-response curve to ensure actual toxicity exists. This failure puts dischargers at risk of non-compliance without adequate justification.

- 4) The proposed TST procedure does not accurately identify non-toxic samples. When non-toxic method blank data from EPA's Interlaboratory WET Variability Study is re-evaluated using the TST procedure, the number of false positives increases dramatically. Nearly 15% of all non-toxic samples were declared "toxic" in the *Ceriodaphnia dubia* reproduction test - four times more than occurred when using either the NOEC or IC25 method. And, 7.4 % of all non-toxic samples were declared "toxic" using the TST procedure to evaluate Fathead minnow growth. This is double the rate at which similar false conclusions occurred when evaluating the same data with the traditional NOEC or IC25 methods (see Table 1 below and Appendix C).

**Table 1: False Indications of Toxicity in Non-Toxic Method Blank Samples**

Chronic Test Endpoint	TST	NOEC	IC25
<i>C. dubia</i> Reproduction	4 of 27 (14.8%)	1 of 27 (3.7%)	1 of 27 (3.7%)
<i>C. dubia</i> Survival	2 of 27 (7.4%)	0 of 27 (0%)	0 of 27 (0%)
Fathead minnow Growth	2 of 24 (8.3)	1 of 24 (4.2%)	1 of 24 (4.2%)
Fathead minnow Survival	0 of 24 (0%)	0 of 24 (0%)	0 of 24 (0%)

At a workshop before the SWRCB in November of 2010, numerous stakeholders throughout the state cited the above example and requested that the Board direct staff to conduct a new study designed to assess the TST error rate when evaluating known non-toxic (method blank) samples. However, the so-called "test drive" focused exclusively on assessing effluent samples where the true toxicity was unknown or where reference toxicant samples were already known to be toxic. No effort was made to assess the accuracy of the TST technique on method blanks as the State Board members

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<sup>6</sup> Dr. Donald Mount, National Effluent Toxicity Assessment Center, EPA Environmental Research Laboratory - Duluth, MN. NETACommunique, Jan., 1990

<sup>7</sup> Norberg-King, Teresa J., U.S. EPA Environmental Research Laboratory - Duluth, Memorandum to Rob Pederson, EPA Region X, *Review of the Toxicity Results from West Boise and Landers Street POTWs* (June 5, 1989).

promised in the workshop and as EPA had done in the Interlaboratory WET Variability Study to validate the NOEC and IC25. Since this problem has been brought to the Board's attention in previous testimony and numerous comment letters, it is incomprehensible that the issue continues to be ignored. In addition, the absence of such essential information renders the peer review of the proposed policy both incomplete and biased. As a result, the Supplemental Environmental Documentation (SED) prepared by staff fails to meet the minimum standards necessary to adequately analyze the issues and demonstrate "functional equivalence" with CEQA requirements.

- 5) Because of the inherent uncertainties in WET tests and the additional problems with the TST procedures described in this letter, dischargers will be unable to certify TST results on the monthly Discharge Monitoring Reports (DMRs). In March of 2000, U.S. EPA published guidance regarding the certification of WET test results on the DMR wherein EPA stated:

"When a person certifies that the submission of WET testing information is accurate to the best of their knowledge and belief, the person certifies that the results obtained using the WET testing procedures are faithfully and truthfully transcribed on the information submission, and that the results were, in fact results that were obtained using the specified testing procedures."<sup>8</sup>

Since the TST method has not been approved as part of a Part 136 method, dischargers cannot legally certify the results derived from this method. The fact that the TST procedure relies on only two rather than the minimum six test concentrations mandated in the promulgated method also makes it impossible to certify the results. And, finally, the City of San Bernardino would not and could not certify TST results as "true" or "accurate" where the conclusions were inconsistent with those reported using the IC25 procedure that EPA endorsed in the original rule promulgating the existing methods. This is particularly true in light of our inability to confirm the validity of the dose response relationship and the elevated incidence of false positive results observed when using the TST procedure to evaluate non-toxic method blank samples. The City's position is also consistent with the U.S. Court of Appeals finding in the *Amoco* case.<sup>9</sup>

- 6) The proposed requirement to perform two additional accelerated tests in the same calendar month that the original test failure occurred will be virtually impossible to implement. A minimum of 10 days is required to collect, deliver and analyze an effluent

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<sup>8</sup> U.S. EPA. Certification of Accuracy of Information Submissions of Test Results Measuring Whole Effluent Toxicity. Memorandum to Regional Water Management Division Directions, EPA Regions I-X and Regional Enforcement Division Directions, EPA Regions I-X. March 3, 2000.

<sup>9</sup> *Amoco Oil Co. v. EPA*, 501 F.2d 722 (D.C. Cir. 1974) stating in relevant part that the possibility of measurement error "deprives the agency of the power to find a violation of the standard, in enforcement proceedings, where the measured departure from them is within the boundaries of the probable measurement error."

sample. Most POTWs collect and ship the initial sample on the first Monday of the month. The lab initiates the chronic test the following day and the test will conclude 7-8 days later (usually a Tuesday or Wednesday of the following week). The lab will analyze the data and report preliminary results within a day or so. If the test fails, new sample containers must be prepared and new samples must be sent to the lab. This also requires just a day or two. However, the cumulative time that has elapsed is now nearly two weeks. It is difficult, but possible, to perform two tests in a single month. However, it is not possible to conduct three. This is especially true in January, July, November and December when national holidays severely restrict the normal testing schedule.

San Bernardino's existing permit specifies that we conduct regular monthly chronic toxicity testing and already requires us to initiate accelerated monitoring at the first indication of WET test failure. As with the proposed state policy, the City is required to pass the next two tests in order to resume the normal monitoring schedule. However, unlike the proposed policy, we are allowed two months rather than one month to gather the necessary data. This is a reasonable requirement and one we have been able to consistently meet regardless of when the first test is initiated or whether any national holidays occur during the same period.

The only way to comply with the accelerated sampling schedule set forth in the state's proposed policy would be to schedule weekly tests and pre-ship effluent samples in the event they "might" be needed if the initial test fails. Obviously, the follow-on tests could be cancelled if the initial test passes, but the expense of shipping contingency samples and the cancellation penalties imposed by the laboratory would impose unreasonable and unnecessary costs on dischargers. These contingency costs were not considered in the economic analysis commissioned by the SWRCB.

- 7) Like the previous analysis conducted by SAIC, the economic impact analysis prepared by ABT Associates contains numerous errors and severely underestimates the true cost of compliance with the SWRCB's proposed policy. ABT examined all of the WET test data reported on San Bernardino's DMR's between June of 2006 and June of 2008 to estimate the incremental costs likely to occur in the draft policy is approved. However, ABT made this comparison using the incorrect permit. Their analysis was based on NPDES No. CA0105392 not NPDES No. CA800034. The former is a rarely used permit that allows the City to (temporarily) discharge disinfected secondary effluent without Title-22 filtration only when there is 20-to-1 dilution available in the receiving water. The latter is the permit which governs the City's day-to-day discharges from the tertiary filtration facility known as "RIX." The City has only been able to effect discharges under the temporary permit about once in every 10 years when higher rainfall associated with an El Nino winter swell flows in the receiving water. And, even then, the City is rarely able to discharge for more than a week or two before river flows no longer provide the required 20-to-1 dilution. The terms and conditions of Permit No. CA0105392 bear little

relationship to those found in Permit No. CA800034. Thus, ABT's analysis and conclusions with respect to the costs likely to be incurred at San Bernardino are wholly invalid.

In addition, ABT's analysis was done by comparing the NOEC failure rate against the TST failure rate. However, San Bernardino uses IC25, not the NOEC, to calculate the Toxicity Units (TUc) value used to trigger accelerated testing or TIE requirements.<sup>10</sup> This distinction makes a great deal of difference because the final effluent has only failed the IC25 endpoint twice in the last five years, but would have failed five times during the same time period using the TST. The elevated failure rate, likely due to the increased incidence of false toxicity indications, greatly increases the City's total monitoring costs and exposure to enforcement actions and penalties.

ABT also assumed there would be considerable saving associated with running only two test concentrations rather than the six that are currently required. However, as previously noted, federal regulations require that at least five effluent dilutions be run even if the TST uses only data from the control group and the undiluted effluent to estimate compliance. Consequently, ABT was mistaken to assume that a more simplified test design might save the City money. They should have consulted with us prior to drawing such a conclusion from our historic data. We would have also provided them more accurate data on the true cost of WET testing. ABT's estimate is less than half of our actual expense for sample shipping and laboratory testing.

ABT's fundamental lack of understanding for how WET testing works in the real world, and other errors on the detailed requirements in our relevant existing permit, calls into question the validity of their conclusions. If ABT made the same mistakes calculating the incremental cost of compliance for other dischargers as were made when they evaluated San Bernardino's permit, then the analysis is severely flawed and lacks the credibility necessary to demonstrate that the SWRCB made a good faith effort to consider economics as required by §13241 of the California Water Code. At a minimum, ABT's analysis should be audited and peer-reviewed. Based on our direct review of ABT's false statements regarding San Bernardino's discharge, we believe the study should be discarded and redone.

- 8) Many of the QA/QC procedures established by EPA to assure the accuracy and reliability of WET test results become obsolete and irrelevant if the TST method is mandated by the SWRCB. For example, laboratories routinely prepare control charts reporting the results of their reference toxicant tests based on the NOEC or IC25. Neither EPA or the State have established an equivalent control chart metric for the TST. Nor is it clear whether or how the discharger would demonstrate compliance with the existing

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<sup>10</sup> NPDES No. CA8000304. Monitoring and Reporting Program. See §V-A-5 @ pg. E-10 (R8-2006-0052)

requirement to calculate the PMSD (a mandatory measure of test sensitivity) once the TST is enacted.

- 9) To our knowledge, no field studies demonstrate that chronic WET test results derived using the TST procedure are well-correlated with actual instream conditions. Such studies are essential to prove the TST produces results "comparable" to the existing methods that have already been field-validated. Any claim that the TST is "at least as sensitive" as the NOEC or IC25 is based solely on the observation that the TST indicates the presence of toxicity more often than either of those previously promulgated methods. However, more frequent failure is only an indication of greater sensitivity if the results are accurate. As noted above, the TST procedure finds method blank samples to be "toxic" more than twice as often as the NOEC or IC25. Consequently, no reason exists to conclude the proposed method is better than (or even as good as) the current statistical measures. And, there is no basis to believe that TST results will correlate well with the richness and abundance of aquatic organisms downstream of any given discharge. This is particularly true when EPA has admitted that it lacks any field data on the predictive reliability of WET testing for effluent-dependent ecosystems such as the Santa Ana River.<sup>11</sup>

In addition, the correlation between WET test results and instream conditions in EPA's existing field validation studies is based almost entirely on failures induced by excess mortality. More recently, EPA has acknowledged that WET test failures caused solely by changes in growth or reproduction (not survival) may not accurately predict instream impairment.<sup>12</sup>

"The U.S. EPA studies have been criticized for selecting sites with high instream toxicity and known biological impact. Further, none of these studies demonstrated predictive accuracy."<sup>13</sup>

Independent, peer-reviewed scientific studies clearly show that WET tests results are not correlated with the abundance or diversity of species found in aquatic ecosystems after properly controlling for other influential variables such as available habitat. The best such study was performed by the very same expert that developed the TST method for EPA - Dr. Jerry Diamond:

"There is nearly a 50% probability that toxicity exhibited in WET tests may not be reflected instream, even for those effluents exhibiting a relatively

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<sup>11</sup> Letter from Gregory R. Grinder, U.S. EPA Office of Research and Development to Mark T. Pifher, counsel for the Western Coalition of Arid States dated Sept. 11, 1996 in response to FOIA request].

<sup>12</sup> U.S. EPA. A Review of Single Species Toxicity Tests: Are the Tests Reliable Predictors of Aquatic Ecosystem Responses? EPA/600/R-97/114. July, 1999 @ pg. 24

<sup>13</sup> Chapman, P.M. 2000. Whole effluent toxicity testing-usefulness, level of protection, and risk assessment. *Environ. Toxicol. Chem.* 19:3-13



high failure rate (>90%) ... A surprising result of this study was the lack of relationship between Ceriodaphnia acute or chronic WET endpoints and instream biological results."<sup>14</sup>

Therefore, unless the TST procedure can show nearly perfect consistency with the results reported using the NOEC or IC25, the method must be independently validated (in accordance with 40 CFR Part 136.5) before being used as a primary indicator of potential instream impairment. According to EPA's own Administrative Law Judge:

"... the proposed [toxicity] tests must be reasonably related to determining whether the discharge could lead to 'real world' effects. The Clean Water Act objective to prohibit the discharge of 'toxic pollutants in toxic amounts' concerns toxicity in the receiving waters of the United States, not the laboratory tank"<sup>15</sup>

And, this obligation to more fully validate the TST procedure is entirely consistent with EPA's own guidance on the matter:

"A fully validated and standardized method is a method that has been ruggedized by a systematic process and is applicable for its intended use. Ideally, only those methods that have been fully validated and standardized should be used for Agency [EPA] needs. However, due to resource and time constraints, it is not always possible to fully validate and standardization required for a given method depends to some extent on the intended use of the data. For example, methods which will be used extensively for regulatory purposes or where significant decision must be based on the quality of the analytical data normally require more extensive validation and standardization than methods developed to collect preliminary baseline data... Where possible, and in all cases for methods that will have extensive regulatory use, a method should be fully validated and standardized. This increased level of validation verifies that the method is suitable for its intended purpose."<sup>16</sup>  
(emphasis added).

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<sup>14</sup> Diamond, J. and C. Daley. 2000. What is the relationship between whole effluent toxicity and instream biological condition? *Environ. Toxicol. Chem.* 19:158-168 (emphasis added).

<sup>15</sup> Andrew S. Pearlstein. In the Matter of Metropolitan Dade County (Fla.), Miami-Dade Water and Sewer Authority (NPDES Permit No. FL0224805), 1996 EPA ALJ Lexis 80 (Oct. 3, 1996). Also cited in Water Environment and Technology, May 1997, pg. 104) (emphasis added).

<sup>16</sup> Availability, Adequacy, and Comparability of Testing Procedures for the Analysis of Pollutants Established Under Section 304(h) of the Federal Water Pollution Control Act - Report to Congress; EPA/600/9-87/030; September, 1988; p. 3-5 & 3-6

The TST procedure proposed in the SWRCB's draft Policy for Toxicity Assessment and Control has been subjected to only a small fraction of the validation efforts that EPA undertook for the NOEC and IC25. Until such time as EPA promulgates the TST as part of an approved Part 136 method, the SWRCB must provide the validation documentation normally prepared by EPA, or wait until EPA completes this validation. This includes appropriate interlaboratory studies, analysis of method blanks, and confirmation of a correlation to instream conditions. To date, none of this supplemental information has been compiled or submitted to formal Peer Review as required by both state and federal law. As such, the State Board lacks the authority to require use of the TST procedure in lieu of the promulgated methods (NOEC or IC25) for the purpose of imposing, and assessing compliance with, effluent limitations in an NPDES permit.

For the reasons set forth above, the City of San Bernardino encourages the SWRCB to reject the proposed policy as presently drafted.

Respectfully submitted,



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City of San Bernardino  
Municipal Water Department

cc: David Aladjem and Melissa Thorme, Downey Brand LLP  
Tim Moore, Risk Sciences  
Roberta Larson, CASA

**Appendix A:**

**Side-by-Side Comparison of TST vs. NOEC vs. IC25 Methods  
Using Historic WET Test Results for Discharges Made Pursuant to  
NPDES Permit No. CA CA8000304**



**Appendix B:**

**Side-by-Side Comparison of TST vs. NOEC vs. IC25 Methods  
Using Historic WET Test Results for Discharges Made Pursuant by  
Yucaipa Valley Water District  
And  
Inland Empire Utilities Agency**



Facility / Location	Test ID#	Date	Species	Endpoint	Control Mean	Control STD	100% Mean	100% STD	NOEC	% Difference at NOEC	NOEC	TST Result	b	$Y_1 - b \cdot Y_c$	$S_c^2$	$S_c$	$n_c$	$S_c^2/n_c$	$b^2 S_c^2/n_c$	SqRT of $[(S_c^2/n_c) + (b^2 S_c^2/n_c)]$	Calc. t	$[(S_c^2/n_c) + (b^2 S_c^2/n_c)]^2$	$(b^2 S_c^2/n_c)^2$	$n_c - 1$	Degrees of Freedom	Crit. t	
IEUA 001	001Jan10	1/3/2010	C. dubia	Reproduction	25.1	2.726	20.2	3.19	80	19.52	Toxic	Non-Toxic	0.75	1.38	10.18	7.43	10	1.02	0.42	1.20	1.14756	2.06	1.04	0.17	9	15	0.8602
IEUA 001	2nd001Jan10	01/17/10	C. dubia	Reproduction	20.1	3.957	14.9	5.834	100	25.97	Non-Toxic	Toxic	0.75	-0.18	35.21	5.66	10	3.52	0.86	2.10	-0.0634	19.38	12.40	0.78	9	13	0.8702
IEUA 001	001Feb10	2/6/2010	C. dubia	Reproduction	17.1	7.31	18.6	3.836	100	-6.77	Non-Toxic	Non-Toxic	0.75	5.78	14.71	53.44	10	1.47	3.01	2.12	2.72928	20.05	2.17	8.03	9	16	0.8647
IEUA 001	001Mar10	3/7/2010	C. dubia	Reproduction	24.9	3.348	19.4	4.526	90	22.09	Toxic	Toxic	0.75	0.73	20.48	11.21	10	2.05	0.63	1.94	0.44295	7.18	4.20	0.40	9	14	0.8681
IEUA 001	2nd001Mar10	3/21/2010	C. dubia	Reproduction	25.9	3.843	22.7	3.622	90	12.36	Toxic	Non-Toxic	0.75	3.28	13.12	14.77	10	1.31	0.83	1.46	2.23737	4.59	1.72	0.69	9	17	0.8653
IEUA 001	001Apr10	4/4/2010	C. dubia	Reproduction	25.9	9.374	30.7	6.183	100	-18.53	Non-Toxic	Non-Toxic	0.75	11.28	38.23	87.87	10	3.82	4.94	2.96	3.80622	76.84	14.61	24.43	9	18	0.862
IEUA 001	2nd001Apr10	4/18/2010	C. dubia	Reproduction	20.6	6.077	17.1	6.244	100	16.99	Non-Toxic	Non-Toxic	0.75	1.65	38.99	36.93	10	3.90	2.06	2.44	0.97496	35.71	15.20	4.32	9	16	0.8647
IEUA 001	001May10	5/27/2010	C. dubia	Reproduction	22.1	3.315	28.1	1.912	100	-27.15	Non-Toxic	Non-Toxic	0.75	11.53	3.66	10.99	10	0.37	0.62	0.99	11.62	0.97	0.13	0.38	9	17	0.8653
IEUA 001	001Jun10	5/30/2010	C. dubia	Reproduction	23.2	6.179	25.6	3.883	100	-10.34	Non-Toxic	Non-Toxic	0.75	8.20	15.16	38.18	10	1.52	2.15	1.91	4.28435	13.42	2.30	4.61	9	17	0.8653
IEUA 002	002Jan10	1/6/2010	C. dubia	Reproduction	19	2.789	18.6	1.578	100	2.11	Non-Toxic	Non-Toxic	0.75	4.35	2.49	7.78	10	0.25	0.44	0.33	5.24993	0.47	0.06	0.19	9	17	0.8653
IEUA 002	002Feb10	2/14/2010	C. dubia	Reproduction	24.5	4.301	25.9	3.725	100	-5.71	Non-Toxic	Non-Toxic	0.75	7.53	13.98	18.50	10	1.39	1.04	1.56	4.82917	5.90	1.83	1.08	9	16	0.862
IEUA 002	002Mar10	2/28/2010	C. dubia	Reproduction	22	1.784	23.6	2.068	100	-7.27	Non-Toxic	Non-Toxic	0.75	7.10	4.27	3.11	10	0.43	0.18	0.78	9.15162	0.36	0.18	0.03	9	15	0.8662
IEUA 002	002Apr10	4/11/2010	C. dubia	Reproduction	28.2	3.155	19	6.11	80	32.62	Toxic	Toxic	0.75	-2.15	37.33	9.95	10	3.73	0.56	2.07	-1.0377	18.43	13.94	0.31	9	12	0.8728
IEUA 002	002May10	5/6/2010	C. dubia	Reproduction	23.9	6.42	28.5	1.581	100	-19.25	Non-Toxic	Non-Toxic	0.75	10.58	2.50	41.22	10	0.25	2.32	1.90	6.59859	6.80	0.06	5.38	9	11	0.8791
IEUA 002	2nd002May10	5/23/2010	C. dubia	Reproduction	25.8	7.989	23.3	9.228	100	9.69	Non-Toxic	Non-Toxic	0.75	3.95	85.12	63.50	10	8.51	3.57	3.48	1.13629	146.02	72.45	12.76	9	15	0.8662
IEUA 002	002Jun10	6/8/2010	C. dubia	Reproduction	26.3	2.584	26	3.127	100	1.14	Non-Toxic	Non-Toxic	0.75	6.28	9.78	6.68	10	0.98	0.38	1.16	5.39388	1.83	0.86	0.14	9	15	0.8662
IEUA RP5	RPSJan10	1/24/2010	C. dubia	Reproduction	16.1	4.508	15.6	4.169	100	3.11	Non-Toxic	Non-Toxic	0.75	3.53	17.38	20.32	10	1.74	1.14	1.70	2.0767	8.30	3.02	1.31	9	17	0.8653
IEUA RP5	RPSFeb10	2/21/2010	C. dubia	Reproduction	25.3	3.883	24	2.582	100	5.14	Non-Toxic	Non-Toxic	0.75	5.03	6.67	13.66	10	0.67	0.76	1.20	4.20259	2.04	0.44	0.58	9	18	0.862
IEUA RP5	RPSMar10	3/14/2010	C. dubia	Reproduction	16.4	7.648	23.2	4.662	100	-41.46	Non-Toxic	Non-Toxic	0.75	10.90	21.73	58.49	10	2.17	3.29	2.34	4.86323	28.85	4.72	10.83	9	17	0.8653
IEUA RP5	RPSApr10	4/25/1940	C. dubia	Reproduction	24.8	3.259	26.5	4.503	100	-6.85	Non-Toxic	Non-Toxic	0.75	7.90	20.28	10.62	10	2.03	0.60	1.92	4.87566	6.89	4.11	0.36	9	14	0.8681
IEUA RP5	RPSMay10	5/19/2010	C. dubia	Reproduction	22	5.578	26.4	2.459	100	-20	Non-Toxic	Non-Toxic	0.75	9.80	6.05	31.11	10	0.60	1.75	1.53	6.45141	5.55	0.37	3.06	9	15	0.8662
IEUA RP5	RPSJun10	6/13/2010	C. dubia	Reproduction	26.5	2.415	27.5	2.593	100	-3.77	Non-Toxic	Non-Toxic	0.75	7.83	6.72	6.83	10	0.97	0.33	1.90	7.82337	1.00	0.45	0.11	9	16	0.8647
IEUA CC	CCJan10	1/3/2010	C. dubia	Reproduction	19.9	5.763	16.2	6.529	100	18.59	Non-Toxic	Non-Toxic	0.75	1.28	42.83	33.21	10	4.26	1.87	2.48	0.51463	37.59	18.17	3.49	9	16	0.8647
IEUA CC	CCFeb10	2/6/2010	C. dubia	Reproduction	21.1	4.149	17.7	6.667	100	16.11	Non-Toxic	Non-Toxic	0.75	1.88	44.45	17.21	10	4.44	0.97	2.33	0.80569	29.30	19.76	0.94	9	13	0.8702
IEUA CC	CCMar10	3/7/2010	C. dubia	Reproduction	20.9	6.045	10.8	7.885	80	48.33	Toxic	Toxic	0.75	-4.08	59.06	36.54	10	5.91	2.06	2.92	-1.7277	63.38	34.88	4.23	9	15	0.8662
IEUA CC	2ndCCMar10	3/21/2010	C. dubia	Reproduction	17.3	6.001	23.7	1.703	100	-36.99	Non-Toxic	Non-Toxic	0.75	10.73	2.80	36.01	10	0.28	2.03	1.52	7.04765	5.36	0.08	4.10	9	12	0.8728
IEUA CC	CCApr10	4/4/2010	C. dubia	Reproduction	14.4	7.891	27.9	6.367	100	-93.75	Non-Toxic	Non-Toxic	0.75	17.10	40.54	69.15	10	4.05	3.33	2.72	6.29411	54.48	16.43	11.07	9	18	0.862
IEUA CC	2ndCCApr10	4/18/2010	C. dubia	Reproduction	27.4	5.502	27.8	1.989	100	-1.46	Non-Toxic	Non-Toxic	0.75	7.25	3.86	30.27	10	0.40	1.70	1.45	5.00487	4.40	0.16	2.90	9	13	0.8702
IEUA CC	CCMay10	5/27/2010	C. dubia	Reproduction	23.2	3.994	28.3	2.163	100	-21.98	Non-Toxic	Non-Toxic	0.75	10.90	4.68	15.95	10	0.47	0.90	1.17	9.329	1.86	0.22	0.81	9	16	0.8647
IEUA CC	CCJun10	5/30/2010	C. dubia	Reproduction	17.1	4.228	22.4	2.119	100	-30.99	Non-Toxic	Non-Toxic	0.75	9.58	4.49	17.88	10	0.46	1.01	1.21	7.93918	2.12	0.20	1.01	9	16	0.8647

## **Appendix C:**

**Side-by-Side Comparison of TST vs. NOEC vs. IC25 Methods  
Using Historic WET Test Results for Non-Toxic Method Blanks  
from EPA's Interlaboratory WET Variability Study**



Table 1. Summaries of *Ceriodaphnia dubia* reproduction “blank” data from the USEPA Inter-Laboratory Validation Study. Samples that were determined invalidate by USEPA were not included. Fourth or higher broods were excluded in counting.

Row #	Sample ID	Analysis Using the Proposed New TST Method					Current 40 CFR 136 Method	
		Mean Control Response	Mean Sample Response	% Effect	TST Results	Discharger has Reasonable Potential (RP) according to Draft Policy for Toxicity Assessment and Control	NOEC	IC 25
1	9330	25.4	25.0	1.5	Non-Toxic	No	100	>100
2	9332	16.6	16.3	1.8	Non-Toxic	No	100	>100
3	9337	20.1	19.4	3.5	Non-Toxic	No	100	>100
4	9338	24.2	21.3	12.0	Non-Toxic	Yes	100	>100
5	9340	15.3	19.8	-29.4	Non-Toxic	No	100	>100
6	9341	23.5	21.3	9.4	Non-Toxic	No	100	>100
7	9344	11.1	17.0	-53.2	Non-Toxic	No	100	>100
8	9349	30.8	30.3	1.6	Non-Toxic	No	100	>100
9	9350	29.5	22.9	22.4	Toxic	Yes	100	>100
10	9356	24.1	22.4	7.1	Non-Toxic	No	100	>100
11	9367	22.2	16.7	24.8	Non-Toxic	Yes	100	>100
12	9371	19.9	21.3	-7.0	Non-Toxic	No	100	>100
13	9376	20.4	17.8	12.7	Non-Toxic	Yes	100	>100
14	9379	24.9	26.8	-7.6	Non-Toxic	No	100	>100
15	9381	26.5	25.6	3.4	Non-Toxic	No	100	>100
16	9382	26.1	25.7	1.5	Non-Toxic	No	100	>100
17	9384	15.5	18.7	-20.6	Non-Toxic	No	100	>100
18	9402	16.0	16.2	-1.3	Non-Toxic	No	100	>100
19	9409	22.2	26.3	-18.6	Non-Toxic	No	100	>100
20	9410	24.8	22.8	8.1	Non-Toxic	No	100	>100
21	9429	31.0	31.1	-0.3	Non-Toxic	No	100	>100
22	9432	17.0	18.2	-7.1	Non-Toxic	No	100	>100
23	9436	28.1	31.8	-13.2	Non-Toxic	No	100	>100
24	9439	18.9	12.1	36.0	Toxic	Yes	100	>100
25	9445	23.6	22.4	5.1	Non-Toxic	No	100	>100
26	9446	22.2	18.3	17.6	Toxic	Yes	100	>100
27	9450	19.4	4.1	78.9	Toxic	Yes	25	15.9
Summary Statistics	N	27	27	27			27	27
	Min	11.10	4.10	-53.15			25	15.9
	Max	31.00	31.80	78.87			100	>100
	Median	22.20	21.30	1.81			100	>100
	Mean	22.20	21.17	3.29				>100
	# of Blank Samples Incorrectly Declared Toxic				4	7	1	1
	Error Rate for Non-Toxic Blank Samples				14.8	25.9	3.7	3.7

\*Samples 9332, 9350, 9367, and 9450 were previously determined as toxic using data that contain either 4<sup>th</sup> or higher broods.

Table 2. Summaries of *Ceriodaphnia dubia* survival “blank” data from the USEPA Inter-Laboratory Validation Study. Samples that were determined invalidate by USEPA were not included.

Row #	Sample ID	Analysis Using the Proposed New TST Method					Current 40 CFR 136 Method		
		Mean Control Response	Mean Sample Response	% Effect	TST Results	Discharger has Reasonable Potential (RP) according to Draft Policy for Toxicity Assessment and Control	NOEC	LC50	
1	9330	1.0	1.0	0.0	Non-Toxic	No	100	>100	
2	9332	0.8	1.0	-25.0	Non-Toxic	No	100	>100	
3	9337	1.0	1.0	0.0	Non-Toxic	No	100	>100	
4	9338	1.0	1.0	0.0	Non-Toxic	No	100	>100	
5	9340	0.8	0.9	-12.5	Non-Toxic	No	100	>100	
6	9341	1.0	1.0	0.0	Non-Toxic	No	100	>100	
7	9344	1.0	0.9	10.0	Non-Toxic	No	100	>100	
8	9349	1.0	1.0	0.0	Non-Toxic	No	100	>100	
9	9350*	1.0	1.0	0.0	Non-Toxic	No	100	>100	
10	9356	0.9	1.0	-11.1	Non-Toxic	No	100	>100	
11	9367	1.0	0.8	20.0	Toxic	Yes	100	>100	
12	9371	1.0	1.0	0.0	Non-Toxic	No	100	>100	
13	9376	1.0	1.0	0.0	Non-Toxic	No	100	>100	
14	9379	1.0	1.0	0.0	Non-Toxic	No	100	>100	
15	9381	1.0	1.0	0.0	Non-Toxic	No	100	>100	
16	9382	1.0	1.0	0.0	Non-Toxic	No	100	>100	
17	9384	0.9	0.8	11.1	Toxic	Yes	100	>100	
18	9402	1.0	1.0	0.0	Non-Toxic	No	100	>100	
19	9409	0.9	1.0	-11.1	Non-Toxic	No	100	>100	
20	9410	1.0	1.0	0.0	Non-Toxic	No	100	>100	
21	9429	1.0	1.0	0.0	Non-Toxic	No	100	>100	
22	9432	0.9	0.9	0.0	Non-Toxic	No	100	>100	
23	9436	1.0	1.0	0.0	Non-Toxic	No	100	>100	
24	9439*	1.0	0.9	10.0	Non-Toxic	No	100	>100	
25	9445	1.0	1.0	0.0	Non-Toxic	No	100	>100	
26	9446*	1.0	1.0	0.0	Non-Toxic	No	100	>100	
27	9450*	0.9	1.0	-11.1	Non-Toxic	No	100	>100	
Summary Statistics	N	27	27	27			27	27	
	Min	0.80	0.80	-25.00			100	>100	
	Max	1.00	1.00	20.00			100	>100	
	Median	1.00	1.00	0.00			100	>100	
	Mean	0.97	0.97	-0.73			100	>100	
	# of Blank Samples Incorrectly Declared Toxic					2	2	0	0
	Error Rate for Non-Toxic Blank Samples					7.4	7.4	0.0	0.0

Note: Mean response is a survival rate (e.g., 1 = 100% survival).  
 Samples 9350, 9439, 9446, and 9450 were determined toxic for “reproduction endpoint.”

Table 3. Summary of USEPA Inter-laboratory Variability Study for Fathead Minnow Larval Growth Chronic 7-day Test.

Row #	Sample ID	Analysis Using the Proposed New TST Method					Current 40 CFR 136 Method		
		Mean Control Response	Mean Sample Response	% Effect	TST Results	Discharger has Reasonable Potential (RP) according to Draft Policy for Toxicity Assessment and Control	NOEC	IC25	
1	9113	0.38	0.42	-11	Non-Toxic	No	100	>100	
2	9114	0.36	0.46	-27	Non-Toxic	No	100	>100	
3	9117	0.37	0.45	-22	Non-Toxic	No	100	>100	
4	9119	0.51	0.46	10	Non-Toxic	No	100	>100	
5	9123	0.79	0.83	-6	Non-Toxic	No	100	>100	
6	9131	0.38	0.42	-9	Non-Toxic	No	100	>100	
7	9135	0.47	0.53	-12	Non-Toxic	No	100	>100	
8	9136	0.51	0.56	-10	Non-Toxic	No	100	>100	
9	9138	0.38	0.22	43	Toxic	Yes	100	>100	
10	9142	0.36	0.39	-10	Non-Toxic	No	100	>100	
11	9143	0.38	0.35	7	Non-Toxic	No	100	>100	
12	9145	0.29	0.34	-16	Non-Toxic	No	100	>100	
13	9151	0.81	0.77	5	Non-Toxic	No	100	>100	
14	9152	0.38	0.39	-5	Non-Toxic	No	100	>100	
15	9158	0.39	0.29	27	Toxic	Yes	50	94	
16	9160	0.38	0.47	-23	Non-Toxic	No	100	>100	
17	9182	0.66	0.66	0	Non-Toxic	No	100	>100	
18	9186	0.37	0.42	-13	Non-Toxic	No	100	>100	
19	9188	0.77	0.72	7	Non-Toxic	No	100	>100	
20	9192	0.50	0.46	8	Non-Toxic	No	100	>100	
21	9196	0.60	0.64	-6	Non-Toxic	No	100	>100	
22	9197	0.69	0.64	8	Non-Toxic	No	100	>100	
23	9198	0.26	0.29	-9	Non-Toxic	No	100	>100	
24	9209	0.64	1.33	-107	Non-Toxic	No	Inconclusive <sup>1</sup>	>100	
Summary Statistics	N	24	24	24		24	23	24	
	Min	0.26	0.22	-107			50	93.6	
	Max	0.81	1.33	43			100	>100	
	Median	0.39	0.46	-8			100	>100	
	Mean	0.49	0.52	-7				99.7	
	# of Blank Samples Incorrectly Declared Toxic					2	2	1	1
	Error Rate for Non-Toxic Blank Samples					8.3	8.3	4.3	4.2

<sup>1</sup> Results were excluded from summary statistics.

Table 4. USEPA blank data from USEPA Inter-laboratory Variability Study (Fathead Minnow Larval Survival Chronic 7-day Test).

Row #	Sample ID	Analysis Using the Proposed New TST Method					Current 40 CFR 136 Method		
		Mean Control Response	Mean Sample Response	% Effect	TST Results	Discharger has Reasonable Potential (RP) according to Draft Policy for Toxicity Assessment and Control	NOEC	IC25	
1	9113	1	0.95	5.0	Non-Toxic	No	100	>100	
2	9114	0.95	0.95	0.0	Non-Toxic	No	100	>100	
3	9117	0.975	0.975	0.0	Non-Toxic	No	100	>100	
4	9119	1	0.975	2.5	Non-Toxic	No	100	>100	
5	9123	1	1	0.0	Non-Toxic	No	100	>100	
6	9131	1	1	0.0	Non-Toxic	No	100	>100	
7	9135	0.9	0.95	-5.6	Non-Toxic	No	100	>100	
8	9136	0.925	1	-8.1	Non-Toxic	No	100	>100	
9	9138	0.966667	0.9	6.9	Non-Toxic	No	100	>100	
10	9142	0.975	0.975	0.0	Non-Toxic	No	100	>100	
11	9143	0.975	0.925	5.1	Non-Toxic	No	100	>100	
12	9145	0.95	0.95	0.0	Non-Toxic	No	100	>100	
13	9151	1	1	0.0	Non-Toxic	No	100	>100	
14	9152	1	0.925	7.5	Non-Toxic	No	100	>100	
15	9158	0.95	0.825	13.2	Non-Toxic	Yes	100	>100	
16	9160	0.925	0.9	2.7	Non-Toxic	No	100	>100	
17	9182	0.975	0.975	0.0	Non-Toxic	No	100	>100	
18	9186	0.95	0.975	-2.6	Non-Toxic	No	100	>100	
19	9188	1	1	0.0	Non-Toxic	No	100	>100	
20	9192	1	1	0.0	Non-Toxic	No	100	>100	
21	9196	0.975	1	-2.6	Non-Toxic	No	100	>100	
22	9197	0.95	0.975	-2.6	Non-Toxic	No	100	>100	
23	9198	0.9	1	-11.1	Non-Toxic	No	100	>100	
24	9209	1	1	0.0	Non-Toxic	No	100	>100	
Summary Statistics	N	24	24	24	24	24	24	24	
	Min	0.90	0.83	-11			100	>100	
	Max	1.00	1.00	13			100	>100	
	Median	0.98	0.98	0			100	>100	
	Mean	0.97	0.96	0			100	>100	
	# of Blank Samples Incorrectly Declared Toxic					0	1	0	0
	Error Rate for Non-Toxic Blank Samples					0.0	4.2	0.0	0.0