



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

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

Dear Chairman Hoppin and Members:

1 At a workshop held in November of 2010, the State Board agreed to sponsor a "test drive" of EPA's new TST methodology. Several speakers that morning emphasized the importance of ensuring that the study design include a review of TST performance on method blanks as this is the only way to accurately gauge the true error rate for non-toxic samples. Unfortunately, for reasons that have never been clearly defined, the final "test drive" failed to evaluate any method blanks whatsoever. So, we did it for them and the results are presented in this comment letter.

2 Like EPA, we relied on Monte Carlo simulation techniques to determine the performance traits of the TST methodology. We began by constructing a synthetic populations of 20,000 *Ceriodaphnia dubia* with an average reproduction of 26 offspring per female and a defined standard deviation of 7.8 (interreplicate coefficient-of-variation = 30%). This corresponds to the average interreplicate CV reported for control organisms during EPA Interlaboratory WET Variability Study.

A random sample of 10 organisms was collected from the synthetic population and assigned to Group 1 (the simulated control group). If the mean reproduction for Group 1 was less than 15 offspring per female, the sample was discarded and another sample was collected from the synthetic population. This was done to ensure the sample met EPA's minimum Test Acceptance Criteria. Then, another random sample of 10 organisms was collected from the same synthetic population and assigned to Group 2 (the simulated effluent group). The resampling process was repeated one thousand times to simulate the results of 1,000 chronic WET tests conducted on known non-toxic samples.

Although Group 1 is called the "Control Group" and Group 2 is called the "Effluent Group," there is actually no difference between the two groups. Both came from the same general synthetic population and both are intended to represent the normal range of reproduction for *Ceriodaphnia dubia* exposed to non-toxic water.

The average reproduction in Group 2 ("effluent") was compared to the average reproduction in Group 1 ("controls") using the existing promulgated methods (NOEC and IC25) and EPA's newly proposed TST procedure. Results are summarized in Appendix A to this comment letter.

In this simulation of 1,000 non-toxic trials, the NOEC passed 961 of 1000 tests. The IC25 passed 982 of 1000 tests. And, the TST passed 897 of 1000 tests. Thus, the NOEC falsely indicated the presence of toxicity in a non-toxic sample 3.9% of the time. This is very close to the 3.7% estimate reported by EPA during the Interlaboratory WET Variability Study. The IC-25 provided a false indication of toxicity 1.8% of the time. And, the TST incorrectly reported non-toxic samples were toxic in 10.3% of the trials (three times higher than the NOEC and more than five times higher than the IC25). It is also important to note that in 84% of the 113 TST failures the average level of reproduction in Group 2 ("effluent") was actually higher than the Regulatory Management Decision (RMD) threshold ($0.75 \times \text{mean of Group 1 controls}$). The tests "failed" because the null hypothesis, which presumes worse reproductive performance than actually occurred, could not be rejected.

The error rate varies with the inter-replicate coefficient of variation. So, we repeated the same simulation of 1,000 trials three more times with the same mean (26) but three different standard deviations (5.2, 6.5 and 9.1). These three additional simulations represent conditions where the coefficient of variation ranges between 20%, 25% and 35% respectively. Results are provided in Appendices B, C and D attached to this comment letter and are summarized in the table below.

Table 1: % of Trials Where Method Incorrectly Indicated Toxicity in a Non-Toxic Sample

Method	CV = 20%	CV = 25%	CV = 30%	CV = 35%
NOEC	4.3%	3.8%	3.9%	4.5%
IC25	0.0%	0.2%	1.8%	4.4%
TST	0.9%	4.7%	10.3%	16.8%

Re-running the simulations with the exact same statistical parameters produced nearly identical results ($\pm 0.5\%$) as is expected for a Monte Carlo study of 1,000 trials.

It is evident from these simulations that the error rate for the TST only comparable to or better than the NOEC when the inter-replicate CV is less than 25%. The TST is less accurate at correctly identifying non-toxic samples when the CV increases above 25%. And, the TST is substantially less accurate than the IC25 and should not be considered "comparable" to this method promulgated under 40 CFR Part 136 when evaluating data from non-toxic samples.

Results from this Monte Carlo simulation clearly indicate why it was essential to include a dedicated analysis of non-toxic method blanks in the study design for the so-called "test drive." The omission of such an analysis severely compromises the study's conclusions and calls into question the validity of the subsequent peer review process.

Results from this Monte Carlo simulation can be used in lieu of re-doing the test drive study. Or, the State Board can ask its own experts to prepare an independent Monte Carlo analysis. But, in either case, the State Board should not act to approve to require use of the TST in California until there is a robust demonstration of how accurate the method is when evaluating data from non-toxic method blanks.

Copies of the spreadsheet used to perform the Monte Carlo simulation study are attached to this comment letter and delivered electronically to the Clerk of the Board via email transmission. I am available to answer any questions at your convenience.

Respectfully submitted,

A handwritten signature in blue ink, appearing to read "T. Moore", with a long horizontal flourish extending to the right.

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Encl.: four appendices and one electronic spreadsheet

Appendix A

Monte Carlo Simulation of Synthetic Non-Toxic Method Blank Data
(where inter-replicate coefficient of variation = 30%)

Monte Carlo Simulation for *Ceriodaphnia dubia* Reproduction:

Comparing Performance of WET Test Methods Using Non-Toxic Control Data

Parameter	Assigned Value
Number of Replicates in Simulated General Population	20,000 female <i>C. dubia</i>
True Mean Reproduction of Simulated General Population	26.0 offspring per female
True Standard Deviation of Simulated General Population	7.8
Number of Replicates in Random Group 1 (simulated control)*	10 female <i>C. dubia</i>
Number of Replicates in Random Group 2 (simulated effluent)	10 female <i>C. dubia</i>
Number of Monte Carlo Resamples	1,000 simulated tests

*The ten members of group 1 and the ten members of Group 2 were all selected from the same simulated General population, however individual tests were discarded and repeated if group 1 (the simulated control group) failed to meet EPA's minimum Test Acceptance Criteria of at least 15 offspring per female.

TST vs.	NOEC		
	Pass	Fail	Total
Pass	883	4	887
Fail	78	35	113
Total	961	39	1000

TST vs.	IC-25		
	Pass	Fail	Total
Pass	887	0	887
Fail	95	18	113
Total	982	18	1000

TST vs.	Both NOEC & IC-25		
	Pass	Fail	Total
Pass	883	0	883
Fail	77	17	94
Total	960	17	977

NOEC vs.	IC-25		
	Pass	Fail	Total
Pass	960	1	961
Fail	22	17	39
Total	982	18	1000

Method	Pass #	Pass %	Fail #	Fail %	Total
NOEC	961	96.10%	39	3.90%	1000
EC/IC25	982	98.20%	18	1.80%	1000
TST	897	89.70%	103	10.30%	1000

Appendix B

Monte Carlo Simulation of Synthetic Non-Toxic Method Blank Data
(where inter-replicate coefficient of variation = 20%)

Monte Carlo Simulation for *Ceriodaphnia dubia* Reproduction:

Comparing Performance of WET Test Methods Using Non-Toxic Control Data

Parameter	Assigned Value
Number of Replicates in Simulated General Population	20,000 female <i>C. dubia</i>
True Mean Reproduction of Simulated General Population	26.0 offspring per female
True Standard Deviation of Simulated General Population	5.2
Number of Replicates in Random Group 1 (simulated control)*	10 female <i>C. dubia</i>
Number of Replicates in Random Group 2 (simulated effluent)	10 female <i>C. dubia</i>
Number of Monte Carlo Resamples	1,000 simulated tests

*The ten members of group 1 and the ten members of Group 2 were all selected from the same simulated General population, however individual tests were discarded and repeated if group 1 (the simulated control group) failed to meet EPA's minimum Test Acceptance Criteria of at least 15 offspring per female.

TST vs.	NOEC		
	Pass	Fail	Total
Pass	956	35	991
Fail	1	8	9
Total	957	43	1000

TST vs.	IC-25		
	Pass	Fail	Total
Pass	991	0	991
Fail	9	0	9
Total	1000	0	1000

TST vs.	Both NOEC & IC-25		
	Pass	Fail	Total
Pass	956	0	956
Fail	1	0	1
Total	957	0	957

NOEC vs.	IC-25		
	Pass	Fail	Total
Pass	957	0	957
Fail	43	0	43
Total	1000	0	1000

Method	Pass #	Pass %	Fail #	Fail %	Total
NOEC	957	95.70%	43	4.30%	1000
EC/IC25	1000	100.00%	0	0.00%	1000
TST	991	99.10%	9	0.90%	1000

Appendix C

Monte Carlo Simulation of Synthetic Non-Toxic Method Blank Data
(where inter-replicate coefficient of variation = 25%)

Monte Carlo Simulation for *Ceriodaphnia dubia* Reproduction:

Comparing Performance of WET Test Methods Using Non-Toxic Control Data

Parameter	Assigned Value
Number of Replicates in Simulated General Population	20,000 female <i>C. dubia</i>
True Mean Reproduction of Simulated General Population	26.0 offspring per female
True Standard Deviation of Simulated General Population	6.5
Number of Replicates in Random Group 1 (simulated control)*	10 female <i>C. dubia</i>
Number of Replicates in Random Group 2 (simulated effluent)	10 female <i>C. dubia</i>
Number of Monte Carlo Resamples	1,000 simulated tests

*The ten members of group 1 and the ten members of Group 2 were all selected from the same simulated General population, however individual tests were discarded and repeated if group 1 (the simulated control group) failed to meet EPA's minimum Test Acceptance Criteria of at least 15 offspring per female.

TST vs.	NOEC		
	Pass	Fail	Total
Pass	940	13	953
Fail	22	25	47
Total	962	38	1000

TST vs.	IC-25		
	Pass	Fail	Total
Pass	953	0	953
Fail	45	2	47
Total	998	2	1000

TST vs.	Both NOEC & IC-25		
	Pass	Fail	Total
Pass	940	0	940
Fail	22	2	24
Total	962	2	964

NOEC vs.	IC-25		
	Pass	Fail	Total
Pass	962	0	962
Fail	36	2	38
Total	998	2	1000

Method	Pass #	Pass %	Fail #	Fail %	Total
NOEC	962	96.20%	38	3.80%	1000
EC/IC25	998	99.80%	2	0.20%	1000
TST	953	95.30%	47	4.70%	1000

Appendix D

Monte Carlo Simulation of Synthetic Non-Toxic Method Blank Data
(where inter-replicate coefficient of variation = 35%)

Monte Carlo Simulation for *Ceriodaphnia dubia* Reproduction:

Comparing Performance of WET Test Methods Using Non-Toxic Control Data

Parameter	Assigned Value
Number of Replicates in Simulated General Population	20,000 female <i>C. dubia</i>
True Mean Reproduction of Simulated General Population	26.0 offspring per female
True Standard Deviation of Simulated General Population	9.1
Number of Replicates in Random Group 1 (simulated control)*	10 female <i>C. dubia</i>
Number of Replicates in Random Group 2 (simulated effluent)	10 female <i>C. dubia</i>
Number of Monte Carlo Resamples	1,000 simulated tests

*The ten members of group 1 and the ten members of Group 2 were all selected from the same simulated General population, however individual tests were discarded and repeated if group 1 (the simulated control group) failed to meet EPA's minimum Test Acceptance Criteria of at least 15 offspring per female.

TST vs.	NOEC		
	Pass	Fail	Total
Pass	822	1	823
Fail	133	44	177
Total	955	45	1000

TST vs.	IC-25		
	Pass	Fail	Total
Pass	823	0	823
Fail	133	44	177
Total	956	44	1000

TST vs.	Both NOEC & IC-25		
	Pass	Fail	Total
Pass	822	0	822
Fail	124	35	159
Total	946	35	981

NOEC vs.	IC-25		
	Pass	Fail	Total
Pass	946	9	955
Fail	10	35	45
Total	956	44	1000

Method	Pass #	Pass %	Fail #	Fail %	Total
NOEC	955	95.50%	45	4.50%	1000
EC/IC25	956	95.60%	44	4.40%	1000
TST	832	83.20%	168	16.80%	1000