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:

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.

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 * 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 (±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,

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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 *C. dubia*
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 *C. dubia*
Number of Replicates in Random Group 2 (simulated effluent)	10 female *C. dubia*
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 simiulated General population, however individual tests were discardedand 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 *C. dubia*
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 *C. dubia*
Number of Replicates in Random Group 2 (simulated effluent)	10 female *C. dubia*
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 simiulated General population, however individual tests were discardedand 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 *C. dubia*
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 *C. dubia*
Number of Replicates in Random Group 2 (simulated effluent)	10 female *C. dubia*
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 simiulated General population, however individual tests were discardedand 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 *C. dubia*
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 *C. dubia*
Number of Replicates in Random Group 2 (simulated effluent)	10 female *C. dubia*
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 simiulated General population, however individual tests were discardedand 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

Control Mean	Control STD	100% Mean	100% STD	NOEC	NOECPF	% Diff	EC/IC25	TST Result
27.888	3.9998	22.231	6.0773	Fail	0	20.28	Pass	Fail
28.094	4.4777	21.750	6.2719	Fail	0	22.58	Pass	Fail
30.327	6.4201	23.675	5.4282	Fail	0	21.94	Pass	Fail
27.216	5.387	22.099	5.0715	Fail	0	18.80	Pass	Fail
29.652	4.8299	23.819	5.9595	Fail	0	19.67	Pass	Fail
29.249	3.2084	23.644	6.0528	Fail	0	19.16	Pass	Fail
26.333	4.6578	21.246	4.6908	Fail	0	19.32	Pass	Fail
30.056	1.6151	24.069	5.9507	Fail	0	19.92	Pass	Fail
28.294	5.7781	24.527	3.2552	Fail	0	13.31	Pass	Pass
27.117	5.2295	22.658	4.3321	Fail	0	16.44	Pass	Pass
27.795	6.1309	22.720	5.0525	Fail	0	18.26	Pass	Pass
29.330	5.8497	25.102	4.4487	Fail	0	14.41	Pass	Pass
27.598	5.0346	23.851	4.2788	Fail	0	13.58	Pass	Pass
28.450	4.7658	24.458	4.7783	Fail	0	14.03	Pass	Pass
26.618	6.3076	22.738	3.0172	Fail	0	14.58	Pass	Pass
28.734	3.413	25.018	4.9475	Fail	0	12.93	Pass	Pass
26.447	3.758	21.883	4.3219	Fail	0	17.26	Pass	Pass
26.741	4.0327	22.062	4.9667	Fail	0	17.50	Pass	Pass
27.188	4.4441	23.841	3.1682	Fail	0	12.31	Pass	Pass
28.411	4.301	24.107	3.1103	Fail	0	15.15	Pass	Pass
29.500	3.0787	25.705	5.8815	Fail	0	12.86	Pass	Pass
30.467	4.0869	26.397	4.7463	Fail	0	13.36	Pass	Pass
28.740	4.7071	25.377	3.6451	Fail	0	11.70	Pass	Pass
26.521	6.1241	21.864	4.1748	Fail	0	17.56	Pass	Pass
27.035	3.1701	23.336	3.3288	Fail	0	13.68	Pass	Pass
27.457	5.5199	22.581	4.3969	Fail	0	17.76	Pass	Pass
30.310	3.7478	24.669	5.9456	Fail	0	18.61	Pass	Pass
27.206	4.784	23.161	5.0685	Fail	0	14.87	Pass	Pass
26.011	4.8836	22.909	2.6945	Fail	0	11.92	Pass	Pass
27.130	4.2771	22.791	5.9244	Fail	0	15.99	Pass	Pass
26.769	3.1431	22.979	4.3588	Fail	0	14.16	Pass	Pass
27.255	3.0281	23.528	4.6736	Fail	0	13.68	Pass	Pass
27.361	5.432	22.943	5.3529	Fail	0	16.15	Pass	Pass
30.782	3.1411	27.014	5.0242	Fail	0	12.24	Pass	Pass
28.997	6.3296	23.547	3.4374	Fail	0	18.80	Pass	Pass
28.408	3.9065	24.047	4.8009	Fail	0	15.35	Pass	Pass
27.803	3.9461	24.017	5.0106	Fail	0	13.62	Pass	Pass
29.744	5.0453	25.313	5.3969	Fail	0	14.90	Pass	Pass
28.138	6.174	23.425	5.563	Fail	0	16.75	Pass	Pass
29.489	7.0869	24.441	3.5383	Fail	0	17.12	Pass	Pass
27.080	4.9894	22.356	2.5578	Fail	0	17.45	Pass	Pass
27.836	4.8371	23.548	5.2891	Fail	0	15.40	Pass	Pass
27.112	5.0432	23.674	3.5094	Fail	0	12.68	Pass	Pass
28.085	5.9436	23.433	7.5595	Pass	1	16.56	Pass	Fail
26.237	6.7218	26.018	5.3986	Pass	1	0.83	Pass	Pass
21.158	3.7885	27.789	6.0991	Pass	1	-31.34	Pass	Pass
27.998	5.5132	24.886	5.3032	Pass	1	11.11	Pass	Pass
23.507	5.8688	26.868	4.1029	Pass	1	-14.30	Pass	Pass
24.509	6.7193	24.377	6.2782	Pass	1	0.54	Pass	Pass
27.101	4.2613	27.289	5.1285	Pass	1	-0.69	Pass	Pass
25.945	5.1158	23.784	4.3162	Pass	1	8.33	Pass	Pass
27.812	6.1501	26.140	4.0056	Pass	1	6.01	Pass	Pass
25.284	3.8327	26.230	6.9053	Pass	1	-3.74	Pass	Pass
25.784	4.6387	23.937	3.7361	Pass	1	7.16	Pass	Pass
29.267	3.89	26.886	6.4976	Pass	1	8.14	Pass	Pass
27.373	3.6794	25.461	6.3602	Pass	1	6.99	Pass	Pass
28.997	6.768	26.315	5.7658	Pass	1	9.25	Pass	Pass
26.466	4.4441	25.831	4.1922	Pass	1	2.40	Pass	Pass
25.769	6.8313	25.777	6.8749	Pass	1	-0.03	Pass	Pass
29.384	4.3472	26.415	5.861	Pass	1	10.10	Pass	Pass
24.583	5.9013	23.327	7.9961	Pass	1	5.11	Pass	Pass
25.403	5.2472	25.572	3.7475	Pass	1	-0.66	Pass	Pass
26.161	3.0543	25.024	6.7806	Pass	1	4.35	Pass	Pass
26.228	3.0955	24.987	4.821	Pass	1	4.73	Pass	Pass
24.993	6.6671	26.122	4.7594	Pass	1	-4.52	Pass	Pass
24.487	3.1542	25.516	8.1887	Pass	1	-4.20	Pass	Pass
30.038	4.636	27.025	5.7983	Pass	1	10.03	Pass	Pass
23.918	6.0473	25.665	5.7708	Pass	1	-7.30	Pass	Pass
28.413	4.3276	25.666	4.6629	Pass	1	9.67	Pass	Pass
25.296	6.6497	26.203	3.5029	Pass	1	-3.59	Pass	Pass
27.331	6.3177	24.274	3.2618	Pass	1	11.19	Pass	Pass
24.390	4.5039	22.947	7.2475	Pass	1	5.92	Pass	Pass