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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

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

Dear Chairman Hoppin and Members:

San Bernardino Valley Municipal Water District ("Valley District") manages wholesale water supply for agricultural and municipal use by nearly two million people in the Inland Empire area of Southern California. We do not operate any wastewater treatment facilities and, as such, have not been previously required to perform routine toxicity tests. And, while we support the State Board's goal of reducing toxic discharges to the environment, the proposed Policy for Toxicity Assessment and Control will likely have several unintended consequences that merit serious consideration.

Valley District has a unique perspective because our supply plan depends heavily on harvesting stormwater and recharging recycled water to meet future water demands. For obvious reasons, we have a keen interest in providing clean safe water to our customers. Nevertheless, Valley District is concerned that the State Board's proposed policy will make it more difficult to divert or recharge some surface waters and far more difficult to use recycled water in the future. The reasons for our concern are as follows:

1) The proposed policy does not explain that the Whole Effluent Toxicity ("WET") test methods are intended to assess the potential for toxicity to aquatic organisms, not people. Once a sample has been found to be "toxic," the public may mistakenly perceive this to mean that the particular source water poses a threat to human health. Since the new policy now proposes to conduct regular WET tests on stormwater runoff, receiving waters, recycled water, and unpermitted channelized discharges (e.g. State

Project Water), any negative result will severely undermine public trust and acceptance of these potential raw water sources. And, there is no way that agricultural operators will agree to use recycled water for irrigation where even a single adverse toxicity test might taint the perceived quality of their produce. Therefore, at a minimum, the State Board must make it absolutely clear that WET test results were never designed to, and should never be used to, suggest that there is any potential threat to human health.

2) Significant risk of product liability may make it legally and economically impossible to serve or recharge any water where the state-mandated test procedure indicates that a sample is "toxic." The proposed policy enacts new numeric objectives, instead of merely adopting an official method for translating the narrative objective found in most basin plans into a numeric water quality target. And, it is probable that the results of such new objectives and new testing protocols (the "Test of Significant Toxicity" or "TST") will be used to determine whether or not a given water body should be added to the 303(d) list because it appears to be "toxic." It is likely that at least one-third of all surface waters in the state that undergo routine WET testing will be mistakenly listed as impaired for reasons more closely related to the natural biological variability of the test method than any actual toxicity in the aquatic environment.[1]

Heretofore, toxicity tests have only rarely been as general tools of assessment and investigation in receiving waters. The new policy now makes each test failure a potential violation of the regional water quality control plans. Consequently, agencies like ours could be exposed to considerable legal liability if we elected to serve or recharge any such water until the "source" of toxicity was identified and eliminated. It not uncommon for typical Toxicity Identification Evaluations (TIE) to take many months or even years to resolve. In the meantime, perfectly good water may go unused in contravention of key provisions in the state constitution that prohibit the "waste of water."

3) Naturally elevated mineral concentrations, common to waters in our area, may increase the risk of test failure as even small differences in the balance between major anions and cations can interfere with the normal reproductive cycle of sensitive invertebrate species.[2] EPA's official WET test method manual also warns that ionic imbalance is a well-known source of interference in the standard chronic toxicity test method.[3]

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[1] A more detailed mathematical analysis of how intrinsic error in the WET test method will cause not toxic waters to be listed as "impaired" is provided in the comment letter submitted by the California Association of Sanitation Agencies (CASA). See Comment #5 on pages 7 & 8 of CASA's letter. We agree with their estimate and include it by reference in our own comment letter.

[2] See, for example, Chapman, P.M. "Whole effluent toxicity testing-usefulness, level of protection, and risk assessment." *Environ. Toxicol. Chem.* 19:3-13 (2000).

[3] US. EPA. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms-4th Ed. EPA-821-R-02-013; October, 2002 (see §4.13.1 @ pg. 12). See also U.S. EPA. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the NPDES Program. EPA-833-R-00-003 (June, 2000); pg. D-6 & D-7. See also U.S. EPA. "Clarifications Regarding Flexibility in 40 CFR Part 136 Whole Effluent Toxicity (WET) Test Methods" Memorandum from Tudor T. Davies, Director

Despite this fact, existing federal guidance does not yet provide any instruction on how to distinguish ionic interference from true toxicity when conducting a TIE. And, even well-qualified labs have struggled with this issue for years.[4]

Elevated hardness, alkalinity, conductivity and TDS may increase the risk of false test failures by as much as ten times the normal error rate (e.g. 50% vs. 5%) regardless of whether one uses the NOEC, IC-25 or TST to make the determination (see appendix A)[5]. There is no doubt that if a particular water body fails 10-50% of all toxicity tests that state authorities and the general public will conclude that it is "toxic" when it is merely more mineralized than EPA's recipe for non-toxic control water. That formula is based on the general ionic composition of Lake Superior because EPA's laboratory is located in Duluth, MN. But, the mineral structure of natural water supplies in the arid west don't bear much chemical resemblance to Lake Superior.

So serious is the problem of ionic interference that some samples will also "fail" the WET test when the receiving water is "too clean." Low levels of hardness, alkalinity and conductivity can also inhibit growth and reproduction to a level that may be mistaken for "toxicity."[6] This phenomena may greatly complicate our ability to rely on state-of-the-art treatment technologies (such as reverse osmosis and microfiltration) to produce ultra-clean water from seawater, municipal effluent, seawater or captured stormwater if doing so all but assures the subsequent WET test will fail. Even rain water, collected in sterile containers, has been shown to fail the chronic WET test due to low pH and conductivity (see Appendix C).

How will suppliers demonstrate to the Department of Water Resources or the Department of Public Health that our product water is "safe" if such water can "fail" a toxicity test due to the relative deficiency of some minerals? Differences in reproduction or growth among the standard test organisms must first account for the confounding effects of any possible mineral imbalances before concluding that a particular sample is actually "toxic." The economic analyses performed by SAIC and ABT Associates did not consider the cost of special adjustments and additional testing needed to address the ionic interference problems that are likely to occur when WET testing is applied more widely throughout the state.

Office of Science and Technology to Water Management Division Directors, Regions I-X Environmental Services Division Directors, Regions I-X; April 10, 1996.

[4] Goodfellow, W.L., et al. 2000. "Major ion toxicity in effluents: a review with permitting recommendations." *Environ. Toxicol. Chem.* 19:175-82.

[5] Expert testimony by Timothy F. Moore in an affidavit submitted to U.S. Court of Appeals - D.C. Circuit in Case No. 96-1062. June, 2004.

[6] Lasier, P.J. & P.V. Winger & I.R. Hardin. "Effects of hardness and alkalinity in culture and test waters on reproduction of *Ceriodaphnia dubia*." Environ. Toxicol. Chem. Vol. 25, No. 10; pp. 2781-2786 (Oct., 2006). See, also, Harmon, S.M. & G.T. Chandler (Univ. of So. Carolina) and W.L. Specht (WSRC) "A Comparison of the Daphnids, *Ceriodaphnia dubia* and *Daphnia ambigua*, for their Utilization in Routine Toxicity Testing in the Southeastern United States. WSRC-MS-2003-00099. http://sti.srs.gov/fulltext/ms2003099/ms2003099.html

4) The draft policy proposes use of a new statistical procedure (the "TST") as the primary method for assessing potential toxicity in a water sample. A key element of that procedure is the initial presumption (called a "null hypothesis") that the exposure to the sample will cause unacceptably poor survival, reproduction or growth until a test demonstrates otherwise. The assumption that all water is toxic until shown to be non-toxic ("guilty until proven innocent") is scientifically unfounded and a public relations disaster for the State of California. No doubt our competitors in Nevada, Arizona, Texas and other states will exploit this self-imposed presumption (that the State's waters are toxic) at every opportunity. What business would want to locate in a state where government authorities assume all the water is toxic? Even more troubling is the fact that the new procedure resolved any statistical uncertainty in favor of continuing to conclude the water sample is toxic. This is utterly unacceptable and an insult to all agencies such as ours that are dedicated to providing clean safe water to all of our customers.

5) It will be impossible to meet the statewide goals of a 20% increase in the use of recycled water by 2020 if all the new testing shows widespread evidence of potential toxicity in such water. Even if the ionic interference problems are overcome, all WET test methods are known to produce inaccurate indications of toxicity in at least 5% of all tests. While such an error rate might appear to be low, the actual number of false failures will be quite large given the huge number of tests that must be routinely performed under the proposed policy and the fact that one-in-twenty tests will fail regardless of whether the water sample is actually toxic or not (see Appendix B). The intrinsic error rate creates a real problem when combined with the state's existing statistical procedure for adding or deleting waterbodies from the 303(d) list. At a minimum, the SWRCB should perform a formal peer-reviewed mathematical analysis to assess the impact of a 5% error rate on the 303(d) impaired waterways listing/de-listing procedures. Until then, WET test results should not be used to make such listings.

6) The draft Policy for Toxicity Assessment and Control imposes numerous new obligations that are not required by the federal Clean Water Act (CWA). In particular, the CWA applies only to "waters of the U.S." Flood control channels and percolation ponds are not waters of the U.S. but may be considered "waters of the state." Therefore, any requirement to perform WET testing on these intrastate waters can only be made pursuant to state authority and, in many cases, could be considered an Unfunded Mandate.

7) The TST procedure appears to fail more frequently than the existing EPA-recommended methods: the NOEC or IC25.[7] And, the economic analyses performed by SAIC and ABT

[7] The error rate for the TST method is up to three times higher (15% instead of 5%) when evaluating known non-toxic (method blank) samples. The evidence supporting this claim was described in Comment #8 on pages 10 & 11 of the written comment letter submitted by CASA. Additional documents summarizing a reanalysis of EPA's method blank data was also provided as Appendix C to the written comments submitted by the City of San

Associates failed to consider the cost or consequences of these more frequent failures, including enforcement actions and implementation of any compliance options. Nor do these economic analyses consider the added cost of performing tests on three different species (fish, invertebrate and plant) until the most sensitive single species is identified.

In sum, we believe that it is premature and imprudent to use test methods that were originally intended for assessing Whole Effluent Toxicity on waters other than municipal and industrial effluents. The results may unfairly characterize such waters as "toxic" when, in fact, they are not. The proposed policy's initial presumption that all samples are toxic until proven otherwise will needlessly undermine confidence in the public water supply.

WET testing is a useful and valuable tool for investigating potential toxicity; but, it should be just that, a tool. It was never intended to be the last word on guilt or innocence and, given the known error rate for the test method, should not be applied in such a manner. No other state has adopted EPA's untried and unapproved WET test methods. And, California will be severely disadvantaged if the State Board elects to go first.

Respectfully submitted,

Douglas D. Headrick

Doug Headrick
General Manager
San Bernardino Valley Municipal Water District

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

Encl.: Three documents identified as "Appendix A," "Appendix B" and "Appendix C."

Bernardino Water Department. We agree with analysis given in both of these letters hereby include the same documentation, by reference, in our own comment letter.

Appendix A:

Summary of Interlaboratory Method Blank Study

Analyzing synthetic freshwater samples that were formulated to match the typical ionic chemistry (elevated hardness, alkalinity, conductivity, and TDS) of western waters.

Performed by the Western Coalition of Arid States

Submitted as an evidentiary exhibit to the U.S. Court of Appeals

2004

Also includes a spreadsheet summary reanalyzing all of the original study data using EPA's new TST procedure.

Interlaboratory Evaluation of Chronic WET Test Precision Using Non-Toxic Matrix Blank Samples Synthesized to Represent the Natural Ionic Composition of Freshwaters in Western States.

Timothy F. Moore, Risk Sciences, ©2004

(submitted as an affidavit to the U.S. Court of Appeals in Edison Electric, et al v. U.S. EPA)

Executive Summary

Whole effluent toxicity (WET) tests were performed on 17 identical split samples, by 14 different laboratories, using EPA's approved chronic method for Ceriodaphnia dubia (freshwater fleas). The non-toxic sample water was prepared so that the general chemical structure was very similar to municipal effluent discharged in arid west states. Such water is often naturally higher in minerals and conductivity than the control water that is traditionally used to culture test organisms and dilute samples during WET tests.

Nine of the 16 valid tests (56%) "failed" and the samples were reported as toxic. The remaining 7 valid tests (44% of the total) passed and the laboratory reported the sample was not toxic. The reported results ranged from 1 chronic toxicity unit, indicating the sample was non-toxic, to more than 16 chronic toxicity units (indicating that that toxicity was still present even after diluting the sample by a ratio of 15-to-1). One of the 17 tests (6%) was deemed invalid because the control organisms failed to meet EPA's mandatory minimum performance criteria.

On average, Ceriodaphnia dubia exposed to undiluted samples of the synthetic western freshwater matrix produced 30% fewer offspring compared to control organisms. Water fleas assigned to the control group were exposed to synthetic freshwater, prepared in accordance with EPA's recommended formula for moderately-hard dilution water. The conductivity of synthetic western freshwater samples was approximately five times higher than the conductivity of EPA's moderately-hard recipe.

Since no known toxic chemicals were introduced when the synthetic western freshwater samples were synthesized, any reduction in reproduction can only be explained by the relative difference in natural mineral concentrations. Consequently, using standard moderately-hard control water when performing WET tests on samples collected in arid west states poses substantial risk of biasing the results toward over-estimating the potential for chronic toxicity in the effluent.

Background

WET tests are performed by exposing living organisms to effluent samples and measuring the effect (if any) on survival, growth or reproduction. The baseline for comparison is determined by exposing other living organisms from the same species to synthetic freshwater known to be non-toxic. The formula for preparing that synthetic freshwater is provided by the U.S. Environmental Protection Agency ("EPA").

If effluent-exposed test organisms die more frequently, grow smaller or reproduce less than the control organisms, then such results imply the potential presence of toxic substances in the effluent sample. Such an inference is warranted provided that all other relevant factors and experimental conditions are held constant throughout the WET test.

Ceriodaphnia dubia (a freshwater flea) is the most common invertebrate species used in WET testing. EPA selected Ceriodaphnia dubia because it is very sensitive to a wide range of toxic chemicals, is relatively easy to culture, and normally reproduces very quickly.

As the chronic test procedures were being developed, many experiments were performed to determine how best to culture Ceriodaphnia dubia. After reviewing the results of these studies, EPA recommended specific formulae for preparing synthetic freshwater to serve as the control and diluent during WET tests. Today, the vast majority of bioassay laboratories culture Ceriodaphnia dubia using the recipe for moderately-hard synthetic freshwater found in EPA's official test methods manual.

EPA's test manuals[1] and related guidance[2] warn that test success and precision can be significantly affected by the hardness, salinity and conductivity of dilution water. Similar findings are reported in the peer-reviewed scientific literature.[3]

Because Ceriodaphnia dubia are very sensitive to small changes in the ionic composition of freshwater, unique problems may occur when the species is used to evaluate the potential for toxicity in samples with naturally elevated concentrations of certain minerals. The presence of such minerals may make it difficult to determine whether observed reductions in reproduction are due to toxic chemicals or to test interference caused by ionic imbalance.

The primary purpose of this study was to determine if changes in mineral concentrations might interfere with the conduct and interpretation of WET tests when Ceriodaphnia dubia reproduction is used as the surrogate measure for toxicity. The secondary purpose was to quantify the level of test precision when identical non-toxic samples are analyzed using the revised WET methods recently promulgated by EPA.[4]

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[1] Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms-3rd Ed. EPA/600/4-91/002; July, 1994; (section 4.13.1) p. 14

[2] Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the NPDES Program. EPA-833-R-00-003 (June, 2000); p. D-6 & D-7

[3] Goodfellow, W.L., et al. 2000. Major ion toxicity in effluents: a review with permitting recommendations. *Environmental Toxicology & Chemistry*. 19:175-82.

[4] See 67 Fed. Reg. 223 @ 69952 (November 19, 2002)

Methodology

A large volume of synthetic western freshwater (SWFW) was prepared using sterilized instruments and containers. Reagent grade mineral salts were added to de-ionized water to achieve the target concentrations and the desired level of conductivity. In general, the procedures were identical to those recommended in EPA's official WET test methods manual except that the amount of mineral salts added were increased so that the final chemical composition more closely matched the natural ionic structure of western waters (see Table 1). A detailed description of the sample preparation process is provided as Appendix A to this report.

Table 1: Ionic Composition of Synthetic Freshwaters

Ion	EPA's Mod-hard Formula	Western Water Formula
Alkalinity	69 mg/L	180 mg/L
Calcium	14 mg/L	95 mg/L
Chloride	2 mg/L	293 mg/L
Conductivity	279 µhmos/cm	1502 µhmos/cm
Hardness	90 mg/L	357 mg/L
Magnesium	12 mg/L	49 mg/L
Potassium	2.1 mg/L	8.4 mg/L
Sodium	26 mg/L	245 mg/L
Sulfate	81 mg/L	325 mg/L

The chemical formula was selected after reviewing the average annual concentration of various mineral ions in effluent analyses performed by several representative metropolitan wastewater agencies in arid west states, including: Phoenix, Tucson, Las Vegas, San Bernardino and Riverside. Based on data published in EPA's recommended water quality criteria documents, none of the ions was expected to be present in concentrations likely to cause toxicity to Ceriodaphnia dubia.

Several preliminary experiments were performed to identify the best method for preparing the SWFW before making the final batch that would be used in the actual study. Chemical analyses were performed throughout the entire study process to confirm that the ionic characteristics remained stable at the intended levels. This was further confirmed by chemical analyses reported by each of the bioassay laboratories that conducted a WET test on one of the SWFW samples.

Sterilized Cubitaners® were filled with identical aliquots of the SWFW. Three containers, each containing one gallon of the artificial western water matrix, were shipped to municipal dischargers who agreed to sponsor the study by bearing the cost of one chronic WET test.

Over a period of one week, the dischargers sent each of the three Cubitaners to the bioassay laboratory they normally use to perform WET tests to demonstrate compliance with monitoring requirements found in their NPDES permits.[5] New chain-of-custody forms were created to ensure that the laboratories did not know that they were part of a special study by obscuring the true origin of the special sample water.

The laboratories were instructed to perform a chronic whole effluent toxicity test using Ceriodaphnia dubia in accordance with the official protocol specified in 40 CFR Part 136.[6] Laboratories were asked to report the No-Observed-Effect Concentration (NOEC) for survival and reproduction and the 25% Inhibition Concentration (IC25) for reproduction. Laboratories were expected to follow the statistical flow-chart recommended by EPA when analyzing data from each WET test.

The laboratories were also asked to report various other water quality parameters routinely recorded during WET tests (e.g. alkalinity, hardness, conductivity, dissolved oxygen, pH, temperature, chlorine, ammonia, etc.). Copies of laboratory benchsheets and relevant reference toxicant test data were also required.

At no time were the laboratories informed that the samples were part of a coordinated round-robin research project. Nor were the laboratories given any information about the true chemical composition of the special synthetic western freshwater matrix especially that the sample was formulated, from the outset, to be non-toxic despite containing elevated mineral concentrations.

Each of the laboratories was certified to perform WET tests in their respective states and several had previously participated in EPA's own round-robin after undergoing a rigorous prequalification process.[7] Although minor deviations from official protocols occurred at several laboratories during the study, none were reported as so significant as to warrant invalidating the tests.

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[5] Five of the 14 laboratories received samples directly from the laboratory that prepared the synthetic western freshwater matrix rather than routing the sample through a municipal discharger first. In these cases, the laboratories were told that the "client" needed a split-sample as part of the normal QA/QC review process and that the samples would be collected and shipped to the testing laboratory by the wastewater agency's "local lab." Similar check samples are routinely analyzed by laboratories during the normal course of business so our explanation aroused no suspicion in this instance

[6] Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms-4th Ed. EPA-821-R-02-013; October, 2002. Method 1002.0

[7] See "Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods;" EPA-821-B-01-004; September, 2001

All of EPA's normal mandatory Test Acceptance Criteria were applied to the results from each laboratory. A test was considered valid if at least 80% of the control organisms survived and produced an average of at least 15 offspring per female. Only one test failed to meet the minimum control performance standards. That test was invalidated and excluded from all subsequent analyses.

Results

In all, 17 WET tests were performed at 14 different laboratories. Three laboratories analyzed two samples because the municipal wastewater agencies sponsoring the study happened to use the same bioassay laboratory as one of the other sponsors. All but 16 of the 17 tests met EPA's mandatory minimum control performance criteria. The other test was deemed invalid.

In 7 of the 16 valid tests (44%), there was no statistically-significant difference in the rate of survival or reproduction when Ceriodaphnia dubia exposed to undiluted samples of the synthetic western freshwater were compared to control organisms exposed only to moderately hard dilution water (see Table 2).

Table 2: Results for Chronic Tests that Passed

Test ID	Lab ID	NOEC[8] for Survival	NOEC for Reproduction	IC25[9] for Reproduction	Reported Result
6	E	100%	100%	>100%	Not Toxic
8	G	100%	100%	>100%	Invalid
10	I	100%	100%	>100%	Not Toxic
11	G	100%	100%	>100%	Not Toxic
12	J	100%	100%	>100%	Not Toxic
14	K	100%	100%	>100%	Not Toxic
15	L	100%	100%	>100%	Not Toxic
16	M	100%	100%	>100%	Not Toxic

Nine of the 16 valid tests (56%) "failed" because there was a statistically-significant reduction in the rate of survival or reproduction observed among organisms exposed to the synthetic western freshwater sample (see Table 3).

[8] No Observed Effect Concentration (NOEC) is the highest tested concentration at which there was no statistically-significant difference in survival or reproduction compared to control organisms.

[9] IC25 is the estimated concentration at which the tested sample is likely to reduce Ceriodaphnia dubia reproduction by 25% when the organisms are exposed to that concentration for approximately 7 days.

Most of the failures were due to differences in reproduction. Only 1 of the 16 valid tests (6%) showed a statistically-significant increase in mortality among organisms exposed to samples of the synthetic western freshwater matrix.

Results were mixed for the 3 laboratories that evaluated more than one sample. Laboratory A reported both samples as toxic. Laboratory G reported both samples as non-toxic; however, one of the samples was deemed invalid due to poor control performance. And, Laboratory K reported one sample as toxic and the other sample as non-toxic.

Table 3: Results for Chronic Tests that Failed

Test ID	Lab ID	NOEC for Survival	NOEC for Reproduction	IC25 for Reproduction	Reported Result
1	A	100%	50%	64%	Toxic
2	B	100%	25%	42%	Toxic
3	A	100%	50%	53%	Toxic
4	C	90%	<6%	4%	Toxic
5	D	100%	100%	44%	Toxic
7	F	100%	50%	65%	Toxic
9	H	100%	50%	65%	Toxic
13	K	100%	50%	54%	Toxic
17	N	100%	50%	>100%	Toxic

In two instances, there were also inconsistencies between the different statistical methods EPA has approved for assessing potential toxicity. In test #5, the NOEC measure indicated the sample was not toxic but the IC25 endpoint indicated that the sample was toxic. In test #17, the reverse was true. The NOEC measure showed the sample was toxic but the IC25 metric showed that the sample was not toxic. Both statistical endpoints are deemed to be accurate and reliable measures of toxicity despite such inconsistencies.

Since all of the samples analyzed by the laboratories were identical aliquots of the same synthetic western freshwater matrix, inconsistent results cannot be explained by any actual differences in water quality. Nor can the fact that half the labs reported the sample was toxic while the other half concluded that it was not be explained by differences in test sensitivity (see Table 4)[10]

[10] Test sensitivity is measured and reported as "Percent Minimum Significant Difference" (PMSD). It is the smallest detectable change in reproduction that would be deemed to be a statistically-significant reduction were it to occur. PMSD is calculated using a formula supplied by EPA in the WET test methods manual.

The average sensitivity for tests that passed was 29%; while the mean sensitivity for tests that failed was 24%. Although passing tests appear to be about 19% less sensitive than failing tests, the small difference was not statistically-significant ($p>0.16$). In only one case (#11) were the results close to being considered toxic had the test been more sensitive.

Table 4: Relative Sensitivity for Chronic Tests

Test ID	Lab ID	Reported Result	Toxicity Units[11]	Sensitivity (PMSD)	Pct. Effect in 100% SWFW[12]
1	A	Toxic	1.6 TUc	15.5%	86%
2	B	Toxic	2.4 TUc	14.3%	50%
3	A	Toxic	1.9 TUc	20.0%	79%
4	C	Toxic	25.0 TUc	31.3%	100%
5	D	Toxic	2.3 TUc	40.8%	5%
6	E	Not Toxic	1.0 TUc	8.7%	7%
7	F	Toxic	1.5 TUc	27.4%	56%
9	H	Toxic	1.5 TUc	23.1%	33%
10	I	Not Toxic	1.0 TUc	52.5%	8%
11	G	Not Toxic	1.0 TUc	37.4%	22%
12	J	Not Toxic	1.0 TUc	27.8%	1%
13	K	Toxic	1.9 TUc	28.2%	56%
14	K	Not Toxic	1.0 TUc	32.3%	7%
15	L	Not Toxic	1.0 TUc	23.9%	15%
16	M	Not Toxic	1.0 TUc	24.4%	0%
17	N	Toxic	1.0 TUc	14.1%	19%

[11] Chronic Toxicity Units (TUc) are calculated using the following formulas: 100 / NOEC or 100 / IC25. Zero detected toxicity is represented as 1 TUc.

[12] Percent Effect is calculated by subtracting the average number of offspring produced by control organisms from the average number of offspring produced by Ceriodaphnia dubia exposed to undiluted synthetic western freshwater and dividing by the average number of control offspring. The result is expressed as a percent reduction compared to control performance.

There was a large difference in the level of adverse effect observed in tests that passed versus tests that failed (see Table 5). That difference was statistically-significant ($p<0.001$).

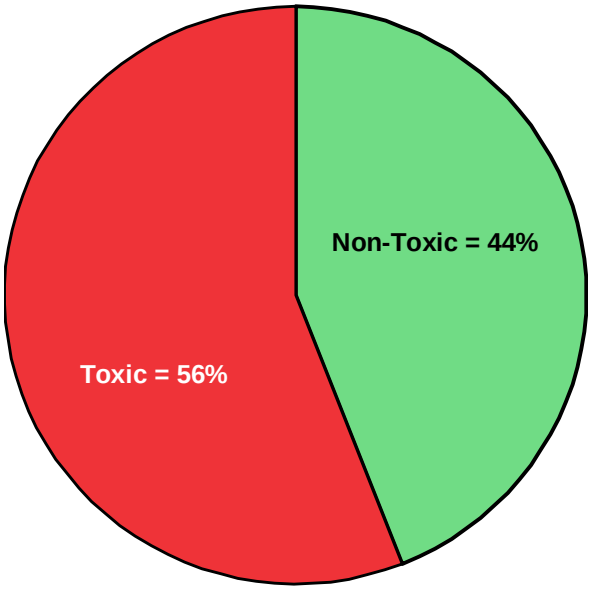
Table 5: Comparison Between Passing Tests and Failing Tests

Reported Result	Avg. TUc	Mean % Effect
Passed (Not Toxic)	1.0 TUc	7%
Failed (Toxic)	4.3 TUc	54%
All Valid Tests	2.9 TUc	33%

Discussion

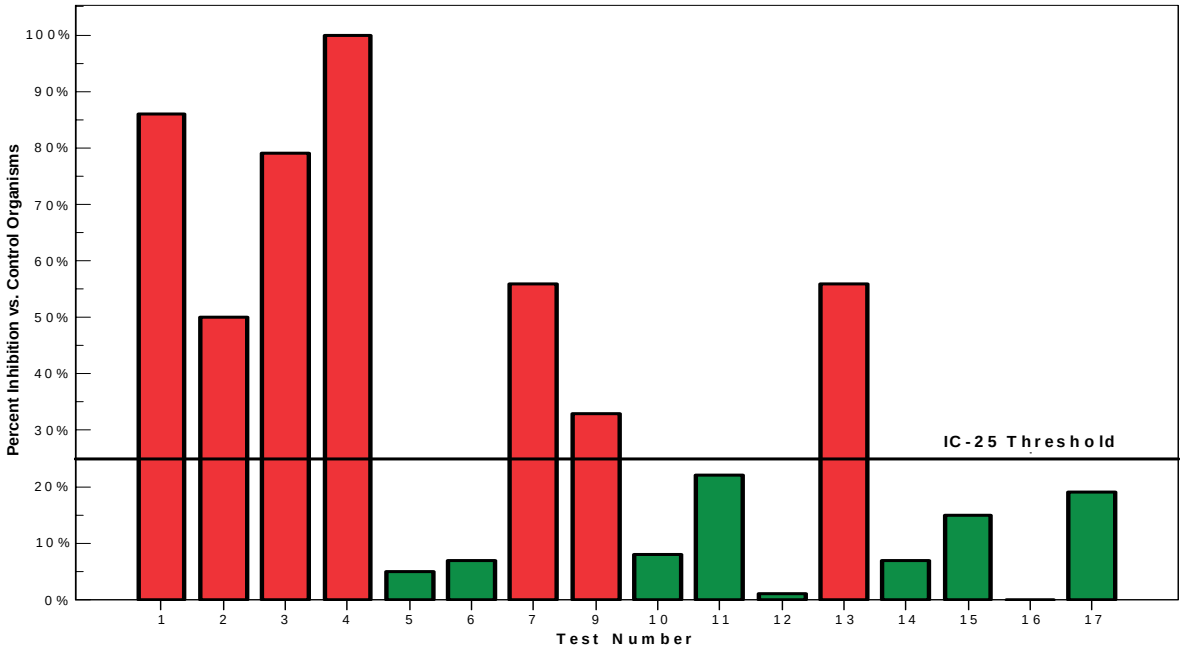
Based on the results from 17 independent WET tests performed at 14 different laboratories it is impossible to discern whether the synthetic western freshwater matrix is toxic or not (see Fig. 1).

Fig. 1: Inconsistency Between Toxicity Tests of Identical Samples



Fifteen of the 16 valid tests agreed that exposure to the synthetic western freshwater matrix caused no statistically-significant increase in Ceriodaphnia dubia mortality. However, estimates of the effect on reproduction ranged from a 100% inhibition in test # 4 to a 10% stimulation in test #16 (see Fig. 2).

Fig. 2: Range of Reported Effects on Ceriodaphnia dubia Reproduction



Note: values above 25% are deemed "toxic," values below 25% are considered Not-Toxic.

Among all tests that passed, inhibition on reproduction ranged from zero to 22%. And, among all the tests that failed, inhibition on reproduction ranged from 19% to 100%. There is no pattern to suggest why tests failed at one lab but passed at another when the samples and methods were identical throughout the study (see Table 6).

Table 6: Range of Reported Results for Chronic Ceriodaphnia dubia Toxicity Test

Test Endpoint	Min.	Max.	Mean	Median	CV
Survival	1 TUc	2 TUc	1.06 TUc	1.0 TUc	24%
Reproduction	1 TUc	16 TUc	2.88 TUc	1.3 TUc	205%

Note: TUc = "Chronic Toxicity Units" 1 TUc = Non-Toxic

Results from this investigation are similar to those reported in previous interlaboratory studies using non-toxic blank samples. Moore, et al found that the false positive rate was nearly 40% when identical split samples of moderately-hard synthetic freshwater samples were analyzed, in the blind, using the chronic test method for Ceriodaphnia dubia.[13] EPA also analyzed identical split samples of a moderately-hard synthetic freshwater sample during a large-scale interlaboratory study.[14] The false positive rate was 33%.[15]

It is evident from the data that increased conductivity may inhibit reproduction in Ceriodaphnia dubia. The magnitude of effect may vary depending on the initial health of the cultured test organisms or other factors within the laboratory.

Although ionic interference is a source of biological stress on standard test organisms, it is questionable whether this constitutes "toxicity" as generally defined by most states. The "stress" observed during a WET test occurs when Ceriodaphnia dubia that are cultured in a specific ionic matrix are suddenly switched to a different ionic matrix without any opportunity to reacclimate. Consequently, the adverse impact on reproduction may be more an artifact of the test design than a true indicator of potential toxicity in the receiving water.

Laboratories in Virginia, North Carolina and South Carolina have observed a similar reduction in reproduction caused by the very low hardness of natural freshwater streams in those states. Many of those laboratories have attempted to compensate for the interference problem by breeding special genetic strains of Ceriodaphnia dubia that are more tolerant of low conductivity waters. Results, to date, have been mixed.

Where ionic interference is expected, EPA recommends using "dual controls" when performing WET tests. Unfortunately, the second control (with higher conductivity) often fails to meet the minimum performance standards and the test is frequently deemed invalid.

Comparing reproduction rates for organisms exposed to the typical ionic matrix found in western streams to the reproduction among control organisms exposed to moderately-hard control water is mixing apples and oranges. The experimental conditions no longer properly control all of the relevant variables during the test. Consequently, it is difficult to interpret the data properly.

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[13] T.F. Moore, S.P. Canton & M. Grimes. "Investigating the incidence of Type-I errors for chronic whole effluent toxicity testing using Ceriodaphnia dubia." Environmental Toxicology & Chemistry. Vol. 19 No. 1 (January, 2000) pg. 118-122.

[14] 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.

[15] The sample was originally intended to be a reference-toxicant. However, due to errors in sample preparation, non-toxic samples were actually shipped to the laboratories. Two-thirds of the laboratories, including 82% of those performing the most sensitive tests, reported that the sample was not toxic. One-third of the laboratories, generally those with the least sensitive tests, reported that the sample was non-toxic.

Although EPA is aware of, and warns about, these ionic interference problems the current test method and related guidance do not describe how to avoid or account for the adverse influence on WET test results. Until this deficiency is remedied, Ceriodaphnia dubia reproduction data may be of limited utility for assessing potential toxicity in western waters. Dischargers to ephemeral streams must demonstrate compliance at the "end-of-pipe" without benefit of dilution. However, given the demonstrated error band and expected false positive rate it is unlikely that the chronic Ceriodaphnia dubia reproduction test can make accurate and reliable determinations under such circumstances.

Table Z. SUMMARY OF WESTCAS 2004 STUDY TESTS USING CERIODAPHNIA DUBIA.

Lab 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 %	Reported Result
1		26.3	3.7	86	**Toxic**	**Yes**	50	64	**Toxic**
2		24.7	12.4	50	**Toxic**	**Yes**	25	42	**Toxic**
3		25.4	5.4	79	**Toxic**	**Yes**	50	53	**Toxic**
4		32.5	0.8	98	**Toxic**	**Yes**	<6	4	**Toxic**
5		15.4	11.9	23	**Toxic**	**Yes**	100	44	**Toxic**
6		32.4	30.0	7	Not-toxic	No	100	>100	Non-toxic
7		31.7	16.3	49	**Toxic**	**Yes**	50	65	**Toxic**
8		Invalid							
9		25.3	17.0	8	**Toxic**	**Yes**	50	65	**Toxic**
10		15.4	14.1	22	Not-toxic	No	100	>100	Non-toxic
11		22.3	17.4	1	**Toxic**	**Yes**	100	>100	Non-toxic
12		29.9	29.6	56	Not-toxic	No	100	>100	Non-toxic
13		32.8	14.3	56	**Toxic**	**Yes**	50	54	**Toxic**
14		31.3	29.0	7	Not-toxic	No	100	>100	Non-toxic
15		23.6	20.0	15	Not-toxic	**Yes**	100	>100	Non-toxic
16		22.1	24.2	-10	Not-toxic	No	100	>100	Non-toxic
17		36.2	29.3	19	Not-toxic	**Yes**	50	>100	**Toxic**
Summary Statistics	N	16	16	16			16	16	16
	Min	15.4	0.8	-9.5			25.0	4.0	
	Max	36.2	30.0	97.5			100.0	65.0	
	Median	25.9	16.7	22.4			100.0	53.5	
	Mean	26.7	17.2	35.4			75.0	48.9	
	# of Blank Samples Incorrectly Declared Toxic				**9**	**11**	**8**	**8**	**9**
	Error Rate for Non-Toxic Blank Samples				**56.3%**	**68.8%**	**50.0%**	**50.0%**	**56.3%**

Appendix B:

Cumulative Risk of False Indications of Toxicity in WET Testing

Most end-users of WET test results, including many state and federal regulators, mistakenly expect non-toxic samples to fail few if any WET tests because the risk of Type-1 error is very low for any individual test. [1] However, while the risk of error is relatively low for any single test, it is very high when are large number of statistical analyses are performed even if such errors are expected to occur only once in every twenty tests.

Table 1 illustrates the cumulative risk of error when monthly chronic toxicity tests are performed on three species (fish, invertebrate & plant) during a normal 5-year permit term. Such a monitoring program requires 300 statistical analyses to be performed and assumes that the Type I error rate is only 5%.

Table 1: Cumulative Probability of False Failures in Monthly Test Regime Using 3 Species

# of Type-1 Errors Observed in 300 Statistical Analyses	Probability of Observing EXACTLY as many Type-1 Errors	Probability of Observing AT LEAST as many Type-1 Errors
0 errors in 300 tests	.00002%	---
1 error in 300 tests	.0003%	99.99998%
2 errors in 300 tests	.003%	99.9997%
3 errors in 300 tests	.01%	99.997%
4 errors in 300 tests	0.1%	99.98%
5 errors in 300 tests	0.2%	99.93%
6 errors in 300 tests	0.4%	99.77%
7 errors in 300 tests	0.9%	99.34%
8 errors in 300 tests	1.8%	98.4%
9 errors in 300 tests	3.1%	96.6%
10 errors in 300 tests	4.7%	93.5%
11 errors in 300 tests	6.6%	88.8%
12 errors in 300 tests	8.3%	82.2%
13 errors in 300 tests	9.7%	73.9%
14 errors in 300 tests	10.5%	64.2%
15 errors in 300 tests	10.5%	53.7%
16 errors in 300 tests	9.9%	43.2%
17 errors in 300 tests	8.7%	33.3%
18 errors in 300 tests	7.1%	24.7%

Note: monthly testing fill evaluate a total of 60 effluent samples over the course of 5 years. Each month five separate statistical analysis are performed: fish survival, fish growth, invertebrate survival, invertebrate reproduction and plant growth. Therefore a total of 300 separate statistical analysis will be conducted. And, each biological endpoint is statistically independent from all the others which is an appropriate assumption when assessing known non-toxic samples (such as method blanks).

[1] This statistical phenomena is discussed in relation to reference toxicant tests in USEPA. 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 4.16.5 @ pg. 16 but is true for any random sample of non-toxic waters also.

The plain English explanation for Table 1 is a follows: if 1,000 dischargers perform monthly chronic toxicity tests on three species, the probability of passing all 300 statistical analyses is 0.00002% (1 in 5 million) even if the effluent is chemically identical to the non-toxic control water used by the laboratory. Nearly 75% of the permittees will record 13 or more false failures during the 5-year monitoring period

It is essential to recognize that all analytical tests, including both WET and chemical methods, contain a measure of irreducible error. Because such errors are beyond the discharger's control, it is inappropriate to conclude that test failures are prima facie evidence of a permit violation. This is particularly important where a large number of samples will be analyzed over time. Even if the total number of tests is reduced from 300 to 100 (as would be the case if a discharger must perform a chronic WET test on only the single most sensitive species each month) , it remains a virtual certainty that all dischargers will experience at least one false failure indicating toxicity. But, more than half of all agencies obliged to monitor at the same frequency will record at least one false test failure per year. And, 1 in every 6 agencies that perform 100 chronic WET tests will be the victims of 8 or more false test failures.

Table 2: Cumulative Probability of False Failures in Monthly Test Regime Using One Species*

# of Type-1 Errors Observed (False Positives)	Probability of Observing AT LEAST as many Type-1 Errors
1 or more in 100 analyses	99.4%
2 or more in 100 analyses	96.3%
3 or more in 100 analyses	88.2%
4 or more in 100 analyses	74.2%
5 or more in 100 analyses	56.4%
6 or more in 100 analyses	38.4%
7 or more in 100 analyses	23.4%
8 or more in 100 analyses	12.8%
9 or more in 100 analyses	6.3%
10 or more in 100 analyses	2.8%

Note: each toxicity test requires a statistical analysis of two biological endpoints: a survival metric and a sublethal metric (such as growth or reproduction). In a non-toxic sample, these endpoints are independent of one another Thus, running a monthly chronic WET test on a single species actually results in 120 different statistical comparisons (60 months x 2 endpoints). So, the actual number of false test failures observed by those that perform one test per month on a single species for a period of five years will be slightly higher than shown in Table 2.

Appendix C:

Excerpts from a report prepared by:

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Brentwood, TN

and

Risk Sciences
Rockvale, TN

on behalf of

General Electric, Inc.
Rome, GA

Formally submitted to Georgia Dept. of Environmental Protection
2006

Abstact: rain water samples were collected in sterile containers that were elevated ten feet off the ground and analyzed using the chronic WET method for Ceriodaphnia dubia. All organisms exposed to undiluted rain water were dead within 48 hours.

Table 3: Acute Toxicity Test Results for a Sample of Natural Rain (May 1st)
(# of surviving organisms / # of organisms tested)

Sample Concentration	0% (control)	20%	40%	60%	80%	100% (undiluted)
Fathead minnows	20/20	20/20	20/20	20/20	20/20	0/20
Ceriodaphnia dubia	20/20	20/20	20/20	20/20	5/20	0/20

The pH was measured in the rain water sample and found to range between 4.24 and 4.85 s.u. By contrast, pH in laboratory control water ranges between 7.71 and 8.18. Because pH is reported on a logarithmic scale, the observed values imply that the rain water sample was 1000-times more acidic than the control water.

In addition, the conductivity of the rain water sample was measured at 43-68 umhos/cm. By contrast, laboratory control water used to culture test organisms ranged between 302-322 uhmos/cm. So, for all practical purposes, the rain water was nearly de-ionized. Aquatic organisms require a certain concentration of ions in freshwater in order to maintain an equilibrium of osmotic pressure at the gill interface; failure to do so will cause the animal to die.[1] Figure 1 graphically illustrates the dose-response relationship between exposure to various rain water concentrations and acute mortality among toxicity test organisms.

Table 4: Acute Toxicity Test Results for a Sample of Natural Rain (April 12)
(# of surviving organisms/ # of organisms tested)

Sample Concentration	0% (control)	6.25%	12.7%	25%	50%	75%	100% (undiluted)
Ceriodaphnia dubia	20/20	20/20	20/20	20/20	15/20	2/20	0/20

In their official method manual, EPA warns:

> *"Mortality or impairment of growth or reproduction due to pH alone may occur if the pH of the sample falls outside the range of 6.0 -9.0"*[2]

Therefore, the mortality observed among organisms exposed to rain water is predictable given the low pH. But, EPA also states that:

> *"Test organisms should not be subjected to changes of more than … 2 units of pH in any 24-hour period."*[3]

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[1] Goodfellow, W.L., et al. Major ion toxicity in effluents: a review with permitting recommendations. *Environmental Toxicology and Chemistry.* Vol. 19; January, 2000; p. 175-182.

[2] Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms; EPA/600/4-91/002; July, 1994, pg. 33

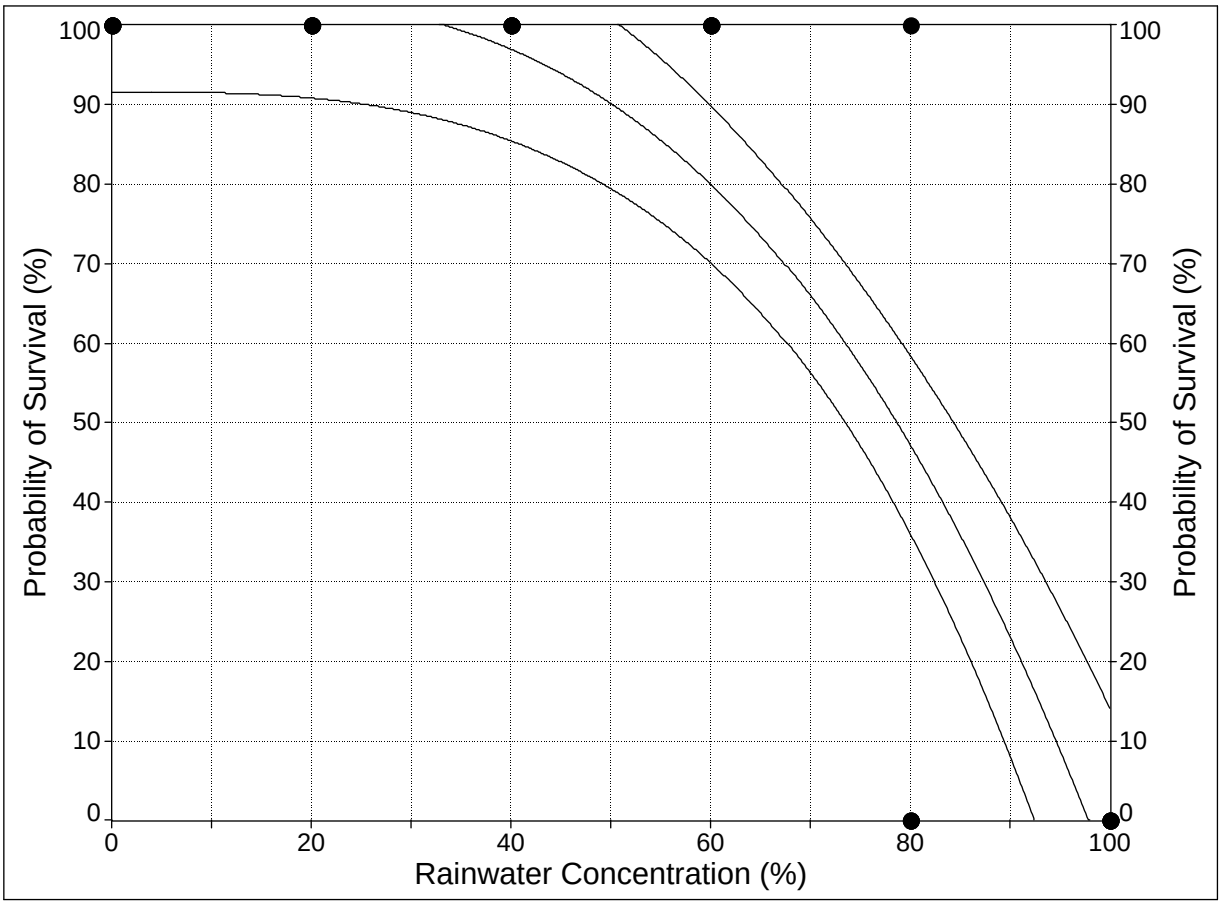
[3] Ibid, p. 23

Thus, the abnormally low pH of rain water and the shock associated with transferring test organisms from the culture water to the sample water adds an additional element of stress on the organism. In addition, the extremely low hardness, conductivity and alkalinity act synergistically with the low pH in rain water to magnify the adverse biological impact on Ceriodaphnia dubia. Once again, EPA warns:

> *In addition, parameters such as TDS (hardness, salinity, conductivity), turbidity, DO, pH, micronutrients, and bacteria counts can impact test organism physiology, sensitivity, and biological response.*[4]

Fig. 1: Probability of Survival for Organisms Exposed to Undiluted Rain Water

48-hour Acute Mortality for Ceriodaphnia dubia



[4] Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the NPDES Program. EPA-833-R-00-003 (June, 2000); p. D-7