Whole Effluent Toxicity Test Drive Analysis of the Test of Significant Toxicity (TST)

U.S. Environmental Protection Agency
EPA Region 9
1001 I Street
Sacramento, CA 95814

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EXECUTIVE SUMMARY

The Test of Significant Toxicity (TST) is a statistical approach developed by the U.S. Environmental Protection Agency (EPA) for analyzing whole effluent (WET) and ambient toxicity data, and is being proposed in the State Water Resources Control Board (State Water Board) draft Toxicity Policy. EPA has demonstrated the advantages of the TST approach using WET and ambient toxicity data across the U.S. The State Water Board recommended conducting a "test drive" comparing results obtained using TST with results obtained using the current WET statistical approach based on comments raised at the November 16, 2010 workshop. The test drive had two specific objectives: (1) Evaluate and compare resulting interpretations of WET data analyzed using TST and the No Observed Effect Concentration (NOEC) statistical approach currently being used in California's WET program; and (2) Determine how many (if any) additional within-test replicates for the control and IWC would be needed to declare samples non-toxic that were initially identified as toxic using TST with a mean effect less than TST regulatory management decision (RMD).

TST Regulatory Management Decisions (RMDs)

- The sample is declared toxic if there is greater than or equal to a 25% effect in chronic tests or is greater than or equal to 20% effect in acute tests at the permitted instream waste concentration (IWC). (referred to as the toxic RMD)
- The sample is declared non-toxic if there is less than or equal to 10% effect at the IWC in acute or chronic tests. (referred to as the non-toxic RMD)

Valid WET data from over 25 dischargers were compiled and analyzed in this test drive representing both stormwater and wastewater effluents from a variety of facilities, including small facilities from underprivileged communities. A total of 837 tests were compiled in this test drive representing the majority of WET test methods and endpoints used in California's toxicity program. Some of the tests received did not meet test method test acceptability criteria and were therefore not used. Additionally, some tests could not be used because the test did not include a concentration at or near the facilities' instream waste concentration (IWC). A total of 775 valid, usable tests were analyzed in this test drive.

Each valid test was analyzed using both TST and the current NOEC approach and a determination made as to whether the sample is toxic or not using each approach. In addition, this study evaluated the effect of adding simulated replicates to those tests declared toxic using TST and had less than 25% effect in a chronic test or less than 20% effect in an acute test.

Results of the test drive are as follows:

1. TST analysis declared 2.9% of all tests as toxic which had a mean effect at the IWC less than 25% for chronic methods or less than 20% for acute methods, while NOEC analysis declared 5.3% of those tests as toxic. (see Table E-1; Figure E-1)
2. TST analysis declared 0.1% of all tests as toxic which had an effect less than or equal to 10%, while NOEC analysis declared 2.6% of those tests as toxic. These results, combined with those in #2 above, demonstrate that truly non-toxic samples were more often declared non-toxic using TST than using the NOEC approach. (see Table E-1)
3. TST appeared to perform better than the current NOEC approach for those tests exhibiting significant toxicity at the IWC. For chronic tests with a mean effect greater than or equal to 25%, the NOEC analysis declared a significantly higher percentage of these tests not toxic as compared to TST (14.3% and 0.1% for NOEC and TST, respectively; Figure E-2). Thus, NOEC analysis missed declaring truly toxic samples (effects greater than or equal to 25%) more often. It is desired that our statistics declare a sample as toxic at or above the respective RMD.
4. In the few cases, where TST detected toxicity at effects less than 25% in chronic tests or less than 20% in acute tests, this was due to high variability between replicates in the controls and/or IWC treatments. Addition of a minimal number of replicates to these tests usually resulted in the sample being declared non-toxic using the TST procedure. These results provide useful

information to permittees, laboratories, and the State Water Board regarding within-test variability and demonstrate an advantage to the permittee of using more than the minimum required number of replicates in certain cases.

5. Test results using both TST and the current NOEC approach were generally the same overall, indicating that the use of TST is not expected to change the number of enforcement actions over the current status (Table E-1, Figure E-1).

Table E-1. Summary of all WET method tests declared toxic and non-toxic regardless of percent mean effect and those declared toxic with a percent mean effect at the IWC less than 25% and less than or equal to 10% for each analysis method grouped by test type. Numbers represent the percentage based on all tests for a given method type.

Method Type	Percent of Tests Declared Non-Toxic		Percent of Tests Declared Toxic [1]		Percent of Tests Declared Toxic with less than 25% (20% for Acute) Effect at IWC[2]		Percent of Tests Declared Toxic with less than or equal to 10% Effect at IWC	
	TST	NOEC	TST	NOEC	TST	NOEC	TST	NOEC
Chronic Marine	89.4	83.6	10.6	16.4	2.3	8.9	0	4.6
Chronic Freshwater	80.1	82.3	19.9	17.7	4.9	4.0	0	1.8
Acute Marine	100	100	0	0	0	0	0	0
Acute Freshwater	96.8	98.9	3.2	1.1	1.6	0	0.5	0
All Methods	**88.6**	**87.2**	**11.4**	**12.8**	**2.9**	**5.3**	**0.1**	**2.6**

1 This includes tests which are truly toxic above the RMD of 20% for acute or 25% for chronic, as well as those tests with effects below the respective RMDs.

2. This includes tests with effects less than or equal to the non-toxic RMD of 10% effect at the IWC.

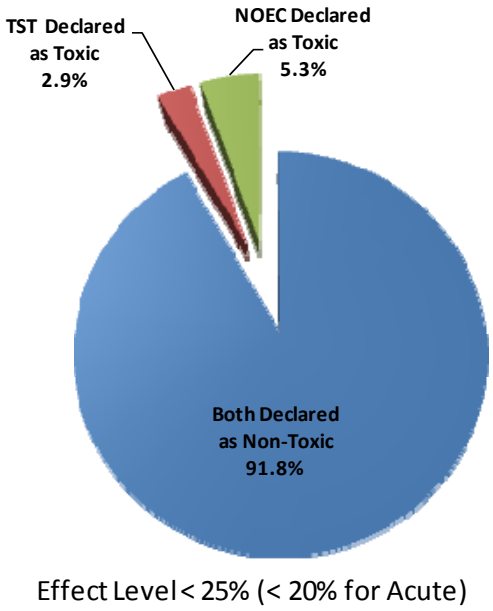

Figure E-1. Summary of the tests from all methods that were declared toxic using TST and NOEC analysis with a mean effect at the IWC less than the toxic RMD of 25% for chronic or 20% for acute tests. These percentages include those tests having effects at the IWC less than or equal to the non-toxic RMD of 10%.

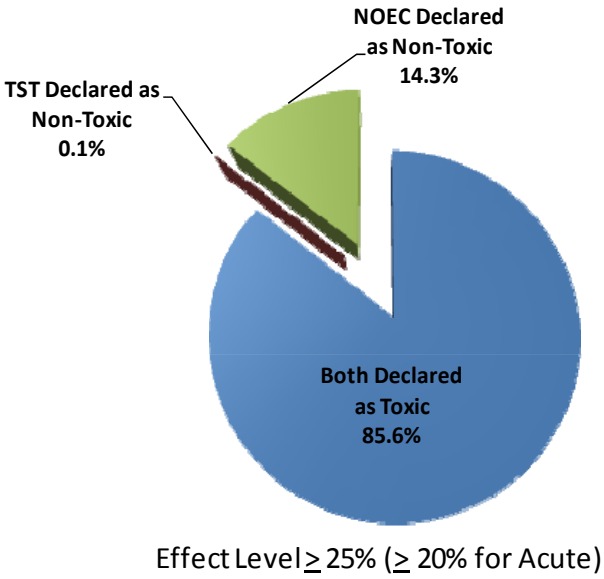

Figure E-2. Summary of the tests that were declared non-toxic using TST and NOEC analysis with a mean effect at the IWC greater than or equal to the toxic RMD of 25% for chronic or 20% for acute tests.

1.0 INTRODUCTION

At the public workshop held on November 16, 2010 in Sacramento, CA, the State Water Resources Control Board (State Water Board) recommended that the State Water Board staff and its contractors conduct a "test drive" of the Test of Significant Toxicity (TST). The TST is a statistical approach developed by U.S. Environmental Protection Agency (EPA) for analyzing whole effluent (WET) and ambient toxicity data (USEPA 2010; Denton et al. 2011), and is being proposed in the State Water Board's draft WET Policy. While EPA has demonstrated the advantages of the TST approach using WET and ambient toxicity data across the U.S., to address concerns raised at the Board workshop, additional comparisons of results obtained using TST with results obtained using the current WET statistical approach was recommended. The test drive had two specific objectives:

(1) Evaluate and compare resulting interpretations of WET data analyzed using TST and the No Observed Effect Concentration (NOEC) statistical approach currently being used in California's WET program.

(2) Determine how many (if any) additional within-test replicates for the control and IWC would be needed to declare samples non-toxic that were initially identified as toxic using TST and had and a mean effect less than the toxic TST regulatory management decision (RMD) for unacceptable toxicity (25% for chronic and 20% for acute tests).

Using data from a number of sources, the first objective identified the number of tests passing or failing, the range of effects associated with passing or failing, and the within-test variability associated with these tests using the TST and the NOEC approach. This information is useful because it describes the comparison of WET results using the two different statistical analysis approaches, and demonstrates why differences are observed. The second objective further addresses how many replicates could be added for those tests which were declared toxic below the toxic RMD, to increase the probability of declaring those tests non-toxic.

2.0 METHODS

2.1 WET Data Collection

Valid WET data from wastewater dischargers were compiled and analyzed in this test drive. To ensure that representative WET data were used, data were obtained from both stormwater and wastewater effluents from a variety of facilities including small facilities from underprivileged communities. Facilities represented in this test drive encompassed a range of instream waste concentrations (<5% - 100%), various treatment types (e.g., various forms of secondary treatment), a range of population sizes served (very small–very large), and wide geographic range (northern and southern California). In addition, WET data were generated by many commercial laboratories including WET laboratories in California. A total of 837 tests were compiled in this test drive. Each discharger was assigned a code letter to maintain anonymity. To increase the number of tests available for certain WET methods WET data were provided by the State of Washington for numerous dischargers (e.g., *Daphnia* acute test). All of the WET data from the dischargers in Washington were grouped into one discharger code (referred to as Facility I in this report).

2.2 WET Data Analysis and Database Construction

WET data were provided in one of the following formats: CETIS export database files, Microsoft Excel files, PDF image files of lab reports, and printed copies of CETIS and ToxCalc report datasheets. Data obtained included organism response data for each replicate and effluent concentration as required by the EPA method. In addition, the facility instream waste concentration (IWC) was obtained so that pass or fail could be determined for each test using the current NOEC approach and TST.

As data files were received they were either imported (CETIS export files) or hand entered (Excel files, PDF files, and hard copy reports) into a unified CETIS database. All WET test data were then analyzed in CETIS to generate an NOEC value for both lethal and sub-lethal endpoints. Test Acceptability Criteria (TAC) were also examined for each test using CETIS. Tests that did not meet all TAC for a given method (43 tests, or 5.1% of the 837 tests received) were not used in analysis.

Some of the tests examined, including those from Washington, did not use the IWC as one of the test concentrations. In these cases, the nearest effluent concentration tested was used in the analysis, provided it was within 5% of that facility's IWC. Tests that did not have test concentrations within 5% of the facility's IWC (19 tests, or 2.3% of the 837 tests) were not analyzed in this Test Drive. Therefore, the total number of valid, usable tests analyzed in this test drive was 775.

NOEC, mean organism response, coefficient of variation (CV) and standard deviation in the control and IWC for each endpoint were exported from CETIS and imported into a Microsoft Excel spreadsheet which was created to store all test records. CETIS analytical reports were also printed for each WET test including all viable endpoints. The CETIS reports were then used to hand enter the control and IWC replicate data for each test and endpoint into EPA's TST calculator (version 1.4) for TST analysis. The results of the TST analysis and mean percent

effect were then hand entered from the TST calculator into the project Microsoft Excel spreadsheet. All hand-entered data were double checked by an independent reviewer to ensure data accuracy of analyses.

2.3 Analysis Using Additional Replicates

TST is designed to nearly always declare a chronic test toxic when the mean percent effect at the IWC is ≥ 25% compared to the control or ≥ 20% effect in an acute test, and nearly always pass the test when the mean percent effect at the IWC is ≤ 10% compared to the control. At effect levels between these boundaries (10 and 25% effect for chronic tests and 10 and 20% effect for acute tests), TST is designed to pass most tests if within-test variability is at or below the national average for the method. One way to lower within-test variability is for laboratories to test additional replicates. Testing additional replicates beyond the minimum required in a method often provides more certainty in results using TST. This study evaluated the effect of adding replicates to those tests declared toxic using TST and had < 25% effect in a chronic test or < 20% effect in an acute test. Results of this analysis provide useful information to permittees, laboratories, and the State Water Board regarding within-test variability and demonstrate an advantage to the permittee of using more than the minimum required number of replicates in certain cases.

Additional replicates were simulated using an automated integer-based number generator in Excel where the random number generator function was bounded by the minimum and maximum organism response value observed in the control and in the IWC. For example, if the control minimum and maximum values in a *Ceriodaphnia* reproduction test were 16 and 30 neonates, respectively, and the IWC minimum and maximum were 12 and 29, the formula would be applied individually to the control and IWC using these minimum and maximum values. Random number generation was used to simulate additional replicates because this is an unbiased and objective procedure. Replicates were added one at a time to both the control and IWC groups and analyzed using TST until either the result of the TST analysis declared the test non-toxic, or double the number of replicates were added.

3.0 RESULTS

3.1 *Haliotis rufescens* Larval Development Test

A total of 115 *Haliotis rufescens* larval development tests were evaluated representing three different facilities. Median mean percent effect values at the IWC averaged 2.6%, 7.8%, and 0.7% for facilities A, B, and D, respectively.

TST analysis resulted in 17 tests declared toxic from all facilities combined, while NOEC analysis resulted in 24 tests declared toxic (Table 3-1). One test (from Facility A) had a mean percent effect > 25% at the IWC (31.2% effect) and was declared non-toxic using the NOEC analysis method. This test was declared toxic using TST. For tests with a mean percent effect < 25% at the IWC, two (2.0%) were declared toxic using TST analysis and ten (9.8%) were declared toxic using NOEC analysis (Table 3-1). The tests declared toxic using TST analysis had percent mean effect values of 15.4% and 20.2%, while the tests found toxic using NOEC analysis had percent mean effect values between 5.4% and 20.2%. TST analysis did not declare any tests toxic with a mean percent effect ≤ 10% at the IWC; however, NOEC analysis declared five tests toxic with mean percent effect values ≤ 10% at the IWC (Table 3-1).

Table 3-1. Summary of *Haliotis rufescens* tests declared toxic and non-toxic regardless of mean percent effect and those tests declared toxic with a mean percent effect at the IWC < 25% and ≤ 10% for each analysis method grouped by facility. Numbers in parenthesis represent the percentage based on all tests for a given facility.

Facility	N	Number (Percent) of Tests Declared Non-Toxic		Number (Percent) of Tests Declared Toxic		Number (Percent) of Tests Declared Toxic with < 25% Effect at IWC		Number (Percent) of Tests Declared Toxic with ≤ 10% Effect at IWC	
		TST	NOEC	TST	NOEC	TST	NOEC	TST	NOEC
A	27	19 (70)	19 (70)	8 (30)	8 (30)	2(7)	3 (11)	0 (0)	0 (0)
B	25	16 (64)	9 (36)	9 (36)	16 (64)	0 (0)	7 (28)	0 (0)	5 (20)
D	63	63 (100)	63 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Control variability for the two tests declared toxic using TST analysis with a mean percent effect < 25% was between the 25th and 50th percentile based on the national distribution (USEPA, 2010) (Table 3-2). The IWC in both of these tests had at least double the 90th percentile control variability reported in the national distribution at the IWC (Table 3-2).

For the two tests declared toxic using TST with a mean percent effect < 25% at the IWC, replicates were added to control and IWC to determine if this resulted in the test being declared non-toxic. The test that had a mean percent effect of 15.4% needed one additional replicate to be declared non-toxic, while the test that had a mean effect of 20.2% was declared toxic using TST with up to five additional replicates (Table 3-3). This latter test had a high within-test variability (SD = 0.009 and 0.208 for control and IWC, respectively).

Table 3-2. Range of standard deviations (SD) observed in the control and IWC for the *Haliotis rufescens* tests declared toxic using TST and NOEC when the mean percent effect < 25% by facility and compared to the national distributions from USEPA, 2010 (NA = No tests were declared toxic with < 25% effect at the IWC).

Analysis Method / Concentration	Facility	Minimum SD	Median SD	Maximum SD	25th Percentile SD (EPA, 2010)	50th Percentile SD (EPA, 2010)	75th Percentile SD (EPA, 2010)	90th Percentile SD (EPA, 2010)
TST / Control	A	0.01	0.02	0.03	0.01	0.03	0.04	0.06
	B	NA	NA	NA				
	D	NA	NA	NA				
TST / IWC	A	0.11	0.16	0.21				
	B	NA	NA	NA				
	D	NA	NA	NA				
NOEC / Control	A	0.01	0.01	0.03				
	B	0.01	0.02	0.04				
	D	0.02	0.02	0.02				
NOEC / IWC	A	0.00	0.05	0.11				
	B	0.02	0.03	0.05				
	D	0.05	0.05	0.05				

Table 3-3. Effect of adding additional replicates on results of *Haliotis rufescens* tests declared toxic when the mean effect was < 25% for facility A. NC = no change in result using up to 5 additional replicates (i.e., 10 replicates for IWC and control).

Test	Mean Effect at IWC (%)	Found Non-Toxic With Additional Replicates	Number of Additional Replicates Needed
1	15.40	Yes	1
2	20.20	No	NC

3.2 *Macrocystis pyrifera* Germination and Germ-tube length Test

3.2.1 Germination

A total of 43 *Macrocystis pyrifera* germination tests were evaluated representing three different facilities. Mean percent effect values at the IWC averaged 0%, 1.5%, and 2.3% for facilities D, E, and G, respectively and maximum mean percent effect values observed were 5.6%, 4.5%, and 9.9% for facilities D, E, and G, respectively. No tests were declared toxic using either TST or NOEC analysis.

3.2.2 Germ-tube length

A total of 43 *Macrocystis pyrifera* germ-tube length tests were evaluated representing three different facilities. Mean percent effect values at the IWC averaged 0%, 0%, and 3.9% for facilities D, E, and G, respectively. All four tests from facility E had a mean percent effect of 0. Maximum mean percent effect values from facilities D and G were 15.1% and 25.2%, respectively.

TST and NOEC analysis resulted in one test declared toxic based on all facilities combined (Table 3-4). No tests were declared toxic using TST analysis with a mean percent effect < 25% and one test was deemed toxic using NOEC analysis (Table 3-4). The test found toxic using NOEC analysis had a mean percent effect of 15.1% at the IWC. Neither analysis method declared any test toxic with a mean percent effect ≤ 10% at the IWC (Table 3-4).

Table 3-4. Summary of *Macrocystis pyrifera* germ-tube length tests declared toxic and non-toxic regardless of mean percent effect and those tests declared toxic with a mean percent effect at the IWC < 25% and ≤ 10% for each analysis method grouped by facility. Numbers in parenthesis represent the percentage based on all tests for a given facility.

Facility	N	Number (Percent) of Tests Declared Non-Toxic		Number (Percent) of Tests Declared Toxic		Number (Percent) of Tests Declared Toxic with < 25% Effect at IWC		Number (Percent) of Tests Declared Toxic with ≤ 10% Effect at IWC	
		TST	NOEC	TST	NOEC	TST	NOEC	TST	NOEC
D	29	29 (100)	28 (97)	0(0)	1 (3)	0(0)	1 (3)	0(0)	0(0)
E	4	4 (100)	4 (100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
G	10	9 (90)	10 (100)	1(10)	0 (0)	0(0)	0(0)	0(0)	0(0)

One test with a mean percent effect > 25% was declared non-toxic using the NOEC analysis method. The test was from facility G and had a mean percent effect of 25.2%. TST analysis found this test to be toxic, as it should.

3.3 Urchin Fertilization Test

A total of 61 urchin fertilization tests were evaluated representing three different facilities. Mean percent effect values for the IWC averaged 0.1%, 0.3%, and 2.0% for facilities M, N, and O, respectively. Maximum mean percent effect values observed at facilities M, N, and O were 60.2%, 15.9%, and 99.4%, respectively.

TST analysis declared 16 tests toxic based on all facilities combined while NOEC analysis declared 21 tests toxic (Table 3-5). For tests with a mean percent effect < 25% at the IWC, four tests (8.2%) were declared toxic using TST and nine (18.4%) were declared toxic using NOEC (Table 3-5). The four tests found toxic using TST analysis had mean percent effect values of 15.9%, 19.2%, 24.2%, and 24.3% while the nine tests found toxic using NOEC analysis had mean percent effect values ranging between 1.1% and 24.3%. TST analysis did not declare any tests toxic with a mean percent effect ≤ 10% at the IWC. NOEC analysis declared three tests as toxic with effect values at the IWC ≤ 10% (Table 3-5).

Table 3-5. Summary of urchin fertilization tests declared toxic and non-toxic regardless of percent mean effect and those tests declared toxic with a percent mean effect at the IWC < 25% and ≤ 10% for each analysis method grouped by facility. Numbers in parenthesis represent the percentage based on all tests for a given facility.

Facility	N	Number (Percent) of Tests Non-Toxic		Number (Percent) of Tests Toxic		Number (Percent) of Tests Declared Toxic with < 25% Effect at IWC		Number (Percent) of Tests Declared Toxic with ≤ 10% Effect at IWC	
		TST	NOEC	TST	NOEC	TST	NOEC	TST	NOEC
M	12	10 (83)	10 (83)	2 (17)	2 (17)	0 (0)	0 (0)	0 (0)	0 (0)
N	12	11 (92)	12 (100)	1 (8)	0 (0)	1 (8)	0 (0)	0 (0)	0 (0)
O	37	24 (65)	18 (49)	13 (35)	19 (51)	3 (8)	9 (24)	0 (0)	3 (8)

For the four tests declared toxic using TST with mean percent effect < 25% at the IWC, SDs ranged from < 25th to > 90th percentiles based on the national distribution (Table 3-6). The addition of two replicates to the test with a 15.9% effect resulted in declaring the test non-toxic using TST (Table 3-7). The remaining three tests with effect levels of 19.2%, 24.2%, and 24.3% were still declared toxic using TST analysis, even with the addition of up to five more replicates (Table 3-7) due to relatively high within-test variability and an effect close to the toxic RMD using TST (25% effect).

Table 3-6. Range of standard deviations (SD) observed in the control and IWC for the urchin fertilization tests declared toxic using TST and NOEC when the mean percent effect < 25% by facility. (NA = No tests were declared toxic with < 25% effect at the IWC).

Analysis Method / Concentration	Facility	Minimum SD	Median SD	Maximum SD	25th Percentile SD (EPA, 2010)	50th Percentile SD (EPA, 2010)	75th Percentile SD (EPA, 2010)	90th Percentile SD (EPA, 2010)
TST / Control	M	NA	NA	NA	0.01	0.02	0.04	0.09
	N	0.01	0.01	0.01				
	O	0.01	0.01	0.02				
TST / IWC	M	NA	NA	NA				
	N	0.12	0.12	0.12				
	O	0.07	0.09	0.11				
NOEC / Control	M	NA	NA	NA				
	N	NA	NA	NA				
	O	0.00	0.02	0.03				
NOEC / IWC	M	NA	NA	NA				
	N	NA	NA	NA				
	O	0.01	0.04	0.11				

Table 3-7. Effect of adding additional replicates on the result of the urchin fertilization tests declared toxic when the mean effect is < 25% for facilities N and O. NC = no change in result using up to five additional replicates.

Test	Mean Effect at IWC (%)	Found Non-Toxic With Additional Replicates	Number of Additional Replicates Needed
1	15.94	Yes	2
2	19.22	No	NC
3	24.20	No	NC
4	24.29	No	NC

3.4 Chronic *Americamysis bahia* Survival and Growth Test

3.4.1 Survival

A total of 46 *Americamysis bahia* chronic tests were evaluated for survival, all from facilities in Washington State (Facility I). The median, 75th percentile, and maximum mean percent effect values were 0%, 4.6%, and 14.3%, respectively. TST and NOEC analysis did not declare any of these tests toxic based on survival (Table 3-8).

Table 3-8. Summary of chronic *Americamysis bahia* survival tests declared toxic and non-toxic regardless of percent mean effect and those tests declared toxic with a percent mean effect at the IWC < 25% and ≤ 10% for each analysis method at facility I. Numbers in parenthesis represent the percentage based on all tests for a given facility.

Facility	N	Number (Percent) of Tests Declared Non-Toxic		Number (Percent) of Tests Declared Toxic		Number (Percent) of Tests Declared Toxic with < 25% Effect at IWC		Number (Percent) of Tests Declared Toxic with ≤ 10% Effect at IWC	
		TST	NOEC	TST	NOEC	TST	NOEC	TST	NOEC
I	46	46 (100)	46 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

3.4.2 Growth

For the same 46 tests as noted for survival the median, 75th percentile, and maximum percent mean effect values based on growth were 0.5%, 5.0%, and 20.6%, respectively.

TST and NOEC analysis each declared one test toxic (Table 3-9). The test declared toxic using TST had a mean percent effect of 20.6% at the IWC while the test declared toxic using NOEC had a mean percent effect of 16.3%. No test with an effect ≤ 10% at the IWC was declared toxic using either analysis method (Table 3-9).

Control SD for the test declared toxic using TST was comparable to the 90th percentile based on the national distribution (USEPA, 2010; Table 3-10). SD at the IWC for this same test was near the 75th percentile based on the national distribution (Table 3-10). The addition of seven

replicates (total of 15 replicates) to this test changed the result to non-toxic using TST analysis (Table 3-11).

Table 3-9. Summary of chronic *Americamysis bahia* growth tests declared toxic and non-toxic regardless of percent mean effect and those tests declared toxic with a percent mean effect at the IWC < 25% and ≤ 10% for each analysis method at facility I. Numbers in parenthesis represent the percentage based on all tests for a given facility.

Facility	Number (Percent) of Tests Declared Non-Toxic		Number (Percent) of Tests Declared Toxic		Number (Percent) of Tests Declared Toxic with < 25% Effect at IWC		Number (Percent) of Tests Delcared Toxic with ≤ 10% Effect at IWC	
	TST	NOEC	TST	NOEC	TST	NOEC	TST	NOEC
I	45 (98)	45 (98)	1 (2)	1 (2)	1 (2)	1 (2)	0 (0)	0 (0)

Table 3-10. Standard deviations (SD) observed in the control and IWC for chronic *Americamysis bahia* growth tests declared toxic using TST and NOEC when the mean percent effect < 25% for facility I and compared to the national distribution from USEPA, 2010.

Analysis Method / Concentration	Facility	SD	25th Percentile SD (EPA, 2010)	50th Percentile SD (EPA, 2010)	75th Percentile SD (EPA, 2010)	90th Percentile SD (EPA, 2010)
TST / Control	I	0.084	0.03	0.04	0.06	0.08
TST / IWC	I	0.064				
NOEC / Control	I	0.036				
NOEC / IWC	I	0.058				

Table 3-11. Effect of additional replicates on the result of chronic *Americamysis bahia* growth tests declared toxic when the mean effect is < 25% for facility I.

Test	Mean Effect at IWC (%)	Found Non-Toxic With Additional Replicates	Number of Additional Replicates Needed
1	20.61	Yes	7

3.5 *Mytilus* sp. Larval Development Test

A total of 29 *Mytilus sp.* larval development tests were evaluated representing five different facilities. Mean percent effect values for the IWC averaged 0.4%, 4.3%, 2.3%, 1.4%, and 0.3% for facilities I, F, P, Q, and R, respectively. No facility had a mean percent effect value that exceeded 12.6%.

TST did not declare any tests toxic, while NOEC analysis declared nine tests toxic based on all facilities combined (Table 3-12). For tests with a mean percent effect of < 25%, 9 (31.0%) tests

were declared toxic using NOEC analysis (Table 3-12). The tests declared toxic using NOEC analysis had mean percent effect values at the IWC that ranged from 2.0% to 12.6%. NOEC analysis declared eight tests with a mean effect at the IWC ≤ 10% as toxic (Figure 3-12).

Table 3-12. Summary of *Mytilus sp.* larval development tests declared toxic and non-toxic regardless of percent mean effect and those tests declared toxic with a percent mean effect at the IWC < 25% and ≤ 10% for each analysis method grouped by facility. Numbers in parenthesis represent the percentage based on all tests for a given facility.

Facility	N	Number (Percent) of Tests Declared Non-Toxic		Number (Percent) of Tests Declared Toxic		Number (Percent) of Tests Declared Toxic with < 25% Effect at IWC		Number (Percent) of Tests Declared Toxic with ≤ 10% Effect at IWC	
		TST	NOEC	TST	NOEC	TST	NOEC	TST	NOEC
I	4	4 (100)	4 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
F	9	9 (100)	3 (33)	0 (0)	6 (67)	0 (0)	6 (67)	0 (0)	6 (67)
P	6	6 (100)	3 (50)	0 (0)	3 (50)	0 (0)	3 (50)	0 (0)	2 (33)
Q	5	5 (100)	5 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
R	5	5 (100)	5 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

3.6 Chronic *Atherinops affinis* Survival and Growth Test

3.6.1 Survival

A total of 54 *Atherinops affinis* chronic tests were examined for the survival endpoint representing two different facilities. Mean percent effect values for the IWC averaged 0% for both facilities and maximum mean percent effect values were 17% and 36% for facilities D and I, respectively.

TST analysis declared one test toxic based on both facilities combined, while NOEC analysis did not declare any tests as toxic (Table 3-13). The test declared toxic by TST analysis had a mean percent effect of 36% (Facility I). No tests with a mean percent effect < 25% were declared toxic using TST (Table 3-13).

Table 3-13. Summary of chronic *Atherinops affinis* survival tests declared toxic and non-toxic regardless of percent mean effect and those tests declared toxic with a percent mean effect at the IWC < 25% and ≤ 10% for each analysis method grouped by facility. Numbers in parenthesis represent the percentage based on all tests for a given facility.

Facility	N	Number (Percent) of Tests Declared Non-Toxic		Number (Percent) of Tests Declared Toxic		Number (Percent) of Tests Declared Toxic with < 25% Effect at IWC		Number (Percent) of Tests Declared Toxic with ≤ 10% Effect at IWC	
		TST	NOEC	TST	NOEC	TST	NOEC	TST	NOEC
D	11	11 (100)	11 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
I	43	42 (98)	43 (100)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

3.6.2 Growth

This endpoint measures biomass, which is the final dry weight divided by the original number of fish in each test chamber. Mean percent effect values for biomass at the IWC averaged 0.7% and 0.8% for facilities D and I, respectively, and maximum mean percent effect values observed for both facilities did not exceed 25.5%.

TST analysis declared two tests toxic based on both facilities combined, while NOEC analysis declared one test toxic (Table 3-14). One test with a mean percent effect > 25% (25.5% effect Facility I) was declared non-toxic using NOEC. TST analysis declared this test to be toxic. For tests with a mean percent effect < 25% at the IWC, one (2.0%) test was declared toxic using either analysis method (Table 3-14). The test declared toxic using TST analysis had a mean percent effect of 19.1%, while the test found toxic using NOEC analysis had a mean percent effect of 14.7%. No tests with a mean percent effect ≤ 10% at the IWC were declared toxic using either analysis method (Table 3-14). The control SD for the test found toxic using TST with a mean percent effect < 25% was near the 75th percentile based on the distribution observed in California (Table 3-15). The SD at the IWC for this test was at the 90th percentile based on the national distribution (Table 3-15). Adding two replicates to this test resulted in declaring the test non-toxic (Table 3-16).

Table 3-14. Summary of chronic *Atherinops affinis* growth tests declared toxic and non-toxic regardless of percent mean effect and those tests declared toxic with a percent mean effect at the IWC < 25% and < 10% for each analysis method grouped by facility. Numbers in parenthesis represent the percentage based on all tests for a given facility.

Facility	N	Number (Percent) of Tests Declared Non-Toxic		Number (Percent) of Tests Declared Toxic		Number (Percent) of Tests Declared Toxic with < 25% Effect at IWC		Number (Percent) of Tests Declared Toxic with ≤ 10% Effect at IWC	
		TST	NOEC	TST	NOEC	TST	NOEC	TST	NOEC
D	11	11 (100)	11 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
I	43	41 (95)	42 (98)	2 (5)	1 (2)	1 (2)	1 (2)	0 (0)	0 (0)

Table 3-15. Standard deviations (SD) observed in the control and IWC for chronic *Atherinops affinis* growth tests declared toxic using TST and NOEC when the mean percent effect < 25% by facility. (NA = No tests were declared toxic with < 25% effect at the IWC).

Analysis Method / Concentration	Facility	SD	25th Percentile SD (EPA, 2010)	50th Percentile SD (EPA, 2010)	75th Percentile SD (EPA, 2010)	90th Percentile SD (EPA, 2010)
TST / Control	D	NA	0.10	0.15	0.22	0.27
	I	0.22				
TST / IWC	D	NA				
	I	0.30				
NOEC / Control	D	NA				
	I	0.09				
NOEC / IWC	D	NA				
	I	0.26				

Table 3-16. Effect of additional replicates on the result of *Atherinops affinis* growth tests declared toxic when the mean effect is < 25% for facility I.

Test	Mean Effect at IWC (%)	Found Non-Toxic With Additional Replicates	Number of Additional Replicates Needed
1	19.06	Yes	2

3.7 Chronic *Ceriodaphnia dubia* Reproduction Test

A total of 84 chronic *Ceriodaphnia dubia* reproduction tests were evaluated representing five different facilities. Maximum mean percent effect values at the IWC did not exceed 21.0% at facilities H, J, and K, while facilities I and L had maximum effect values of 65.0% and 61.4%, respectively at the IWC.

TST analysis declared ten tests toxic based on all facilities combined, while NOEC analysis declared nine tests toxic (Table 3-17). Two tests with a mean percent effect > 25% at the IWC were declared non-toxic using the NOEC (both from facility L) and both had mean percent effect values of 30.9%. TST analysis declared these tests toxic. Three (3.9%) tests with a mean percent effect < 25% were declared toxic using TST, and four (5.2%) tests were declared toxic using NOEC analysis (Table 3-17). The tests declared toxic using TST had mean percent effect values of 19.4%, 20.6%, and 20.8% at the IWC, while the tests declared toxic using the NOEC had mean percent effect values of 5.0%, 5.7%, 9.9%, and 19.4% at the IWC. TST did not declare any tests with a mean percent effect ≤ 10% as toxic; however, NOEC analysis declared three tests toxic with effect values ≤ 10% at the IWC (Table 3-17).

For those tests declared toxic using TST analysis with a mean percent effect < 25%, control SDs were near the 75th percentile based on the national distribution (USEPA, 2010; Table 3-18). SDs at the IWC for these same tests were ≥ 90th percentile of the national distribution (USEPA, 2010; Table 3-18).

Table 3-17. Summary of *Ceriodaphnia dubia* reproduction tests declared toxic and non-toxic regardless of percent mean effect and those tests declared toxic with a percent mean effect at the IWC < 25% and ≤ 10% for each analysis method grouped by facility. Numbers in parenthesis represent the percentage based on all tests for a given facility.

Facility	N	Number (Percent) of Tests Declared Non-Toxic		Number (Percent) of Tests Declared Toxic		Number (Percent) of Tests Declared Toxic with < 25% Effect at IWC		Number (Percent) of Tests Declared Toxic with ≤ 10% Effect at IWC	
		TST	NOEC	TST	NOEC	TST	NOEC	TST	NOEC
H	27	27 (100)	27 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
I	7	6 (86)	6 (86)	1 (14)	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)
J	15	14 (93)	13 (87)	1 (7)	2 (13)	1 (7)	2 (13)	0 (0)	2 (13)
K	15	14 (93)	13 (87)	1 (7)	2 (13)	1 (7)	2 (13)	0 (0)	1 (7)
L	20	13 (65)	16 (80)	7 (35)	4 (20)	1 (5)	0 (0)	0 (0)	0 (0)

Table 3-18. Range of standard deviations (SD) observed in the control and IWC for the *Ceriodaphnia dubia* reproduction tests declared toxic using TST and NOEC when the mean percent effect < 25% by facility and compared to the national distribution from EPA, 2010 (NA = No tests were declared toxic with < 25% effect at the IWC).

Analysis Method / Concentration	Facility	Minimum SD	Median SD	Maximum SD	25th Percentile SD (EPA, 2010)	50th Percentile SD (EPA, 2010)	75th Percentile SD (EPA, 2010)	90th Percentile SD (EPA, 2010)
TST / Control	H	NA	NA	NA	2.64	3.79	5.82	8.41
	I	NA	NA	NA				
	J	5.02	5.02	5.02				
	K	4.84	4.84	4.84				
	L	5.27	5.27	5.27				
TST / IWC	H	NA	NA	NA				
	I	NA	NA	NA				
	J	10.70	10.70	10.70				
	K	10.15	10.15	10.15				
	L	8.48	8.48	8.48				
NOEC / Control	H	NA	NA	NA				
	I	NA	NA	NA				
	J	9.51	9.96	10.41				
	K	3.66	4.25	4.84				
	L	NA	NA	NA				
NOEC / IWC	H	NA	NA	NA				
	I	NA	NA	NA				
	J	3.16	5.58	7.99				
	K	3.30	6.73	10.15				
	L	NA	NA	NA				

For two of those three tests in which TST declared the test toxic and there was < 25% mean effect at the IWC, the addition of up to 10 more replicates (total of 20 replicates each for the IWC and control) did not change the results to non-toxic due to high within-test variability (Table 3-19). The test with an effect of 20.6% was found non-toxic with seven additional replicates (total of 17 replicates; Table 3-19).

Table 3-19. Effect of adding additional replicates on the result of *Ceriodaphnia dubia* reproduction tests declared toxic when the mean effect is < 25% for facility I. NC = no change in result using up to ten additional replicates (total of 20 replicates).

Facility	Mean Effect at IWC (%)	Found Non-Toxic With Additional Replicates	Number of Additional Replicates Needed
J	20.83	No	NC
K	19.44	No	NC
L	20.59	Yes	7

3.8 Chronic *Pimephales promelas* Survival and Growth Test

3.8.1 Survival

A total of 83 chronic *Pimephales promelas* tests were evaluated representing three different facilities. Mean percent effect values for the IWC averaged zero for survival for all three facilities. No facility had a maximum mean percent effect that was ≥ 25%. The maximum mean percent mean values observed at facilities H and J were 10.3 and 2.5, respectively.

TST analysis declared three tests toxic for survival while NOEC analysis declared one test toxic from all facilities combined (Table 3-20). For tests with a mean percent effect < 25% at the IWC, two (0.3) were declared toxic using TST analysis and one (1.2%) was declared toxic using NOEC analysis (Table 3-20). The mean percent effect values for the two tests declared toxic using TST were 19.4% and 23.1%. The test found toxic using NOEC analysis was one of the tests found toxic using TST with a 23.1% mean effect. Both TST and NOEC analysis did not declare any tests with ≤ 10% effect at the IWC as toxic (Table 3-20).

Table 3-20. Summary of the *Pimephales promelas* chronic survival tests declared toxic and non-toxic regardless of mean percent effect and those tests declared toxic with a mean percent effect at the IWC < 25% and ≤ 10% for each analysis method grouped by facility. Numbers in parenthesis represent the percentage based on all tests for a given facility.

Facility	N	Number (Percent) of Tests Non-Toxic		Number (Percent) of Tests Toxic		Number (Percent) of Tests Toxic with ≤ 25% Effect at IWC		Number (Percent) of Tests Toxic with ≤ 10% Effect at IWC	
		TST	NOEC	TST	NOEC	TST	NOEC	TST	NOEC
H	13	13 (100)	13 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
I	43	41 (95)	42 (98)	2 (5)	1 (2)	2 (5)	1 (2)	0 (0)	0 (0)
J	27	27 (100)	27 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

For the two tests declared toxic using TST with a mean percent effect < 25%, control SDs were between the 50th and 90th percentile based on the national distribution (USEPA, 2010; Table 3-21). SDs at the IWC for these same two tests was between the 50th and nearly double the 90th percentile based on the national distribution (USEPA, 2010; Table 3-21). For these two tests one and three additional replicates, respectively, resulted in declaring the test non-toxic using TST (Table 3-22).

Table 3-21. Range of standard deviations (SDs) observed in the control and IWC for the *Pimephales promelas* chronic survival tests declared toxic using TST and NOEC when the mean percent effect < 25% by facility and compared to the national distributions for Pimephales promelas growth values from USEPA, 2010 (NA = No tests were declared toxic with < 25% effect at the IWC).

Analysis Method / Concentration	Facility	Minimum SD	Median SD	Maximum SD	25th Percentile SD (EPA, 2010)	50th Percentile SD (EPA, 2010)	75th Percentile SD (EPA, 2010)	90th Percentile SD (EPA, 2010)
TST / Control	H	NA	NA	NA	0.03	0.05	0.08	0.11
	I	0.05	0.06	0.08				
	J	NA	NA	NA				
TST / IWC	H	NA	NA	NA				
	I	0.06	0.13	0.21				
	J	NA	NA	NA				
NOEC / Control	H	NA	NA	NA				
	I	0.05	0.05	0.05				
	J	NA	NA	NA				
NOEC / IWC	H	NA	NA	NA				
	I	0.06	0.06	0.06				
	J	NA	NA	NA				

Table 3-22. Effect of adding additional replicates on the result of *Pimephales promelas* survival tests declared toxic when the mean effect is < 25% for facility I.

Test	Mean Effect at IWC (%)	Found Non-Toxic With Additional Replicates	Number of Additional Replicates Needed
1	19.44	Yes	1
3	23.08	Yes	3

3.8.2 Growth

Similar to the *Atherinops* chronic test, this endpoint measures biomass. A total of 83 chronic *Pimephales promelas* chronic tests were evaluated for the biomass (weight) endpoint representing three different facilities. Median mean percent effect values for the IWC averaged 1.2%, 2.2%, and 2.7% for facilities H, I, and J, respectively. Maximum effect values were 7.0%, 18.8%, and 10.2% for facilities H, I, and J, respectively.

TST analysis declared three tests toxic (all from facility I), while NOEC declared no tests toxic for this endpoint (Table 3-23). All three tests had a mean percent effect < 25% at the IWC (18.8%, 17.4%, and 14.8%, Table 3-23). Both TST and NOEC analysis did not declare any tests with ≤ 10% effect at the IWC as toxic (Table 3-23).

Among those tests declared toxic using TST with a mean percent effect < 25%, control SDs were between 25th and > 90th percentile based on the national distribution (USEPA, 2010; Table 3-24). SDs for the IWC in these three tests were between the 50th and > 90th percentile based on the national distribution (USEPA, 2010; Table 3-24). For the three tests, two were declared not toxic using TST analysis with the addition of one replicate (Table 3-25). These two tests had mean percent effects of 17.4% and 14.8%. The test that had a mean effect of 18.8% was declared toxic with up to five additional replicates (Table 3-25). The SDs in the control and IWC for this test at Facility I were both at the 50th percentile.

Table 3-23. Summary of chronic *Pimephales promelas* growth tests declared toxic and non-toxic regardless of percent mean effect and those tests declared toxic with a percent mean effect at the IWC < 25% and ≤ 10% for each analysis method grouped by facility. Numbers in parenthesis represent the percentage based on all tests for a given facility.

Facility	N	Number (Percent) of Tests Declared Non-Toxic		Number (Percent) of Tests Declared Toxic		Number (Percent) of Tests Declared Toxic with ≤ 25% Effect at IWC		Number (Percent) of Tests Declared Toxic with ≤ 10% Effect at IWC	
		TST	NOEC	TST	NOEC	TST	NOEC	TST	NOEC
H	13	13 (100)	13 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
I	43	40 (93)	43 (100)	3 (7)	0 (0)	3 (7)	0 (0)	0 (0)	0 (0)
J	27	27 (100)	27 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 3-24. Range of standard deviations (SD) observed in the control and IWC for chronic *Pimephales promelas* growth tests declared toxic using TST and NOEC when the mean percent effect < 25% by facility and compared to the national distributions from USEPA, 2010 (NA = No tests were declared toxic with < 25% effect at the IWC).

Analysis Method / Concentration	Facility	Minimum SD	Median SD	Maximum SD	25th Percentile SD (EPA, 2010)	50th Percentile SD (EPA, 2010)	75th Percentile SD (EPA, 2010)	90th Percentile SD (EPA, 2010)
TST / Control	H	NA	NA	NA	0.03	0.05	0.08	0.11
	I	0.03	0.05	0.13				
	J	NA	NA	NA				
TST / IWC	H	NA	NA	NA				
	I	0.05	0.05	0.13				
	J	NA	NA	NA				
NOEC / Control	H	NA	NA	NA				
	I	NA	NA	NA				
	J	NA	NA	NA				

Table 3-24. Continued.

Analysis Method / Concentration	Facility	Minimum SD	Median SD	Maximum SD	25th Percentile SD (EPA, 2010)	50th Percentile SD (EPA, 2010)	75th Percentile SD (EPA, 2010)	90th Percentile SD (EPA, 2010)
NOEC / IWC	H	NA	NA	NA				
	I	NA	NA	NA				
	J	NA	NA	NA				

Table 3-25. Effect of adding additional replicates on the result of *Pimephales promelas* growth tests declared toxic when the mean effect is < 25% for facility I. NC = no change in result using up to five additional (total of 9) replicates.

Test	Mean Effect at IWC (%)	Found Non-Toxic With Additional Replicates	Number of Additional Replicates Needed
1	17.40	Yes	1
2	14.79	Yes	1
3	18.83	No	NC

3.9 *Selenastrum capricornutum* Growth Test

A total of 45 *Selenastrum capricornutum* growth tests were evaluated representing two different facilities. Maximum mean percent effect values were 23.4% and 89.4% for facilities H and C, respectively.

TST analysis declared 24 tests toxic based on both facilities combined, while NOEC analysis declared 25 tests toxic (Table 3-26). For tests with a mean percent effect < 25% at the IWC, TST analysis declared one test (4.6%) as toxic while NOEC analysis declared two (9.1%) tests as toxic (Table 3-26). The test declared toxic using TST had a mean percent effect of 23.4% at the IWC and the two tests declared using NOEC had a mean percent effect of 11.4% and 12.7%. No tests with a mean percent effect ≤ 10% at the IWC were declared toxic using either analysis method (Table 3-26).

Table 3-26. Summary of *Selenastrum capricornutum* growth tests declared toxic and non-toxic regardless of percent mean effect and those tests declared toxic with a percent mean effect at the IWC < 25% and ≤ 10% for each analysis method grouped by facility. Numbers in parenthesis represent the percentage based on all tests for a given facility.

Facility	N	Number (Percent) of Tests Declared Non-Toxic		Number (Percent) of Tests Declared Toxic		Number (Percent) of Tests Declared Toxic with < 25% Effect at IWC		Number (Percent) of Tests Declared Toxic with ≤ 10% Effect at IWC	
		TST	NOEC	TST	NOEC	TST	NOEC	TST	NOEC
C	31	8 (26)	6 (19)	23 (74)	25 (81)	0 (0)	2 (5)	0 (0)	0 (0)
H	14	13 (93)	14 (100)	1 (7)	0 (0)	1 (7)	0 (0)	0 (0)	0 (0)

Control SD for the one test declared toxic using TST with a mean percent effect < 25% was nearly double the 90th percentile based on the national distribution (USEPA, 2010; Table 3-27). The SD at the IWC was over four times the 90th percentile based on the national distribution (USEPA, 2010; Table 3-27). The use of up to five additional replicates for this test did not change the result using TST; the test was still declared toxic (Table 3-28) due to the very high within-test variability.

Table 3-27. Range of standard deviations (SD) observed in the control and IWC for *Selenastrum capricornutum* growth tests declared toxic using TST and NOEC when the mean percent effect < 25% by facility and compared to the national distributions from USEPA, 2010 (NA = No tests were declared toxic with < 25% effect at the IWC).

Analysis Method / Concentration	Facility	Minimum SD	Median SD	Maximum SD	25th Percentile SD (EPA, 2010)	50th Percentile SD (EPA, 2010)	75th Percentile SD (EPA, 2010)	90th Percentile SD (EPA, 2010)
TST / Control	C	NA	NA	NA	135154.20	309232.90	447446.50	583299.40
	H	963200	963200	963200				
TST / IWC	C	NA	NA	NA				
	H	2800000	2800000	2800000				
NOEC / Control	C	25900	28650	31400				
	H	NA	NA	NA				
NOEC / IWC	C	24500	31600	38700				
	H	NA	NA	NA				

Table 3-28. Effect of additional replicates on the result of *Selenastrum capricornutum* growth tests declared toxic when the mean effect is < 25% for facility H. NC = no change in result with up to five additional (total of 10) replicates.

Test	Mean Effect at IWC (%)	Found Non-Toxic With Additional Replicates	Number of Additional Replicates Needed
1	23.39	No	NC

3.10 Acute Daphnid Survival Test

A total of 82 acute daphnid survival tests using either *Daphnia pulex* or *Ceriodaphnia dubia* were evaluated for several facilities in Washington State. Mean percent effect was 0% for 75% of the tests and the maximum percent effect observed was 100%.

TST analysis declared four tests toxic from all facilities combined while NOEC analysis declared one test toxic (Table 3-29). One test was declared non-toxic using NOEC analysis with a mean percent effect > 20% in the IWC (21.0% effect). TST declared this test toxic. For tests with a mean percent effect < 20% at the IWC, two (2.5%) were declared toxic using TST analysis and no tests were declared toxic using the NOEC analysis (Table 3-29). The two tests declared toxic

using TST had mean percent effect values of 10.5% and 15.0%. Neither analysis method declared any tests toxic with a mean percent effect ≤ 10% (Table 3-29).

For the two tests found toxic using TST analysis at a mean percent effect < 20%, one test had a control standard deviation equal to the 90th percentile based on the national distribution (USEPA, 2010) (Table 3-30). SDs at the IWC for these two tests were either equal to or nearly double the 90th percentile of the national distribution (USEPA, 2010) (Table 3-30). Adding one or two replicates to either of these two tests resulted in TST declaring the tests non-toxic (Table 3-31).

Table 3-29. Summary of acute daphnid survival tests declared toxic and non-toxic regardless of the mean percent effect and those tests declared toxic with a mean percent effect at the IWC < 20% and ≤ 10% for each analysis method. Numbers in parenthesis represent the percentage based on all tests for a given facility.

Facility	N	Number (Percent) of Tests Declared Non-Toxic		Number (Percent) of Tests Declared Toxic		Number (Percent) of Tests Declared Toxic with < 20% Effect at IWC		Number (Percent) of Tests Declared Toxic with ≤ 10% Effect at IWC	
		TST	NOEC	TST	NOEC	TST	NOEC	TST	NOEC
I	82	78 (95)	81 (99)	4 (5)	1 (1)	2 (2)	0 (0)	0 (0)	0 (0)

Table 3-30. Range of standard deviations (SD) observed in the control and IWC for acute daphnid survival tests declared toxic using TST and NOEC when the mean percent effect was < 20% compared to the national distributions from, 2010b (NA = No tests were declared toxic with < 20% effect at the IWC).

Analysis Method / Concentration	Facility	Minimum SD	Median SD	Maximum SD	25th Percentile SD (EPA, 2010)	50th Percentile SD (EPA, 2010)	75th Percentile SD (EPA, 2010)	90th Percentile SD (EPA, 2010)
TST / Control	I	0.00	0.05	0.10	0.00	0.00	0.00	0.10
TST / IWC	I	0.10	0.15	0.19				
NOEC / Control	I	NA	NA	NA				
NOEC / IWC	I	NA	NA	NA				

Table 3-31. Effect of adding additional replicates on the result of the acute daphnid tests declared toxic when the mean effect is < 20% for facility I.

Test	Mean Effect at IWC (%)	Found Non-Toxic With Additional Replicates	Number of Additional Replicates Needed
1	15.00	Yes	1
2	10.53	Yes	2

3.11 Acute Four Replicate Fish Survival Test

A total of 99 acute, four replicate fish survival tests using *Pimephales promelas*, *Atherinops affinis*, *Oncorhynchus mykiss*, or *Menidia beryllina* were evaluated representing one facility in California (D), as well as several in Washington State (I). Facility D had a mean percent effect at the IWC between 0% and 5.0%. The Washington facilities had mean percent effect values ranging between 0 and 10.0%.
TST analysis resulted in one test declared toxic while NOEC analysis declared no tests toxic (Table 3-32). For tests with a mean percent effect < 20% at the IWC, one (1.0%) test was declared toxic using TST analysis and no tests were declared toxic using NOEC analysis (Table 3-32). The test found toxic using TST analysis had a mean percent effect value of 10.0% and a SD in the IWC near the 90th percentile control SD found in USEPA, 2010 (Table 3-33).

Table 3-32. Summary of the acute 4 replicate fish survival tests declared toxic and non-toxic regardless of percent mean effect and those tests declared toxic with a percent mean effect at the IWC < 20% and ≤ 10% for each analysis method grouped by facility. Numbers in parenthesis represent the percentage based on all tests for a given facility.

Facility	N	Number (Percent) of Tests Declared Non-Toxic		Number (Percent) of Tests Declared Toxic		Number (Percent) of Tests Declared Toxic with ≤ 20% Effect at IWC		Number (Percent) of Tests Declared Toxic with ≤ 10% Effect at IWC	
		TST	NOEC	TST	NOEC	TST	NOEC	TST	NOEC
D	15	15 (100)	15 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
I	84	83 (99)	84 (100)	1 (1)	0 (0)	1 (1)	0 (0)	1 (1)	0 (0)

Table 3-33. Standard deviations (SD) observed in the control and IWC for the 4 replicate fish acute survival tests declared toxic using TST and NOEC when the mean percent effect < 20% by facility and compared to the national distributions from USEPA, 2010 (NA = No tests were declared toxic with < 20% effect at the IWC).

Analysis Method/ Concentration	Facility	SD	25th Percentile SD (EPA, 2010)	50th Percentile SD (EPA, 2010)	75th Percentile SD (EPA, 2010)	90th Percentile SD (EPA, 2010)
TST / Control	D	NA	0.00	0.00	0.00	0.18
	I	0				
TST / IWC	D	NA				
	I	0.14				
NOEC / Control	D	NA				
	I	NA				
NOEC / IWC	D	NA				
	I	NA				

For the one test declared toxic using TST with a mean percent effect < 20%, one additional replicate was sufficient to declare the test non-toxic (Table 3-34).

Table 3-34. Effect of adding additional replicates on the result of acute fish tests declared toxic when the mean effect is < 20% for facility I.

Test	Mean Effect at IWC (%)	Found Non-Toxic With Additional Replicates	Number of Additional Replicates Needed
1	10.00	Yes	1

3.12 Acute Four Replicate *Americamysis bahia* Survival Test

A total of 18 acute *Americamysis bahia* survival tests were evaluated representing two different facilities. Maximum percent effect values from facilities D and I did not exceed 7.9%. TST and NOEC analysis did not declare any of these tests toxic (Table 3-35).

Table 3-35. Summary of acute *Americamysis bahia* survival tests declared toxic and non-toxic regardless of percent mean effect and those tests declared toxic with a percent mean effect at the IWC < 20% and ≤ 10% for each analysis method grouped by facility. Numbers in parenthesis represent the percentage based on all tests for a given facility.

Facility	N	Number (Percent) of Tests Declared Non-Toxic		Number (Percent) of Tests Declared Toxic		Number (Percent) of Tests Declared Toxic with < 20% Effect at IWC		Number (Percent) of Tests Declared Toxic with ≤ 10% Effect at IWC	
		TST	NOEC	TST	NOEC	TST	NOEC	TST	NOEC
D	10	10 (100)	10 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
I	8	8 (100)	8 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

3.13 Small Facility Results – All Methods

A total of 16 WET tests were provided from three smaller facilities (from underprivileged communities) in California. Data included one *Selenastrum capricornutum* growth test, six *Ceriodaphnia dubia* reproduction tests, seven *Pimephales promelas* survival and growth tests, and two acute *Ceriodaphnia dubia* survival tests.

TST analysis resulted in ten tests declared toxic while NOEC analysis declared six tests toxic (Table 3-36). For tests with a mean percent effect < 25% for chronic tests or <20% for acute tests at the IWC, five (27.8%) tests were declared toxic using TST analysis and two (11.1%) tests were declared toxic using NOEC analysis (Table 3-36). One chronic *P. promelas* test with a mean percent effect value of 7.9% was declared toxic based on survival using NOEC analysis, while TST analysis did not declare any tests with a effect ≤ 10% as toxic (Table 3-36).

The three *Ceriodaphnia dubia* chronic tests declared toxic using TST analysis had mean percent effect values of 11.8%, 18.3%, and 13.2%. The control SD for the test with an effect of 18.3% fell in the 25th to 50th percentile range of values reported in USEPA, 2010, while the IWC SD for this test was > 75th percentile (Table 3-37). Control SD for the other tests was considerably > 90th percentile reported in the national distribution (USEPA, 2010).

Table 3-36. Summary of the small facility tests declared toxic and non-toxic regardless of percent mean effect and those tests declared toxic with a percent mean effect at the IWC <25% (20% for acute) and ≤ 10% for each analysis method grouped by WET test method. Numbers in parenthesis represent the percentage based on all tests for a given facility.

WET Test Method	Number (Percent) of Tests Declared Non-Toxic		Number (Percent) of Tests Declared Toxic		Number (Percent) of Tests Declared Toxic with < 25% Effect (Chronic tests) or 20% Effect (Acute tests) at IWC		Number (Percent) of Tests Declared Toxic with ≤ 10% Effect at IWC	
	TST	NOEC	TST	NOEC	TST	NOEC	TST	NOEC
Selenastrum Growth	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)
C. dubia Reproduction	0 (0)	4 (67)	6 (100)	2 (33)	3 (50)	0 (0)	0 (0)	0 (0)
P. promelas Chronic Survival	6 (86)	5 (71)	1 (14)	2 (29)	1 (14)	2 (29)	0 (0)	1 (14)
P. promelas Growth	6 (86)	7 (100)	1 (14)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)
C. dubia Acute Survival	1 (50)	1 (50)	1 (50)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)

Table 3-37. Range of standard deviations (SD) observed in the control and IWC for tests declared toxic using TST and NOEC when the mean percent effect < 25% compared to the national distributions from USEPA, 2010 (NA = No tests were declared toxic with < 25% effect at the IWC).

Analysis Method / Concentration	Test Endpoint	Minimum SD	SD	Maximum SD	25th Percentile SD (EPA, 2010)	50th Percentile SD (EPA, 2010)	75th Percentile SD (EPA, 2010)	90th Percentile SD (EPA, 2010)
TST / Control	C.dubia reproduction	3.23	12.28	12.98	2.64	3.79	5.82	8.41
	P. promelas chronic survival	0.06	0.06	0.06	0.03	0.05	0.08	0.11
	P. promelas chronic growth	0.02	0.02	0.02	0.03	0.05	0.08	0.11
TST / IWC	C.dubia reproduction	7.01	7.75	9.80	2.64	3.79	5.82	8.41
	P. promelas chronic survival	0.39	0.39	0.39	0.03	0.05	0.08	0.11
	P. promelas chronic growth	0.14	0.14	0.14	0.03	0.05	0.08	0.11

Table 3-37. Continued

Analysis Method / Concentration	Test Endpoint	Minimum SD	SD	Maximum SD	25th Percentile SD (EPA, 2010)	50th Percentile SD (EPA, 2010)	75th Percentile SD (EPA, 2010)	90th Percentile SD (EPA, 2010)
NOEC / Control	C.dubia reproduction	NA	NA	NA	2.64	3.79	5.82	8.41
	P. promelas chronic survival	0.00	0.03	0.06	0.03	0.05	0.08	0.11
	P. promelas chronic growth	0.02	0.02	0.02	0.03	0.05	0.08	0.11
NOEC / IWC	C.dubia reproduction	NA	NA	NA	2.64	3.79	5.82	8.41
	P. promelas chronic survival	0.05	0.07	0.10	0.03	0.05	0.08	0.11
	P. promelas chronic growth	0.03	0.03	0.03	0.03	0.05	0.08	0.11

One *Pimephales promelas* survival and growth test was declared toxic using TST analysis for both endpoints with mean effect values of 18.4% and 13.1%, respectively (Table 3-37). Control SD for each endpoint was $\leq 50^{th}$ percentile value observed in the national distribution (Table 3-37; USEPA, 2010). However, IWC variability for both endpoints was $\geq 90^{th}$ percentile value reported in the national distribution (Table 3-37).

For all five chronic tests that were declared toxic using TST and had mean percent effect < 25%, no more than three additional replicates were needed to declare the test not toxic (Table 3-38). In all but one of the tests, only one additional replicate was sufficient to declare the test not toxic.

Table 3-38. Effect of adding additional replicates on TST results for WET tests initially declared toxic (mean percent effect at the IWC was < 25% for these chronic tests).

Test	Endpoint	Mean % Effect at IWC	Found Non-Toxic with Additional Replicates	# of Additional Replicates Needed
1	C. dubia Reproduction	11.84	Yes	1
2	C. dubia Reproduction	18.32	Yes	1
3	C. dubia Reproduction	13.21	Yes	3
4	P. promelas Growth	18.42	Yes	1
5	P. promelas Survival	13.07	Yes	1

4.0 SUMMARY

Based on the 775 valid WET tests evaluated, including all methods commonly used in California, there was high concordance between results obtained using either the TST or the NOEC analysis approach (Table 4-1, Figure 4-1). Both approaches declared a similar percentage of the tests non-toxic or toxic (Table 4-1). For those tests which had a mean effect at the IWC less than the toxic Regulatory Management Decision of 25% for chronic methods or 20% for acute methods, TST analysis declared fewer (2.9%) of those tests as toxic compared to the NOEC approach (5.3%; Table 4-1; Figure 4-1). In addition, TST analysis declared a very low percentage (0.1%) of all tests as toxic which had an effect less than or equal to the non-toxic RMD of 10%, while NOEC analysis declared 2.6% of those tests as toxic (Table 4-1). Thus truly non-toxic samples were more often declared non-toxic using TST than using the NOEC approach. In the few cases where TST detected toxicity at effects less than the toxic RMD but above the non-toxic RMD, this was due to high variability between replicates in the controls and/or IWC treatments. Addition of a minimal number of replicates to these tests usually resulted in the sample being declared non-toxic using the TST procedure.

Table 4-1. Summary of all WET method tests declared toxic and non-toxic regardless of percent mean effect and those declared toxic with a percent mean effect at the IWC < 25% and ≤ 10% for each analysis method grouped by test type. Numbers represent the percentage based on all tests for a given method type.

Method Type	Number (Percent) of Tests Declared Non-Toxic		Number (Percent) of Tests Declared Toxic [1]		Number (Percent) of Tests Declared Toxic with < 25% (20% for Acute) Effect at IWC[2]		Number (Percent) of Tests Declared Toxic with ≤ 10% Effect at IWC	
	TST	NOEC	TST	NOEC	TST	NOEC	TST	NOEC
Chronic Marine	311 (89.4)	291 (83.6)	37 (10.6)	57 (16.4)	8 (2.3)	31 (8.9)	0 (0)	16 (4.6)
Chronic Freshwater	181 (80.1)	186 (82.3)	45 (19.9)	40 (17.7)	11 (4.9)	9 (4.0)	0 (0)	4 (1.8)
Acute Marine	18 (100)	18 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Acute Freshwater	177 (96.8)	181 (98.9)	6 (3.2)	2 (1.1)	3 (1.6)	0 (0)	1 (0.5)	0 (0)
All Methods	**687 (88.6)**	**676 (87.2)**	**88 (11.4)**	**99 (12.8)**	**22 (2.9)**	**40 (5.3)**	**1 (0.1)**	**20 (2.6)**

1 This includes tests which are truly toxic above the RMD of 20% for acute or 25% for chronic, as well as those tests with effects below the respective RMDs.

2 This includes tests with effects less than or equal to the non-toxic RMD of 10% effect at the IWC

For tests with a mean effect at the IWC greater than or equal to the toxic RMD, TST analysis resulted in a much lower rate of non-toxic tests than the NOEC analysis (Figure 4-2). Thus, TST more consistently identified truly toxic samples than the NOEC analysis.

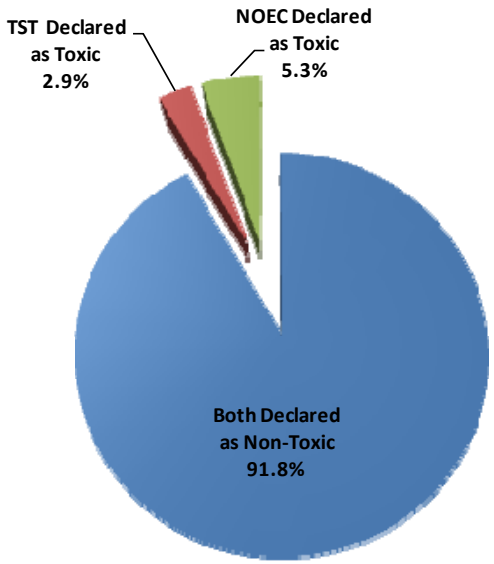

Figure 4-1. Summary of the tests from all methods that were declared toxic using TST and NOEC analysis with a mean effect at the IWC less than the toxic RMD of 25% for chronic or 20% for acute tests. These percentages include those tests having effects at the IWC less than or equal to the non-toxic RMD of 10%.

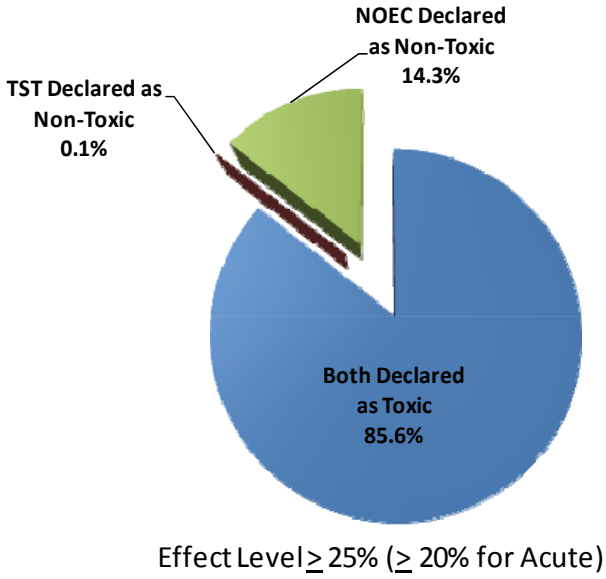

Figure 4-2. Summary of the tests from all methods that were declared non-toxic using TST and NOEC analysis with a mean effect at the IWC greater than or equal to the toxic RMD of 25% for chronic or 20% for acute tests.

5.0 LITERATURE CITED

Denton. D., J. Diamond, and L. Zheng. 2011. Test of significant toxicity: a statistical application for assessing whether an effluent or site water is truly toxic. *Environmental Toxicology and Chemistry* 30:1117-1126.

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