Whole Effluent Acute Toxicity Testing and Toxicity Provisions Species Screening Requirements

Drew Gantner
McCampbell Analytical
April 26, 2022

Webinar Overview

Part I – Acute Toxicity Testing

- Staff training.
- Root cause analysis for failed bioassays.
- Normalizing reference toxicant results.
- Common types of reference toxicants used.

Part II – Report Review and Upcoming Toxicity Provisions

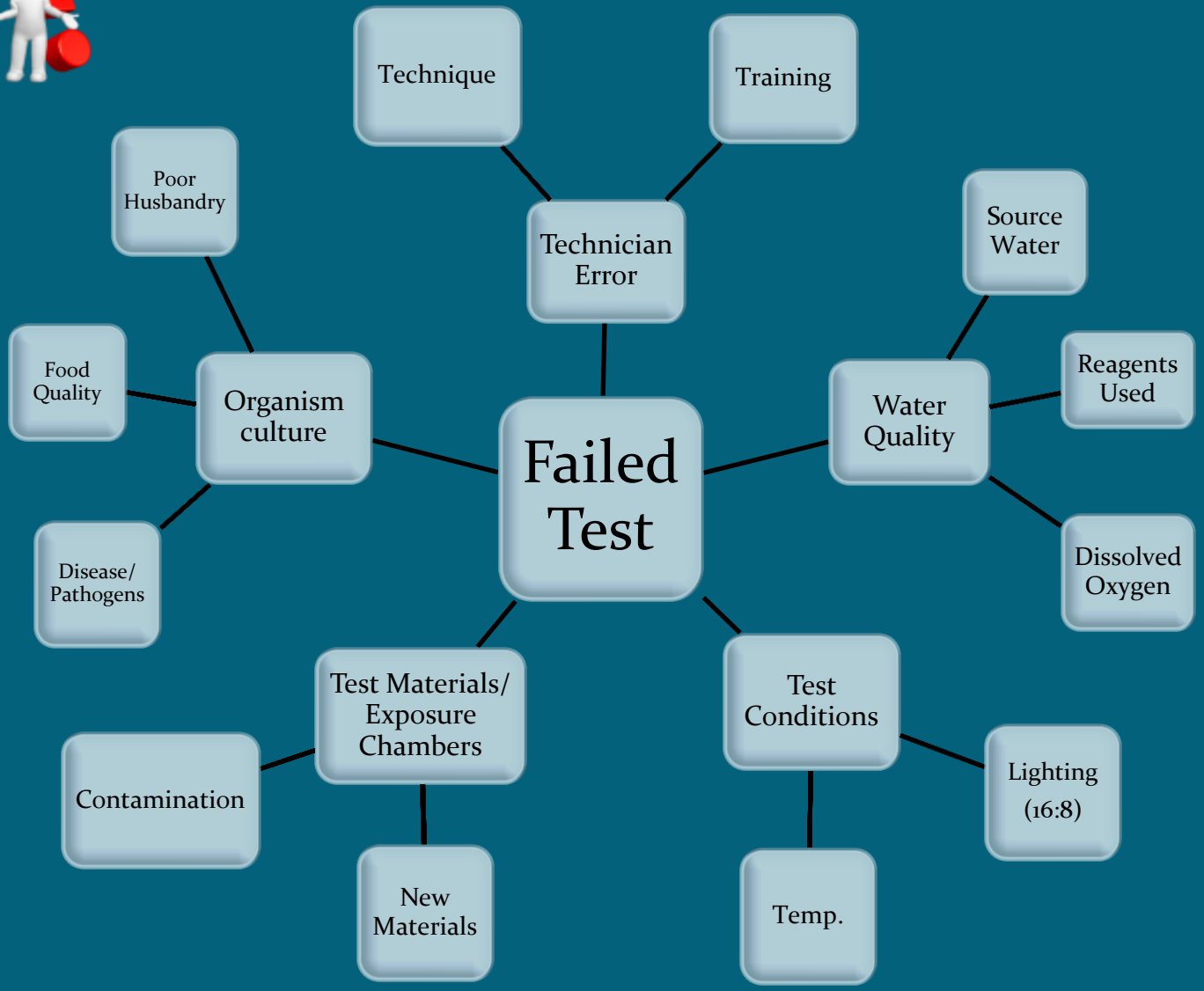
- Report Review Process.
- Extracting acute data from chronic bioassays.
- Toxicity Provisions and what they mean for POTWs.
- Species screening process under the new Toxicity Provisions.

Staff Training

Is group training acceptable under TNI Module 7?

- As long as the analyst(s) participate in all critical aspects of the testing.
  - Initiation, maintenance, termination.
  - Sometimes requires multiple tests for alternating shifts.
  - Ongoing DOC does not need to be the same test and can be split between multiple tests, however analyst proficiency is based on the earliest date one aspect of the test is performed.
  - MAI has separate DOCs for water chemistry parameters (e.g. pH, D.O. Alkalinity, Hardness, etc.)

Root Cause Analysis



Reference Toxicant Sensitivity and "Saving" Data

- If a RT response is outside of ±2SD, it does not automatically invalidate the test.

4.15.6 Reference toxicant test results should not be used as a *de facto* criterion for rejection of individual effluent or receiving water tests. Reference toxicant testing is used for evaluating the health and sensitivity of organisms over time and for documenting initial and ongoing laboratory performance. While reference toxicant test results should not be used as a *de facto* criterion for test rejection, effluent and receiving water test results should be reviewed and interpreted in the light of reference toxicant test results. The reviewer should consider the degree to which the reference toxicant test result fell outside of control chart limits, the width of the limits, the direction of the deviation (toward increased test organism sensitivity or toward decreased test organism sensitivity), the test conditions of both the effluent test and the reference toxicant test, and the objective of the test.

- Reference toxicant QC charts can be adjusted to reflect national averages

4.15.5 If the toxicity value from a given test with the reference toxicant falls well outside the expected range for the test organisms when using the standard dilution water, the laboratory should investigate sources of variability, take corrective actions to reduce identified sources of variability, and perform an additional reference toxicant test during the same month. Performance should improve with experience, and the control limits for point estimates should gradually narrow. However, control limits of ±2S, by definition, will be exceeded 5% of the time, regardless of how well a laboratory performs. Highly proficient laboratories which develop a very narrow control limit may be unfairly penalized if a test which falls just outside the control limits is rejected *de facto*. For this reason, the width of the control limits should be considered in determining whether or not a reference toxicant test result falls "well" outside the expected range. The width of the control limits may be evaluated by comparing the calculated CV (i.e., standard deviation / mean) of the LC50 for the 20 most recent data points to the distribution of laboratory CVs reported nationally for reference toxicant testing (Table 3-3 in USEPA, 2000b). In determining whether or not a

- Per US EPA 2000b. "Understanding and accounting for method variability in whole effluent toxicity applications under the NPDES program." Section 5.3.1.1:
  - "If a laboratory's CV exceeds the 75th percentile CV from Tables 3-2 through 3-4, EPA recommends calculating warning and control limits based on the 75th and 90th percentiles, respectively, of CVs for the method and endpoint. (Tables 3-2 and 3-3 and Appendix Tables B-1 and B-2)."
  - Recommendations if normalization of QC charts is required:
    - Set control limits using 90th percentile
    - Set warning limits using 75th percentile
    - Promptly take actions to bring the results to be within the control limits.
    - Attempt to bring results within the warning limits in 3-12 months.

NOTE: Normalizing the data only works for species listed in the tables and for test methods that have three or more labs reporting.

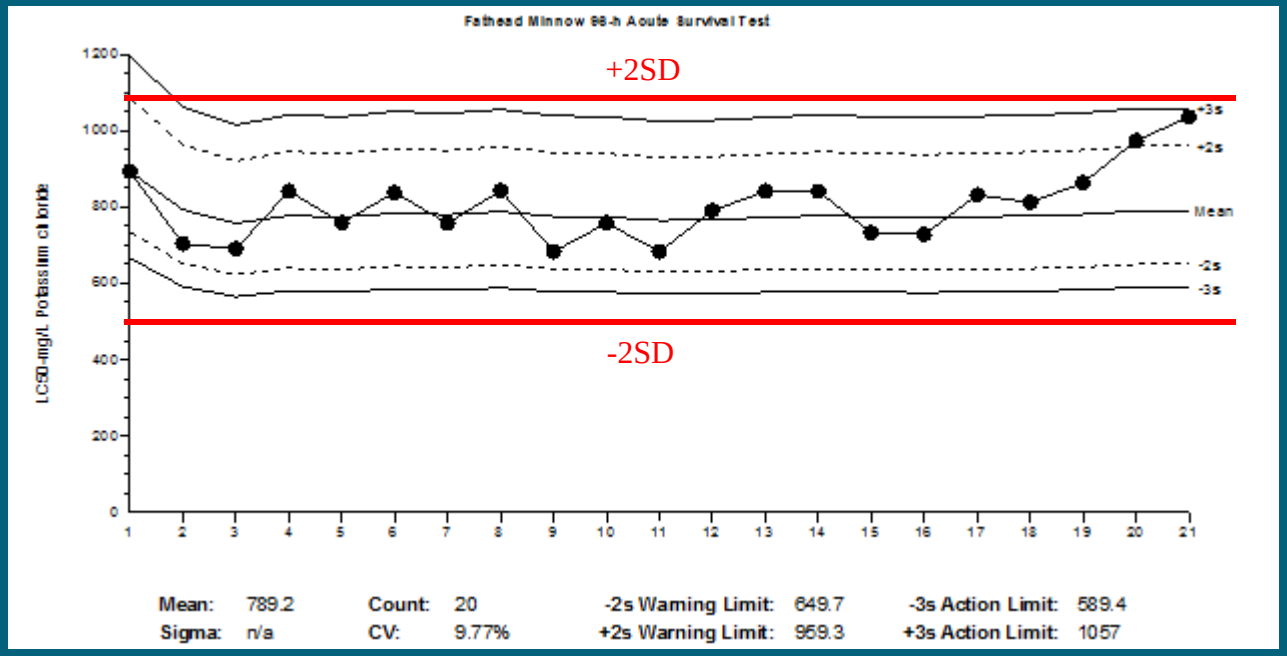
Table 3-3. Quartiles (25th and 75th) and Median (50th) of the Within-Laboratory Values of CV for LC50

Test Method[a]	Test Method No.	Endpoint	No. of Labs	Percentiles of CV		
				25th	50th	75th
Freshwater Methods for Chronic Toxicity[c]						
Fathead Minnow Larval Survival & Growth	1000.0	S	19	0.15	0.23	0.31
Ceriodaphnia (Cd) Survival & Reproduction	1002.0	S	33	0.10	0.16	0.29
Sheepshead Minnow Larval Survival & Growth	1004.0	S	5	0.07	0.08	0.12
Inland Silverside Larval Survival & Growth	1006.0	S	16	0.16	0.28	0.35
Mysid (Ab) Survival, Growth, & Fecundity	1007.0	S	10	0.16	0.26	0.27
Methods for Acute Toxicity[d,e]						
Fathead Minnow Larval Survival	2000.0	S	21	0.10	0.16	0.19
Ceriodaphnia (Cd) Survival	2002.0	S	23	0.11	0.19	0.29
Sheepshead Minnow Survival	2004.0	S	5	0.12	0.14	0.21
Inland Silverside Larval Survival	2006.0	S	5	0.15	0.16	0.21
Mysid (Ab) Survival	2007.0	S	3	0.17	0.25	0.26
Mysid (Hc) Survival	2011.0	S	2	0.27	0.30	0.34
Rainbow Trout Survival	2019.0	S	1	0.23	0.23	0.23
Daphnia (Dm) Survival	2021.0	S	5	0.07	0.22	0.24
Daphnia (Dp) Survival	2022.0	S	6	0.19	0.21	0.27

Table B-2. Percentiles of the Within-Laboratory Values of CV for EC50"

Test Method1*	Test Method No.c	End-point*	No. of Labs	CV				
				P10	P25	P50	P75	P90
Acute								
Fathead Minnow Larval Survival	**2000.0**	S	21	0.08	0.10	0.16	0.19	0.33
Ceriodaphnia (Cd) Survival	**2002.0**	S	23	0.06	0.11	0.19	0.29	0.34
Sheepshead Minnow Survival	2004.0	S	**5**	0.11	0.12	0.14	0.21	0.37
Inland Silverside Larval Survival	2006.0	S	5'	0.07	0.15	0.16	0.21	0.44
Mysid (Ab) Survival	2007.0	S	3	0.17	0.17	0.25	0.26	0.26
Mysid (He) Survival	2011.0	S	**2**	0.27	0.27	0.30	0.34	0.34
Rainbow Trout Survival	2019.0	S	1	0.23	0.23	0.23	0.23	0.23
Daphnia (Dm) Survival	2021.0	S	5	0.05	0.07	**0.22**	0.24	0.46
Daphnia (Dp) Survival	**2022.0**	S	**6**	0.15	0.19	0.21	0.27	0.48

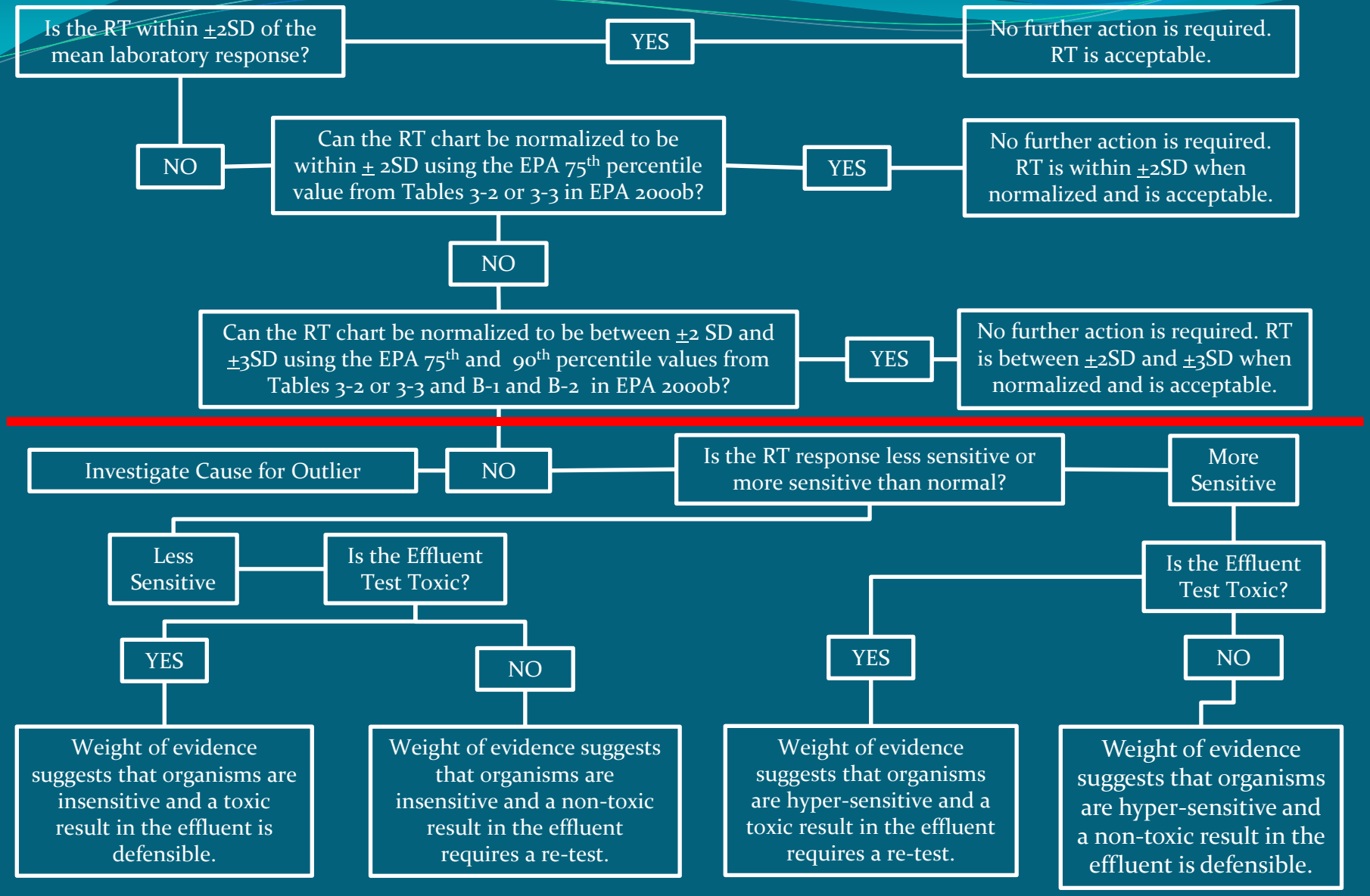
Calculating ±2 Standard Deviations



EPA 75th percentile LC_{50} = 0.19
Control Chart Mean = 789.2 mg/L
1SD Calculation: (789.2 mg/L) x (0.19) = 149.9 mg/L
2SD Calculation: (149.9 mg/L) x (2) = 299.8 mg/L

NORMALIZED (2 Standard Deviations)
-2SD Warning Limit: 798.2 - 299.8 = 498.4 mg/L
+2SD Warning Limit: 798.2 + 299.8 = 1098 mg/L

Reference Toxicant Acceptability Criteria Decision Tree



USEPA. 2000b. Understanding and accounting for method variability in whole effluent toxicity applications under the national pollutant discharge elimination system program. Office of Wastewater Management, U.S. Environmental Protection Agency, Washington, D.C. 20460. EPA/833/R-00/003.

Reference Toxicants

Toxicant	Pros	Cons
Sodium Chloride	• Not very potent • Good for use with sensitive organisms • Good conductivity signature • Tight RT Control charts	• Requires lots of salt for some species • Not too efficient
Potassium Chloride	• Potent • Does not require much salt • Good conductivity signature	• Control charts can be more variable than NaCl
Metals (Copper/Zinc)	• Consistent using the same water type.	• No conductivity signature. • Toxicity is pH dependent.
Sodium Dodecyl Sulfate (SDS)	• N/A	• N/A

Part 2:Report Review and Upcoming Toxicity Provisions

- Extracting Acute Data from Chronic Test Data
- Report Review
- Toxicity Provisions
  - Species Screening Process

Extracting Acute Survival Data from Chronic Bioassays

- It is acceptable to do as long as:
  - Discharger has written authorization to do so from regulators.
  - Acute and chronic compliance species are the same and can utilize the same test design (e.g. temp, replication, organisms per rep).
  - Acute and Chronic sample requirements are the same (e.g. grab vs. composite).
  - Chronic renewal is performed within the correct time window at 96-hrs.

Report Review

- Acute Tests:
  - Primarily looking at survival endpoint.

Summary of Acute Fathead Minnow Test Results		
	Lab Control	100% Effluent
Percent Survival	100%	100%

  - Permits rarely **require** the reporting of reference toxicant tests for acute toxicity bioassays.
  - MAI does report acute RT data upon request from clients.
  - Under the upcoming Toxicity provisions, acute tests will be evaluated as PASS/FAIL using the TST statistical method.

- Chronic Tests:
  - Calculated TUc, % effect, or PASS/FAIL (TST method)
  - RT tests **must meet TAC** and dose responses are acceptable as discussed earlier.
  - PMSD is acceptable and data is normalized if PMSD fails low to remove any false positives. High PMSD values can invalidate the test.
  - TST analysis should alleviate false positive problem.

TABLE 6. VARIABILITY CRITERIA (UPPER AND LOWER PMSD BOUNDS) FOR SUBLETHAL HYPOTHESIS TESTING ENDPOINTS SUBMITTED UNDER NPDES PERMITS.[1]

Test Method	Endpoint	Lower PMSD Bound	Upper PMSD Bound
Method 1000.0, Fathead Minnow Larval Survival and Growth Test	growth	12	30
Method 1002.0, *Ceriodaphnia dubia* Survival and Reproduction Test	reproduction	13	47
Method 1003.0, *Selenastrum capricornutum* Growth Test	growth	9.1	29

[1] Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b).

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	TU	PMSD	S
09-8435-2069	7d Survival Rate	Fisher Exact/Bonferroni-Holm Test		4000	>4000	n/a		n/a	1
00-6318-2481	Reproduction	Steel Many-One Rank Sum Test		~~250~~	~~500~~	353.6		11.1%	1

500 1,000

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	mg/L	95% LCL	95% UCL	TU	S
12-7519-2288	Reproduction	Linear Interpolation (ICPIN)		IC5	209	98.6	511		1

Test Acceptability

				TAC Limits			
Analysis ID	Endpoint	Attribute	Test Stat	Lower	Upper	Overlap	Decision
09-8435-2069	7d Survival Rate	Control Resp	1	0.8	>>	Yes	Passes Criteria
00-6318-2481	Reproduction	Control Resp	33.5	15	>>	Yes	Passes Criteria
12-7519-2288	Reproduction	Control Resp	33.5	15	>>	Yes	Passes Criteria
00-6318-2481	Reproduction	PMSD	0.111	0.13	0.47	Yes	Below Criteria

Reproduction Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LW	10	33.5	31.4	35.6	28	37	0.922	2.92	8.70%	0.00%
250		10	31.5	29	34	24	36	1.09	3.44	10.92%	5.97%
500		10	29.9	28.1	31.7	26	34	0.809	2.56	8.56%	10.75%
1000		10	19.6	14.9	24.3	10	28	2.06	6.5	33.17%	41.49%
2000		10	0.2	-0.252	0.652	0	2	0.2	0.632	316.23%	99.40%
4000		10	0	0	0	0	0	0	0		100.00%

Per the US EPA, test concentrations with a percent effect less than the lower PMSD bound should not be considered statistically significant. Therefore, the test NOEL and LOEL are 500 mg/L and 1,000 mg/L, respectively.

Toxicity Provisions

- What to expect, (although not fully adopted yet):
  - Take effect upon permit reissuance.
  - Chronic species screening will require quarterly testing using one Tier I plant, one invertebrate, one vertebrate.
  - Species Selection is based on receiving water salinity.
    - Freshwater species: RW is <1.0ppt 95% of the time.
    - Marine Species: RW is >1.0ppt 95% of the time.
    - All other instances are up to the permitting authority.
  - Dilution series are required and bracket the IWC.
    - This is not clear in the provisions.
    - The 100% effluent concentration should be included in the event that the IWC is a low concentration.

Species Selection

U.S. EPA Toxicity Test Method	Tier	RMD (b)	β Error	α Error
Chronic Freshwater Methods				
Ceriodaphnia dubia (water flea) Survival and reproduction	I	0.75	0.05	0.20
Pimephales promelas (fathead minnow) Survival and growth	I	0.75	0.05	0.25
Selenastrum capricornutum (green alga) Growth	I	0.75	0.05	0.25
Chronic West Coast Marine Methods				
Atherinops affinis (topsmelt) Survival and growth	I	0.75	0.05	0.25
Dendraster excentricus (sand dollar); *Strongylocentrotus purpuratus* (purple urchin) Fertilization	I	0.75	0.05	0.05
Dendraster excentricus (sand dollar); Strongylocentrotus purpuratus (purple urchin) Larval development	I	0.75	0.05	0.05
Haliotis rufescens (red abalone) Larval development	I	0.75	0.05	0.05
Mytilus sp. (mussels); *Crassostrea gigas* (oyster) Larval development	I	0.75	0.05	0.05
Macrocystis pyrifera (giant kelp) Germination and germ-tube length	I	0.75	0.05	0.05
Chronic East Coast Marine Methods				
Menidia beryllina (inland silverside) Survival and growth	II	0.75	0.05	0.25
Americamysis bahia (mysid) Survival and growth	II	0.75	0.05	0.15

Species Screening Process

- Generate a screening proposal.
  - MAI provides these services.
  - Includes proposed test species, dilutions, frequency, etc.
  - This will be reviewed by regional water board staff for approval.
- Implement species screening process.
  - Requires careful coordination between the laboratory, client and organism suppliers.
- Produce summary report identifying the most sensitive Tier I species.