

Toxicity 101: Species Screening and Toxicity Report Review

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Objective of Presentation

- ◆ Provide an overview of species screening
- ◆ Provide recommended toxicity test review approach in support of an independent validation of toxicity test data

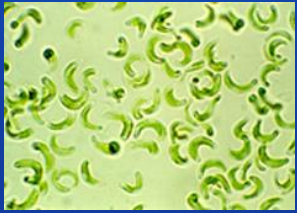
Species Screening

Species Screening Requirements

- NPDES permits in some regions require the performance of species screening prior to permit renewal
- Species Screening Study Plan required in most new permits
- Objective is to select the most sensitive species for future compliance monitoring
- Is an element in the State Board draft toxicity policy

Typical NPDES Chronic Test Species

Algae



Invertebrates



Fish



Species Screening Requirements

- Species selection for Species Screening can be based on:
 - Ocean Plan
 - Inland Surface Waters, Enclosed Bays, and Estuaries Plan
 - the permit
- Marine/Estuarine - typically 5 species (freshwater and marine), including at least one “plant”, one invertebrate, and one fish in Round 1, and then 3 species in Rounds 2 & 3
- Freshwater - typically 3 species in Rounds 1-3
- May include acute species screening as well
- Most sensitive species = compliance monitoring species

Toxicity Report Review

Fundamentals of Toxicity Testing



Overview of Review Steps

- ◆ Sampling and Sample Handling
- ◆ Test Acceptability Criteria (TAC)
- ◆ Test Conditions
- ◆ Statistical Methods
- ◆ Concentration Response Relationships
- ◆ Reference Toxicant Tests
- ◆ Test Variability (e.g., PMSD)

Sampling and Sample Handling

- ◆ Hold time is 36 hours for initial use;
 - ◆ Use for test solution renewals for up to 72 hours after first use for renewals
(need COC and test data sheets to review)
- ◆ Chilled to 0-6°C during or immediately after collection
- ◆ Store at 0-6°C



Overview of Review Steps

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Test Acceptability Criteria

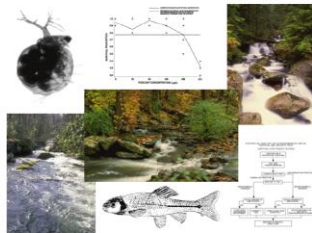
- ◆ Test must meet method specified Test Acceptability Criteria (TAC) to be valid



Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms

Fourth Edition

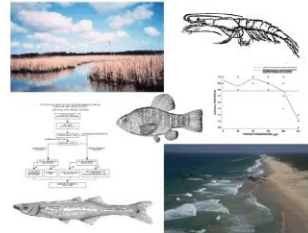
October 2002



Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms

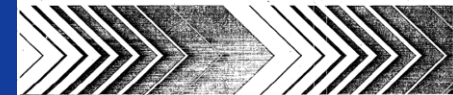
Third Edition

October 2002



United States Environmental Protection Agency Office of Research and Development Washington DC 20460 EPA/600/R-90/136 August 1990

Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms



Test Acceptability Criteria

Species	Endpoint	TAC ¹
<i>Selenastrum capricornutum</i>	Growth (Cell Density)	<ul style="list-style-type: none"> • $\geq 1.0 \times 10^6$ cells/mL in control • $\leq 20\%$ CV in control
<i>Ceriodaphnia dubia</i>	Survival	<ul style="list-style-type: none"> • $\geq 80\%$ control survival
	Reproduction ²	<ul style="list-style-type: none"> • 3-brood reproduction avg. ≥ 15 neonates/female in control • 3-broods in 60% of females in 8 days (need datasheets to review) • ≤ 2 males in control³
<i>Pimephales promelas</i>	Survival	<ul style="list-style-type: none"> • $\geq 80\%$ control survival
	Growth ²	<ul style="list-style-type: none"> • ≥ 0.25 mg average weight per surviving fish in control

1 - Test acceptability criteria are summarized in the corresponding summary of test conditions and test acceptability criteria table for each test method. These tables are included in the handout

2 – Based on number of surviving organisms

3 – Not in in the corresponding summary of test conditions and test acceptability criteria table, in protocol narrative

Test Acceptability Criteria

Chronic *Ceriodaphnia dubia* Test

Reproduction Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	10	36	32.6	39.4	28	44	1.52	4.81	13.4%	0.0%
100		10	28.6	24.6	32.6	20	35	1.79	5.66	19.8%	20.6%

Survival Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	10	1	1	1	1	1	0	0	0.0%	0.0%
100		10	1	1	1	1	1	0	0	0.0%	0.0%

Test Acceptability Criteria

Chronic *Ceriodaphnia dubia* Test

Pacific EcoRisk

Environmental Consulting and Testing

Short-Term Chronic 3-Brood *Ceriodaphnia dubia* Survival & Reproduction Test Data

Client: [REDACTED] Material: Effluent Test Date: 10-15-15
 Project #: 24778 Test ID: 64789 Randomization: 10,5,7 Control Water: Modified EPAMH w/ 5% Ambient Water

Day	pH		D.O.		Cond. (µS/cm)	Temp (°C)	Survival / Reproduction												SIGN-OFF			
	New	Old	New	Old			A	B	C	D	E	F	G	H	I	J	Date:	New WQ:	Test Init. Time:			
0	7.88		8.5		318	24.6	0	0	0	0	0	0	0	0	0	0	0	0	0	Date: 10/15/15	New WQ: YJ WC	Test Init. Time: 1355
1	8.10	7.79	8.8	7.8	321	24.2	0	0	0	0	0	0	0	0	0	0	0	0	0	Date: 10/16/15	New WQ: RL	Counts: XJ
2	7.74	8.38	8.6	8.2	321	24.3	0	0	0	0	0	0	0	0	0	0	0	0	0	Date: 10/15/15	New WQ: WC	Counts: 00
3	7.68	7.83	8.6	7.6	326	24.7	5	5	5	5	5	6	5	6	5	6	5	6	5	Date: 10/15/15	New WQ: TL	Counts: 23B
4	7.74	7.80	8.6	6.3	323	24.8	11	0	7	10	0	12	10	0	9	0	0	0	0	Date: 10/15/15	New WQ: DM	Counts: 00D
5	7.66	7.77	8.6	8.8	333	24.8	0	13	0	0	15	15	17	15	0	9	0	0	0	Date: 10/15/15	New WQ: TL	Counts: 142S
6	-	8.00	-	7.7	380	24.5	16	26	27	23	17	20	0	20	14	21	0	0	0	Date: 10/21/15	New WQ: -	Counts: R0
7																				Date:	New WQ:	Counts:
8																				Date:	Old WQ:	Time:
Total=							32	44	39	38	37	53	32	41	28	36	Mean Neonates/Female = 38.0					
Day	pH		D.O.		Cond. (µS/cm)	Temp (°C)	Survival / Reproduction												SAMPLE ID			
	New	Old	New	Old			A	B	C	D	E	F	G	H	I	J	Date:	New WQ:	Counts:			
0	7.72		8.9		1775		0	0	0	0	0	0	0	0	0	0	0	0	0			40039
1	7.97	7.94	9.0	8.2	1793		0	0	0	0	0	0	0	0	0	0	0	0	0			40039
2	7.57	8.15	9.5	8.4	1756		0	0	0	0	0	0	0	0	0	0	0	0	0			40050
3	7.59	7.75	9.9	8.0	1769		6	6	5	5	5	6	5	7	5	0	0	0	0			40050
4	7.58	7.86	10.1	7.1	1770		12	0	10	11	0	0	7	0	10	6	0	0	0			40050
5	7.49	7.61	8.1	8.2	1725		0	13	3	13	12	12	0	15	0	12	0	0	0			40063
6	-	7.86	-	7.6	1913		15	16	10	0	15	14	10	12	5	3	0	0	0			-
7																						
8																						
Total=							33	35	28	29	32	32	22	34	20	21	Mean Neonates/Female = 28.6					



Overview of Review Steps

- ◆ Sampling and Sample Handling
- ◆ Test Acceptability Criteria (TAC)
- ◆ **Test Conditions**
- ◆ Statistical Methods
- ◆ Concentration Response Relationships
- ◆ Reference Toxicant Tests
- ◆ Test Variability (e.g., PMSD)

Test Conditions

- ◆ Test method protocol specifies “required” and “recommended” test conditions
 - ◆ Required test conditions must be met, or test is invalid:
 - ◆ Test type, duration, age or organisms, test endpoints, solution renewal frequency, etc.
 - ◆ Recommended test conditions should be met; degree of departure *may* invalidate test.
 - ◆ These conditions include test chamber size, solution volume, light intensity, etc.

Test Conditions

- ◆ Required and recommended designations are clearly identified for test conditions summarized in the table
- ◆ Test conditions not found in the summary of test conditions table:
 - ◆ D.O. ≥ 4 mg/L, pH 6-9 (not in table)
- ◆ Need test data sheets to evaluate test conditions

Note: There is a difference between invalidation and qualification of a test

Report Review Examples

Example #1: Test Conditions

- ◆ Toxicity to chronic *C. dubia* repro. in 100% effluent
- ◆ D.O. < 4 mg/L on 2 consecutive days
 - ◆ Chronic *C. dubia* test cannot be aerated while in-progress
- ◆ Nothing else notable uncovered during report review, all test acceptability criteria were met
- ◆ Since effluent toxicity cannot be distinguished from artifactual toxicity due to low D.O., test is invalid

Overview of Review Steps

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Statistical Methods

A point estimate value (e.g. EC50, IC25) is calculated only if a dilution series is performed

In Region 2, $TUc = 100/EC25$

NOEC: The highest effluent concentration where there is not a statistically significant reduction compared to the control

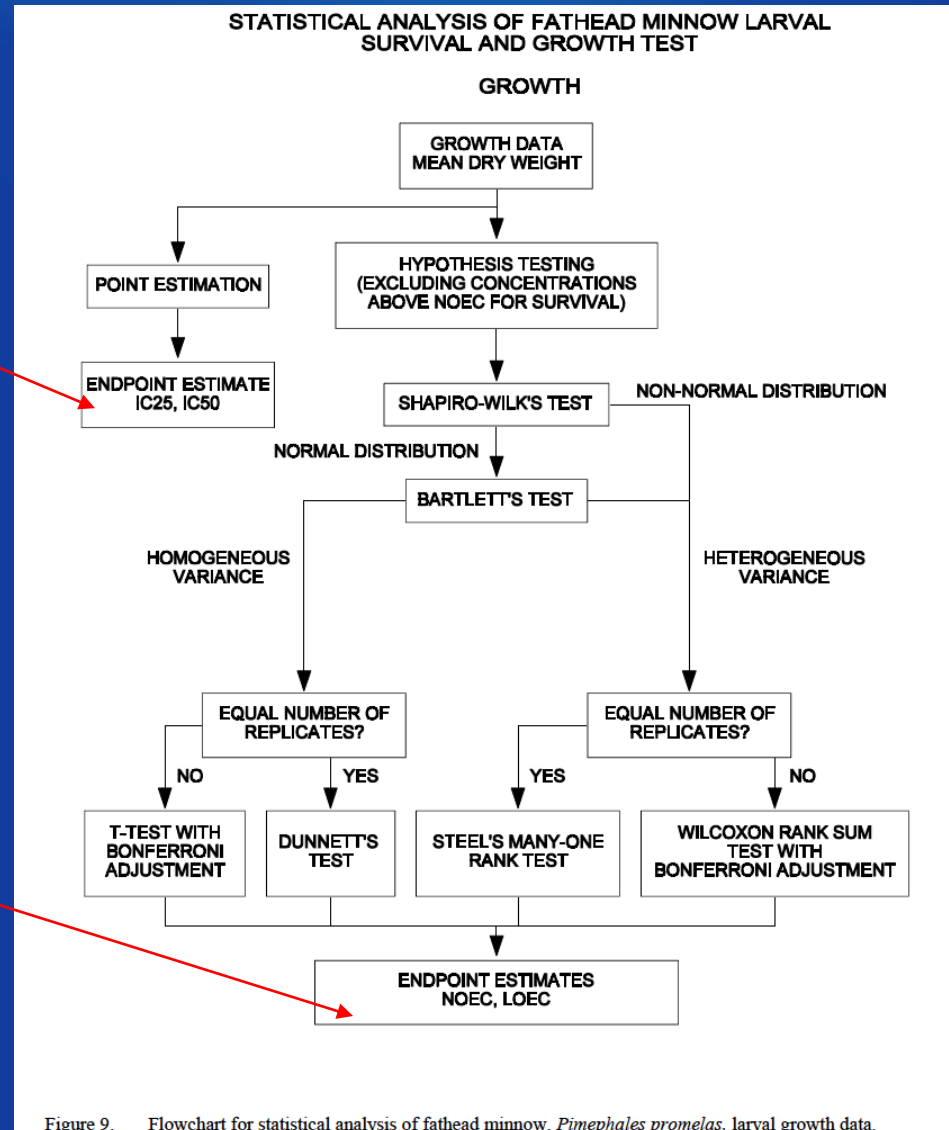


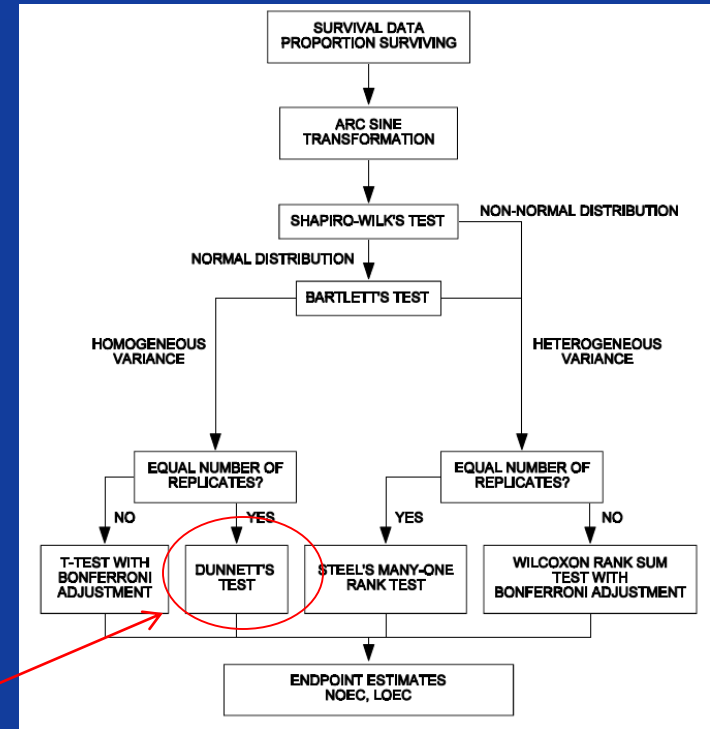
Figure 9. Flowchart for statistical analysis of fathead minnow, *Pimephales promelas*, larval growth data.

Statistical Methods

- ◆ Verify the recommended statistical flow chart was followed
- ◆ CETIS default is to follow flow chart

CETIS Summary Report				Report Date:	11 Feb-15 11:23 (p 1 of 2)		
				Test Code:	60859 01-8430-2899		
Chronic Larval Fish Survival and Growth Test				Pacific EcoRisk			
Batch ID:	14-5454-9595	Test Type:	Growth-Survival (7d)	Analyst:	Michelle Fong		
Start Date:	03 Feb-15 14:20	Protocol:	EPA-821-R-02-013 (2002)	Diluent:	Laboratory Water		
Ending Date:	10 Feb-15 09:00	Species:	Pimephales promelas	Brine:	Not Applicable		
Duration:	6d 19h	Source:	Aquatox, AR	Age:	1		
Sample ID:	09-1748-9763	Code:	Effluent	Client:			
Sample Date:	02 Feb-15 08:00	Material:	Effluent	Project:			
Receive Date:	02 Feb-15 12:35	Source:					
Sample Age:	30h (4.1 °C)	Station:	Eff-001				
Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
13-5710-5485	7d Survival Rate	100	>100	NA	19.1%	1	Dunnett Multiple Comparison Test
20-7614-9119	Mean Dry Biomass-mg	100	>100	NA	18.7%	1	Steel Many-One Rank Sum Test

Distributional Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision (α=1%)	
Variances	Bartlett Equality of Variance	4.93	15.1	0.4248	Equal Variances	
Distribution	Shapiro-Wilk W Normality	0.92	0.884	0.0579	Normal Distribution	



Ex: chronic fathead minnow; survival

Statistical Methods

- ◆ TST statistic is a new tool that can be used to qualify results of hypothesis testing (i.e. NOEC)
 - ◆ Has begun to appear in Region 1 & 5 permits:
 - ◆ IWC vs. Control
 - ◆ May yield different results than NOEC, beneficial for chronic tests with high precision and effects <25%

Report Review Examples

Example #2: Statistics

Single Comparison Summary											
Analysis ID	Endpoint	Comparison Method		P-Value	Comparison Result						
21-0725-7670	Reproduction	TST-Welch's t Test		0.1763	100% passed reproduction						
20-6901-1014	Reproduction	Wilcoxon Rank Sum Two-Sample Test		0.0061	100% failed reproduction						
06-2198-5862	Survival	Fisher Exact Test		1.0000	100% passed survival						
Reproduction Summary											
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LW	10	35.8	30.6	41	21	42	2.31	7.3	20.39%	0.00%
100		10	29.1	25.5	32.7	19	35	1.59	5.04	17.33%	18.72%
Survival Summary											
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LW	10	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
100		10	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
Reproduction Detail											
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	LW	36	37	41	42	38	40	38	41	24	21
100		32	25	24	30	28	33	32	35	33	19
Survival Detail											
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	LW	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
100		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Survival Binomials											
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	LW	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
100		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C > T	100% failed reproduction	13.59%

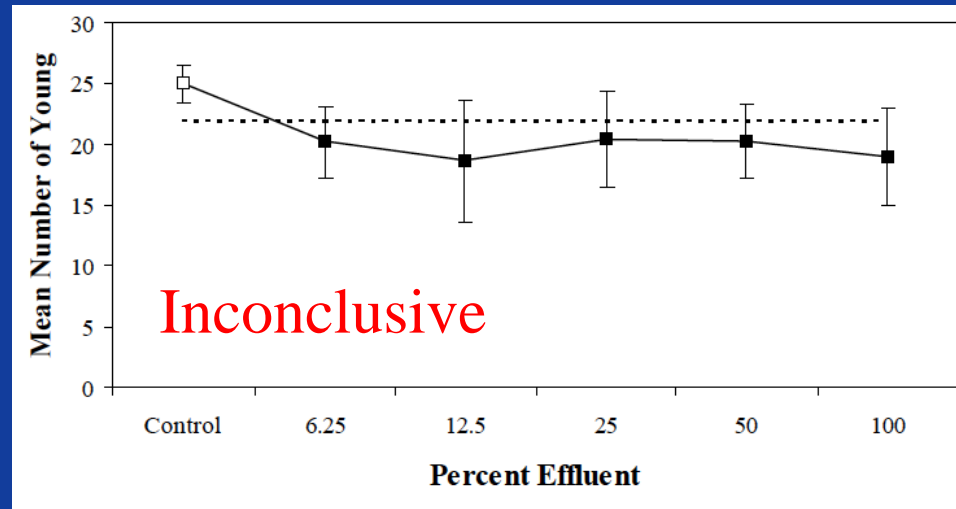
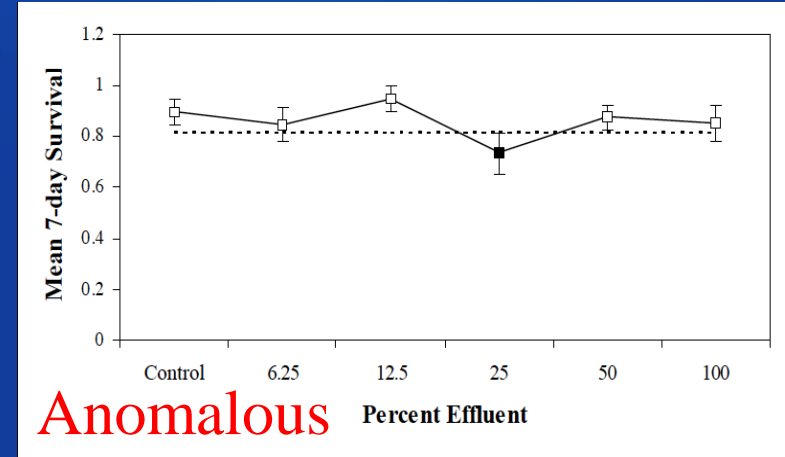
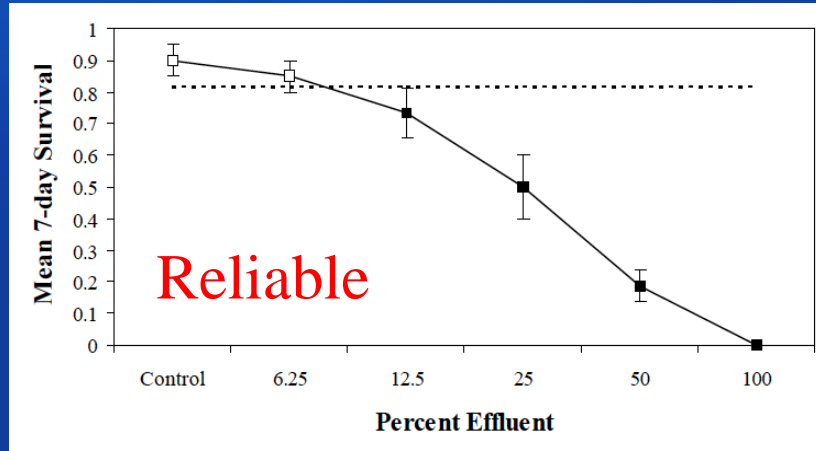
Overview of Review Steps

- ◆ Sampling and Sample Handling
- ◆ Test Acceptability Criteria (TAC)
- ◆ Test Conditions
- ◆ Statistical Methods
- ◆ **Concentration Response Relationships**
- ◆ Reference Toxicant Tests
- ◆ Test Variability (e.g., PMSD)

Concentration Response Relationships

- ◆ If testing with a dilution series, concentration response relationship must be reviewed to ensure calculated results are interpreted correctly (per EPA 821-B-00-004)
- ◆ 3 potential outcomes
 - ◆ Concentration-response curves are reliable and should be reported
 - ◆ Concentration-response curves are anomalous and should be explained
 - ◆ Concentration-response curves are inconclusive and test my require being repeated

Concentration Response Relationships



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Reference Toxicant Tests

- ◆ Reference toxicant tests represent a “positive control”
 - ◆ Often performed concurrently, as required by SIP
- ◆ Organisms exposed to standard concentrations of the selected toxicant
- ◆ Organism response evaluated against testing laboratory’s 20-test performance history
 - ◆ Response outside control limits indicates unusual or anomalous sensitivity

Reference Toxicant Test

- ◆ Reference toxicant test results plotted on control chart
 - ◆ Results falling outside of control chart limits (± 2 and ± 3 SD) are to be evaluated to determine validity of associated effluent test
 - ◆ Reference toxicant test should not to be used as *de facto* criterion of effluent test validity
- ◆ Should consider:
 - ◆ Degree result fell outside of control limits
 - ◆ Width of limits (long- term precision)
 - ◆ Direction of deviation
 - ◆ TAC and test conditions

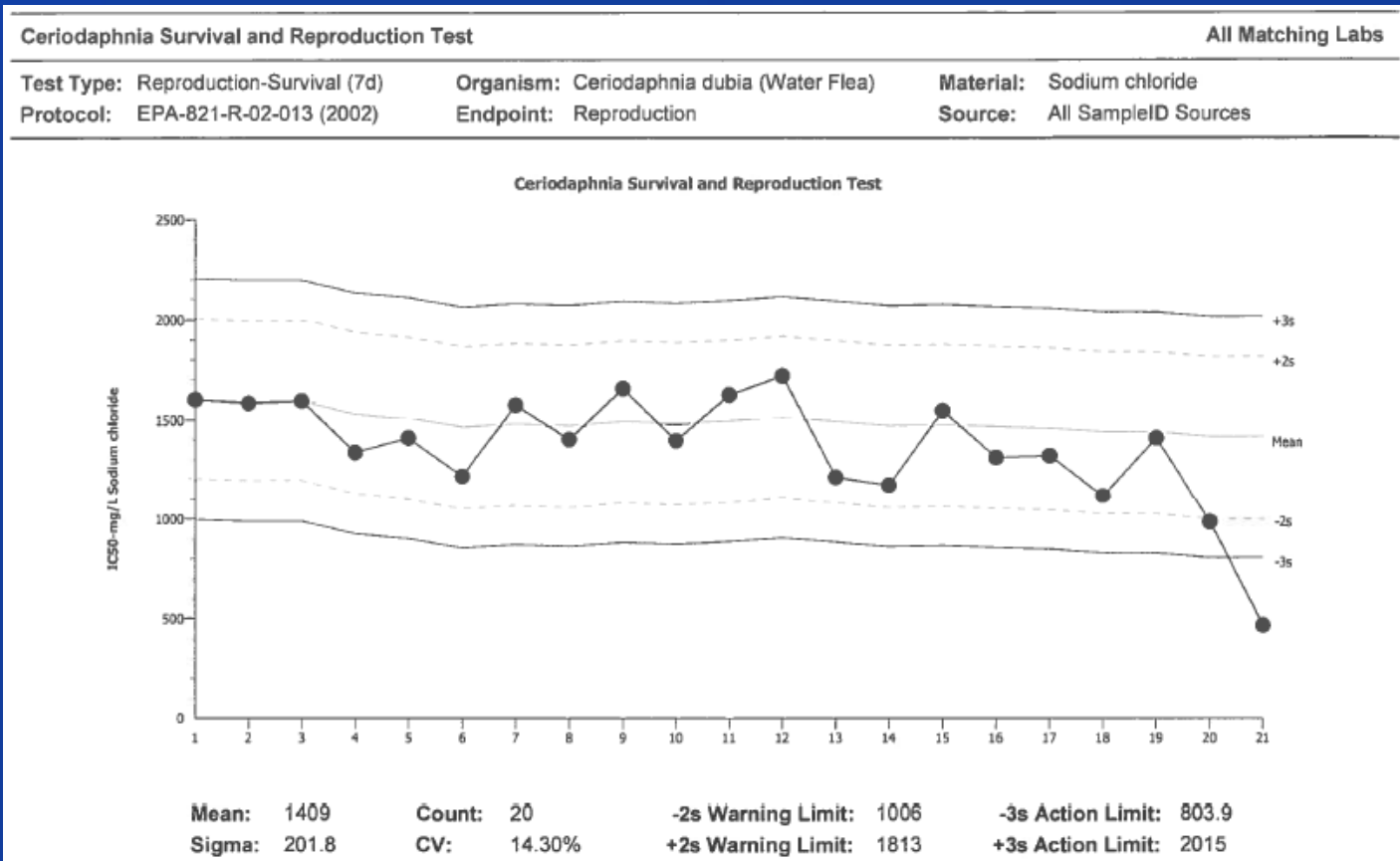
Report Review Examples

Example #3 Reference Toxicant Test

- ◆ Toxicity to chronic *C. dubia* repro. in 100% effluent
- ◆ Nothing else notable uncovered during report review, all test acceptability criteria were met.
- ◆ Concurrent reference toxicant test indicated that the organisms are hypersensitive
- ◆ The effluent test results would be qualified, but it is still a valid test and should be reported. Retest is recommended based our best professional judgement

Report Review Examples

Example #3 Reference Toxicant Test



Overview of Review Steps

- ◆ Sampling and Sample Handling
- ◆ Test Acceptability Criteria (TAC)
- ◆ Test Conditions
- ◆ Statistical Methods
- ◆ Concentration Response Relationships
- ◆ Reference Toxicant Test
- ◆ Test Variability (e.g., PMSD)

Test Variability

- ◆ Must review test variability when using sublethal hypothesis test endpoints
- ◆ Calculate percent minimum significant difference (PMSD)
 - ◆ The PMSD is a test statistic that is to be compared to criteria thresholds established by the EPA

Test Variability

- ◆ PMSD is $< 10^{\text{th}}$ percentile, too sensitive
 - ◆ If toxic (statistically significant), but relative response is less than lower PMSD, consider sample not toxic
 - ◆ If not toxic, accept test
- ◆ PMSD is $> 90^{\text{th}}$ percentile, too insensitive
 - ◆ If not toxic, retest
 - ◆ If toxic, accept test

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, <i>Ceriodaphnia dubia</i> Survival and Reproduction Test	reproduction	13	47
Method 1003.0, <i>Selenastrum capricornutum</i> 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).

Report Review Examples

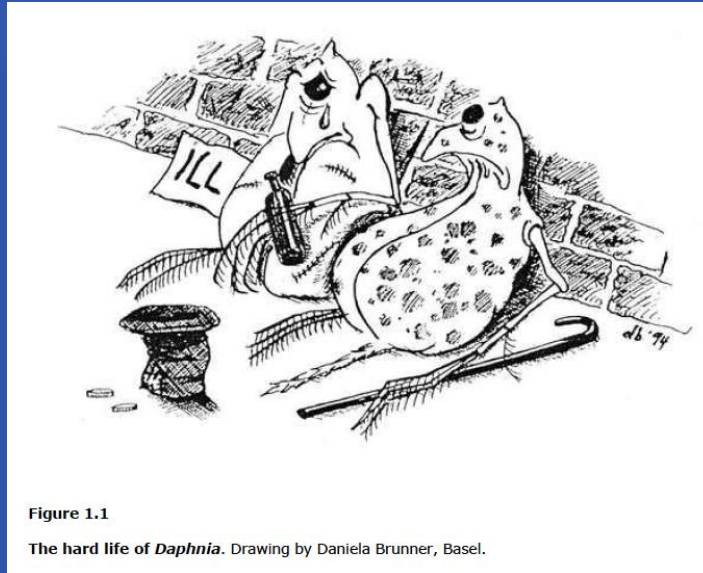
Example #4 Test Variability

Chronic <i>Ceriodaphnia dubia</i> Test Data			
Effluent Treatment	% Survival	Reproduction (# neonates /female)	% Effect
Lab Control	100	36.9	-
12.5%	100	36.8	0.27
25%	100	34.4	6.78
50%	100	36.1	2.17
75%	100	33.3 ^a	9.76
100%	100	32.4 ^a	12.20
Summary of Statistics			
NOEC =	100% effluent	100% effluent	
TUc (100/NOEC) =	1	1	
Survival EC25 or Reproduction IC25 =	>100% effluent ^b	>100% effluent	
Survival EC50 or Reproduction IC50 =	>100% effluent ^b	>100% effluent	
PMSD		12.8%	

a - Although the statistical analysis of the test data indicates that the response at this test treatment is significantly less than the Control treatment response, it is not to be considered toxic as the test PMSD and relative difference from the Control treatment response are less than the EPA's 10th percentile PMSD limit (i.e., 13%).

b - Due to the absence of significant mortalities, the EC point estimates could not be calculated, but can be determined by inspection to be >100% effluent.

Questions?



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