

Whole Effluent Toxicity Testing- Quality Control and Data Review

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What is a WET Test?

- WET tests are used to determine the toxicity of an effluent or receiving water over a certain period of time.
- Whole effluent toxicity is measured as opposed to chemical specific toxicity
- Also known as “bioassay” or “biomonitoring”

Common Types of WET Tests

- Acute toxicity tests
 - Measures lethality in a 24 – 96 hour period
 - Can be static or flow-through
 - Screen or Definitive
 - Renewal or non-renewal
- Chronic Toxicity tests
 - Measures toxicity over a 7-8 day period
 - Measures lethal and sub lethal (non lethal) effects
 - Screen or Definitive
 - Daily renewals required

TEST SPECIES

Freshwater Acute

Daphnia pulex or
Daphnia magna
(water flea)



Pimephales promelas
(fathead minnow)



Freshwater Chronic

Ceriodaphnia dubia
(water flea)



Pimephales promelas
(fathead minnow)



Marine Acute & Chronic

Mysidopsis bahia
(Mysid shrimp)



Menidia beryllina
(inland silverside minnow)



Test Endpoints

- LC_{50} : Lethal Concentration-the concentration of sample that kills 50% of the test organisms.
- NOEL (NOEC): No Observed Effect Level (Concentration)- the highest effluent concentration that is not significantly different from the control based on statistical analysis.
- LOEL (LOEC): Lowest Observed Effect Level (Concentration) – the lowest effluent concentration that is significantly different from the control based on statistical analysis.
- IC25: Inhibition Concentration 25%- the effluent concentration that shows a 25% reduction in toxicity. For the biomass values which are combined effects of survival/growth, survival/reproduction, survival/fecundity

Critical Dilution

- The effluent dilution, expressed as a percentage, representative of the dilution afforded a wastewater discharge according to the appropriate Q^* -dependent mixing zone.
- Q^* - the ratio of regulatory effluent flow to the regulatory receiving water flow
- The test must pass at the critical dilution

Critical Dilution

- A critical dilution of 100% = undiluted effluent.
- A critical dilution of 75.0% = 750 milliliters effluent diluted with 250 milliliters of dilution water
- A critical dilution of 4.0% = 40 milliliters of effluent diluted with 960 milliliters of dilution water

Ethical Statement

- “It is unlawful and a violation of this permit for a permittee or the designated agent to manipulate test samples in any manner, to delay sample shipment, or to terminate or cause to terminate a toxicity test. Once initiated, all toxicity tests must be completed unless specific authority has been granted by _____(DEQ)”

Data Review

- Test Acceptability Criteria (TAC)
 - “MUST” – where conditions must be met, test invalid if aren’t met.
 - “SHOULD”- test may be suspect if multiple “shoulds” violated

Data Review (cont.)

- Study based on standard methodology
- Sampling correct and holding time met
- Test organisms
 - Organisms correct for test type
 - Test organisms were correct age:
 - Acute tests
 - C.dubia/D.pulex/D.magna - <24 hours old
 - Fathead minnow – 1-14 days old (24 hour range)
 - Mysid shrimp – 1-5 days old (24 hour range)
 - Silverside minnow – 9-14 days old (24 hour range)

Data Review (cont.)

- Test organisms (cont.)

- Age of Test organisms

- Chronic Test

- C.dubia- <24 hours old (8-hour period, known parentage from individual cultures of adults that have had 3 broods)
 - Fathead minnow - <24 hours old (< 48 hours max.)
 - Mysid shrimp – 5 to 7 days old

- Silverside minnow – 7-11 days old (24 hour)

- All test organisms were from the same source and were appropriately acclimated prior to starting the test.

Data Review (cont.)

- Test Conditions and Design
 - Dilution water type and chemical characteristics are consistent with protocol
 - Test solution temperature was held at the appropriate level:
 - Acute – $25 \pm 1^{\circ}$ Celsius or $20 \pm 1^{\circ}$ Celsius
 - Chronic - $25 \pm 1^{\circ}$ Celsius

Data Review (cont.)

- Test vessels were of appropriate substance and size
 - C.dubia/D.pulex/D.magna- 30 ml plastic or glass
 - Minnows/mysid shrimp- minimum 500 ml plastic or glass
 - Menidia – minimum 600 ml plastic or glass

Data Review (cont.)

- Test Termination Criteria (Control)
 - Acute tests – 90% survival
 - Chronic tests
 - C.dubia – 80% survival; 60% animals had 3 broods; average in control 15 neonates per surviving female
 - Fathead minnow – 80% survival; ≥ 0.250 mg dry weight per surviving larvae
 - Mysid shrimp – 80% survival; ≥ 0.200 mg per surviving mysid and 50% egg production.
 - Menidia – 80% survival; 0.500 mg dry weight when dried immediately after termination or ≥ 0.430 mg when preserved.

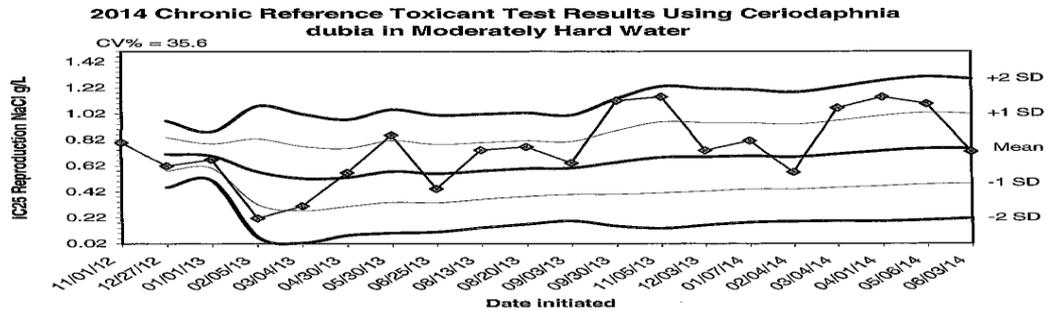
Determining Broods in C.dubia Chronic Test

<u>Replicate</u>	<u>Day 3</u>	<u>Day 4</u>	<u>Day 5</u>	<u>Day 6</u>	<u>Day7</u>	<u>Total</u>	<u># Broods</u>
A	3	8	0	10	12	21	4
B	0	4	9	1	12	26	3
C	2	0	7	0	15	24	3
D	0	4	0	10	20	34	3
E	3	0	5	0	20	28	3
F	3	0	7	10	0	20	3
G	4	0	7	0	20	31	3
H	0	3	8	0	21	32	3
I	0	4	9	18	0	31	3
J	0	5	0	10	18	33	3
					Average	28	

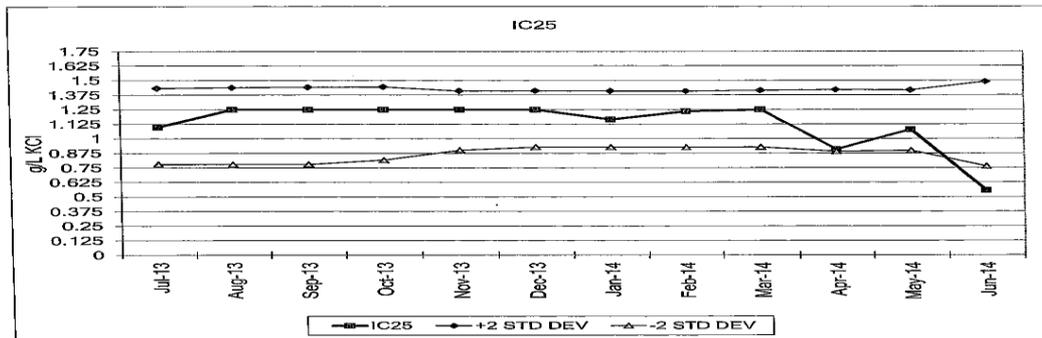
Data Review (cont.)

- Quality Control- Practices that must address all activities that affect quality of final toxicity data
 - Effluent sampling and handling
 - Source and condition of test organisms
 - Condition and operation of equipment
 - Test conditions
 - Instrument calibration
 - Use of reference toxicants
 - Record keeping
 - Data Evaluation

Quality Control Charts



Pimephales promelas



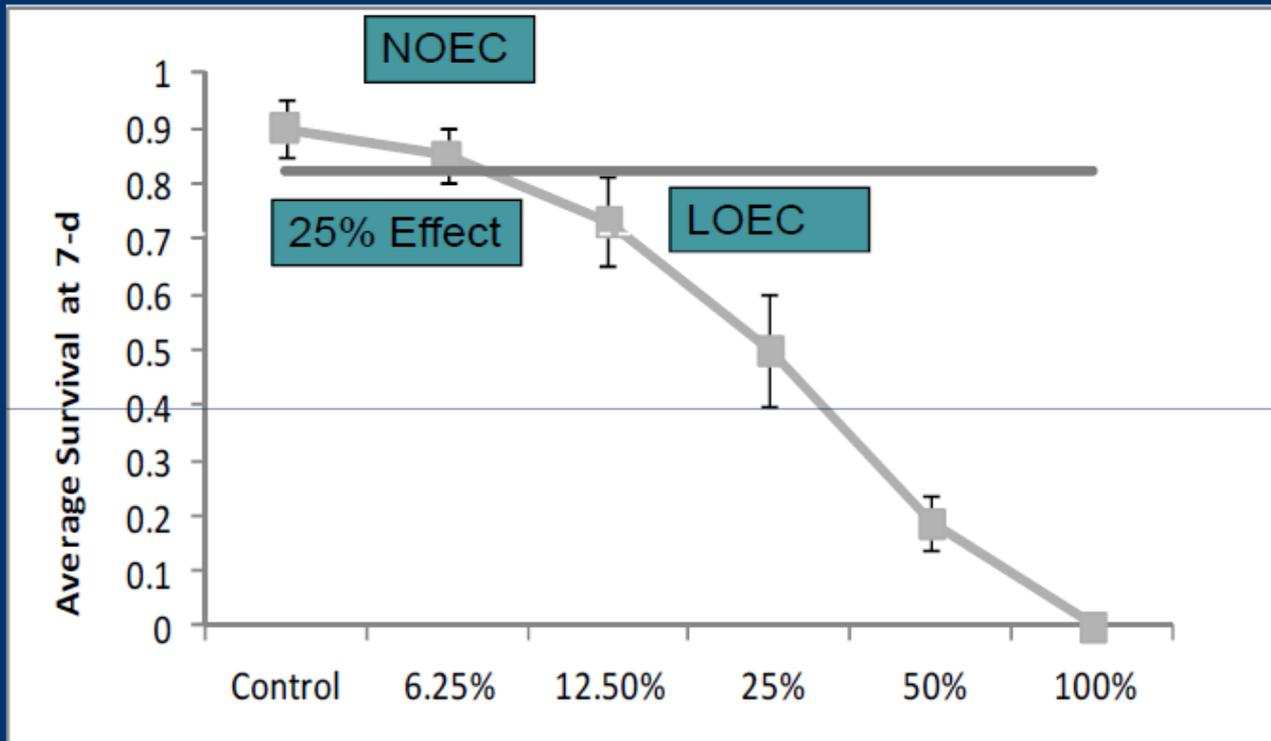
Data Review (cont.)

- It is required to always evaluate the concentration response for each of the chronic sub-lethal test methods when determining the NOEC (NOEL) and LOEC (LOEL) values.
- In Region 6, the % coefficient of variation value is determined and must be $\leq 40\%$ in the control and in the passing critical dilution for the test to be valid. Measures the variability among test replicates

Data Review (cont.)



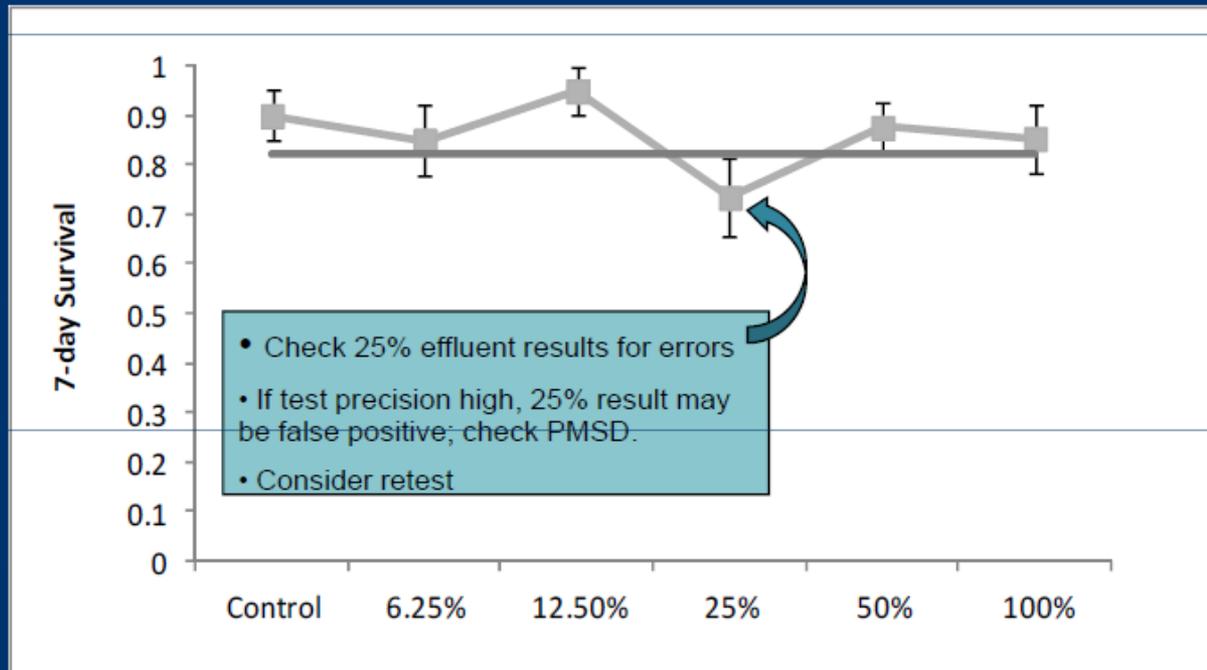
Classic Concentration-Response Relationship: Endpoints are Reliable



Data Review (cont)



Interrupted Concentration-Response: Endpoint may or may not be reliable



Data Review- Test Variability

- PMSD
 - Measures within-test variability; represents the difference from the control response that can be detected statistically
 - PMSD is expressed as a percentage of the control response
($\text{PMSD} = \text{Minimum significant difference (MSD)}/\text{control mean} \times 100$)

Data Review- Test Variability

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).

Data Review- Test Variability

- If the PMSD is \leq the upper bound criterion, the test's variability lies within normal bounds and the NOEC and LOEC values would normally be accepted
- If the PMSD is $>$ upper bound criterion:
 - Toxicity found at the critical dilution, the result is accepted
 - Toxicity not found at the critical dilution, test not accepted
 - Lower Bound Evaluation – used to avoid penalizing labs with unusually high precision; variability document states few labs can achieve unusually high precision on a regular basis. Concentration not toxic if relative difference from the control $<$ normal bounds
 - $\text{Control mean-treatment mean/control mean} \times 100$

IC25 vs. NOEC/LOEC

- Point estimate instead of hypothetical result.
- Can also be used to determine endpoint when NOEC/LOEC difficult to determine (not a true dose response)

TAKE HOME

- Understanding of WET test
- Develop routine procedures to evaluate EVERY WET TEST

Questions?

