#### TRACEABILITY IN WHOLE EFFLUENT TOXICITY TESTING (WETT)

ERIN "GINGER" BRIGGS PRESIDENT BIO-ANALYTICAL LABORATORIES 3240 SPURGIN ROAD DOYLINE, LA 71023 (318) 745-2772 BAL@BIOANALYTICALLABS.COM



WWW.BIOANALTYICALLABS.COM



#### WHAT IS A WET TEST?

- AN IMPORTANT COMPONENT OF EPA'S INTEGRATED APPROACH TO PROTECT SURFACE WATERS FROM POLLUTANTS.
- WET IS "THE AGGREGATE TOXIC EFFECT OF AN EFFLUENT MEASURED DIRECTLY BY A TOXICITY TEST FOR ACUTE AND CHRONIC EFFECTS".
- WET TESTS ARE USED TO DETERMINE THE TOXICITY OF AN EFFLUENT OVER A CERTAIN PERIOD OF TIME.
- WHOLE EFFLUENT TOXICITY IS MEASURED AS OPPOSED TO CHEMICAL SPECIFIC TOXICITY.
- TYPICALLY INCLUDED IN NPDES PERMITS.



#### WHY USE WETT?

- DIRECTLY IMPLEMENTS EPA'S AND STATES' AQUATIC LIFE PROTECTION NARRATIVE CRITERIA OF "NO TOXICS IN TOXIC AMOUNTS."
- WATER-QUALITY-BASED-EFFLUENT LIMIT (WQBEL) IS NEEDED.
- COST EFFECTIVE APPROACH



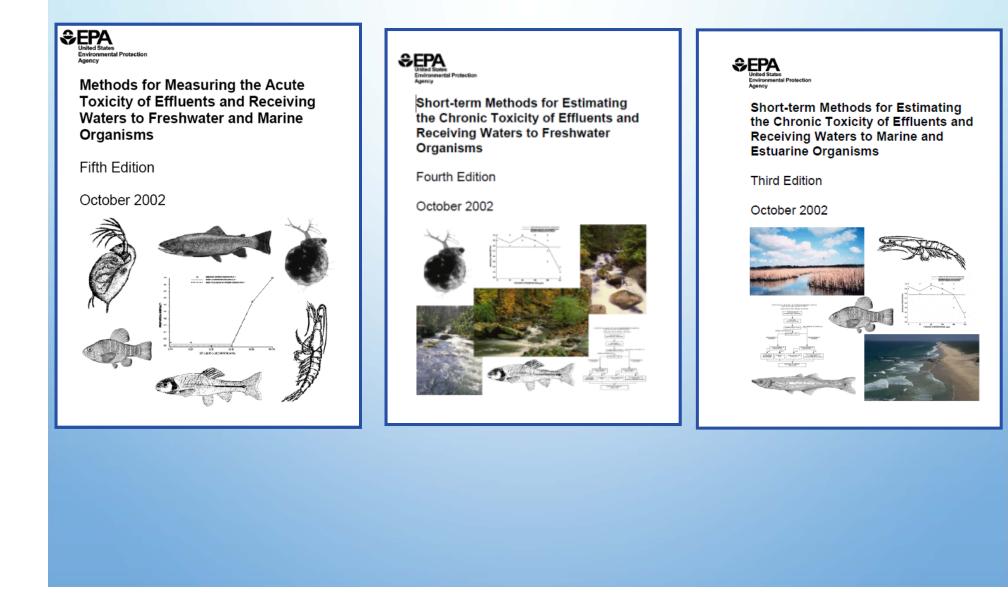
#### THE WETT METHODS LISTED BELOW ARE CODIFIED AT 40 CFR 136.3, TABLE IA

ACUTE TOXICITY - FRESHWATER, MARINE, AND ESTUARINE METHODS Method Title No. ACUTE TOXICITY, FRESHWATER ORGANISMS 2000.0 Fathead Minnow, Pimephales promelas, and Bannerfin shiner, Cyprinella leedsi 2002.0 Daphnia, Ceriodaphnia dubia 2019.0 Rainbow trout, Oncorhynchus mykiss, and Brook trout, Salvelinus fontinalis 2021.0 Daphnia pulex and Daphnia magna ACUTE TOXICITY, ESTUARINE/MARINE ORGANISMS OF THE ATLANTIC OCEAN AND GULF OF MEXICO 2004.0 Sheepshead minnow, Cyprinodon variegatus 2006.0 Silverside, Menidia beryllina, Menidia menidia, and Menidia peninsulae 2007.0 Mysid, Americamysis bahia CHRONIC TOXICITY, FRESHWATER ORGANISMS 1000.0 Fathead minnow, Pimephales promelas, larval survival and growth 1001.0 Fathead minnow, Pimephales promelas, larval survival and teratogenicity Daphnia, Ceriodaphnia dubia, survival and reproduction 1002.0 1003.0 Green alga, Selenastrum capricornutum, growth CHRONIC TOXICITY, ESTUARINE/MARINE ORGANISMS OF THE ATLANTIC OCEAN AND GULF OF MEXICO Sheepshead minnow, Cyprinodon variegatus, larval survival and growth 1004.0 1005.0 Sheepshead minnow, Cyprinodon variegatus, embryo-larval survival and teratogenicity 1006.0 Inland silverside, Menidia beryllina, larval survival and growth 1007.0 Mysid, Americamysis bahia, survival, growth and fecundity Sea urchin, Arbacia punctulata, fertilization 1008.0



https://www.epa.gov/cwa-methods/whole-effluent-toxicity-methods

#### **PROMULGATED MANUAL REFERENCES**



# **ACUTE TEST SPECIES**

#### **Codified Freshwater**



DAPHNIA PULEX OR

DAPHNIA MAGNA (METHOD 2021.0)

CERIODAPHNIA DUBIA (METHOD 2002.0) (WATER FLEA) (ADULTS IN THE PHOTOS) Pimephales promelas (fathead minnow) Method 2001.0

Oncorhynchus mykiss (Rainbow trout) **or** Salvelinus fontinalis (brown trout) Method 2019.0







#### **Codified Marine/Estuarine**

Americamysis bahia (formerly Mysidopsis bahia, mysid shrimp) Method 2007.0



Cyprinodon variegatus (Sheepshead minnow) Method 2004.0



Menidia beryllina, M. menidia, M. peninsulae (silverside minnows) Method 2006.0

# SHORT-TERM CHRONIC SPECIES



#### **Codified Freshwater**

Ceriodaphnia dubia (water flea) Method 1002.0





Pimephales promelas (fathead minnow) Method 1000.0, 1001.0



Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum (freshwater algae) Method 1003.0



Americamysis bahia (Mysid shrimp) Method 1007.0



**Codified Marine/Estuarine** 

Menidia beryllina (inland silverside minnow) Method 1006.0



*Cyprinodon variegatus* (Sheepshead minnow) Method 1004.0, 1005.0

Arbacia punctulata (sea urchin) Method 1008.0

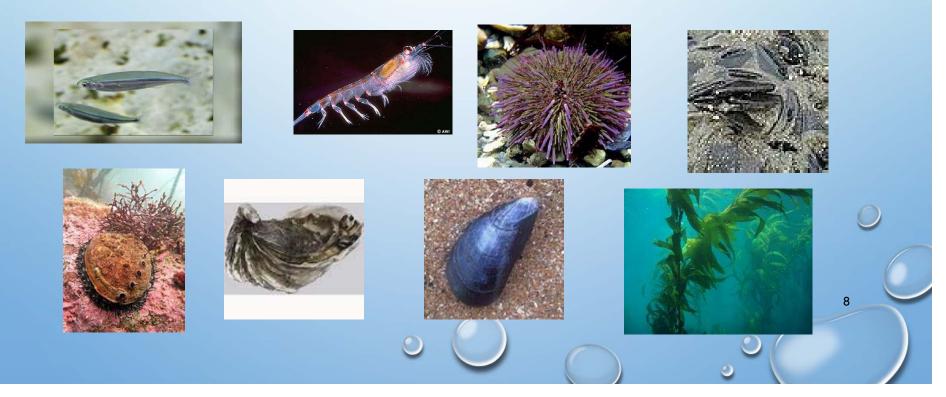


Champia parvula (Red microalgae) Method 1009.0; not listed in CFR Table 1A



### SPECIALIZED TOXICITY TESTS WEST COAST METHODS

 SHORT-TERM METHODS FOR ESTIMATING THE CHRONIC TOXICITY OF EFFLUENTS AND RECEIVING WATERS TO WEST COAST MARINE AND ESTUARINE ORGANISMS. EPA/600/R-95/136, 1995.



### SPECIALIZED TOXICITY TESTS SOIL TOXICITY

- DRILLING FLUID TESTING FLUIDS USED DURING DRILLING OPERATIONS (COOLING AND LUBRICATING THE DRILL BIT, WELL CONTROL, ETC.).
- DRILLING MUD TESTING A MIXTURE OF THE DRILLING FLUIDS WITH THE SOIL EXTRACTED DURING DRILLING OPERATIONS.
  - ORGANISMS USED FOR THIS TESTING CAN BE EITHER SWIMMING OR BURROWING ORGANISMS.
  - FOR EXAMPLE, THE FRESHWATER AMPHIPOD, HYALELLA AZTECA.

#### **SEDIMENT TEST REFERENCES**

- METHODS FOR MEASURING THE TOXICITY AND BIOACCUMULATION OF SEDIMENT-ASSOCIATED CONTAMINANTS WITH FRESHWATER INVERTEBRATES.
- NPDES GENERAL PERMIT 290000 FOR DISCHARGES FROM THE OFFSHORE SUBCATEGORY OF THE OIL AND GAS EXTRACTION CATEGORY FOR THE WESTERN PORTION OF THE OUTER CONTINENTAL SHELF OF THE GULF OF MEXICO OFF THE COASTS OF LOUISIANA AND TEXAS.

#### **FRESHWATER SEDIMENT METHODS**

#### SHORT-TERM CHRONIC TESTS WITH INVERTEBRATES:

- MIDGE, CHIRONOMUS DILUTUS.
  - SURVIVAL AND GROWTH AT 10 DAYS.
  - SURVIVAL, GROWTH, REPRODUCTION, HATCHABILITY.
- AMPHIPOD, HYALELLA AZTECA
  - SURVIVAL AND GROWTH AT 10 DAYS.
  - SURVIVAL, GROWTH, REPRODUCTION.



#### **TEST ENDPOINTS**

- LC50 LETHAL CONCENTRATION
  - THE CONCENTRATION OF SAMPLE THAT KILLS 50% OF THE TEST ORGANISMS.
- NOEC (NOEL) <u>NO</u> <u>OBSERVED</u> <u>EFFECT</u> <u>CONCENTRATION (LEVEL)</u>
  - THE HIGHEST EFFLUENT CONCENTRATION THAT IS NOT SIGNIFICANTLY DIFFERENT FROM THE CONTROL BASED ON STATISTICAL ANALYSIS.
- LOEC (LOEL) LOWEST OBSERVED EFFECT CONCENTRATION (LEVEL)
  - THE LOWEST EFFLUENT CONCENTRATION THAT IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL BASED ON STATISTICAL ANALYSIS.
- ICP INHIBITION CONCENTRATION PERCENTAGE, I.E., IC25%
  - THE EFFLUENT CONCENTRATION THAT SHOWS AN INCREASE IN TOXICITY TO 25% OF THE ORGANISMS OBSERVED FOR THE BIOMASS VALUES WHICH ARE COMBINED EFFECTS OF SURVIVAL/GROWTH, SURVIVAL/REPRODUCTION, SURVIVAL/FECUNDITY.



#### TRACEABILITY



Fathead minnow larvae

### **DEFINITION OF TRACEABILITY**

- "TRACEABILITY IS THE ABILITY TO VERIFY THE HISTORY, LOCATION, OR APPLICATION OF AN ITEM BY MEANS OF DOCUMENTED, RECORDED IDENTIFICATION" –WIKIPEDIA.
- "THE ABILITY TO TRACE THE HISTORY, APPLICATION, OR LOCATION OF AN ENTITY BY MEANS OF RECORDED IDENTIFICATIONS." – THE NELAC INSTITUTE (TNI)



### **GOALS IN TRACEABILITY**

COMPLETENESS OF THE INFORMATION REGARDING EVERY STEP IN A
 PROCESS.

- DOCUMENTATION OF RECORDED INFORMATION.
- GENERATION OF REPRODUCIBLE AND DEFENSIBLE DATA.

# WET VS. CHEMICAL LABORATORIES

#### • SIMILARITIES:

- FOLLOWING REFERENCED METHOD.
- DOCUMENTATION (DOC, SOPS, PREPARATION LOGS, REAGENT LOGS, TEMPERATURE, BALANCES, INSTRUMENTS, ETC.).

- ERROR CORRECTION POLICY REVIEWS.
- TRACEABILITY: REAGENTS, STANDARDS, SOURCE WATER
- INSTRUMENT CALIBRATION AND MAINTENANCE RECORDS.
- CALCULATION REVIEWS.
- QUALITY CONTROL CHARTS.
- PARTICIPATES IN PT TESTING AND EPA DMR-QA

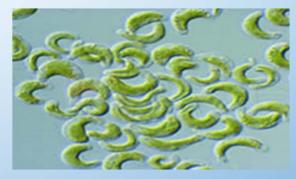
# WETT VS. CHEMICAL LABORATORIES

#### • DIFFERENCES:

- ORGANISM REVIEW: TAXONOMIC VERIFICATION, CULTURING, ORGANISM AGE FOR TESTS, *ETC*.
- TRACEABILITY OF ORGANISMS AND FOOD
- SYNTHETIC/RECONSTITUTED WATER PREPARATION.
- LIGHT CYCLES, INTENSITIES.
- STANDARD REFERENCE TOXICANT TESTS (SRT).
- STATISTICAL ANALYSES REVIEW PER EPA METHODS.
- CHEMICAL DATA (I.E., D.O., PH, SALINITY, CONDUCTIVITY, ETC.) ARE SUPPORTING DOCUMENTATION ONLY.

# **ELEMENTS OF WETT TRACEABILITY**

- TAXONOMY
- TEST ORGANISM HEALTH
  - WATER
  - FOOD
  - REFERENCE TOXICANT TESTING
- SAMPLE INTEGRITY
- PERSONNEL TRAINING.
- TEST DESIGN.



Freshwater algae

#### TAXONOMY

- TAXONOMY
  - THE SCIENCE OF IDENTIFICATION AND/OR VERIFICATION OF SPECIES OF ORGANISMS.
  - MUST BE VERIFIED ANNUALLY OR MORE OFTEN IF CULTURES ARE RE-STARTED.

PHYLUM CLASS

> > GENUS HOMO

- SOURCE PROVIDES TAXONOMIC ID WHEN LAB USES PURCHASED ORGANISMS RATHER THAN IN-HOUSE CULTURES.
  - TAXONOMIC IDENTIFICATION KEPT ON FILE MUST INCLUDE REFERENCE
    - CITATION AND PAGE(S).
    - NAME(S) OF THE TAXONOMIC EXPERT(S).

## **TEST ORGANISM HEALTH**

- ORGANISM HEALTH:
  - GOOD LABS WILL HAVE CULTURE LOGS.
    - SURVIVAL RATE
    - NEONATE PRODUCTION
    - SPAWNING DOCUMENTATION

20



Adult male fathead minnow in spawning substrate

### CERIODAPHNIA DUBIA NEONATE PRODUCTION

- IDENTIFY THE BROOD BOARD NUMBER.
- MAINTAIN RECORDS PROVING THAT THE PARENT FEMALES HAS BEEN DEEMED VALID FOR USE IN TESTING (MUST HAVE 3 BROODS BEFORE USE).
- IDENTIFY THE TIME PERIOD IN WHICH THEY WERE BORN (I.E., 8 HOUR WINDOW).
- MAINTAIN RECORDS THAT THE NEONATES WERE FED PROPERLY PRIOR
  TO USE IN TESTING.



Gravid Ceriodaphnia dubia

#### BROOD BOARD TRACKING FOR CERIODAPHNIA DUBIA

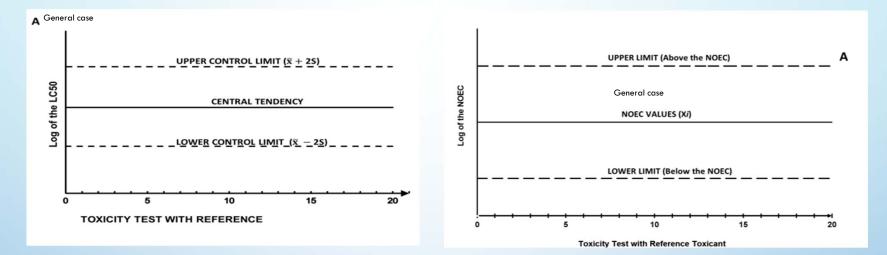


## **STANDARD REFERENCE TOXICANT TESTING**

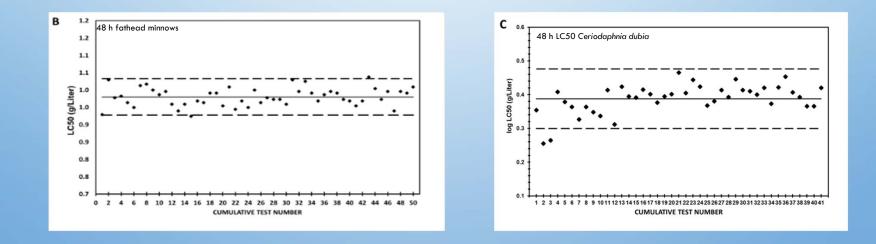
- STANDARD REFERENCE TOXICANT TESTING (SRT) IS PERFORMED
  WITH A KNOWN TOXICANT TO DETERMINE TRENDS IN CULTURE
  HEALTH.
  - MINIMUM OF MONTHLY FOR ALL ORGANISMS RAISED IN-HOUSE.
  - CONCURRENT WITH PERMITTED TEST ON ALL ORGANISMS THAT ARE WILD-CAUGHT.
  - CONCURRENT WITH PERMITTED TEST ON ALL ORGANISMS OBTAINED FROM A COMMERCIAL SUPPLIER, UNLESS THE SUPPLIER PROVIDES CONTROL CHART DATA FROM AT LEAST FIVE MONTHLY REFERENCE TOXICANT TESTS.
  - REGARDLESS OF ABOVE, LABORATORY MUST PERFORM AT LEAST ONE
    ACCEPTABLE SRT PER MONTH FOR EACH TEST CONDUCTED THAT MONTH.



#### **CONTROL CHARTS FOR SRTS**

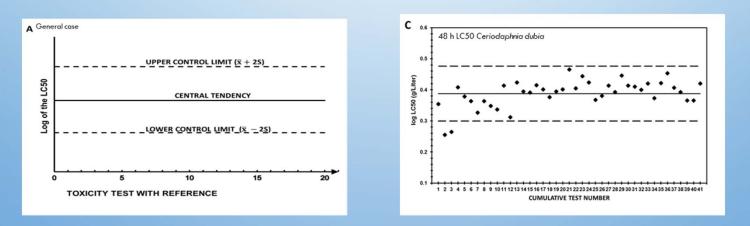


Examples: 48 h acute tests with sodium chloride



#### **CONTROL CHARTS FOR SRTS**

- MAINTAIN CONTROL CHART.
  - USE 2 YEARS OF DATA OR A MINIMUM OF 20 POINTS.
  - ONLY THE LAST 20 DATA POINTS ARE USED TO DETERMINE ACCEPTANCE CRITERIA.
- CONTROL CHARTING PLOTS THE EFFECT (LC, EC, IC, NOEC).
  - ANTI-LOGARITHM VALUES OR LOGARITHM VALUES.
  - CONTROL CHART CANNOT MIX LOG VALUES ON A LOG SCALED Y-AXIS, OR ANTILOG VALUES ON AN ARITHMETIC SCALE Y-AXIS.



#### WATER

- DEIONIZED WATER SYSTEM MAINTENANCE.
  - WEEKLY CHECK OF CONDUCTIVITY, RESISTIVITY, TRCL, NH3.
  - TOXIC ORGANIC AND TOXIC METALS.
- RECONSTITUTED WATER BATCH.



# FOOD

- FOOD SOURCE IDENTIFICATION
- FOOD SOURCE PREPARATION
- NEW BATCH QUALITY CONTROL
  - TOXIC METALS AND ORGANICS
  - FOOD TESTS PRIOR TO USE







- TEMPERATURE MONITORING DATA, SUCH AS HOBO TEMPERATURE LOGGERS.
- LIGHT CYCLES AND INTENSITIES.







#### **SAMPLES: DOCUMENTATION**

- CLEANING OF SAMPLERS
- CHAIN OF CUSTODY DOCUMENTATION
  - SITE ID
  - DATE, TIME, TYPE OF SAMPLE COLLECTED
  - PRESERVATIVE
  - SAMPLER SIGNATURE
  - SAMPLE TRACKING
  - TEMPERATURE UPON ARRIVAL



#### **SAMPLES: DOCUMENTATION**

- SAMPLE TRACKING IN THE LABORATORY:
  - ACCEPTANCE LOGS
  - UNIQUE SAMPLE ID
  - DISPOSAL LOGS
  - MUST BE ABLE TO TRACK SAMPLE FROM THE TIME IT ENTERS THE LABORATORY TO DISPOSAL.



### **PERSONNEL TRAINING**



#### DEMONSTRATION OF CAPABILITY (DOC) 2009 TNI

#### • INITIAL DEMONSTRATION OF CAPABILITY (IDOC).

- EACH ANALYST SHALL MEET THE QUALITY CONTROL REQUIREMENTS AS SPECIFIED IN SECTION 1.7.1.2.
  - NELAC 2003 APPENDIX D2 OR TNI 2009 V1M7 §1.6 (EL-V1M7-2009).
- POSITIVE AND NEGATIVE CONTROLS.
  - SRTS AND CONTROL ORGANISM PERFORMANCE.
- CONTINUING DOC (CDOC).
- DOCUMENTED PROCEDURE DESCRIBING ONGOING DOC.
- ANALYSTS MUST MEET QC REQUIREMENTS OF THE METHOD, LAB SOP, CLIENT SPECIFICATIONS, AND THE STANDARD.
- QC SAMPLE DATA MUST BE REVIEWED TO IDENTIFY PATTERNS FOR INDIVIDUALS OR GROUPS AND MAKE CORRECT ACTIONS.



#### **PERSONNEL TRAINING**

#### TRAINING RECORDS SHOULD INCLUDE:

- GENERAL LABORATORY CHECKLIST.
- ANALYSES SPECIFIC CHECKLIST.
- INITIALS AND SIGNATURE DOCUMENTATION.
- ON-GOING TRAINING ATTENDANCE.
- INITIAL DEMONSTRATION OF CAPABILITY (IDOC).
- CONTINUING DEMONSTRATION OF CAPABILITY (CDOC).
- ETHICS POLICY AGREEMENT.
- JOB RESPONSIBILITIES CHECKLIST.

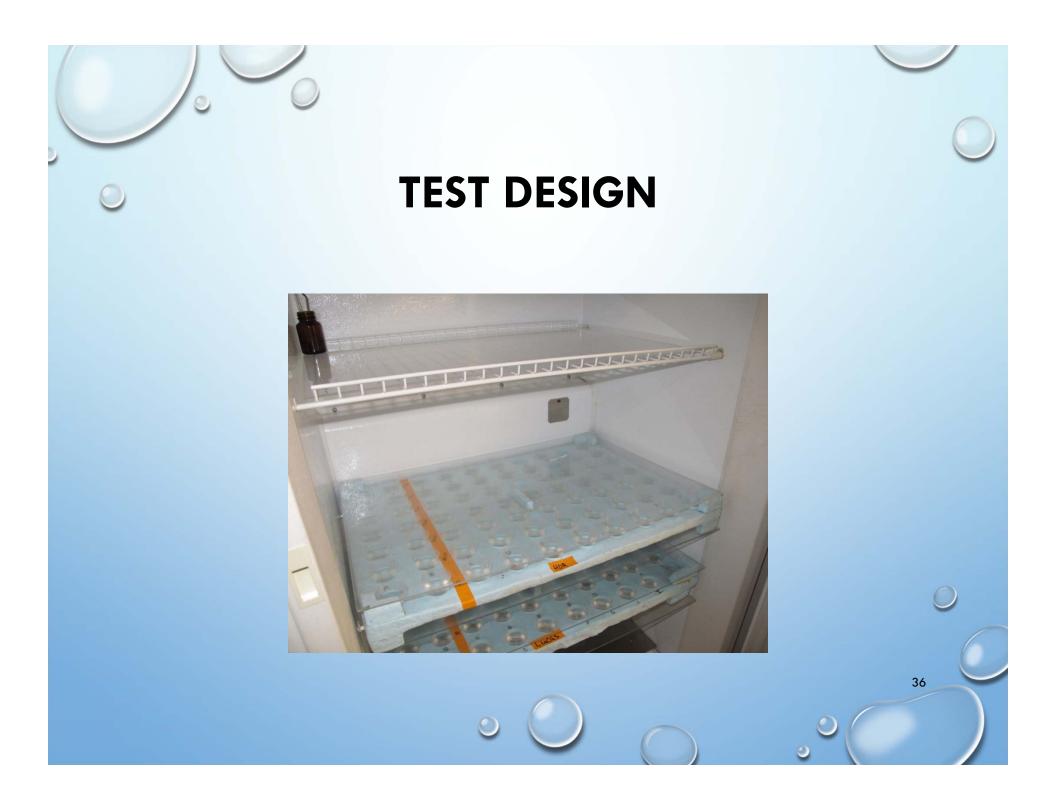


ALL ANALYSTS TRAINED SIMILARLY USING LAB-DEFINED PROTOCOLS, SOPS.

#### **STANDARD REFERENCE TOXICANT (SRT) TESTS**

- SRTS ARE USED FOR EVALUATING THE HEALTH AND SENSITIVITY OF ORGANISMS OVER TIME AND FOR DOCUMENTING INITIAL AND ONGOING LABORATORY PERFORMANCE.
  - INITIALLY NEED 5 OR MORE ACCEPTABLE SRTS FOR EACH TEST METHOD, SPECIES, AND ENDPOINT.
- APPROPRIATE NEGATIVE CONTROLS MUST BE TESTED AT THE FREQUENCY AND DURATION SPECIFIED IN THE TEST METHOD.
- USE CRITERIA OBTAINED FROM CONTROL CHARTS TO DETERMINE GROUP OR ANALYST CAPABILITY.
- ANALYST DOC MAY BE PERFORMANCE WITHIN ESTABLISHED CONTROL LIMITS OR RESULTS OBTAINED ARE THE SAME AS A TRAINED ANALYST.





## **TEST DESIGN: DOCUMENTATION**

TEST DILUTION PREPARATIONS

- DISSOLVED OXYGEN (DO) SHOULD NOT BE SUPERSATURATED OR HAVE A DO CONCENTRATION BELOW 4.0 MG/L.
  - AERATE SAMPLES AFTER HEATING FROM 4° C TO 25° C, BEFORE PREPARING TEST DILUTIONS.
- IF THE SAMPLE CONTAIN WILD ORGANISMS, IT MUST BE FILTERED PRIOR TO USE.
- ANY SAMPLE MANIPULATIONS **MUST** BE DOCUMENTED.

### RANDOMIZATION

- THE DISTRIBUTION OF TEST ORGANISMS AMONG TEST CHAMBERS AND THE ARRANGEMENT OF TREATMENTS AND REPLICATE CHAMBERS.
- PURPOSE IS TO AVOID SITUATIONS WHICH COULD INTRODUCE BIAS INTO THE TEST RESULTS.
- CHRONIC MANUALS REQUIRE RANDOM TEMPLATES.



#### **RANDOM NUMBER TEMPLATE**

Test: LF-Larval Fish Growth and Survival Test Test ID: X6100																
	Species: PP-Pimephales promelas										Protocol: EPAFW02-EPA/821/R-02-013					
Sample ID: LA12345 Sample Type: EFF1-POTW																
Start Date: 7/25/2016 End Date: 7/31/2016										Lab ID: LA00917						
<u>o tart</u>																
	ID		Group		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Total Wgt(	Tare Wgt(	Wgt Count		
66			12.5													
67	9	4	6.25													
68	21	1	50													
69	12	2	12.5											·		
70	28		100													
71	8	3	6.25													
72	2	2	D-Control													
73	23	3	50													
74	29	4	100													
75	1	1	D-Control													
76	20	5	25							<b>F</b>						
77	27	2	100													
78	11	1	12.5													
79	19	4	25													
80	26		100													
81	25	5	50						-	1						
82	13		12.5	i							1					
83		2	6.25													
84		2	25													
85	3		D-Control										· · · ·			
86	10			<u> </u>		1										
87	24									1						
88	<u> </u>		D-Control			1										
89		_	12.5			<u> </u>			<u> </u>	1	· · · ·					
90	6		6.25			<u> </u>	<u> </u>	<u> </u>	<u> </u>							
91	22	2				<u> </u>				<u> </u>						
92	5		D-Control			<u> </u>			<u> </u>	+						
93										+						
94	_										<u> </u>					
95								<u> </u>								
	monte		25			1			<u> </u>	1						

Comments:

#### **RANDOMIZED CHRONIC FISH TEST**



#### BLOCKING BY KNOWN PARENTAGE WHEN INITIATING THE CHRONIC CERIODAPHNIA DUBIA CHRONIC TEST (METHOD 1002.0)

 13.10.2.2 – "THE TEST CHAMBERS MUST BE RANDOMLY ASSIGNED TO A BOARD USING A TEMPLATE (FIGURE 1) OR BY USING RANDOM NUMBERS (SEE APPENDIX A). RANDOMIZING THE POSITION OF THE TEST CHAMBERS AS DESCRIBED IN FIGURE 1 (OR EQUIVALENT) WILL ASSIST IN ASSIGNING TEST ORGANISMS USING BLOCKING BY KNOWN PARENTAGE (SUBSECTION 13.10.2.4)."



#### FIGURE 1: EXAMPLES OF A TEST BOARD AND RANDOMIZING TEMPLATE- CERIODAPHNIA DUBIA CHRONIC TEST

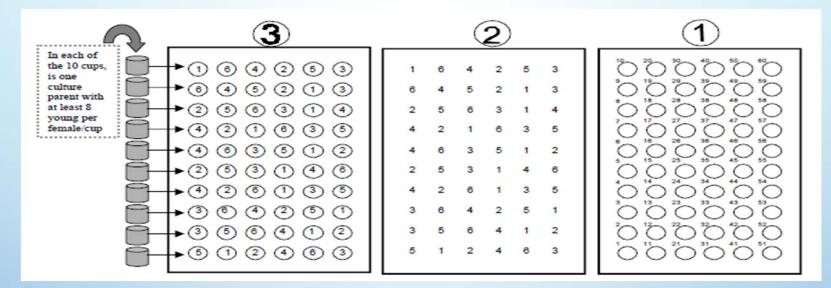


Figure 1. Examples of a test board and randomizing template:

- 1) test board with positions for six columns of ten replicate test chambers with each position numbered for recording results on data sheets,
- 2) cardboard randomizing template prepared by randomly drawing numbers (1-6) for each position in a row across the board, and

3) test board with random locations showing from template.

In practice: test board 1 is placed on top of the 2) the randomizing template, and the test organisms are assigned from one brood cup to each treatment within a given block. Following placement of test chambers, test organisms are allocated using blocking by known parentage.

#### **CLOSING REMARKS**

P EPA HAS RECORDED WEBINARS FOR WHOLE EFFLUENT TOXICITY TESTING (2016) AVAILABLE AT

HTTPS://WWW.EPA.GOV/NPDES/NPDES-TRAINING#WETTRAINING

- MODULE 1: OVERVIEW OF THE NPDES WET PERMITTING PROGRAM
- MODULE 2: NPDES TESTING METHODS FOR WHOLE EFFLUENT TOXICITY
- MODULE 3: NPDES REVIEWING WET TESTS AND WET QA/QC
- MODULE 4: NPDES WET STATISTICAL ANALYSIS AND DATA INTERPRETATION
- MODULE 5: DETERMINING WET REASONABLE POTENTIAL FOR NPDES PERMITTING
- MODULE 6: NPDES WET PERMIT DEVELOPMENT
- MODULE 7: NPDES WET TESTING DECISION-MAKING AND WET PERMIT LANGUAGE REVIEW

- MODULE 8: NPDES WET COMPLIANCE AND ENFORCEMENT
- MODULE 9: NPDES TOXICITY REDUCTION EVALUATIONS AND TOXICITY IDENTIFICATION EVALUATIONS

