



# NPDES PERMIT WHOLE EFFLUENT TOXICITY (WET) REQUIREMENTS, IMPLEMENTATION, AND CASE STUDIES

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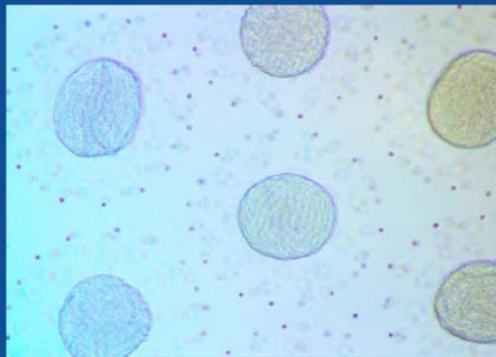
# Presentation Overview

- Background
  - Toxicity Testing 101
  - Regulatory Drivers
- Interpreting Test Results
- Toxicity Reduction Evaluations (TRE/TIE)
- Emerging Issues



# Toxicity Testing 101 - Bioassays

- Whole Effluent Toxicity (WET) test or bioassay is “the aggregate toxic effect of an effluent measured directly by a toxicity test for acute and chronic effects.”
- 40 CFR Part 136 of the Clean Water Act (CWA): EPA promulgated 16 methods for acute and short-term chronic test to estimate acute and chronic toxicity.



72-hours



# Toxicity Testing 101 - Acute

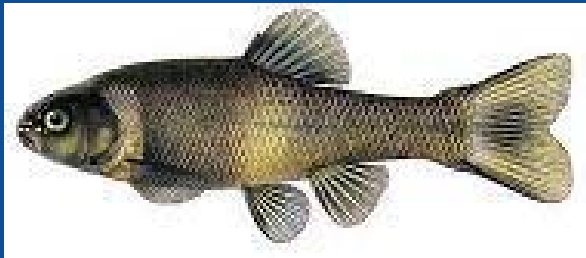
- Expose organisms to effluent and assess mortality

- Basin Plan: (Region 2)

- “Acute toxicity limitation is evaluated by measuring survival of test fishes exposed to effluent for 96 hours”

- Test Species

- *Freshwater vertebrate: Fathead minnow or rainbow trout*



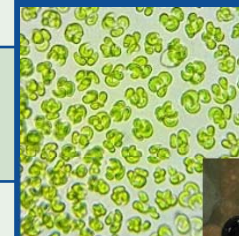
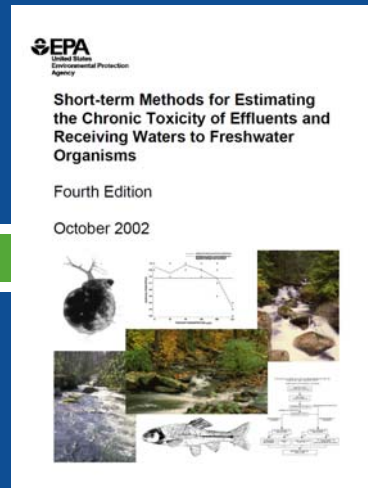
- 3-test median of 90% survival
- Not less than 70% survival more than 10% of the time



# Toxicity Testing 101 - Chronic

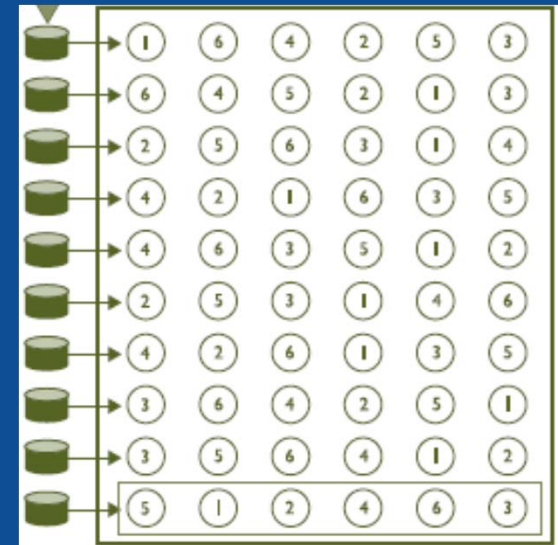
- Duration < 8-days; species dependent
- Endpoints: survival, growth, reproduction

Test Species (examples)	Endpoint
Fathead minnow ( <i>Pimephales promelas</i> )	survival & growth (weight)
Water flea ( <i>Ceriodaphnia dubia</i> )	survival & reproduction (# neonates per female)
Green alga ( <i>Selenastrum capricornutum</i> )	growth (number of cells)
Mussel ( <i>Mytilus</i> sp.)	survival and growth (shell development)
Mysid ( <i>Americamysis bahia</i> )	survival & growth (& reproduction)



# Example with *Ceriodaphnia dubia*

Compare biological response from effluent samples versus control response (e.g., lab water).



- Collect sample(s)
- Chemistry measurements
- Prepare dilution series
- Test species and conditions
- Endpoint
- Statistical test

grab/composite, renewals

DO, pH, EC, alk, hardness, chlorine, NH<sub>3</sub>-N  
 % Effluent: 100, 75, 50, 25, 6.25 + control

e.g., *C. dubia*, temperature, food, light

number of neonates per female, survival

NOEC, EC<sub>25</sub>, TST

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- Toxicity Reduction Evaluations (TRE/TIE)
- Toxicity Provisions / Emerging Issues





# Regulatory Drivers

“It is the national policy that the discharge of toxic pollutants in toxic amounts be prohibited.” *Clean Water Act § 101(a)(3)*

- States adopt water quality standards to ensure protection of Aquatic Life:
  - Chemical-specific monitoring
  - Whole effluent toxicity (WET)
  - Biological criteria





# Regulatory Drivers



## □ California:

- *SWRCB Policy for Implementation of Toxics Standards for Inland Surface Waters, Enclosed Bays, and Estuaries of California (SIP) – Section 4: Chronic Toxicity Control Provisions*
- Basin Plans include a narrative toxicity objective: *All waters shall be maintained free of toxic substances in concentrations that are lethal to or that produce detrimental responses in aquatic organisms (SFBRWQCB 2017).*

# Regulatory Drivers

- SIP – *Section 4: Chronic Toxicity Control Provisions*
  - Requires use of short-term chronic toxicity tests to determine compliance with chronic aquatic life toxicity objectives
  - Requires a chronic toxicity effluent limitation in permits for discharges with “reasonable potential”
  - Requires a Toxicity Reduction Evaluation (TRE) if discharge contributes to chronic toxicity
- SWRCB Proposed Toxicity Provisions (2019)
  - Would supersede the current SIP and Basin Plan toxicity requirements



# Permit Requirements

## □ Chronic Toxicity Test Requirements

- Dilution series (may be required)
- Test frequency
- Test species
- Test endpoints
- Type of dilution water
- Data reporting requirements
- Narrative Toxicity Limits / Numeric Toxicity Limits
- Accelerated testing/ Toxicity Reduction Evaluation (TRE) trigger



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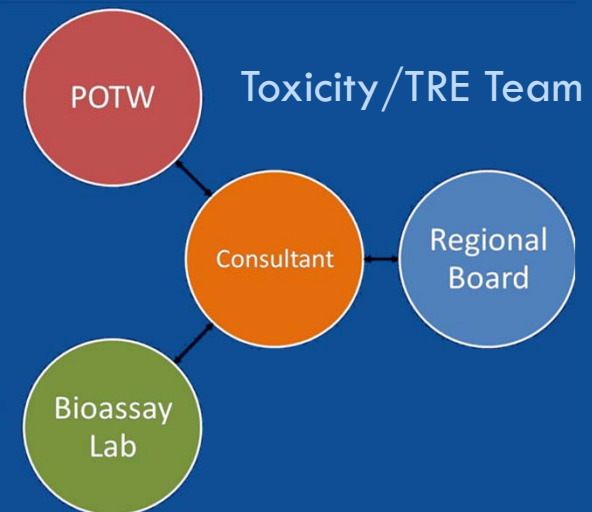
# Reasons for data review

- A good team + review = good results (can avoid an unnecessary TRE)
- Qualified data with low confidence or data that are not representative may prevent it from being used inappropriately for regulatory decisions

- Reasonable potential analysis (RPA)
- Species sensitivity testing

- What to review?





- lab report
- bench sheets
- data analyses (e.g., CETIS output)
- reference toxicity results



# Test Acceptability Criteria



## 1) Does Test meet EPA's test acceptability criteria (TAC)?

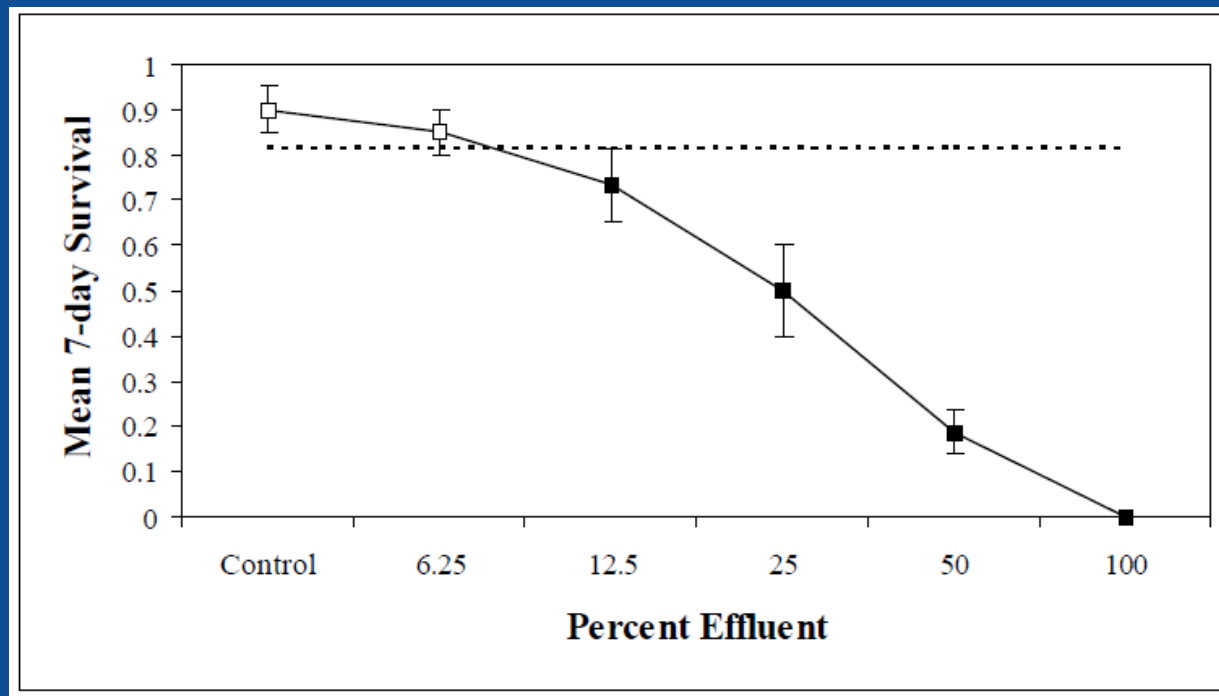
Species	Minimum Test Acceptability Criteria
<i>Pimephales promelas</i>  <a href="http://www.iowadnr.gov">www.iowadnr.gov</a>	<ol style="list-style-type: none"> <li>1. <math>\geq 80\%</math> control survival</li> <li>2. Average dry weight <math>\geq 0.25</math> mg per surviving control organism</li> </ol>
<i>Ceriodaphnia dubia</i> 	<ol style="list-style-type: none"> <li>1. <math>\geq 80\%</math> control survival</li> <li>2. Average of <math>\geq 15</math> young per surviving control females</li> <li>3. <math>\geq 60\%</math> of surviving control females must produce 3 broods (maximum test duration of 8 days)</li> </ol>
<i>Selenastrum capricornutum</i> 	<ol style="list-style-type: none"> <li>1. <math>\geq 1 \times 10^6</math> cells/mL average cell density in control</li> <li>2. <math>\leq 20\%</math> CV in control</li> </ol>
<i>Americamysis (Mysidopsis) bahia</i>  <a href="http://www.calacademy.org">www.calacademy.org</a>	<ol style="list-style-type: none"> <li>1. <math>\geq 80\%</math> control survival</li> <li>2. Average dry weight <math>\geq 0.20</math> mg per surviving control</li> <li>3. <math>\geq 50\%</math> females in controls produce eggs (if fecundity endpoint used)</li> </ol>

# Concentration-Response



2) Evaluate the concentration-response relationship (required; if dilutions)

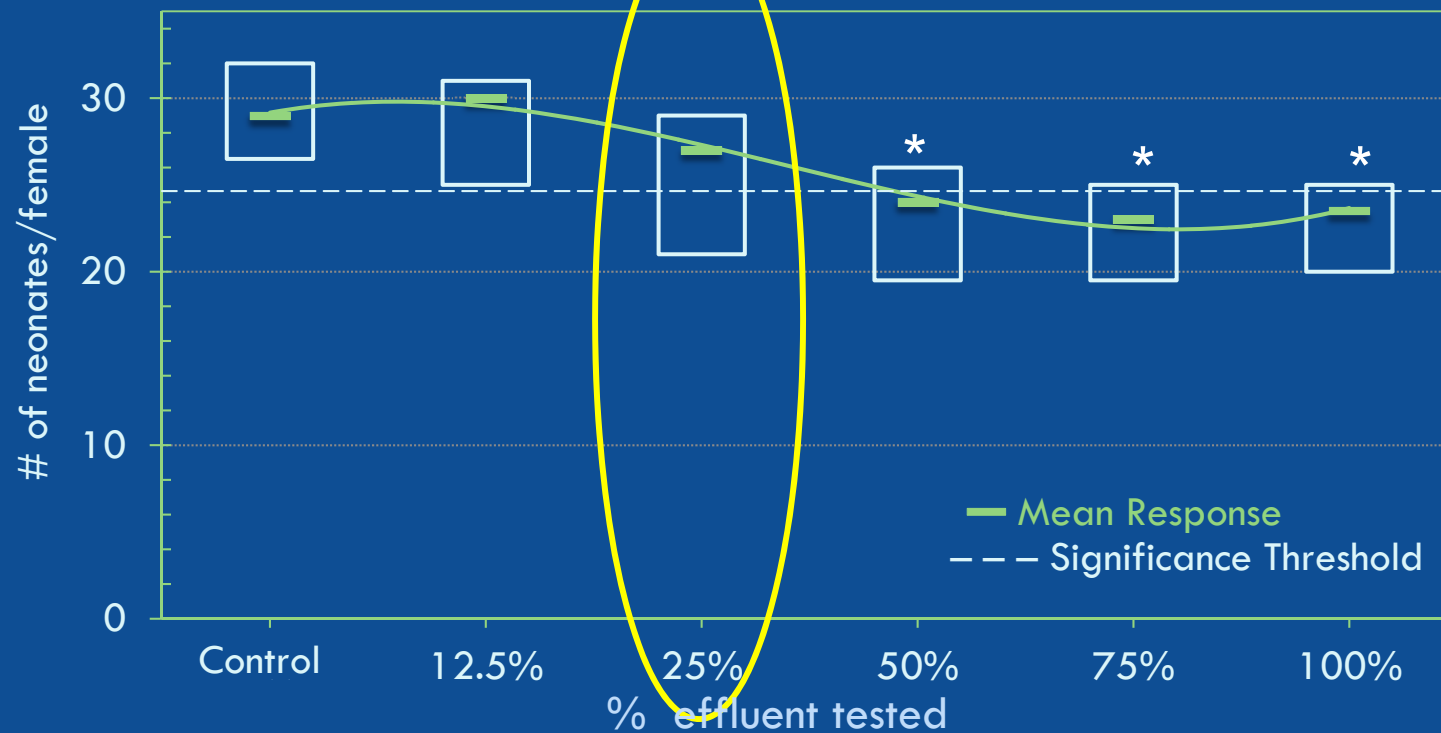
- Characteristics of toxicity – strength, magnitude of effect, abnormal..



1. Ideal concentration-response relationship (EPA 2000)
  - Reliable results can be interpreted from these data

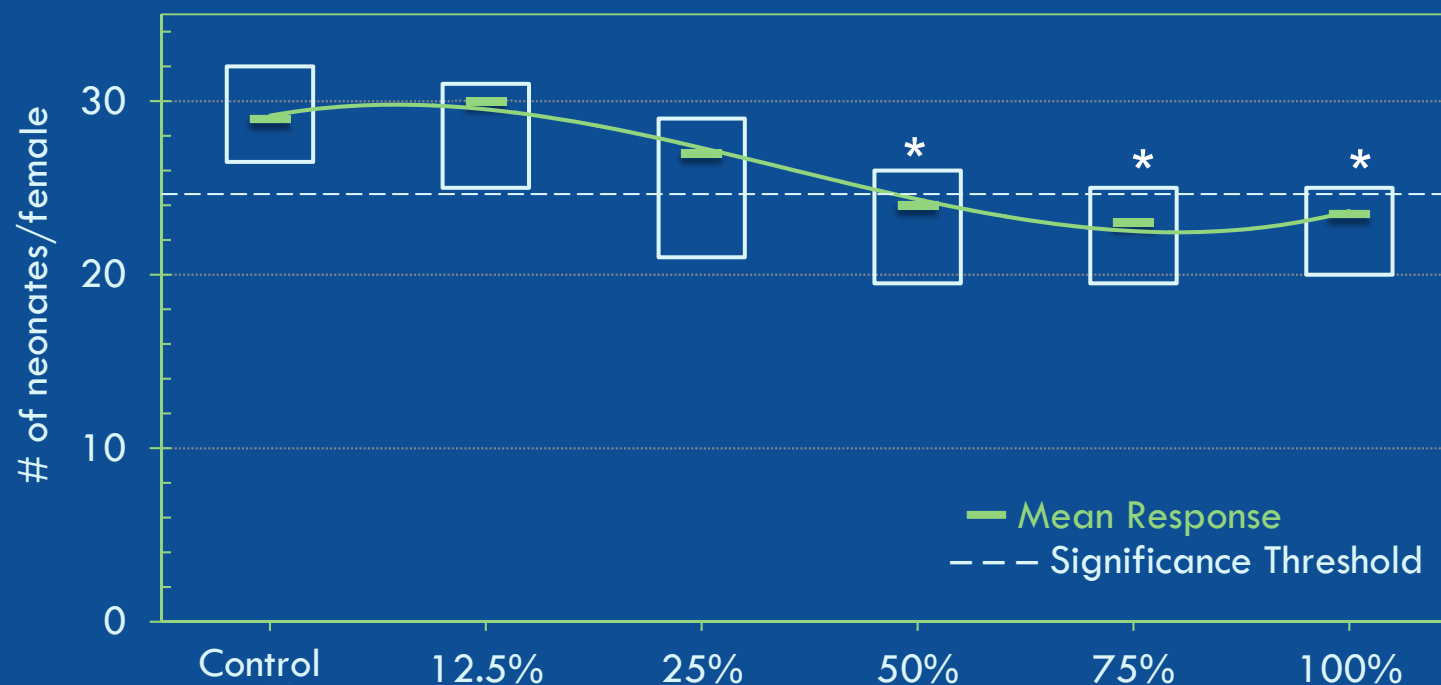


# Toxicity Vocabulary – NOEC



- NOEC = No Observed Effect Concentration; the greatest concentration that is not significantly different from the control
- In this example the 25% effluent concentration is the NOEC
- Chronic Toxic Units =  $TU_c = 100/NOEC$   
 $= 4 TU_c$

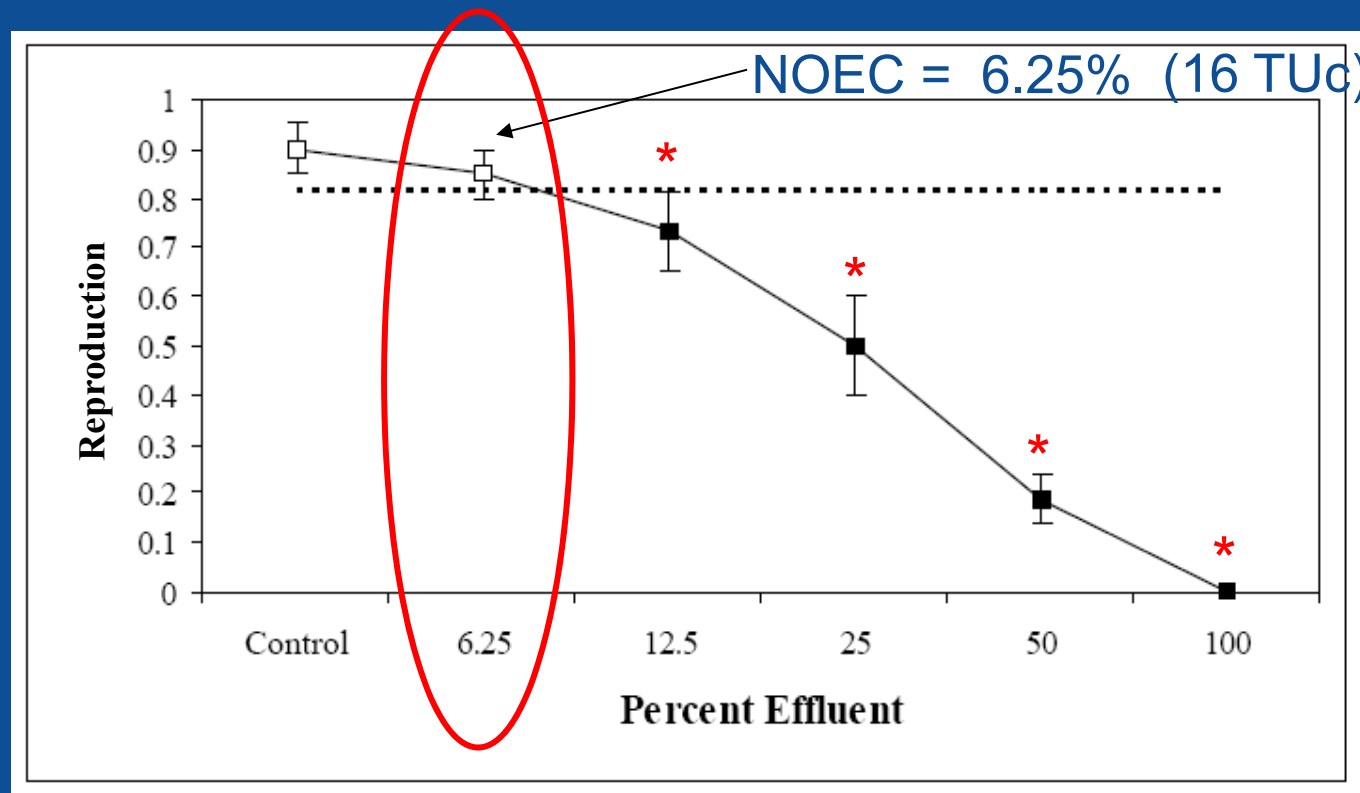
# Toxicity Vocabulary – NOEC



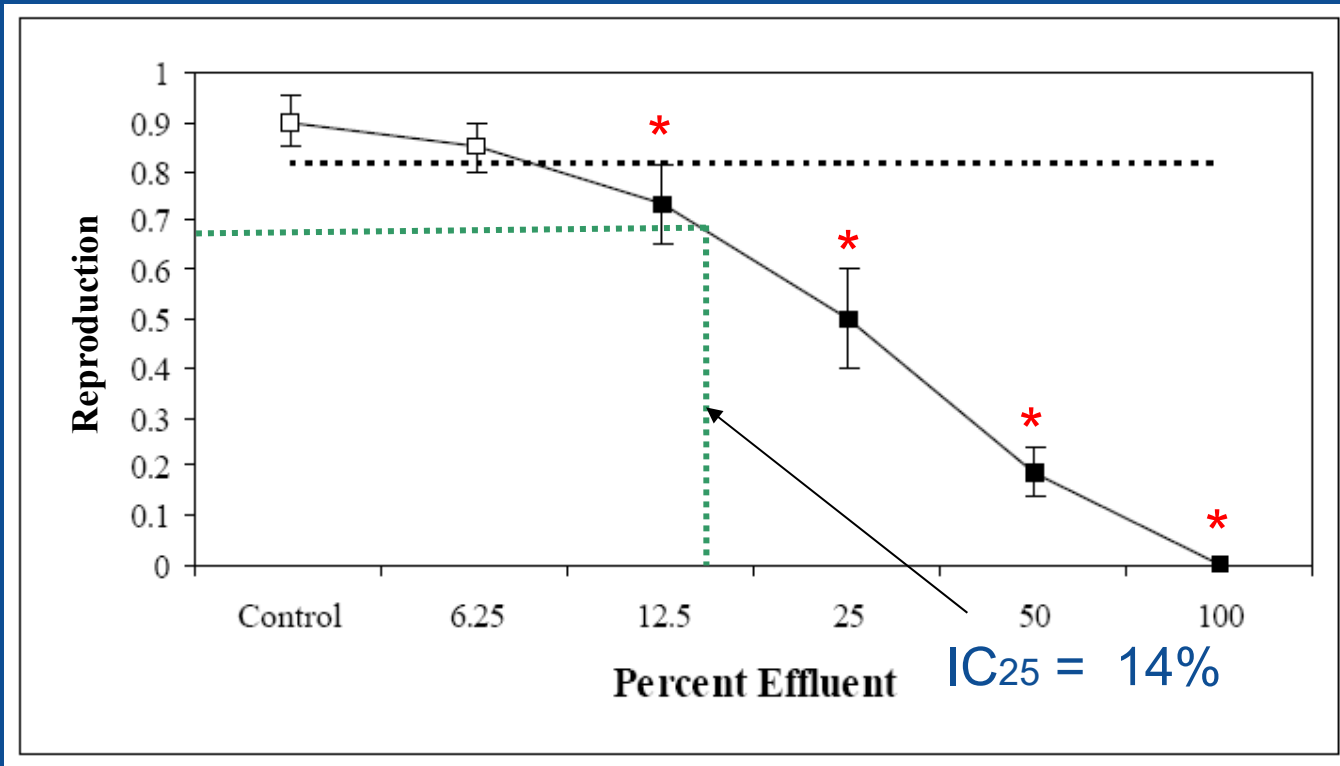
a greater number of TU = more toxicity

NOEC Concentration	Calculation	TUc
100	100/100	1
50	100/50	2
25	100/25	4
12.5	100/12.5	8

# Toxicity Vocabulary – NOEC



# Toxicity Vocabulary – IC<sub>25</sub>

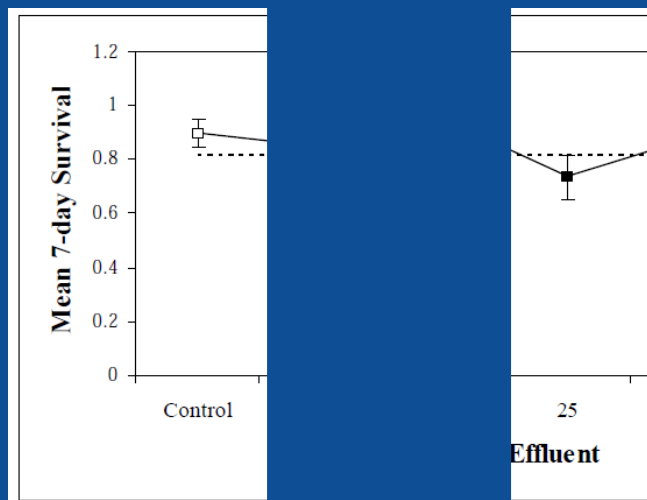


- IC<sub>25</sub> = the concentration causing 25% inhibition (effect) of the response variable (toxicity endpoint).
- $TU_c = 100/IC_{25}$   
= 7  $TU_c$

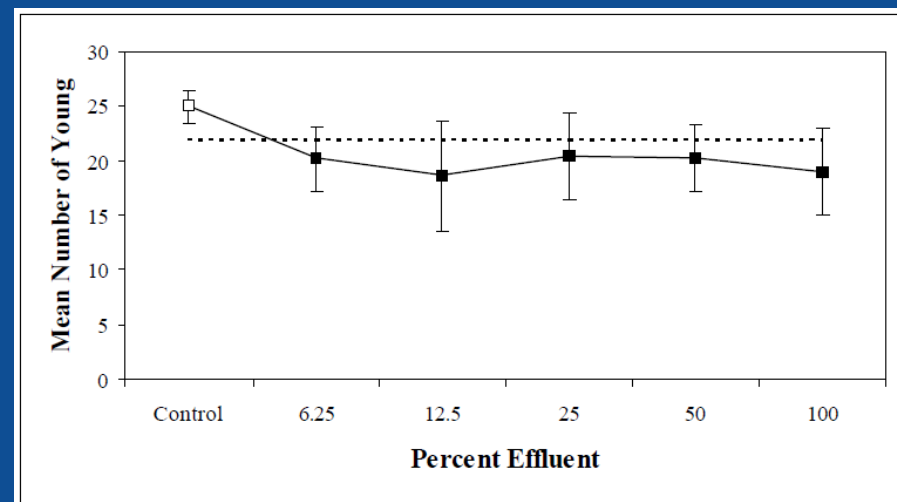
# Concentration-Response



EPA (2000) identified 9 irregular concentration-response types and provides appropriate guidance for each



5. Interrupted – effect bracketed by no effects
- NOEC compromised/interpret carefully
  - IC25 reliable



8. Flat - effects at all concentrations
- NOEC and IC25 compromised
  - interpret carefully

# Percent Minimum Significant Difference

3) Assess variability among replicates and the lab-calculated percent minimum significant difference (PMSD)

- PMSD – smallest percentage decrease in growth or reproduction from the control that could be determined as statistically significant in the test.



Species	Endpoint	Lower PMSD Limit	Upper PMSD Limit
<i>Pimephales promelas</i>	growth	12	30
<i>Ceriodaphnia dubia</i>	reproduction	13	47
<i>Selenastrum capricornutum</i>	growth	9.1	29
<i>Americamysis bahia</i>	growth	11	37

# Percent Minimum Significant Difference

## □ Example 1:

- Fathead minnow growth in 100% effluent 10% lower than the control. Effect was significant. PMSD 8%.
- Result – sample is not toxic because effect is <lower PMSD



## □ Example 2:

- *C. dubia* reproduction in 100% effluent not significantly different from the control. PMSD 52%
- Result – PMSD exceeds upper limit/ poor precision; retest.

Species	Endpoint	Lower PMSD Limit	Upper PMSD Limit
<i>Pimephales promelas</i>	growth	12	30
<i>Ceriodaphnia dubia</i>	reproduction	13	47

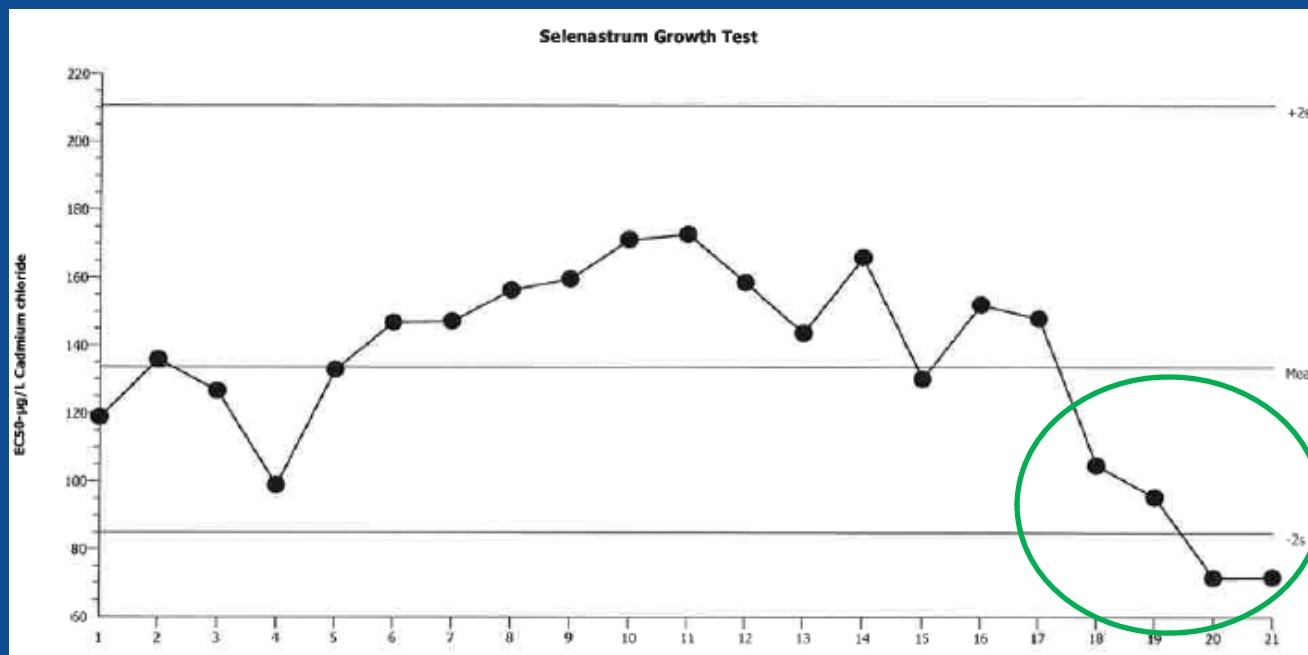


# Reference Toxicity



## 4) Review reference toxicant results and control charts

- Test organism response to a reference toxicant (i.e., IC<sub>50</sub> and EC<sub>50</sub>)
- Tracked over time to evaluate changes in organism sensitivity



135 µg/L

70 µg/L



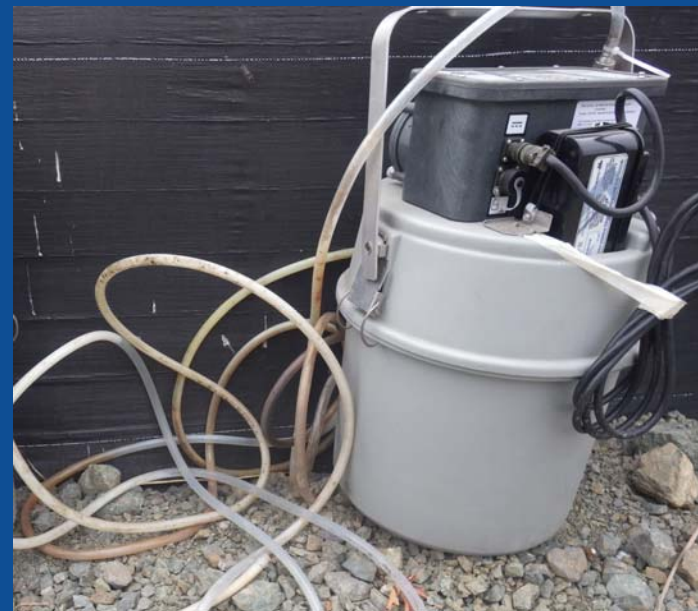
Selenastrum Control Chart

# Reviewing the Report



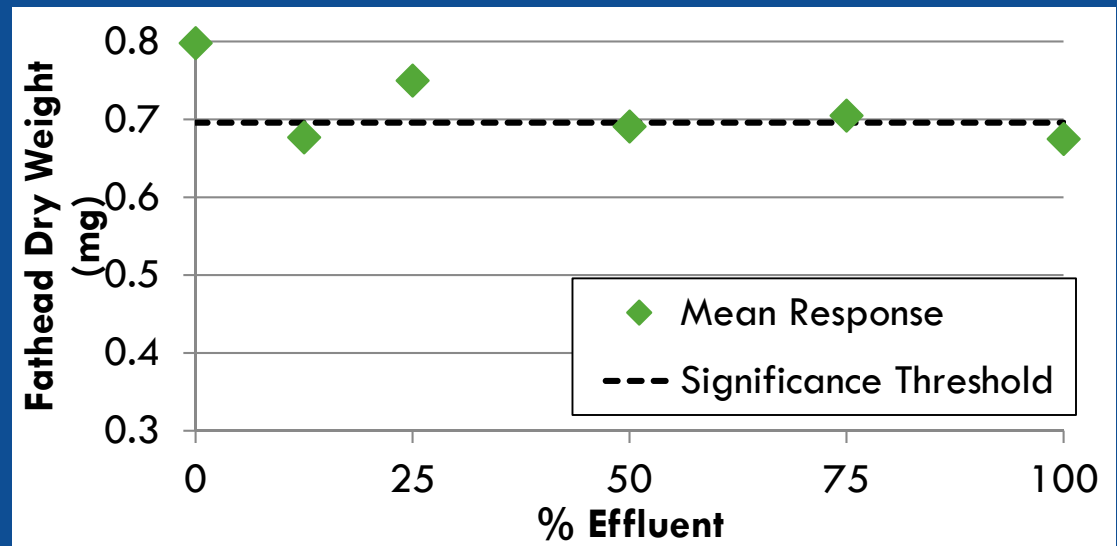
Additional items to consider:

- Review receiving water observations and water quality monitoring results
- Assess sample collection process for sample integrity and possibility of contamination
- Look at the bench sheets
  - Was there a pattern of toxicity?
  - Were calculations made correctly?



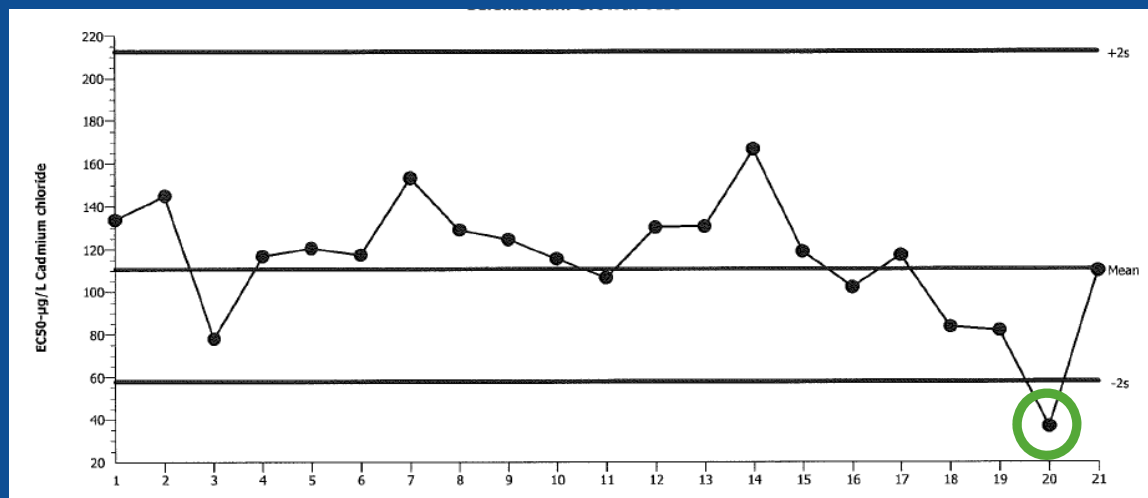
# Bioassay Example 1

- Discharger received 1.3 TUC for fathead minnow (growth)
- Interrupted & flat concentration-response relationship
  - Non-ideal – expect toxicity to increase with % effluent
  - USEPA (2000) WET Testing Guidance – consider pathogen interference
  - Other concurrent fathead tests showed similar results (discussion with bioassay lab)
- Lab “qualified” test results and discharger passed retest and avoided accelerated testing



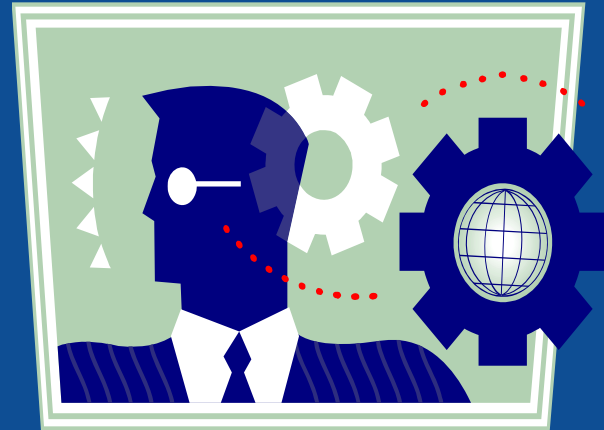
# Bioassay Example 2

- Discharger in accelerated testing with *Selenastrum*
- Fourth test indicated  $>1$  TUc
- Ref. tox. test results  $> 2$  std. dev. beyond long-term mean
  - Evidence of *Selenastrum* culture sensitivity
  - Lab “qualified” effluent test results
- Discharger passed retest and stayed out of TRE



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# What is a TRE?

- Toxicity Reduction Evaluation (TRE)
  - Discharger led study intended to identify the cause of toxicity and control it.



# How is a TRE triggered?

- Routine bioassay results exceed NPDES permit numeric toxicity trigger (e.g.,  $\geq 1$  TUc median / 2 TUc max)
- Accelerated monitoring (e.g., 4 biweekly tests)
- Toxicity in accelerated monitoring leads to TRE initiation
- Only with test species that exhibited toxicity



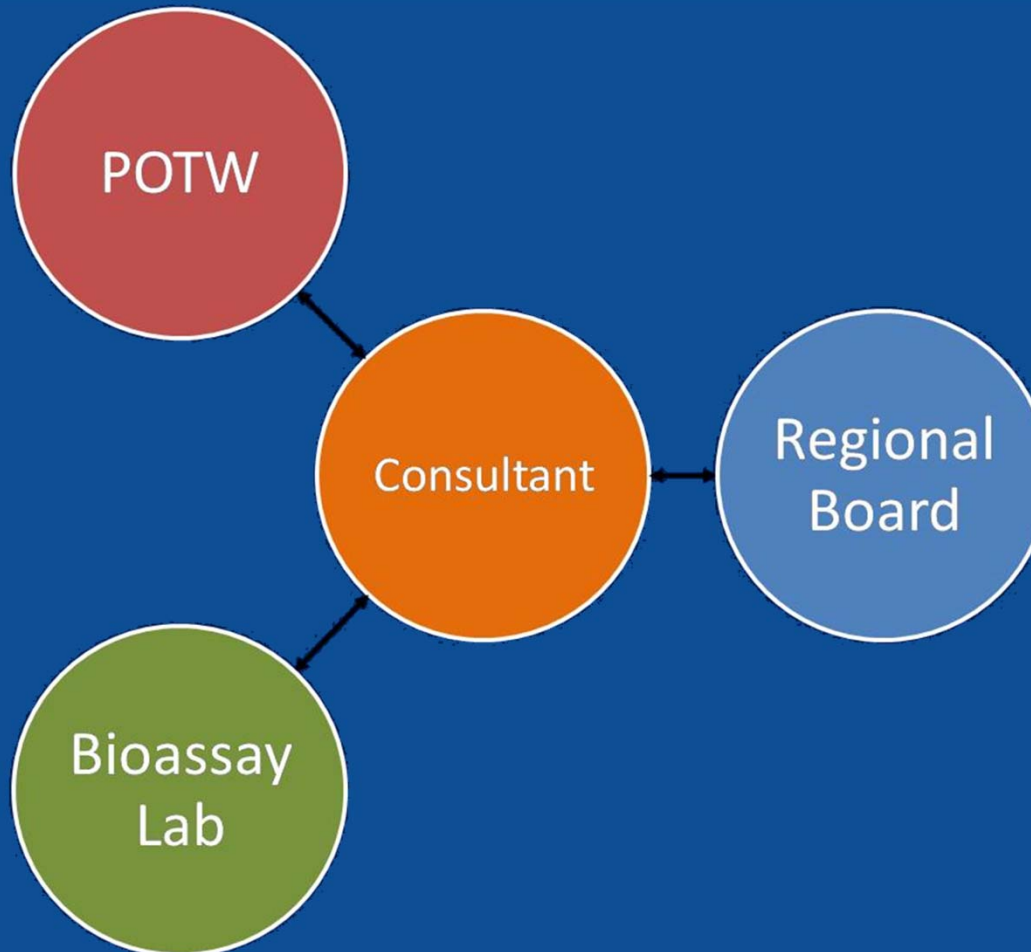


# Communication with Regional Board

- Notify Regional Board within 24 hours of determination sample exceeds trigger (in routine or accelerated monitoring)
- Event-specific TRE Work Plan (30 days after exceedance *during routine testing*)
- Regular updates during TRE

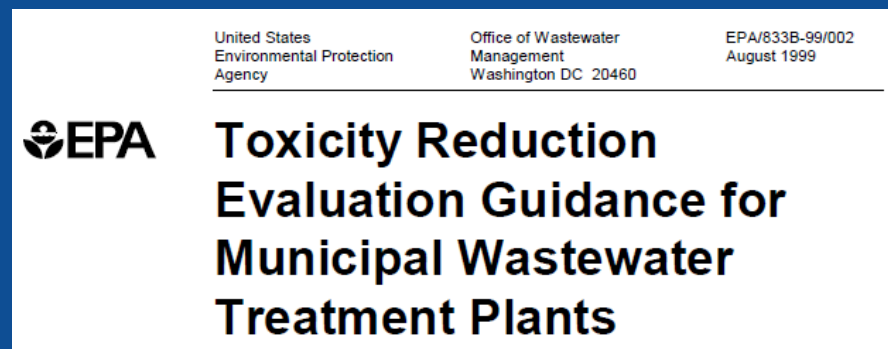


# Project Team



# What does a TRE look like?

- TREs can vary greatly in effort and expense
- EPA provides specific guidance on steps to be taken
  - All steps are not required — A TRE ends when the cause of toxicity is controlled or eliminated
  - Strategy and approach may change as evidence is revealed
  - Experience plays crucial role in guiding future actions



# Elements of TRE Work Plan

- ❑ Tier 1. Information and Data Collection
- ❑ Tier 2. Facility Performance Review
- ❑ Tier 3. Toxicity Identification Evaluation (TIE)
- ❑ Tier 4. Toxicity Source Evaluation
- ❑ Tier 5. Toxicity Control Evaluation
- ❑ Tier 6. Toxicity Control Implementation

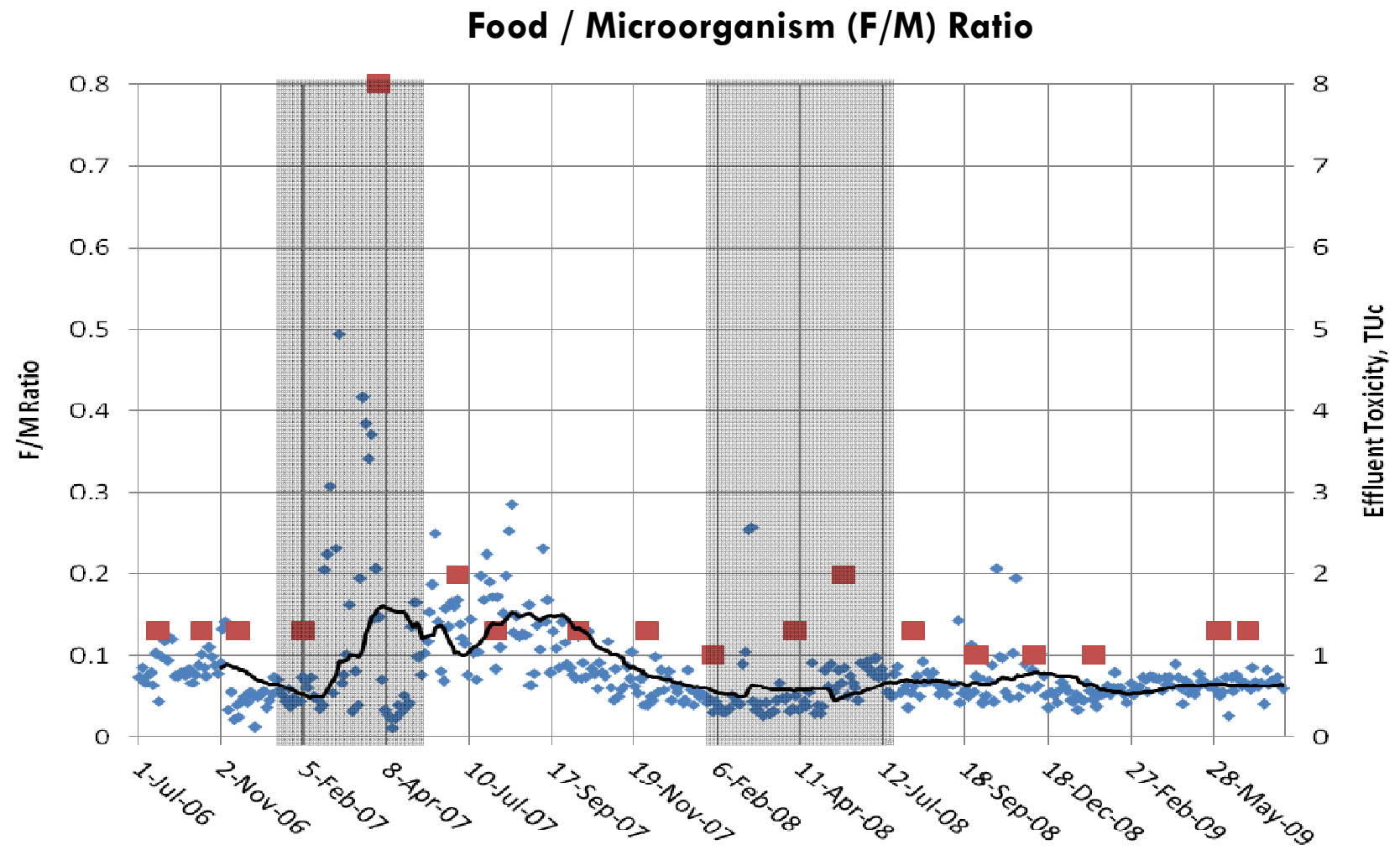


# Tier 2 - Facility Performance Review

- Review available data
  - SMRs and process data
- Process evaluation
  - Primary, Secondary, Tertiary treatment, Chlorination/dechlorination
  - Chemical use/changes
  - Process side-streams
- Important to have everyone “on board”  
(i.e., operators, managers, consultant, lab staff)



# Case Study 1 - Facility Performance Review



# Case Study 1: Who would have thought?

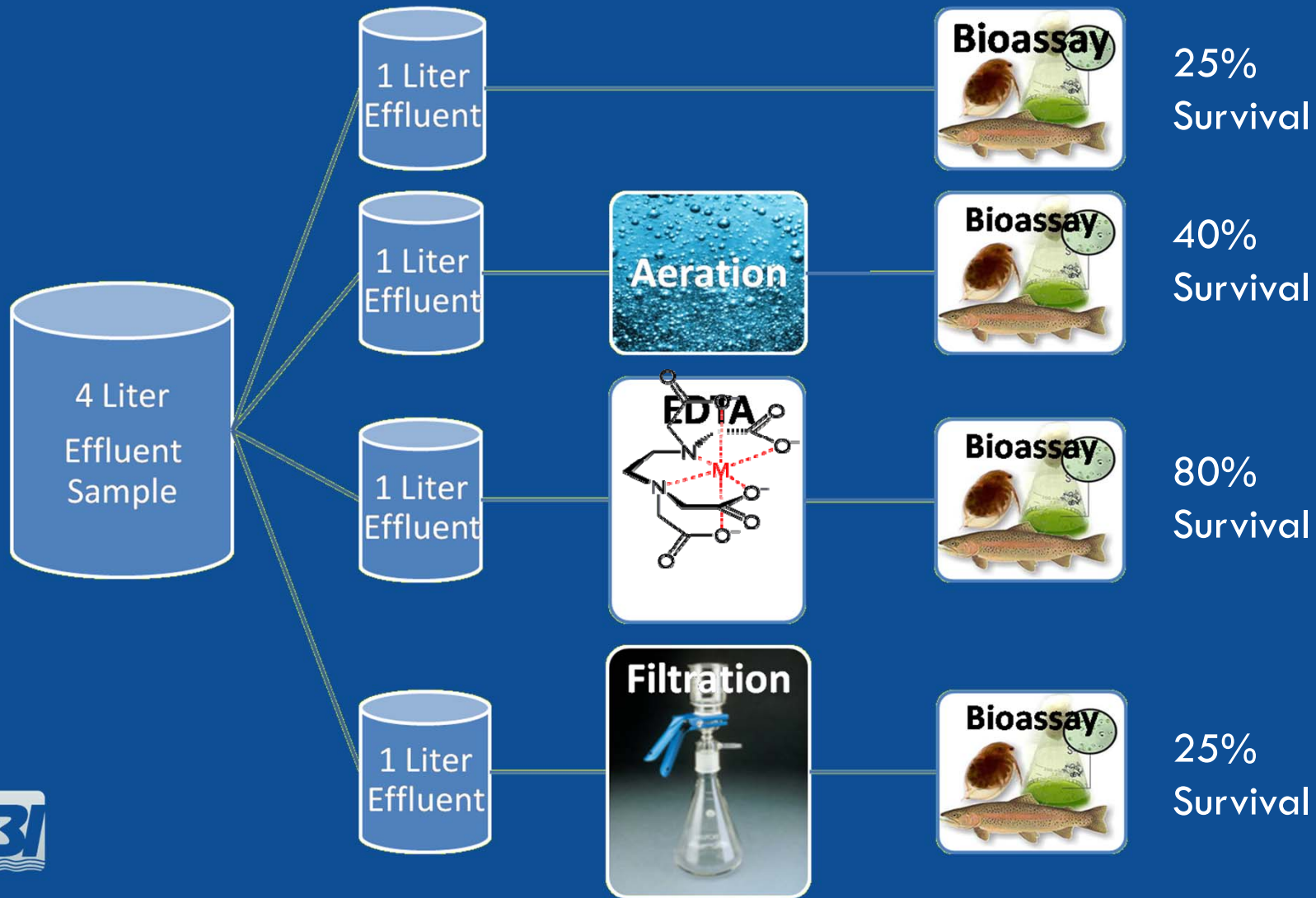
## □ *C. dubia* TRE Lessons Learned:

- Facility performance / integrated team was key
  - Bacterial floc observed in effluent
  - Trialing new chemical in treatment process
- Source Control - Ceased use of new chemical in treatment plant
- Preventative Maintenance – Routinely clean floc from effluent discharge pipeline





# Tier 3 - Toxicity Identification Evaluation (TIE)



# Case Study 2: Moving Target



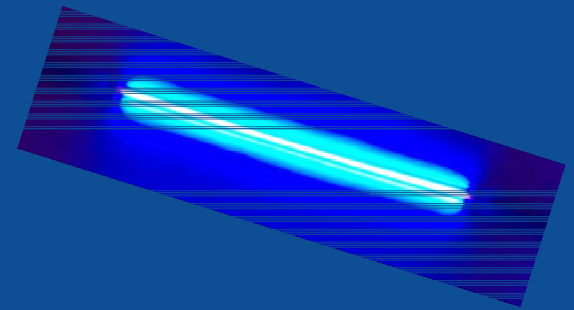
- ❑ *Selenastrum* (algae) TRE
  - ❑ Seasonal toxicity
  - ❑ Low level toxicity (<2 TUc)
- ❑ TRE (can be non-linear and multi-layered)
  - ❑ Tier 2 – Facility Performance Evaluation - inconclusive
  - ❑ Tier 3 – TIEs did not agree with chemistry data - inconclusive
  - ❑ Tier 4 – Toxicity testing with new polymer - inconclusive
  - ❑ Tier 5 – Source control (discontinue new polymer) - ineffective
  - ❑ Tier 4 – Source evaluation - toxicity only after UV treatment
  - ❑ Tier 4 – Toxicity testing of UV tube light cleaning gel – not toxic
  - ❑ Tier 3 – Developed TIE methods to remove peroxide generated by UV - inconclusive



# Case Study 2: Moving Target



- Thinking outside of the box
  - Conducted aquatic insect community surveys in the receiving water
  - Receiving water bioassessment showed no effects from discharge
  - Discharge always had 1:1 dilution with receiving water
- TRE Conclusion: Regional Board adopted new NPDES Permit with 2 TUc trigger for *Selenastrum*
- Key: Focusing on receiving water to determine compliance with the Narrative Toxicity Objective



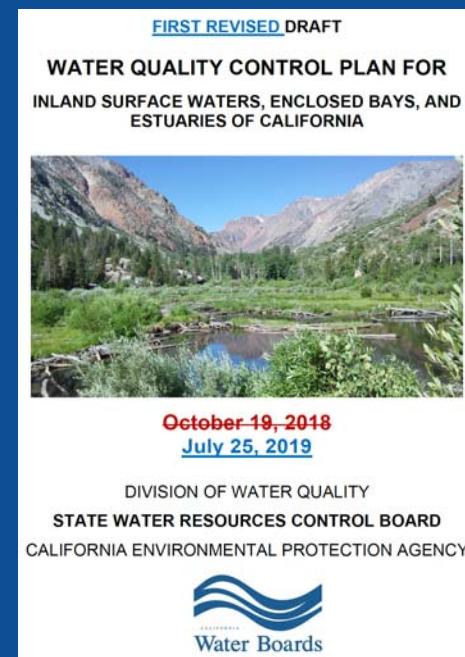
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# Proposed Toxicity Provisions

- Supersedes the SIP
- *“...for chronic toxicity the difference between the organism response in the test water must be greater than or equal to 25 percent compared to the test organism response in the control water”* – chronic toxicity Regulatory Management Decision (RMD)
- Numeric Effluent Limits
- Test methods are unchanged?
- Many comments in 2018
- Workshops scheduled
  - w/ SWRCB Staff August 13<sup>th</sup>, 28<sup>th</sup>
  - w/ Board October 3<sup>rd</sup>

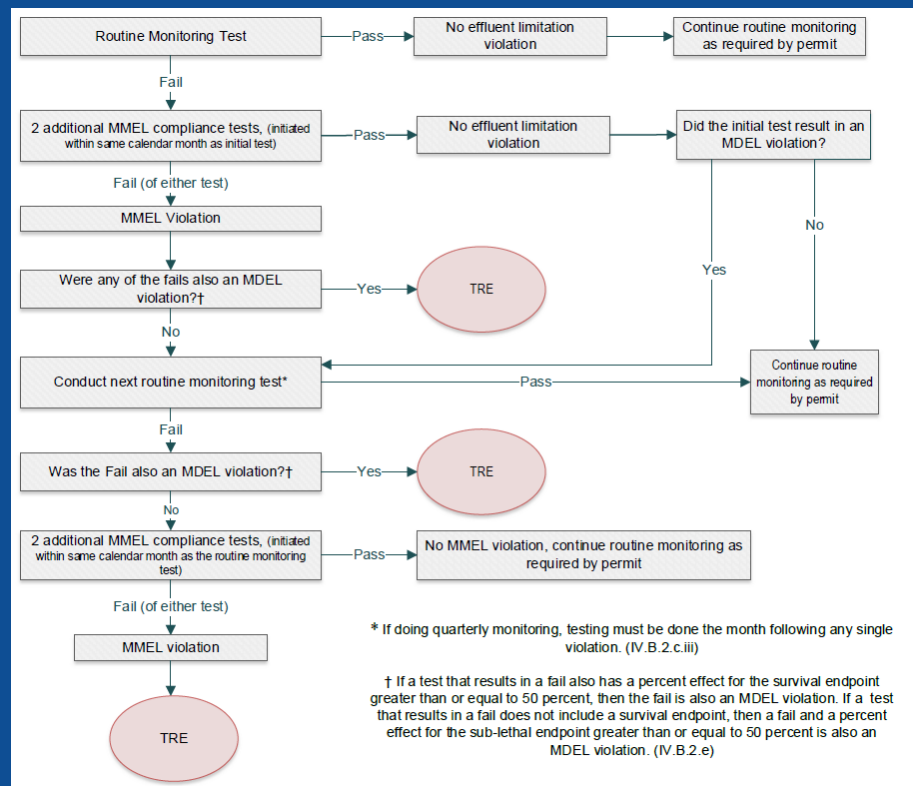


# Test of Significant Toxicity (TST)

- Default is that sample is toxic and statistic determines if sample is not toxic.
- Toxicity evaluated using a different statistic
  - The TST (USEPA 2010) identifies “Pass” / “Fail”
  - Toxicity evaluated only at instream waste concentration (IWC)
  - Atypical concentration-responses may not be considered
  - Greater variability penalized
- *Recall that the RMD is a 25% effect, but.....*
  - There can be a determination of ‘FAIL’ with an effect <25% (chronic; or 20% acute) due to a statistical likelihood that the difference is not <25% (i.e., accept null hypothesis that there is a difference between the treatment and control)

# Proposed Toxicity Provisions

- ❑ Numeric Effluent Limits rather than Triggers
- ❑ Monthly chronic testing (>5 MGD)
- ❑ 2 MMEL compliance tests within 30 days if routine monitoring = “Fail”
- ❑ MMEL violation if either compliance test is a “Fail”
- ❑ TRE required if 2 violations:
  - MMEL violation + MDEL violation (>50% effect)
  - MMEL violation + MMEL violation (consecutive months)



# Questions and Discussion?

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