Interpreting Toxicity Tests: Avoiding Confusion & 'False Positives' in Chronic Bioassay Tests

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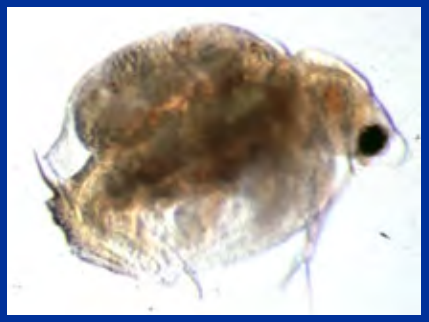

Bay Area Clean Water Association
Toxicity Workshop
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Presentation Overview

- Testing Manuals & Cautionary Principles
- Confounding Factors
  - 'False Positives' due to testing issues
    - Defined here as incorrectly identifying a sample as toxic when in fact it is not
  - Permit-based
  - Concentration-response curve
- Conclusions

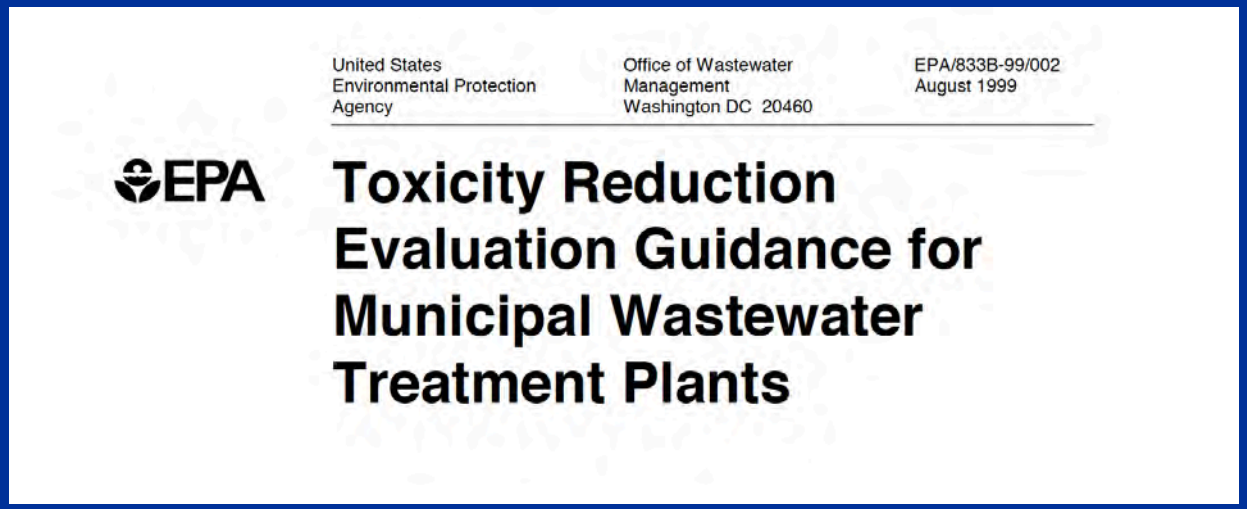
Testing Cautionary Principles

Whole Effluent Testing

- Methods provide considerable flexibility for the labs that can affect the outcome of testing or comparisons among labs, including but not limited to:
  - Dilution water
  - Freshwater algae scoring method
  - “must” vs. “should”
- Much of this science is observation based, so critical observations that can affect test outcome must be addressed

Whole Effluent Testing

- Once an effluent is identified as toxic, most permits require accelerated monitoring, and the implementation of a Toxicity Reduction Evaluation (TRE) if one of the accelerated monitoring tests exceeds the permit limit for toxicity.
- ***This can be costly, and potentially unnecessary***



Whole Effluent Testing: Cautionary Principle

- " Permittee and the regulator should distinguish very early in the process whether an actual toxicity event has occurred or whether the effluent may appear to be toxic but may in fact be the result of an unusual or invalid test" (Ausley *et al*., 2005)
- A detailed review of the test data and laboratory methods will assist in making this determination

Cautionary Principle – Basic Test Review

- Prior to proceeding with reporting that toxicity is present, it is imperative that the laboratory comprehensively review the data to confirm that the testing is valid
  - Have the required test conditions been met?
  - Did the test meet the test acceptability criteria?
  - Were all water quality parameters within an acceptable range for the test species?
  - Is the test variability (PMSD) acceptable? Below the EPA 10th percentile?
  - Is the concentration response curve normal?

Confounding Factors: Testing False Positives

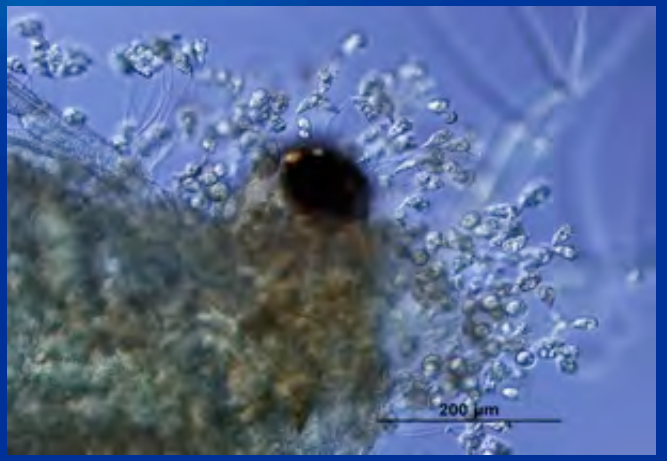
‘False Positives’ – Microbes

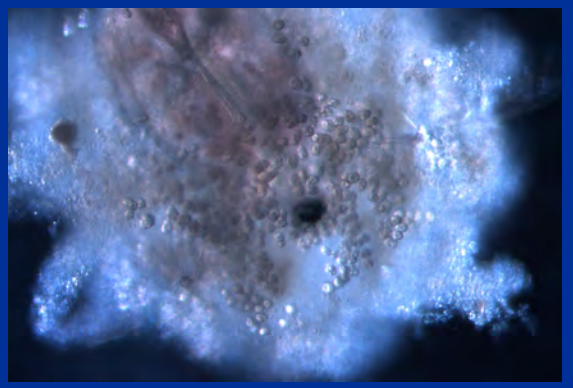
- ‘False Positive’ – defined here as identifying an effluent as toxic when in fact it is not!
- Fathead Minnows – Pathogen Related Mortality

  - Pathogen – *a bacterium, virus, or other microorganism that can cause disease*
  - Easily observed
  - Mitigated by clean techniques, test modification (i.e., increase # replicates with 2 fish per replicate), and/or sample treatment (e.g., UV, filtration, chlorination/dechlorination, and antibiotics)

‘False Positives’ – Microbes

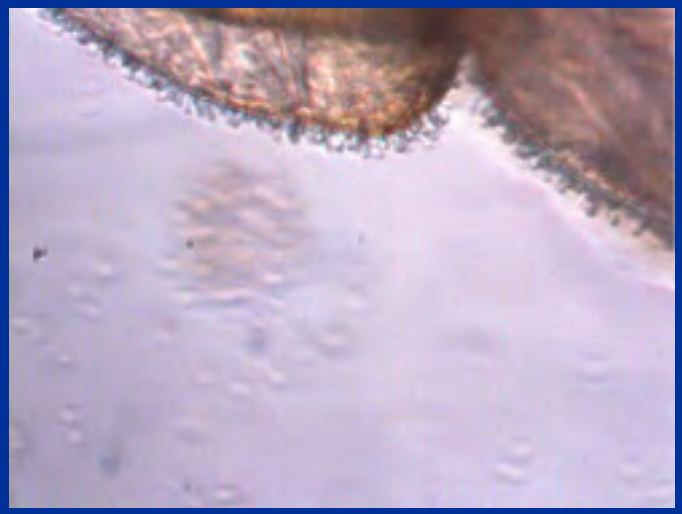
- *Ceriodaphnia dubia*
  - Epibionts – organisms that live on the external surface of another organism
    - Peritrichs (stalked ciliates)
    - Central Valley and SF Regional Boards – acknowledged as a test interference





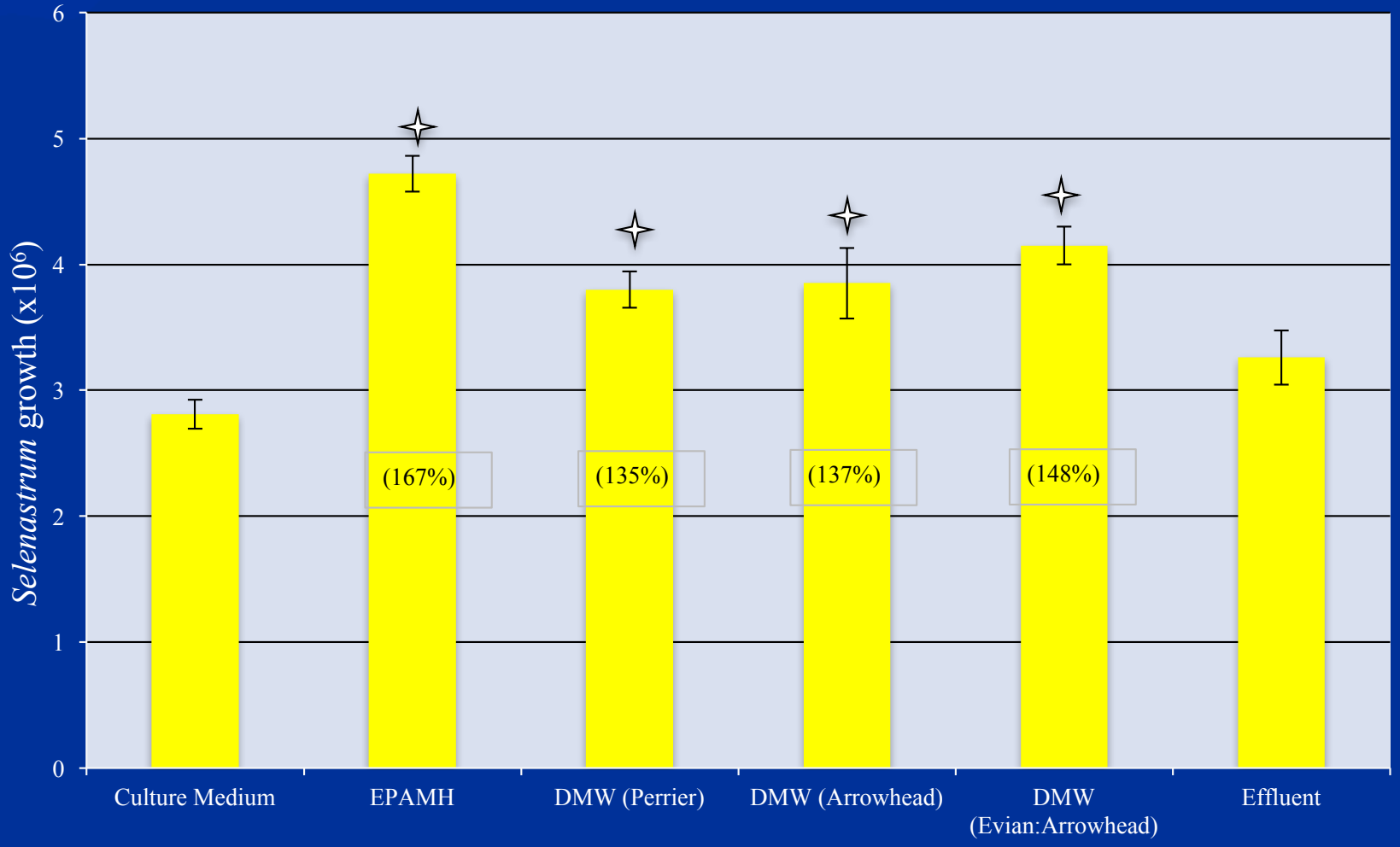
‘False Positives’ – Microbes

- *Ceriodaphnia dubia*
  - Other microbial epibionts
    - Reduced reproduction
- Mitigation measures:
  - Clean sampling equipment
    - Cost one discharger ~ $1 million
  - If TIE required – filtration, UV, chlorination/dechlorination, and antibiotics may be effective



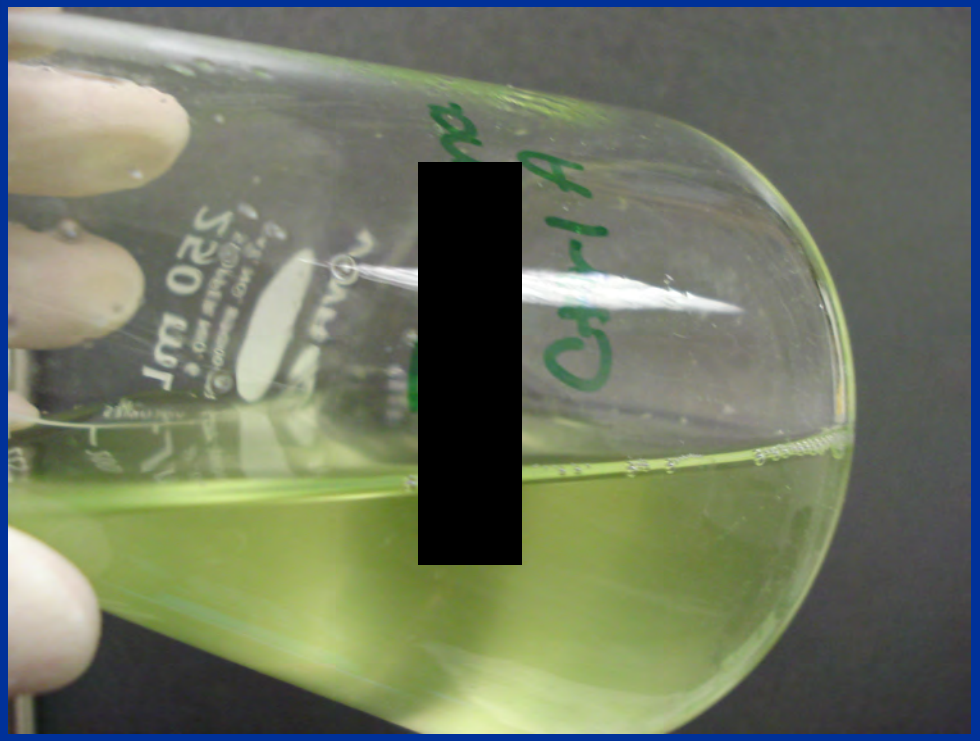
‘False Positives’ – Lab Control Media

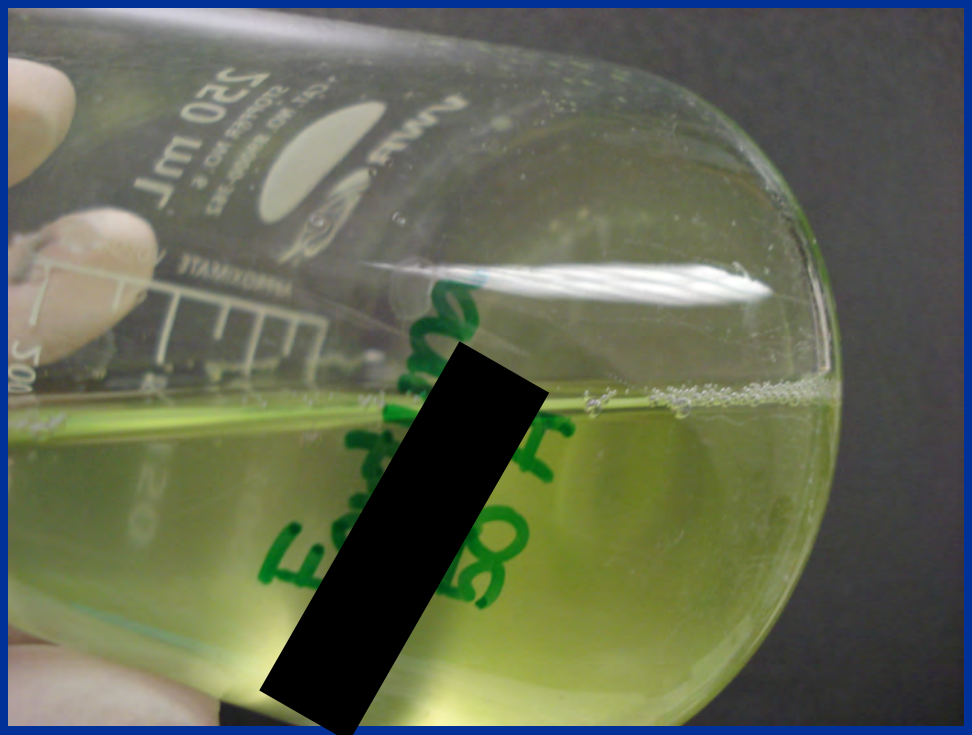
- Lab control treatment media selection can affect the determination of toxicity for the *Selenastrum* test



‘False Positive’ – *Selenastrum*

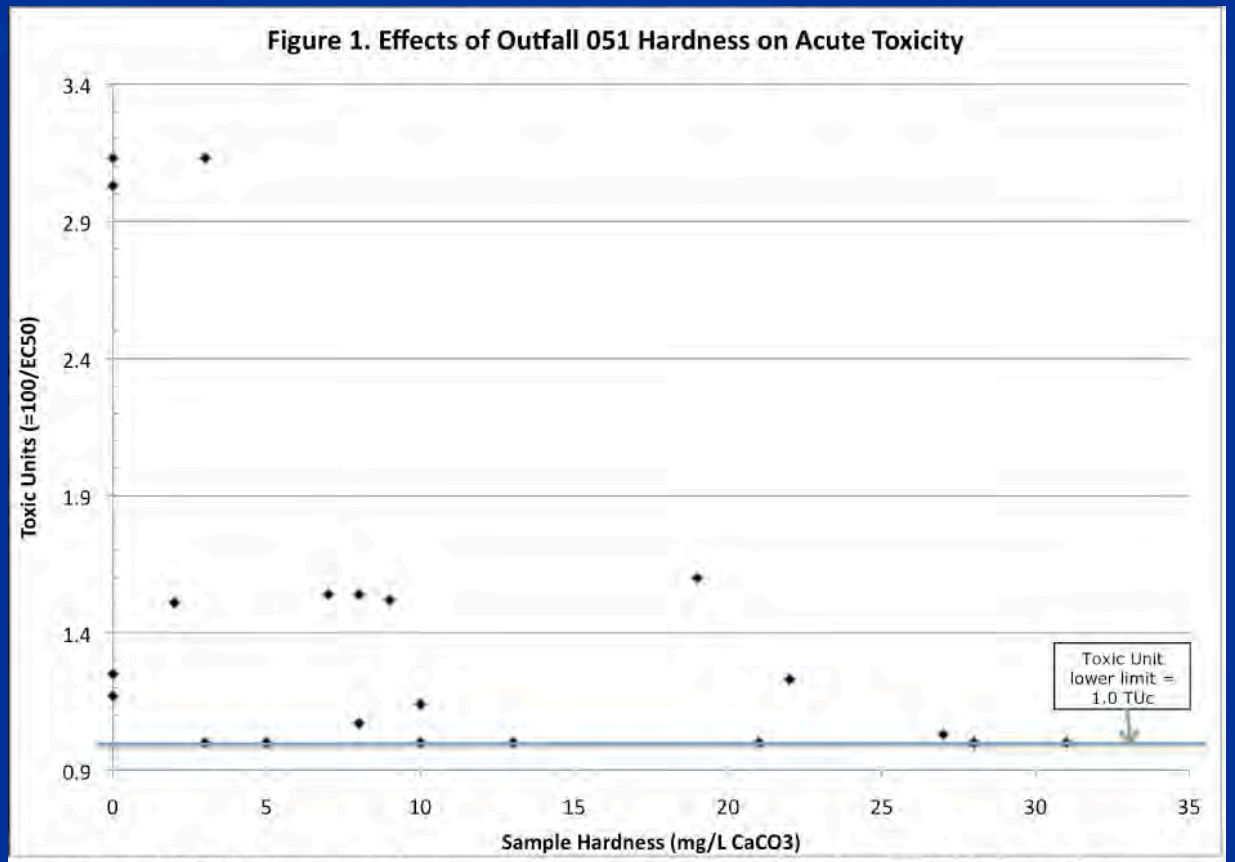
- ‘Plating’ of algae can occur, reducing the algal count
- Need to re-suspend the algae to obtain a count in the flask





‘False Positives’ – Low Hardness

- Low hardness can be detrimental to *Ceriodaphnia* reproduction and survival
- Resolved by amending effluent to a hardness consistent with Receiving Water (or at least ~40-50 mg/L for acute testing)



Note – Toxic Units (TU) are standard measures of the magnitude of toxicity, where TU > 1 indicates the presence of toxicity, with the magnitude of the toxicity increasing as the TU increases.

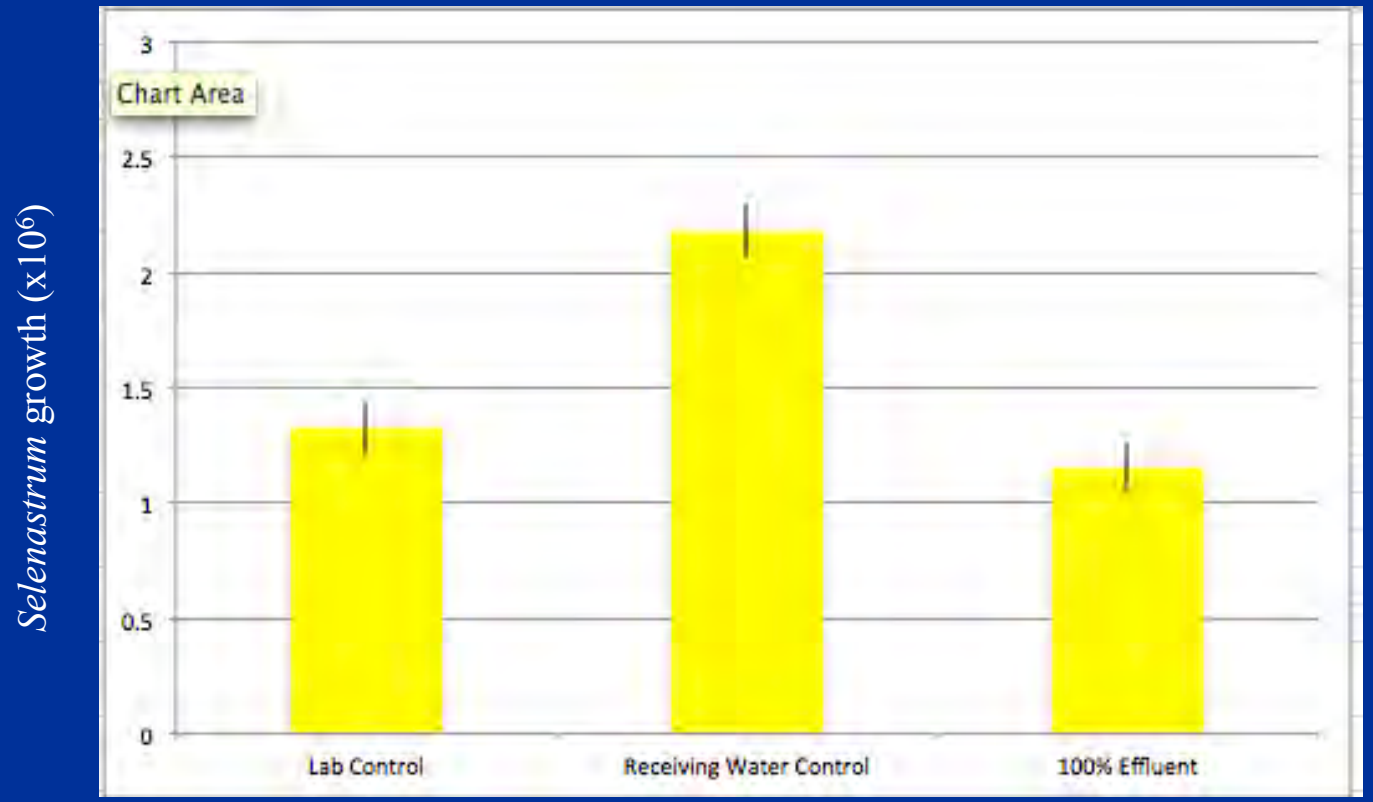
Other Potential 'False Positives'

- Matrix interferences:
  - High hardness waters
    - Can cause cell lysing in *Selenastrum*
    - Can cause low reproduction to mortalities in *Ceriodaphnia*
    - Mine study – high hardness, ion imbalance, or high ionic specific toxicity
  - Can be identified via additional control treatments

Confounding Factors: Permit-Based

‘False Positives’ – RW Control

- Hyper-stimulated receiving water control
  - High nutrients *(Selenastrum)*
  - Additional food (*Ceriodaphnia* and fathead minnow)

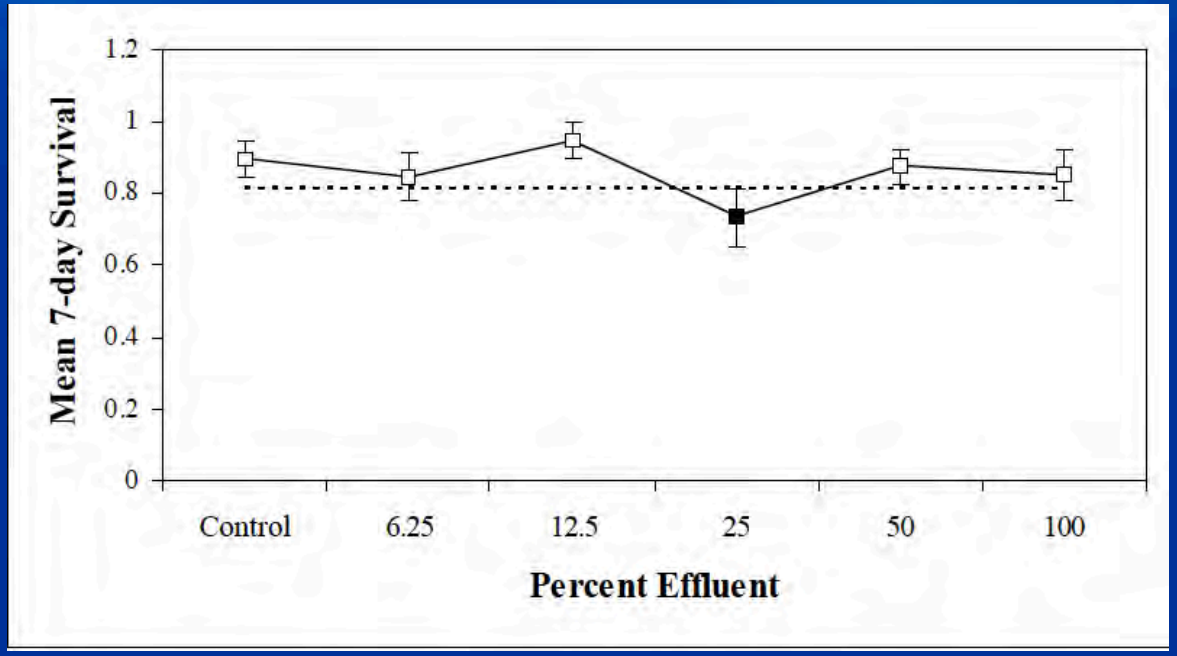


Confounding Factors: Concentration Response Relationship

Concentration Response Relationships Must Be Assessed

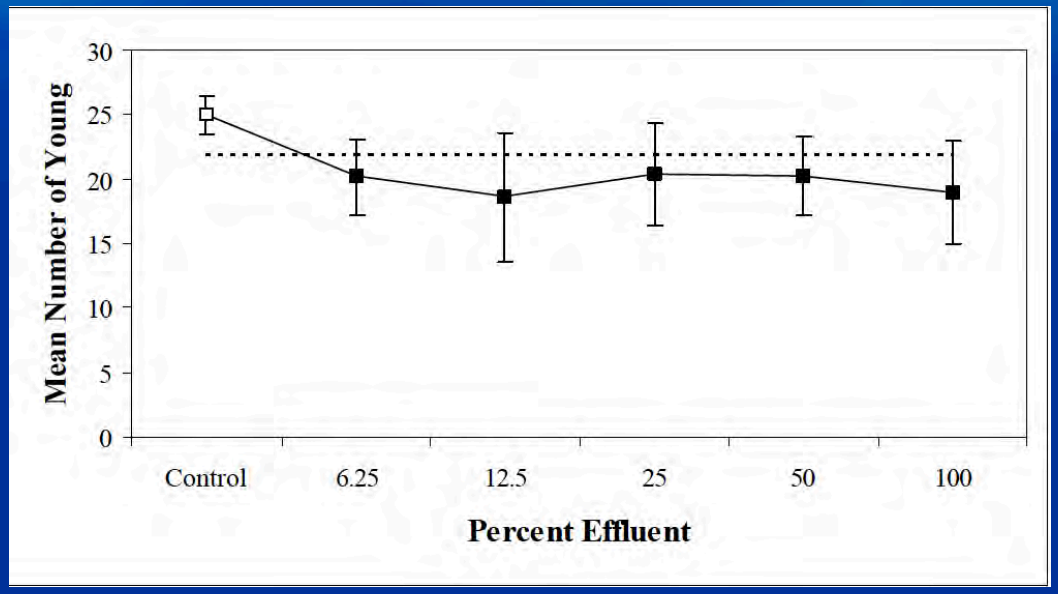
- All or nothing
- Stimulatory at low concentrations and detrimental at higher concentrations
- Stimulatory at low concentrations but no effect at higher concentrations
- Interrupted concentration response - significant effect bracketed by non-significant effect
- Interrupted concentration response - non-significant effect bracketed by non-significant effect
- Significant effects only at highest concentration
- Significant effects at all test concentrations but flat concentration response curve
- Significant effects at all test concentrations with a sloped concentration response curve
- Inverse concentration response relationship

Unusual Response Curve Example



- Must evaluate for procedural errors (e.g., D.O.), within treatment variability, and test sensitivity (PMSD).
- Outcome could be that the 25% effluent treatment in the example above is an outlier and that the NOEC should be 100% effluent (i.e., not toxic) versus the 12.5% effluent (i.e., VERY toxic).

Unusual Response Curve Example



- Must evaluate test sensitivity (PMSD), unusually *high control response*, dilution water (lab vs. receiving water), and ***consider*** pathogen interference
- Be cautious to not jump to conclusions that pathogens are the only driver. If weight of evidence leads to pathogens, perform appropriate treatments (e.g., filtration, UV, chlor/dechlor, and antibiotics) for conclusion.

Conclusions

- It is imperative that a critical evaluation of test data is performed to assure that an effluent preliminarily identified as toxic is in fact toxic (avoid false positives) and use appropriate ‘off ramps’ to avoid unnecessary accelerated monitoring and TIEs
  - Assure that test data have been comprehensively reviewed and that the results are acceptable
  - Assure that test interferences have been eliminated as causative factors for a “toxic” response