April 30, 2026

7:45-8:00 **Login and Opening**

8:00-9:00 **Evaluating and Selecting Contract Laboratories**
Diane Rosseter, Environmental Consultant, Georgia-Pacific

9:00-10:00 **Regulatory Relationships**
Forrest Westall, McGill Associates, P.A.

10:00-10:15 **Break**

10:15-11:15 **Emergency Preparedness**
Brenna Cook, City of Asheville

11:15-11:45 **Lunch Break**

11:45-12:15 **Aquatic Toxicity Unit Updates**
Molly Nicholson, NCDEQ Aquatic Toxicology Branch

12:15-12:45 **Toxicity Studies**
Beth Thompson, BT Solutions, LLC

12:45-1:00 **Break**

1:00-3:00 **Aquatic Toxicity Testing**
Jim Sumner and Jaydon Perez, ETS, Inc.

Evaluating and Selecting a Contract Laboratory

Presented by
Diane Rosseter

Objectives:

- Identify, evaluate, and select a qualified laboratory that will meet your specific analytical, regulatory, and customer service needs.
- Increase confidence in quality and reliability of analytical data

Data generated from collection and analysis of environmental samples provides the decision-making backbone for environmental compliance and assessment activities....

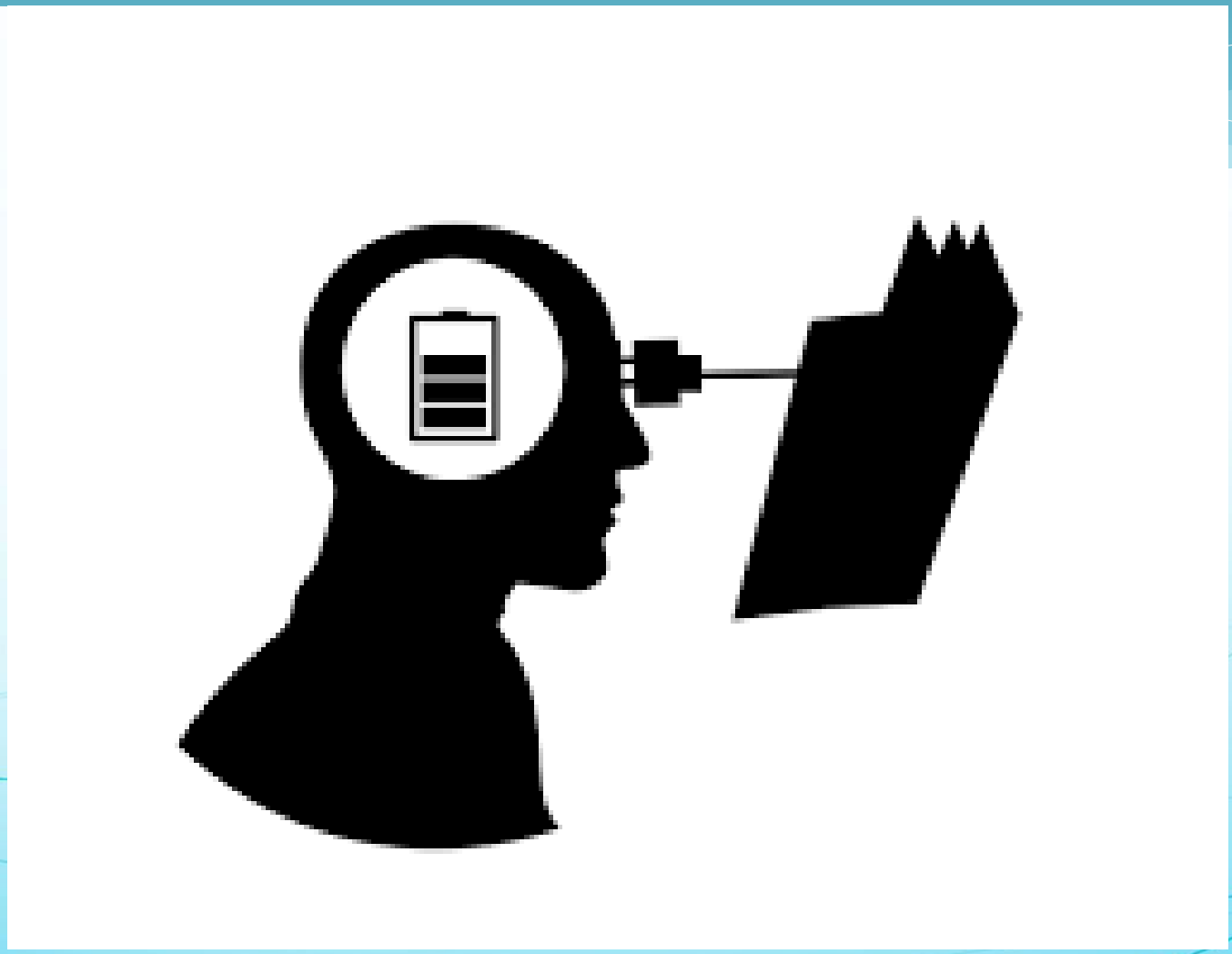
Underlying goal of all environmental sampling activities should be…..

- To understand the fundamental reasons behind sample collection
- To collect and/or generate data that is reliable and usable
- To obtain data and produce reports that are technically sound
- To understand how to accurately interpret and use the data

Start with a few basics

NEVER ASSUME ANYTHING

None of us are mind readers…



Do your homework

- For what purpose is the data being collected?
- What type of data/reporting is required?
- What are my permit requirements?
- Is a Sampling Plan/Quality Assurance Plan required (i.e. regulatory preapproval)?
- Is the sampling routine or special?

Determine the Basics

- Sampling protocols
- Documentation requirements
- Lab / Sampling certification requirements
- Test methods and reporting limits - including parameter lists
- Laboratory deliverables and QA levels
- Reporting requirements

Laboratory Evaluation and Selection

Aren't all labs the same?

* Customer service is not always a priority
* Production-oriented
* Profit vs. quality
* High turnover and lack of training
* Ineptitude and fraudulent practices

Considerations

- All labs are NOT the same (even within the same network)
- You OFTEN get what you pay for
- Not all labs are good at all analyses
- Special analysis = special lab (Drinking water, WET testing)

The sales rep will NOT be analyzing your samples!

Additional Considerations

- "Certified" does not necessarily mean *qualified*
- Differing certification programs and requirements – some states have NONE
- Multitude of QA/QC programs
- Differences in turnaround time, pricing, capabilities, deliverables

Question

True or False:

North Carolina DEQ doesn't require laboratories to be certified to perform analyses for NPDES permit compliance reporting purposes so long as they use EPA-approved test methods.

Environmental professionals take responsibility for the content of documents or reports bearing their signature.

Perform a "Paper" Audit

Ask laboratory to provide….

- **Statement of Qualification (SOQ) or Quality Assurance Plan (QAP)**
- **Copies of all state certifications, including list of certified parameters**
- **Performance audit results for last two years**
- **Results of applicable EPA/state performance evaluation samples for last two years**
- **References**
- **Names of any subcontract labs**

Perform an “On-Site” Audit

Visit the laboratory in person….

- **Scheduled or impromptu**
- **Level of detail based on paper audit**
- **Tour lab and talk to analysts**
- **Inquire about sample check-in/sample tracking procedures**
- **Absolute minimum – meet your project manager and the lab manager**

Appearance isn't everything, but lab should be clean, well organized, and people should be WORKING!

ASK AROUND!

* Reach out to colleagues at other facilities
* Contact the references provided by the laboratory

Establish Minimum Expectations

- Sampling schedule – routine and special
- Lab hours of operation – when they accept certain samples, what are weekend/holiday policies, etc.
- Turnaround time and pricing
- Fees for special services
- Payment terms
- Deliverables and QA/QC requirements
- Reporting requirements

Check your permit!!

Monitoring and recordkeeping requirements are **specified under 40 CFR 122.41(j):**

3) Records of monitoring information shall include:

(i) The date, exact place, and time of sampling or measurements;

(ii) The individual(s) who performed the sampling or measurements;

(iii) The date(s) analyses were performed;

(iv) The individual(s) who performed the analyses;

(v) The analytical techniques or methods used; and

(vi) The results of such analyses.

(4) Monitoring must be conducted according to test procedures approved under 40 CFR Part 136 unless another method is required under 40 CFR subchapters N [Effluent Guidelines] or O [Sewage Sludge].

Establish a Relationship

Once a lab has been selected….

- **Get to know your project manager and make sure he/she gets to know you and your projects**
- **Ask your project manager to pay you a visit. *The sales rep is no substitute!!***
- **Clearly define needs and expectations**
- **Establish and maintain an open line of communication**

Question

Your permit requires you to test for both BOD and COD. The contract lab reported results for BOD that were about 10 times higher than COD during your most recent sampling event. What should you do?

a) **Assume the lab made a mistake, switch the results, and report on the DMR.**

b) **Do nothing. The lab is always right.**

c) **Contact the lab and ask them to verify the results because something doesn't look right.**

Remain Diligent

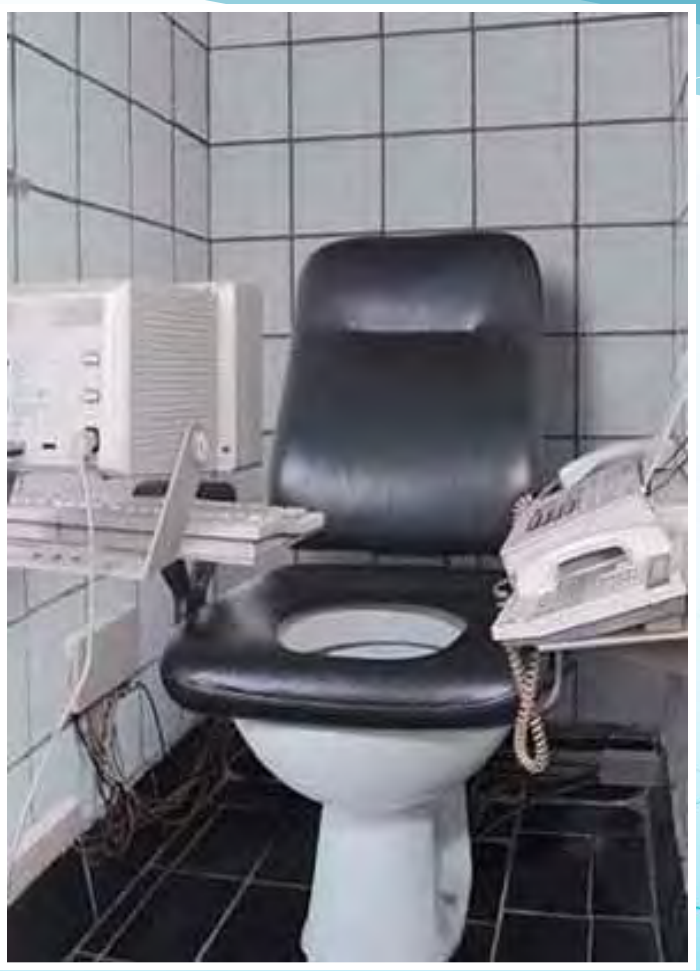
- Realize that Murphy is alive, well, and working in the environmental business – sometimes things just happen
- Never be afraid to ask
- Questionable results deserve satisfactory answers
- Continually challenge - labs can become too comfortable and confident in the relationship

Wrap-up

<u>Using a certified lab with an established QA/QC program does not ensure reliable data.</u>

* ***What am I doing to ensure I receive reliable, usable data?***
* ***Did I get what I asked for and do I have confidence in the results?***
* ***What are the consequences of receiving anything less?***

Typical laboratory Project Manager's office



REGULATORY RELATIONSHIPS: Updated for 2026

Toxicology Workshop, April 30, 2026

Forrest R. Westall, Sr.
McGill Associates, P.A.

mcgill Shaping Communities Together

Don’t let anyone categorize you as a “bad guy.” Being regulated doesn’t mean you are guilty of anything.

Things are getting more difficult: Regulatory controls are moving toward ZERO—What does that mean?

The song is close to the same, but we have new verses!

What we Thought We Knew: PREVIOUS DEFINITION OF A RELATIONSHIP—**IT IS DIFFERENT ALMOST EVERY YEAR**

NOUN

*1) T*he way in which two or more concepts, objects, or people are connected, or the state of being connected:
"the study will assess the relationship between unemployment and political attitudes“.

2) The state of being connected by blood or marriage:
"they can trace their relationship to a common ancestor“.

3) The way in which two or more people or separate groups regard and behave toward each other:
Examples: "the landlord–tenant relationship“; "she was proud of her good relationship with the staff“.

What Happens When the Regulatory Goals are Beyond Technology?

1,4 dioxane
PFAS
New Analytical Science
Nutrient Controls

In the West, We are still dealing with the aftermath of Helene—But the regulations go on! They do for all regulated folks!

The NPDES Permitting Program is a dominate regulatory tool

Question:

NC DWR has limited authority to change National Pollutant Discharge Elimination Permit (NPDES) conditions? True or False

Response: False

NC DWR is delegated the federal Clean Water Act NPDES Permitting program. Permits, after they are issued, are reviewed prior to renewal (for up to 5 years). Previous conditions can be changed and new conditions added. However, new or changed conditions are subject to challenge, administratively or legally. DWR evaluates identified pollutants in accordance with water quality standards and calculate limits. If the model changes, the limits change. New standards and permitting procedures can change and impact old permit conditions.

Even if permits change, the permit holder has the ability to question new or revisied conditions—through DWR contact or filing a contested case (legal challenge)

What we Know or Think we Know Impacts What we do or are Required to do!

- Science is often ahead of our ability to respond
- The public is alarmed about what may be in their water, land, and air
- Regulatory agencies are under lots of pressure
- Lawmakers are divided over what to do
- Emerging Contaminants--PFAS, 1-4, dioxane, and Similar Substances: Pretreatment, Collection System, Effluent Limits, Sludge: Expectations are High!
- Nutrients: Concerns Remain—Requirements are here or coming: Neuse Estuary, Tar River/Pamilco Sound, Albermarle Sound, Jordan Reservoir, Falls Lake, High Rock Lake, Middle Cape Fear River, etc. Over 50% of the state is under some level of nutrient control or targeted for control

Policy and Law Changes—Requirements are in Flux and agency funding is being targeted!

- Pull and tug: NC General Assembly verses the Executive Branch
- Federal changes and pressure on Agencies—the New EPA and the new administration
- Agency staff are concerned and under fire
- Vacancy levels continue to be high
- Statutory changes has restricted some regulatory authority at the state and local level
- However, the enabling laws remain in place (you still have to get permits, approvals, and meet requirements)
- Water and Wastewater, two sides of one coin—control for protection of drinking water—putting water and sewer managers between a rock and a hard place
- Morale is low and those close to retirement are considering their options

Some Proposed NC Legislation—Not Passed

- H 870 would require the General Assembly to approve any memorandum of agreement between the Federal Government and an agency of the State charged with implementation of state or federal environmental law
- H 929 would prohibit introducing fluoride or any chemical containing fluoride to a public water system by any person
- H 876 would require local governments to review and determine whether applications for development approval are complete within two days submittal.
- H 808 would designate water treatment facilities as “critical infrastructure” and require criminal history background checks for applicants to become operators at water treatment facilities
- Several PFAS bills

Even When Things are off Scale, Some aspects do not Change: Relationships are “Dependent” or “Independent”

No matter the current instability: You still have a Regulatory Relationship

The term “Regulatory Relationship” may seem like a contradictory concept.

Maybe, but it is a reality that the regulated community and their agents must deal with.

It is always important to remember that the agency must deal with it as well.

When no one really knows what to do, doing the “right thing” is always a good path.

The Importance of Your Regulatory Relationship

- This relationship is “contractual” because you or your clients have performance requirements under this relationship (rules, permit conditions, other legal obligations, and other agency requirements)
- These “relationships” affect your job and the ability of you or your client to effectively provide “service” or to produce their “product”
- The implications of failing to meet your permit obligations are real, can impact your image, may be monetary and are certainly legally based
- The simple fact that cannot be escaped is that all human interaction is based on conditional relationships

Critical Thinking About Your Regulatory Relationship Requires Point of Reference

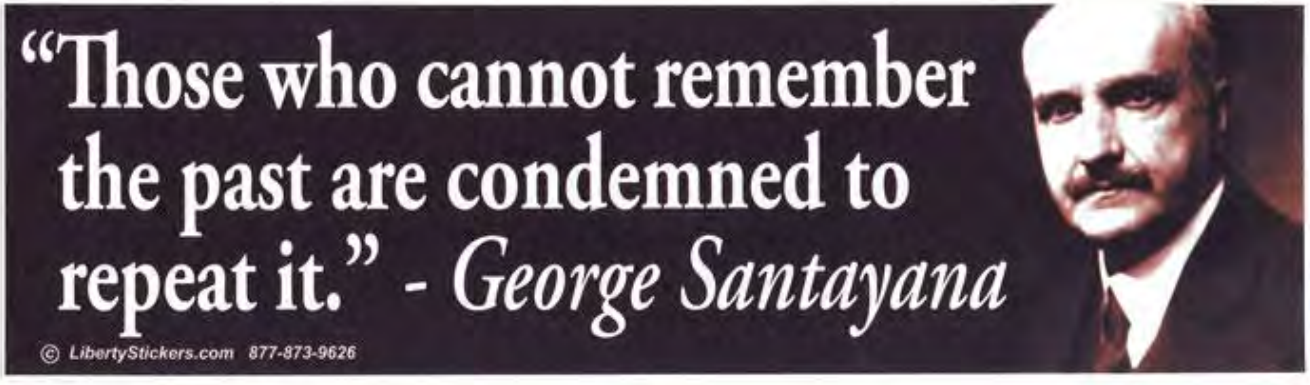
- The Past—Do not Dwell there, but Learn From what **You** and Others have Experienced

- The Present—Making Sure **You** are in the Now

- What is Coming—The most difficult consideration before you—but you have to take care of business

Why the past is important…But the now is the wolf at the door

- Try not to repeat problematic things from the past.
- A child educated only at school is an uneducated child.
- There is no cure for birth and death save to enjoy the interval.



George Santayana
1863-1952

How We Got To Where We Are?

- Pre-1972, Water and Wastewater Management
- The “Watershed” Year of 1972, The Clean Water Act, Safe Drinking Water Act
- How the CWA Changed Everything
- The Criteria For “Success” In Water Pollution Control
- Climate Change: the Weather And Water—Hot And Dry = Worry and Passing New Laws
- COVID and Infrastructure Funding
- Emerging contaminates and the current political landscape
- The world has grinded on to 2026!
- 2026 is not 1972!

Plan for Permit Management think of Now but also the future—You have a say so about your compliance and permit status!

Remember, that regulatory actions, permits and enforcement, allows your input—treat it as a Negotiation. You have options—keep your interests in the forefront

What is Your Need

- Permit Issuance/Revision
- Permit Renewal Concerns
- Enforcement Action
- Permit Compliance Issue(s)
- SOC Issuance/Compliance
- Collection System Permit
- Stream/Wetland Impacts
- Environmental Assessment for a New Project
- Toxicity Reduction Actions
- Pretreatment Issues
- Operational Questions

Which Permit or Approval?

- Municipal WWTP
- Industrial Process WW
- Industrial Stormwater
- Industrial Pretreatment
- Collection System Permit
- NPDES, Stormwater, Pretreatment, Land Application
- Individual Permit or General Permit
- 404/401 Permitting
- Erosion and Sediment Control
- New Facility, Renewal, Expansion
- SEPA/NEPA, EA, EIS FONSI

Which Agency?

- NC Division of Water Resources
- NC Division of Energy, Minerals and Land Resources (DEMLR)
- Army Corps of Engineers (ACOE)
- Wildlife Resources Commission (WRC)
- US Fish and Wildlife Services (USFWS)
- Local Pretreatment Program
- NC Department of Administration (SEPA)
- Federal EPA
- Solid and Hazardous Waste

What Laws and Rules Apply?

- Federal Clean Water Act
- Federal Regulations—What is EPA's Role
- WQ Standards
- Federal Effluent Guidelines
- Stream Classifications
- Groundwater Standards
- NC Environmental Policy Law
- Policy or Procedural Guidance
- Other Requirements

What Part of What Agency?

- DWR-Stream Classifications
- DWR-Nutrient Management
- DWR-Surface Water Protection
- DWR-Wetlands and Stormwater
- DWR-Point-Source
- DWR-Toxicity
- DWR-Pretreatment
- DWR-Aquifer Protection
- DEMLR-Stormwater
- ACOE-Regional Office
- DWQ-Regional Office
- Regional Staff-USFWS
- Regional Staff-WRC
- Municipal/County Governments

Know Your Facts

- Read the Statutes/Rules
- Study the Policy Documents
- Use the Agency Website (Water Resources | NC DEQ)
- Evaluate the Basis of the Agency's Responsibility (under what law and rule are they operating?)
- Talk to the Agency and Ask for Guidance and Information
- Ask for documentation: Get Copies of all Regulatory Documentation (permit file, fact sheet, policy documents, statutory requirements, etc.)
- Before you Proceed, Make Sure that Your Position is Clear Within Your Organization—It Will Not Help If there are Different Stories in Your House
- Trace the Decision Trail and Identify Key Staff

Regardless of what you may think or even what you are told, the permit holder has significant rights to affect the regulatory framework and to question the basis and details of the requirements under which they operate. If roadblocks are set up to an administrative solution, consider legal representation, but do it quickly. You have a window of response. **Do not miss it!**

Finding Solutions to Issues: Informal Negotiation is Usually An Option—Use it when you can

- Permit Conditions are Developed "Based on" Rules, but Can Often be Revised—these "requirements" are often policies and not rules
- Staff Perspective is Vitally Important
- Enforcement Actions are Primarily Based on Self-Monitoring Results—so Remember this When Filing Reports
- Legal Rights Exist and Can be Critical in Establishing Leverage
- Most of the Time, Informal Negotiation is the Best Way to Proceed
- Formal Negotiation Typically Involves Attorneys
- Being Sure Means You Stick to Your Guns
- Mutual Respect is Essential

Legal Challenge is an Option when Your Case is Strong

Reminder of the Things You May be Facing

- Emerging Contaminates: impacts to raw water, effluent limits, biosolids management
- Nutrient Management in the Neuse, Tar, and Cape Fear Basins
- Pretreatment Challenges—what happens when you do not control the situation through treatment?
- Keeping an eye on your permit status—DWR isn't required to notify you if something needs to be done to keep your permit valid
- Moving targets—policies verses rules
- Expected toxic materials: DWR may set limits, even if there is no specific standard in place

From Concept to Reality

Things to Consider as the ORC (Operator in Responsible Charge)

- You have Specific Regulatory Responsibilities
- These Responsibilities are Assigned to You Personally
- Compliance is More than Operational Attention (though that is essential)
- Typically, an ORC is not the Owner or the Responsible Management Official
- Communication to Your Employer is Extremely Important: Inform them of your Needs for Compliance Beyond Operation—Documentation if Essential

Dealing With Enforcement Actions

- Rule number 1, Avoid them if at all Possible
- Stay “Ahead” of the Process—Address Potential Violations When you File Your Report
- Seek to know your status before there is Action
- If the Agency’s Procedure is to Issue an NOV First, Work to Avoid Enforcement after the NOV
- Assessment of Penalties can be Contested, You can Pay them, or Seek Remission
- Find out the Core Objective of an agency Enforcement Action
- The Application of a Schedule of Compliance or Order May Help Avoid Penalties—Chronic Issues

Remind the Regulatory People Who makes sure water/wastewater systems work and protect us and the environment…?

Operators and System Managers

When thanking those who have so greatly improved water quality management in NC, sometimes the agency forgets…

The people who DO the work!

… including the Operator Training and Certification Programs for water and wastewater.

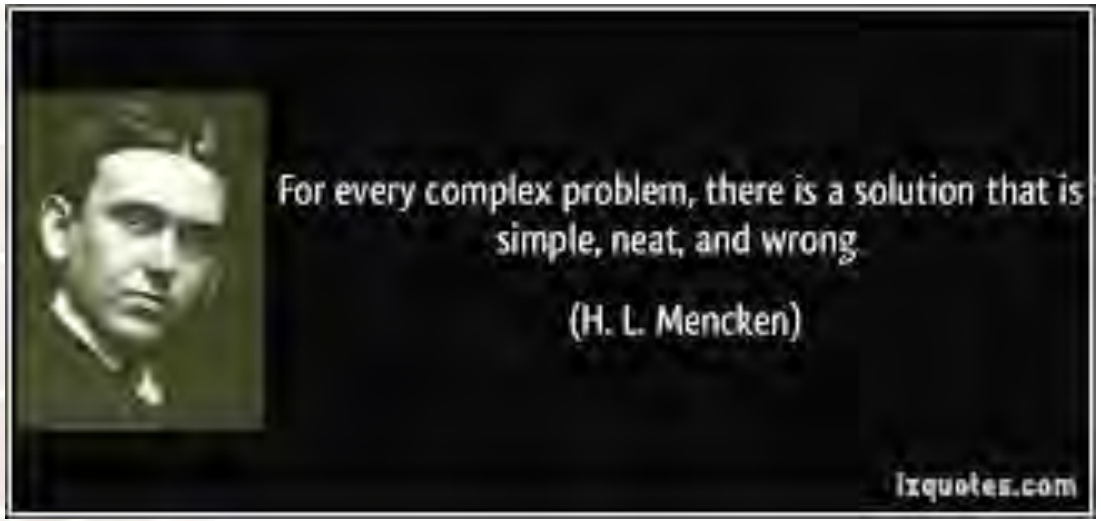
Effective water management requires professional and trained operators and managers

Proper funding and support from system owners allow operators and system managers to do their job effectively

The Future Holds Expansion of Responsibility and Challenges of Finding Personnel Resources.

The World of Water Management is Changing

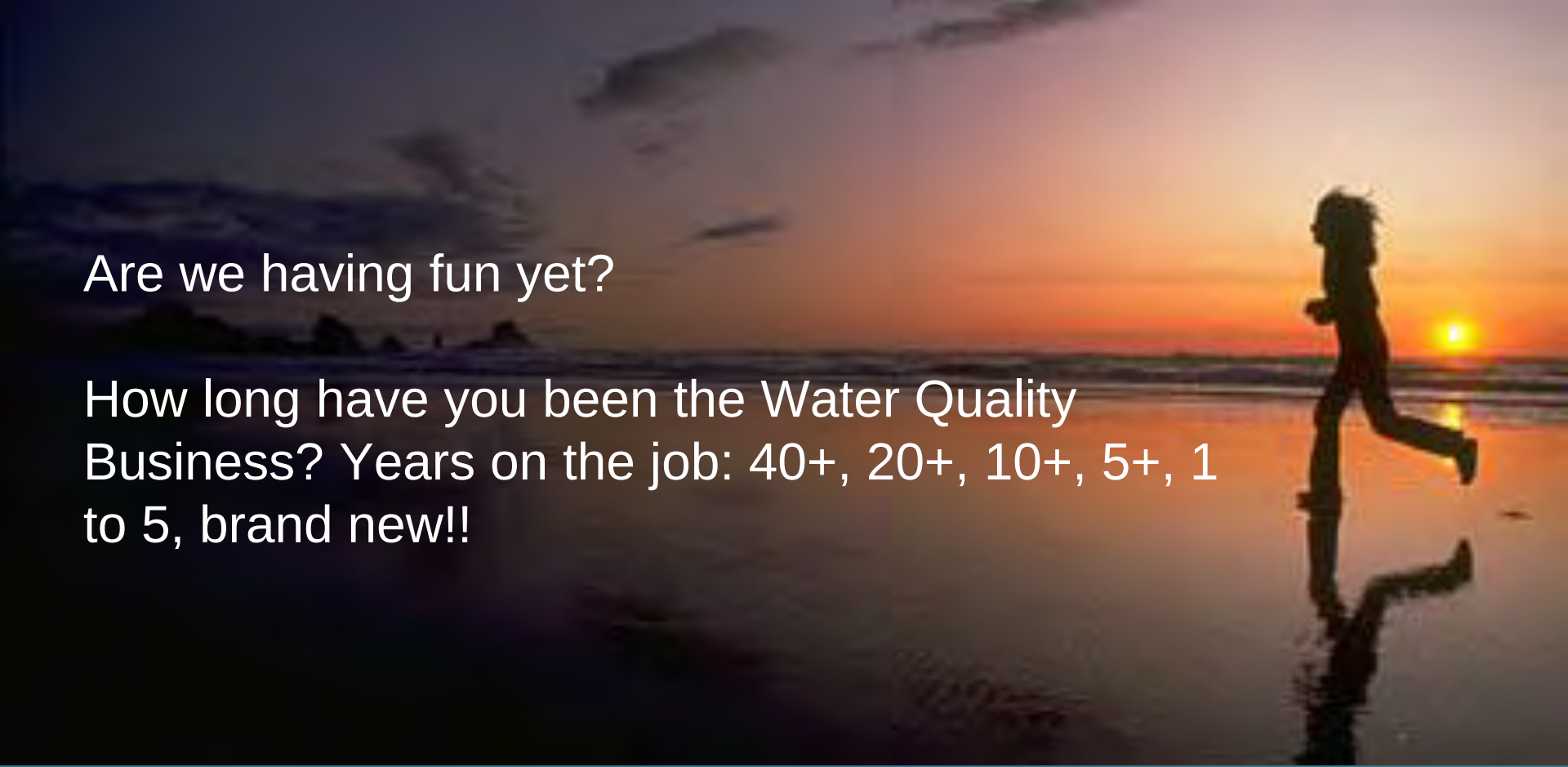
Unfortunately, there are no easy answers. Owners and Operators are on the Bleeding Edge!



Question: True of False

If you have an enforcement action/assessment of penalities against you or your client, you have no choice but to pay. True or False

agency to a violation. Sometimes it follows a Notice of Violation (NOV) and sometimes a penalty is part of the NOV. You typically have three options: 1) pay the penalty (this assumes you accept that you were in violation and agree the agency has the authority to assess you), 2) Contest the assessment under specific provisions of the law that authorizes the agency to enforce permits, rules, and any other statutory or regulatory requirement, or 3) ask for remission (reduction or dismissal of the penalty—but even if the penalty is completely "set aside" agency cost of the enforcement action can and likely will be collected).



Are we having fun yet?

How long have you been the Water Quality Business? Years on the job: 40+, 20+, 10+, 5+, 1 to 5, brand new!!

Forrest R. Westall, Sr., PE, McGill Associates, P.A.
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Asheville Water System:

Hurricane Helene Response and Recovery

04/30/26
2026 Toxicology Workshop

Presented By: Brenna Cook

THE CITY OF ASHEVILLE

Presentation Overview

- Damage to WNC
- Damage Asheville Water System
- Water Preparedness
- Water Response
- Water System Recovery

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Hurricane Helene - WNC Overview

Roads and Bridges:
6,000 miles of roads and over 1,000 bridges were damaged or destroyed, impacting transportation and access to communities.

Water and Sewer Systems:
Over 160 water and sewer systems were damaged, affecting millions of residents and hindering recovery efforts.

Landslides:
The storm triggered 1,400 landslides, further damaging infrastructure and making roads impassable.

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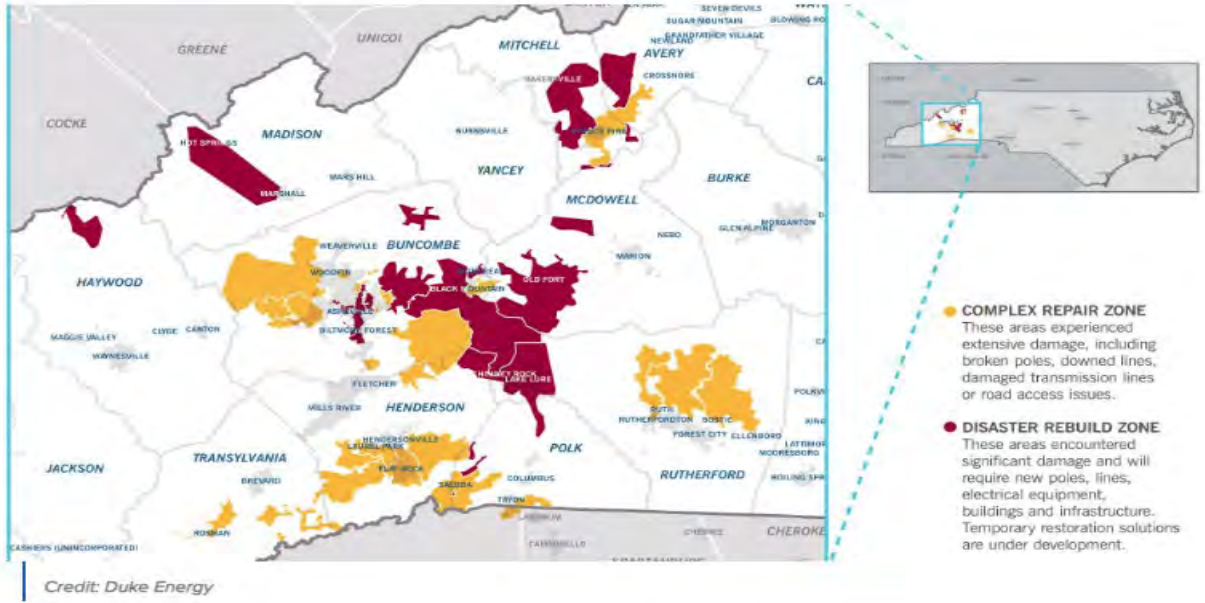
Hurricane Helene - WNC Overview



Credit: Duke Energy

Hurricane Helene 9/27/25

Auxiliary Spillway 9/27/26

Primary Spillway 9/27/26

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City of Asheville Water System Damage from Hurricane Helene

North Fork Water Treatment Facility

- Road way washed out on North Fork-Left Fork Rd to plant
- Turbidity Levels in Burnette Reservoir increased from below 1 NTU to 80 NTU
- Damage to primary spillway at the dyke

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City of Asheville Water System Damage from Hurricane Helene

William DeBruhl Water Treatment Facility

- Turbidity increased from 1 NTU to over 100 NTU (Large landslide into Bee Tree Reservoir)
- Road way to treatment plant washed out blocking access
- Lagoon #2 for wastewater treatment was damaged
- Dam was intact but some sloughing occurred

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North Fork Reservoir
OCT 10


North Fork Reservoir
MARCH 24, 2024


Burnette
Reservoir
Post Helene


Bee Tree
Reservoir
Pre Helene


Bee Tree
Reservoir
Post Helene

North Fork and Bee Tree Reservoirs Pre Helene

North Fork and Bee Tree Reservoirs Post Helene

City of Asheville Water System Damage from Hurricane Helene

Distribution System Damage

- 36”, 24” and 36” bypass transmission lines at North Fork
- 24” line on Old Hwy 70 in Swannanoa
- 12” line at Whitson Avenue, Swannanoa
- 8” line to Black Mountain
- 8” line on Swannanoa River Rd
- 8” line at S. Tunnel and Swannanoa
- 6” line on Old Farm School Rd

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North Fork 36” Bypass Waterline
SEP 30


North Fork 36” Bypass Waterline
OCT 8

North Fork 36” Bypass Waterline

OCT 10

Question 1

When was the 36” bypass repair completed?

a. October 9th
b. October 10th
c. October 11th
d. September 30th

City of Asheville Preparedness

Resilient & Competent Employees

Water Resources ERP

Quarterly ERP Training

City ERP

NIMS/ICS

NC Water Warn

City of Asheville Preparedness

Sept 2004 - Hurricanes Frances

North Fork Dam Improvements 2017-2020

- **Auxiliary Spillway/Parapet Wall on Dam**
- **Seismic Buttressing**

36” Bypass Installation 2019-2021

August 2021 Tropical Storm Fred

Alternatives for Conventional Treatment Study was awarded 9/27/24

Christmas 2022 Water Outage

Auxiliary Spillway

Auxiliary Post Helene

Question 2

True or False:

The auxiliary spillway worked as designed.

Response & Recovery

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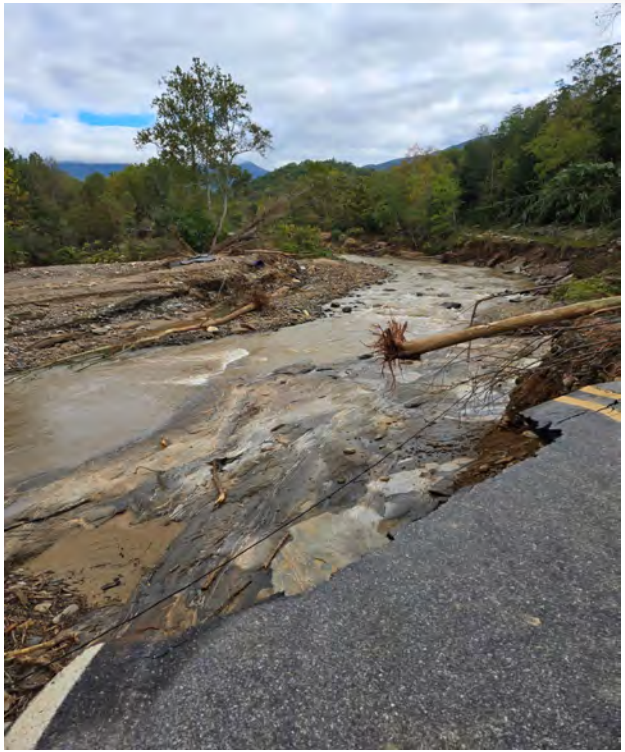
Response at WTPs Before Helene



- Dam EAP was followed
- Staffing was doubled at North Fork and Mills River water plant locations
- Treatment Plant Status Sheet was created documenting flow, clearwell level, raw turbidity and large tanks levels in the system.
- William DeBruhl WTP was shut down at 6:00 pm on 9/26/24 but two operators stayed on site.
- On September 27 at 10:00am NF flow jumped from 25 MDG to 35 MGD
- North Fork slowed operations to keep water flowing through transmission lines

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Response After Helene

- Determined all transmission lines including the 36” bypass were washed away.
- Shut down North Fork and concentrated on the Mills River WTP to keep water in the southern portion of distribution system.
- Water Engineering went to the home(s) of local Asheville area general contractors to help with bypass and transmission line installation.
- T&K, TP Howard and Tennoca showed up on 9/30/24 to review damage at sites and started working that day to repair the roads and start installation of the 36” bypass and 24” line on Old 70 in Swannanoa.

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Response After Helene

- Zombie Apocalypse Desktop scenario involving the installation of the coil due to high turbidity.
- EPA, FEMA and Army Corp of Engineers were onsite by 10/2/24. Consulted with NCDEQ on next steps to provide water for public health and fire protection.
- Installation of spool piece to bypass the plant
- Disinfected water with high chlorine to reduce the chance of pathogens in the distribution system.

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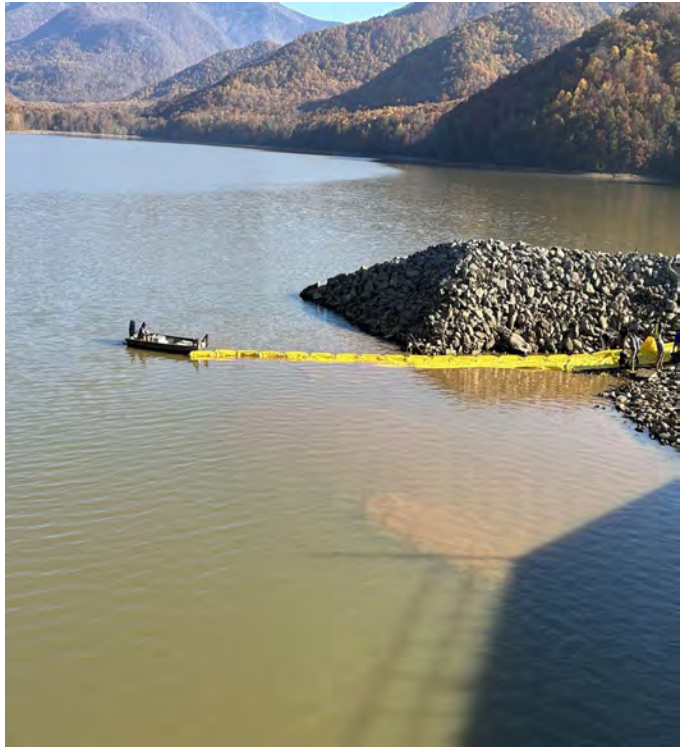
Response After Helene



Enlisted CDM Smith with treatment options in the response to high turbidity in the Burnett Reservoir

- In situ treatment
- In reservoir treatment with Alum and Caustic Soda
- Turbidity Curtain
- Pilot Plant Testing
- Alternative Treatment Systems (DAF)

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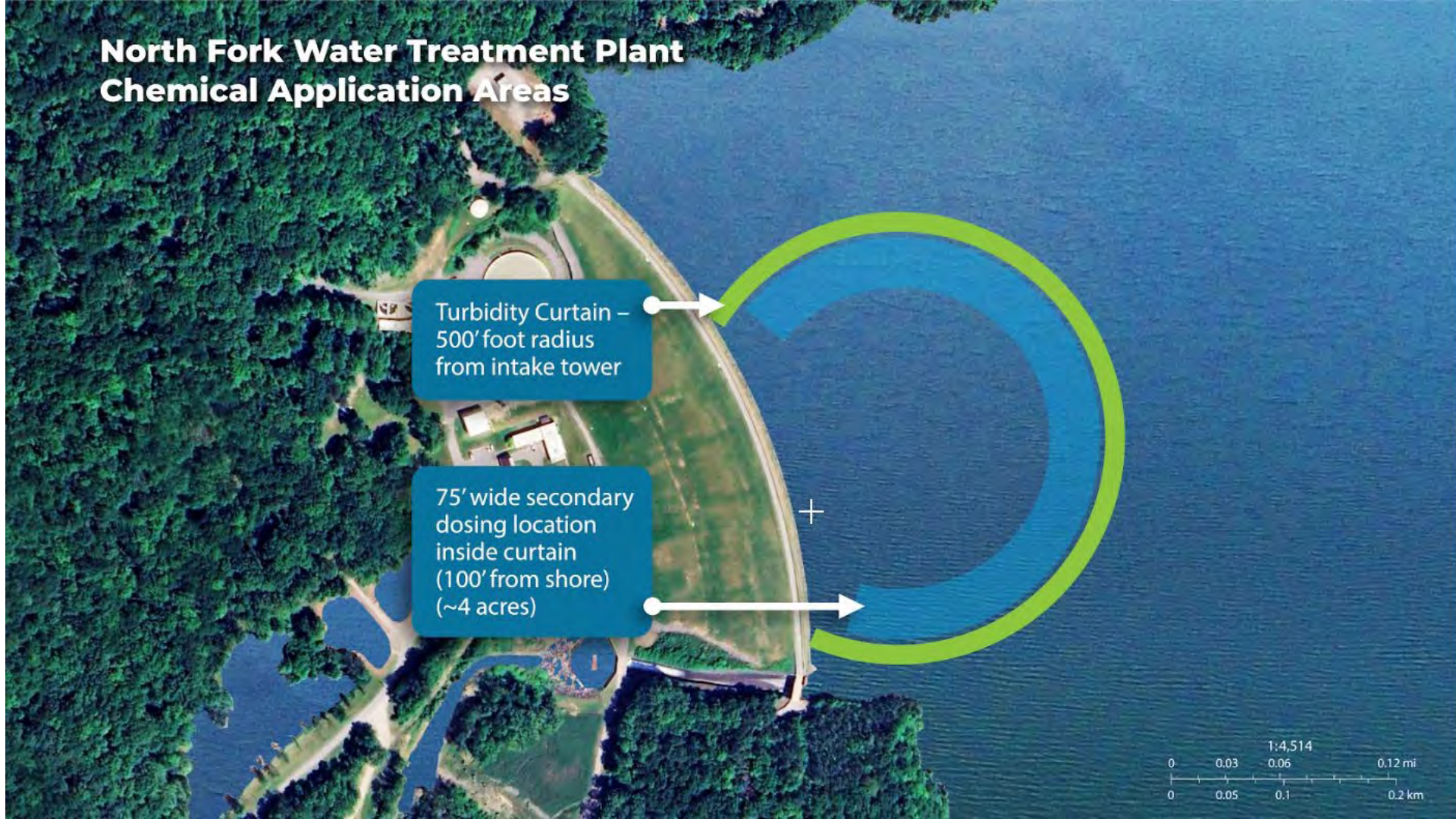
North Fork Water Treatment Plant
Chemical Application Areas
Turbidity Curtain – 500' foot radius from intake tower
75' wide secondary dosing location inside curtain (100' from shore) (~4 acres)
1:4,514
0 0.03 0.06 0.12 mi
0 0.05 0.1 0.2 km

North Fork Turbidity Removal

- FEMA instructed Army Corp to design system to treat high turbidity water
- Army Corp put out a PWS or Performance Work Statement
- Ahtna provided a response in conjunction with CDM Smith which included the use of 13 portable Dissolved Air Flotation (DAF) systems.
- Received NCDEQ approval of pretreatment design

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William DeBruhl Turbidity Removal

- FEMA instructed Army Corp to design system to treat high turbidity water
- Army Corp put out a PWS or Performance Work Statement
- Bering Weston provided a response which included the use of 4 portable Dissolved Air Flotation (DAF) systems, sand filters and bag filters.
- Received NCDEQ approval of pretreatment design

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Dissolved Air Flotation (DAF) Sedimentation Units

Post Helene Recovery

CDM Smith Study released 9/27/24

- Plate Settlers - were chosen to use moving forward
- Dissolved Air Flotation (DAF)
- Super Pulsator
- Ballasted Flocculation (active flow)
- Membrane Filtration

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Question 3

What does the DAF acronym mean?

A: Dissolved Air Flotation
B: Demand Air Flow
C: Dissolved Aeration Flow
D: Decreasing Air Flotation

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Post Helene Recovery

Ideas for Resiliency

- Bypass was reinforced with sheet piles when installed.
- Cross Country Bypass
- Expansion of Mills River Water Treatment Plant - Phase 2 & 3.
- Fourth Water Treatment Plant Location - North/Western Buncombe County

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Q&A

NC DWR Aquatic Toxicology Branch

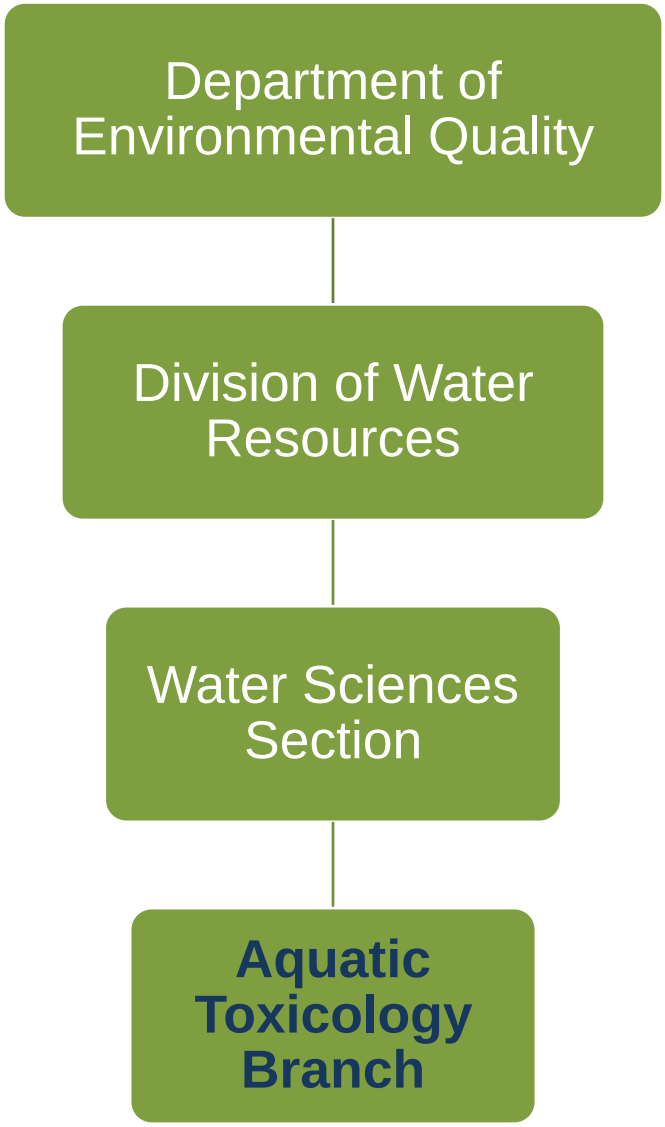
Toxicology Workshop

April 30, 2026

Molly Nicholson

Aquatic Toxicology Branch Supervisor

Aquatic Toxicology Branch (ATB)



Mailing: 1621 MSC
Raleigh, NC 27699-1621

Aquatic Toxicology Branch (ATB)

- ATB has three main functions which support the National Pollutant Discharge Elimination System (NPDES) program.

WET Testing and Biological Organism Culture

Biological Laboratory Certification

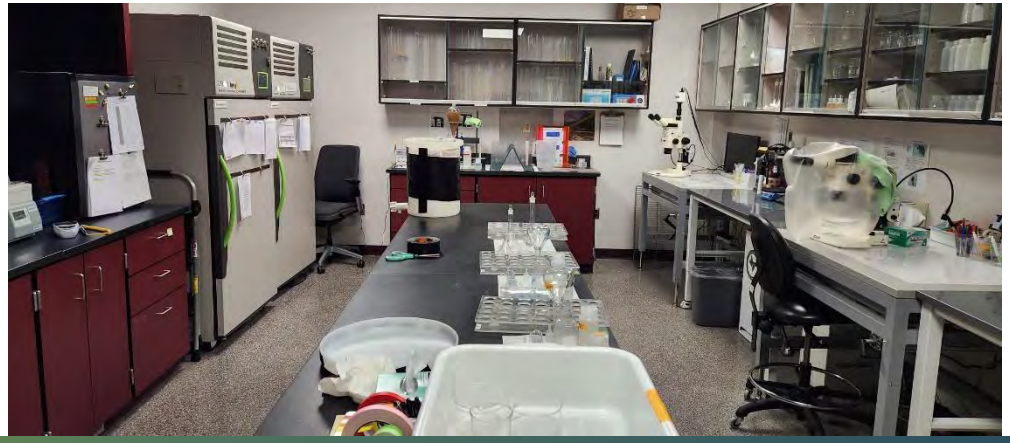
NPDES Compliance and Enforcement

Whole Effluent Toxicity (WET) Testing Laboratory

- Supported by an EPA 106 Grant
  - Regional office staff perform bioassay inspection at facility, pulling a sample for WET testing and sending it to ATB.
  - Annually, ATB tests 10% of all major NC NPDES facilities.

- NC Methods approved by EPA in 1988 and started adding to permits in 1989. EPA promulgated their methods in 1991.

- ATB uses EPA Alternative methods for measuring acute and chronic toxicity of wastewater and surface waters.



Whole Effluent Toxicity (WET) Testing Laboratory

- Culture *Ceriodaphnia dubia* for use in WET testing.
- Other species used in testing are purchased and shipped in to be used for testing.

WET Testing Organisms

Ceriodaphnia dubia
(Cerio, water fleas, bugs)

Pimephales promelas (Fathead minnows)
Menidia menidia (Silverside minnows)

http://www.etsnclab.com/

Americamysis bahia (Mysidopsis bahia)
(Mysid shrimp)

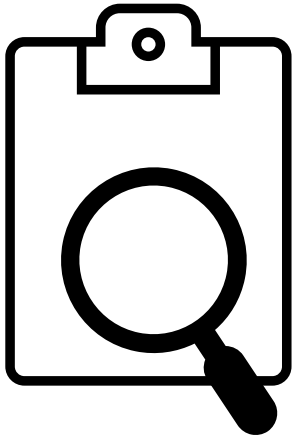
https://aquamania.co.uk/

Biological Laboratory Certification

- Any lab analysis that is used to meet the NC NPDES system.

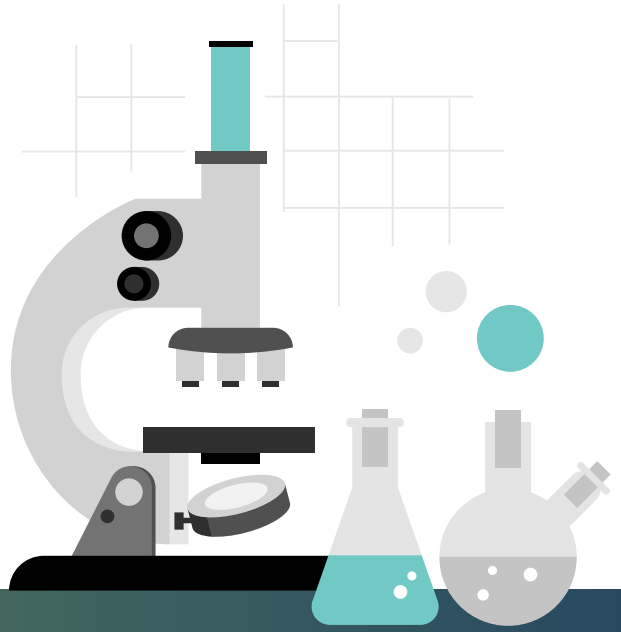
- North Carolina General Statues, G.S. 143-215.3(a)(1) and 143-215.3(a)(10).

- North Carolina Administrative Code, 15A NCAC 2H .0800 & .1100 which details the requirements for both chemical and biological lab certification.



Biological Laboratory Certification & ATB QC

- Conduct lab inspections, track and review data.
- Manage the Annual Proficiency Testing Program mandated by EPA for any facility that submits NPDES tests results.
- Review data and track QC procedures for the ATB lab.
- Monitor and report the EPA 106 Grant data with the help of the regional staff.



NPDES Compliance and Enforcement/Permitting

- Use data from WET testing to track compliance.
- Review toxicity data reported by facilities to verify data quality.
- Ensure compliance on a monthly basis and make enforcement recommendations for non-compliance.
- Provide compliance and enforcement guidance to facilities.
- Review NPDES permits for correct WET requirements.
- Review biocide and PAMS applications.

Biocide & PAMS Applications

Biocide: For any product ultimately discharged into NC surface waters

- Complete biocide application form (Excel or PDF)
- Provide SDS with section 12 data
  - Or perform multiple-concentration chronic *Ceriodaphnia dubia* test

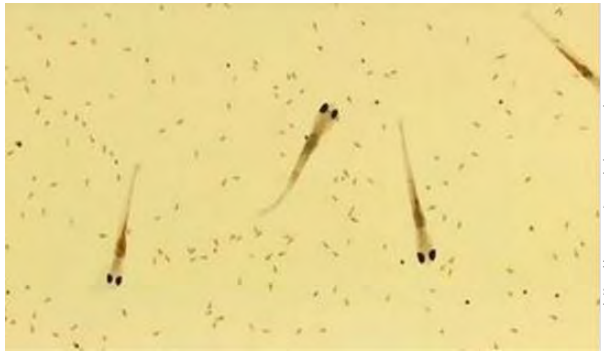
PAMS: Polyacrylamide products used to reduce soil erosion or subsequent sedimentation in streams

- Provide SDS with section 12 data
- Provide information on how product will be applied

Please see ATB Downloads webpage for more info

Second Species Testing (Alternative Species)

- EPA requested that NC major municipal facilities be required to perform 2nd species testing.
- Outlined in EPA form 2A Part E of the permit renewal application.
- Checklist for Municipal Application Requirements: https://deq.nc.gov/water-quality/aquifer-protection/afo/permits/municipal-checklist-2021-dwq-swp-npdes/download
- Second species conditions are included in permits that require it.
- Schedule ahead of time!



MUNICIPAL TYPE	EPA FORM 2A?	IF COMPLETING FORM 2A, DO YOU NEED THE FOLLOWING?					
		PART A - Basic Application Information	**PART B** - Add'l Application Information (expanded testing)	**PART C** - Certification	**PART D** - Priority Pollutant Analysis*	**PART E** - TOX(2nd species)**	**PART F** - SIUs Supplement Info.
Less than 0.1MGD (minor)	No (use NC Short Form A Or Short Form D)	No	No	No	No	No	No
Greater than/equal to 0.1 MGD but less than 1.0 MGD -- without pretreatment	Yes	Yes	Yes	Yes	No	No	No
Greater than/equal to 0.1 MGD but less than 1.0 MGD -- with pretreatment	Yes	Yes	Yes	Yes	Yes	Yes	Yes
1.0 MGD or Greater (major) -- without pretreatment	Yes	Yes	Yes	Yes	Yes	Yes	No
1.0 MGD or Greater (major) -- with pretreatment	Yes	Yes	Yes	Yes	Yes	Yes	Yes

*3 samples with seasonal variation (at least 4 months separation between samples)
**4 samples with seasonal variation (for information on second species toxicity testing, call Aquatic Toxicology Unit at 919-743-8401

Second Species Testing – Permit Renewal

(c.) **2nd-Species Toxicity Testing and Reporting**.

(i.) In addition to the quarterly toxicity tests required in Condition A.(2.) CHRONIC TOXICITY PERMIT LIMIT (QUARTERLY), the Permittee shall perform and report the results of four (4) toxicity tests using the same test methods using a second species of test organism suitable to the tests being conducted.

(ii.) The 2nd species toxicity tests shall be conducted either:

(A) Once per quarter in a single 12-month period (four samples); if this option is chosen, the sample for each 2nd species test shall coincide with the quarterly samples collected for (CHRONIC TOXICITY PERMIT LIMIT (QUARTERLY)); or

(B) Once per 12-month period in the four-and one-half year period prior to the scheduled application for permit renewal (four samples); if this option is chosen, three of the samples for the 2nd species test shall coincide with those for the annual effluent scans and the coincident quarterly toxicity test, and each of the four annual samples shall be collected in a different calendar quarter in order to represent seasonal variation.

(iii.) The results of the toxicity tests shall be submitted to the following address:

North Carolina Division of Water Resources
Water Sciences Section/Aquatic Toxicology Branch
1621 Mail Service Center
Raleigh, North Carolina 27699-1621

Or, results can be sent to the email, ATForms.ATB@deq.nc.gov.

(iv.) Results of the 2nd species tests shall also be summarized in Part E (Toxicity Testing Data) of EPA Municipal Application Form 2A when submitting the permit renewal application to the NPDES Wastewater Program.

The Permittee may contact the Division's Aquatic Toxicology Branch at 919-743-8401 for guidance on conducting the additional toxicity tests and reporting of the results.

MAJOR FACILITIES & MUNICIPALITIES

NORTH CAROLINA
Department of Environmental Quality

Question

Which one of the following statements about second species testing is incorrect?

A) Four second species tests must be completed per permit cycle.

B) Second species tests must be completed prior to applying for permit renewal.

C) All second species tests must be performed with *Ceriodaphnia dubia*.

D) Second species tests must be performed in different seasons.

Answer

Which one of the following statements about second species testing is incorrect?

A) Four second species tests must be completed per permit cycle.

B) Second species tests must be completed prior to applying for permit renewal.

C) All second species tests must be performed with *Ceriodaphnia dubia*. X

D) Second species tests must be performed in different seasons.

Important Toxicity Sections of Permit

PARAMETER	Parameter Code	EFFLUENT LIMITS			MONITORING REQUIREMENTS		
		Monthly Average	Weekly Average	Daily Maximum	Measurement Frequency	Sample Type	Sample Location[2]
Flow	50050	1.15 MGD			Continuous	Recording	I or E
BOD, 5 day, 20°C [3] (Apr 1 - Oct 31)	CO310	5.0 mg/L	7.5 mg/L		2/week [4]	Composite	I and E
BOD, 5 day, 20°C [3] (Nov 1 - Mar 31)	CO310	10.0 mg/L	15.0 mg/L		2/week [4]	Composite	I and E
Total Suspended Solids[3]	CO530	30.0 mg/L	45.0 mg/L		2/week [4]	Composite	I and E
NH_3 as N (Apr 1 - Oct 31)	CO610	1.0 mg/L	3.0 mg/L		2/week[4]	Composite	E
NH_3 as N (Nov 1 - Mar 31)	CO610	2.0 mg/L	6.0 mg/L		2/week[4]	Composite	E
Fecal Coliform (geometric mean)	31616	200/100 mL	400/100 mL		2/week[4]	Grab	E
Dissolved Oxygen	00300	Daily Average ≥ 5.0 mg/L			3/week	Grab	E
pH	00400	Between 6.0 and 9.0 Standard Units			3/week	Grab	E
Temperature (°C)	00010	Monitor and Report			3/week	Grab	E
Total Residual Chlorine [5]	50060			17 µg/L	3/week	Grab	E
Fluoride	00951	1.8 mg/L		5.4 mg/L	Monthly	Composite	E
Total Copper	01042	11.9 µg/L		16.5 µg/L	Monthly	Composite	E
Total Phosphorus (mg/L)	CO665	Monitor and Report			Quarterly	Composite	E
Total Nitrogen [6] (mg/L)	CO600	Monitor and Report			Quarterly	Calculated	E
TKN (mg/L)	00625	Monitor and Report			Quarterly	Composite	E
NO_3-N + NO_2-N (mg/L)	00630	Monitor and Report			Quarterly	Composite	E
Chronic Toxicity [7]	TGP3B	Monitor and Report			Quarterly	Composite	E
Effluent Pollutant Scan [8]	NC01	Monitor and Report			Footnote 8	Footnote 8	E
PFAS[9]	various	Footnote 9			Footnote 9	Grab	E
Hardness[10]-Total as $CaCO_3$ (mg/L)	00900	Monitor and Report			Quarterly	Composite	E
Dissolved Oxygen (mg/L)	00300	Monitor and Report			Variable[2]	Grab	U and D
Temperature °C	00010	Monitor and Report			Variable[2]	Grab	U and D

7. Chronic Toxicity (*Ceriodaphnia dubia*) P/F at 90% with testing in January, April, July and October. See Special Condition A.(2.).

NORTH CAROLINA
Department of Environmental Quality

A. (2.) CHRONIC TOXICITY PERMIT LIMIT (QUARTERLY)
[15A NCAC 02B .0200] [15A NCAC 02B .0500 et seq]

The effluent discharge shall at no time exhibit observable inhibition of reproduction or significant mortality to *Ceriodaphnia dubia* at an effluent concentration of 90%.

The permit holder shall perform at a minimum, **quarterly** monitoring using test procedures outlined in the "North Carolina *Ceriodaphnia* Chronic Effluent Bioassay Procedure," (Revised December 2010, or subsequent versions) or "North Carolina Phase II Chronic Whole Effluent Toxicity Test Procedure" (Revised December 2010, or subsequent versions). The tests will be performed during the months of January, April, July, and October. These months signify the first month of each three-month toxicity testing quarter assigned to the facility. Effluent sampling for this testing must be obtained during representative effluent discharge and shall be performed at the NPDES permitted final effluent discharge below all treatment processes.

If the test procedure performed as the first test of any single quarter results in a failure or ChV below the permit limit, then multiple-concentration testing shall be performed at a minimum, in each of the two following months as described in "North Carolina Phase II Chronic Whole Effluent Toxicity Test Procedure" (Revised-December 2010, or subsequent versions).

All toxicity testing results required as part of this permit condition will be entered electronically using the Division's eDMR system for the months in which tests were performed, using the parameter code **TGP3B** for the pass/fail results and **THP3B** for the Chronic Value. Additionally, DWR Form **AT-3** (original) is to be sent to the following address:

North Carolina Division of Water Resources
Water Sciences Section/Aquatic Toxicology Branch
1621 Mail Service Center
Raleigh, NC 27699-1621

Or, results can be sent to the email, ATForms.ATB@deq.nc.gov.

Completed Aquatic Toxicity Test Forms shall be filed with the Water Sciences Section no later than 30 days after the end of the reporting period for which the report is made.

NORTH CAROLINA
Department of Environmental Quality

Important Toxicity Sections of Permit

A. (2) CHRONIC TOXICITY PASS/FAIL MONITORING (QUARTERLY)
[15A NCAC 02B .0500 et seq.]

The permittee shall conduct **quarterly** chronic toxicity tests using test procedures outlined in the "North Carolina *Ceriodaphnia* Chronic Effluent Bioassay Procedure," (Revised December 2010, or subsequent versions).

The effluent concentration defined as treatment two in the procedure document is **79.22**%. The testing shall be performed as a *Ceriodaphnia dubia* 7-day pass/fail test. The tests will be performed **during the months of March, June, September, and December**. These months signify the first month of each three-month toxicity testing quarter assigned to the facility. Effluent sampling for this testing must be obtained during representative effluent discharge and shall be performed at the NPDES permitted final effluent discharge below all treatment processes.

All toxicity testing results required as part of this permit condition will be entered electronically using the Division's eDMR system for the month in which it was performed, using the parameter code **TGP3B**. Additionally, DWR Form **AT-1** (original) is to be sent to the following address:

NORTH CAROLINA
Department of Environmental Quality

Dual Reporting Requirement

All ATB WET Testing has a dual-reporting requirement

Monthly, quarterly, or annual test results MUST BE:

AT Form emailed (or mailed) to ATB

+

Reported on the eDMR

All toxicity testing results required as part of this permit condition will be entered electronically using the Division's eDMR system for the months in which tests were performed, using the parameter code **TGP3B** for the pass/fail results and **THP3B** for the Chronic Value. Additionally, DWR Form **AT-3** (original) is to be sent to the following address:

North Carolina Division of Water Resources
Water Sciences Section/Aquatic Toxicology Branch
1621 Mail Service Center
Raleigh, NC 27699-1621

Or, results can be sent to the email, ATForms.ATB@deq.nc.gov.

Completed Aquatic Toxicity Test Forms shall be filed with the Water Sciences Section no later than 30 days after the end of the reporting period for which the report is made.

Test data shall be complete, accurate, include all supporting chemical/physical measurements and all concentration/response data, and be certified by laboratory supervisor and ORC or approved designate signature. Total residual chlorine of the effluent toxicity sample must be measured and reported if chlorine is employed for disinfection of the waste stream.

Should there be no discharge of flow from the facility during a month in which toxicity monitoring is required, the Permittee will complete the information located at the top of the aquatic toxicity (AT) test form indicating the facility name, permit number, pipe number, county, and the month/year of the report with the notation of "No Flow" in the comment area of the form. The report shall be submitted to the Water Sciences Section at the address cited above.

Should the Permittee fail to monitor during a month in which toxicity monitoring is required, monitoring will be required during the following month. Assessment of toxicity compliance is based on the toxicity testing quarter which is the three-month time interval that begins on the first day of the month in which toxicity testing is required by this permit and continues until the final day of the third month.

Should any test data from this monitoring requirement or tests performed by the North Carolina Division of Water Resources indicate potential impacts to the receiving stream, this permit may be re-opened and modified to include alternate monitoring requirements or limits.

If a facility experiences:

No flow	→	Submit AT form with "No flow for [month]" written in comments
Missed testing	→	Submit AT form with an explanation written in comments. ATB will ask you to test the following month.

Reasons for Missed Tests:

Invalid Tests

- Poor health of control
- Second effluent sample didn't arrive

Missed Tests

- Shipping error
- Lab capacity issues
- Natural disaster (eg. Hurricane Helene)

NORTH CAROLINA
Department of Environmental Quality

Blank AT Forms

Blank AT forms can be requested by emailing ATForms.ATB@deq.nc.gov

Effluent Toxicity Report Form- Chronic Pass/Fail and Acute LC50

Date _____

Facility _____ NPDES#NC _____ Pipe # _____ County _____

Laboratory Performing Test _____

Signature of Operator in Charge ORC Phone / Email

x _____

Signature of Laboratory Supervisor

Comments _____

MAIL ORIGINAL TO:

Environmental Sciences Section
Div. of Water Resources
N.C. DENR
1621 Mail Service Center
Raleigh, North Carolina 27699-1621

North Carolina *Ceriodaphnia* Chronic Pass/Fail Reproduction Toxicity Test

CONTROL ORGANISMS	1	2	3	4	5	6	7	8	9	10	11	12
# Young Produced												
Adult (L)ive (D)ead												

Effluent% _____

TREATMENT 2 ORGANISMS	1	2	3	4	5	6	7	8	9	10	11	12
# Young Produced												
Adult (L)ive (D)ead												

Chronic Test Results

Calculated t _____
Tabular t _____
% Reduction _____

%Mortality	Avg.Reprod.
Control	Control
Treatment 2	Treatment 2

Control CV

% control organisms producing 3rd brood

PASS FAIL

Check One

pH	1st sample start	1st sample end	1st sample start	1st sample end	2nd sample start	2nd sample end
Control						
Treatment 2						

D.O.	1st sample	1st sample	2nd sample
Control			
Treatment 2			

Complete This For Either Test

Test Start Date / /

Collection (Start) Date
Sample 1 / / Sample 2 / /

Sample Type/Duration

	Grab	Comp.	Duration
Sample 1			
Sample 2			

	Dilution	1st Tox Sample	2nd Tox Sample (Pass/Fail Only)
Hardness(mg/l)			
Spec.Cond.(µmhos)			
Chlorine(mg/l)			
Sample temp. at receipt			

LC50/Acute Toxicity Test

(Mortality expressed as %, combining replicates)

%	%	%	%	%	%	%	%	%	%	Concentration
%	%	%	%	%	%	%	%	%	%	Mortality

LC50=_____%
95% Confidence Limits
_____%-_____%

Method of Determination
Moving Average ☐ Probit ☐
Spearman Karber ☐ Other_____

Organism Tested _____ Duration (hrs)_____

Note: Please Complete This Section Also

	start/end	start/end
Control		
High Conc.		
	pH	D.O.

Question

If a facility is unable to test for toxicity during a required testing month, what should they do?

A) Test the following month.

B) Submit a blank AT form to ATB explaining the issue, then test the following month.

C) Call their regional office, then test the following month.

D) Submit a blank AT form to ATB explaining the issue, then test next quarter.

E) Be proud of yourself for trying, then move on and try again next quarter.

Answer

If a facility is unable to test for toxicity during a required testing month, what should they do?

A) Test the next month.

B) Submit a blank AT form to ATB explaining the issue, then test the following month.

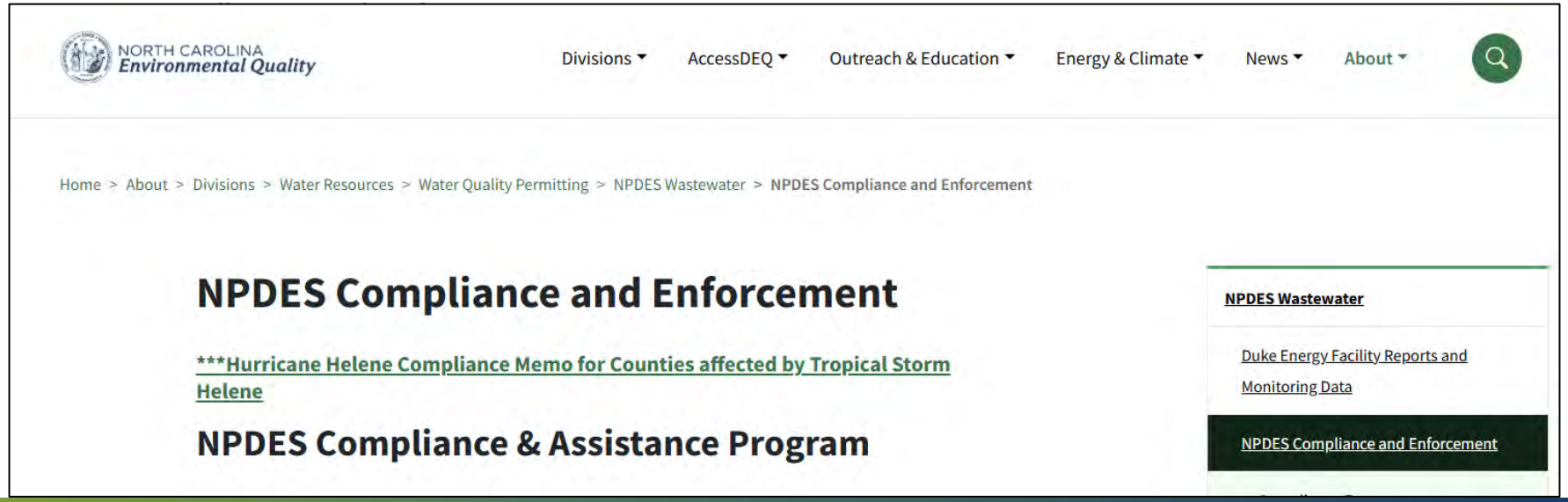
C) Call their regional office, then test the next month.

D) Submit a blank AT form to ATB explaining the issue, then test next quarter.

E) Be proud of yourself for trying, then move on and try again next quarter.

Hurricane Helene

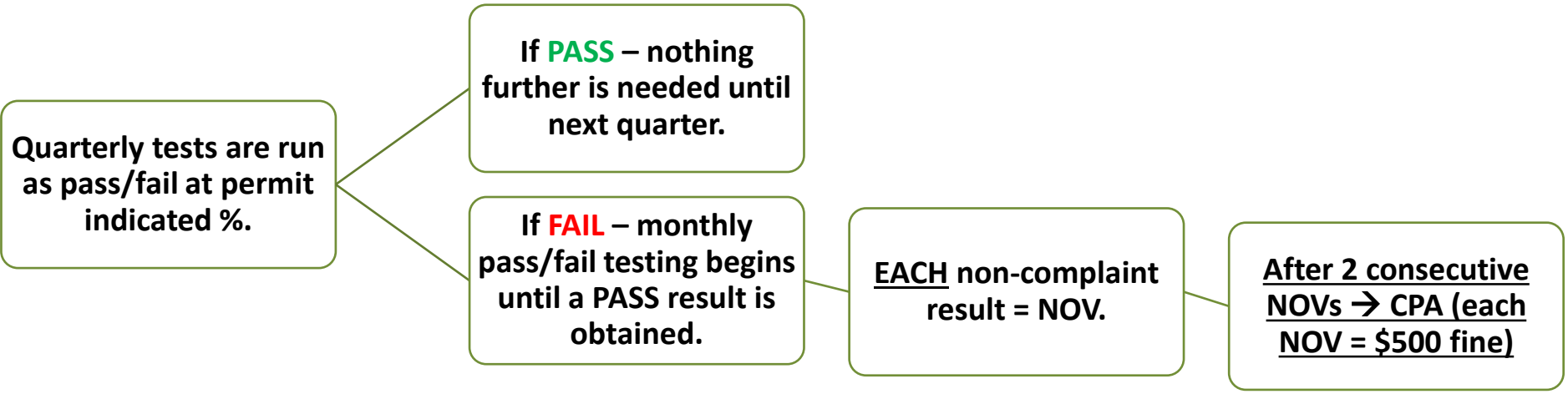
- **Original rules:** No compliance or enforcement through November 30th, 2024, for facilities within 25 counties designated in FEMA Disaster Declaration (9/29/25)
- **Current approach:** Enforcement actions are being approved on a case-by-case basis.
- **Hurricane Helene Compliance Memo:** https://www.deq.nc.gov/about/divisions/water-resources/permitting/npdes-wastewater/npdes-



WET Enforcement Strategy

- All test results reported to ATB are reviewed, processed, and saved.
  - Note: Any tox test performed on a sample from the outfall must be reported.
- Non-compliant tests (fail or ChV below permit limit) result in Notices of Violation.
- The permit specified test (chronic or acute) determines what type of follow-up testing is required.
- The results of the follow-up testing are evaluated to determine if a Civil Penalty Assessment (CPA) will be issued.

Acute Enforcement

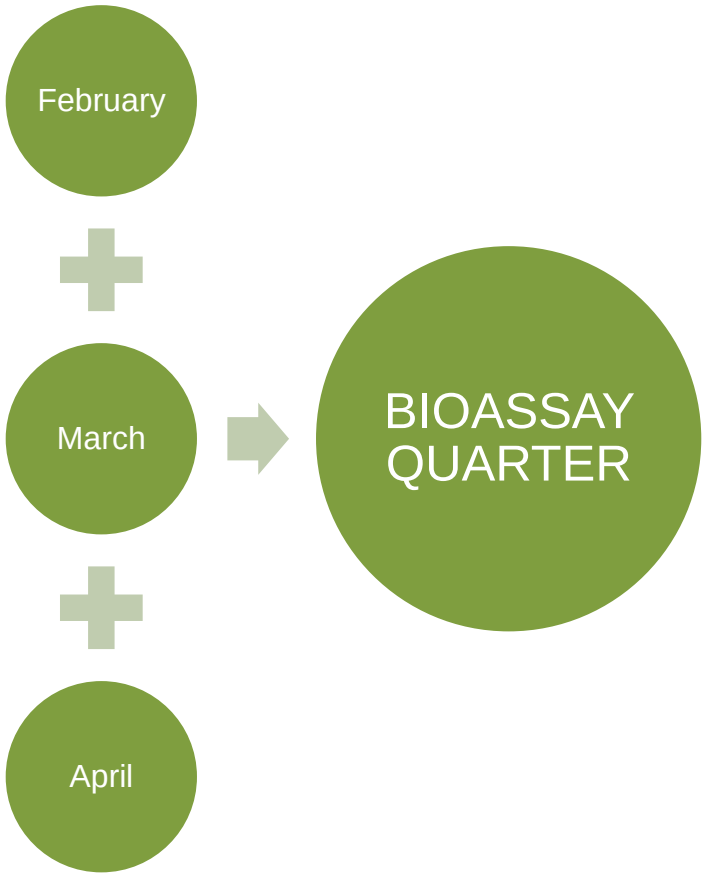


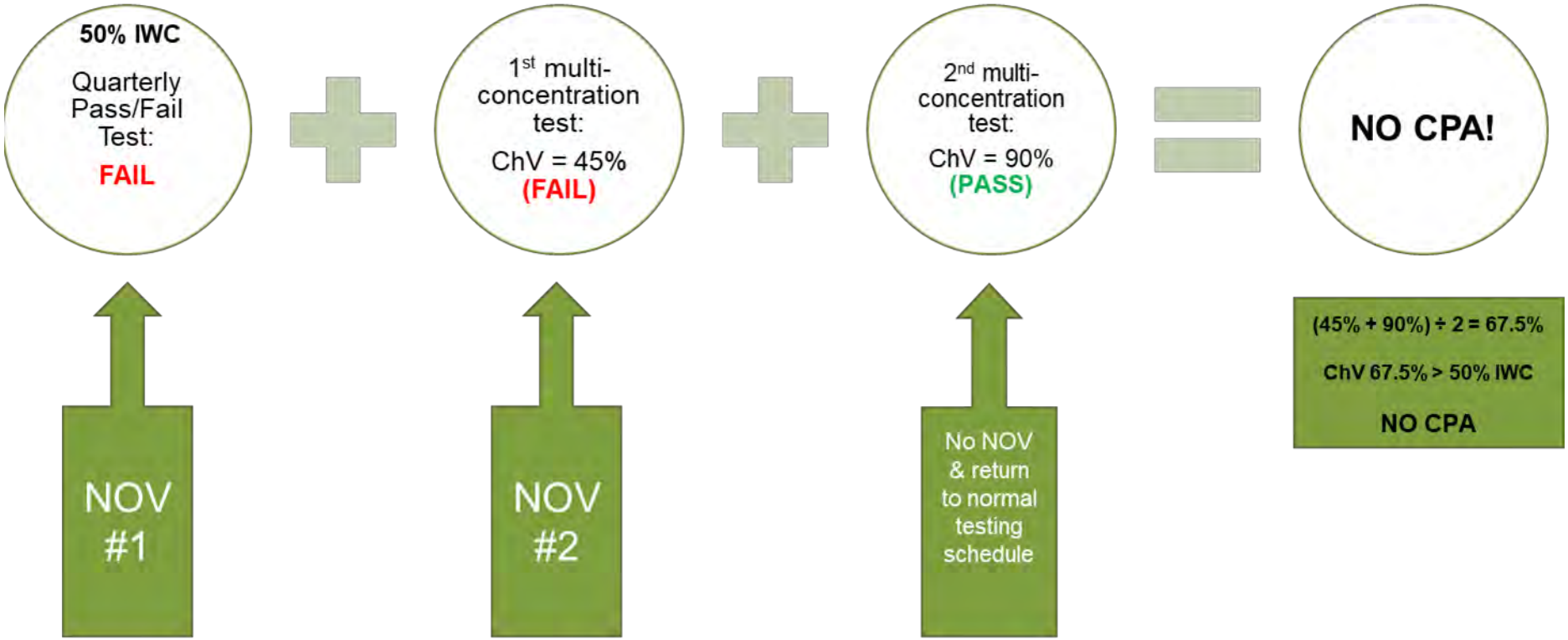
Chronic Enforcement

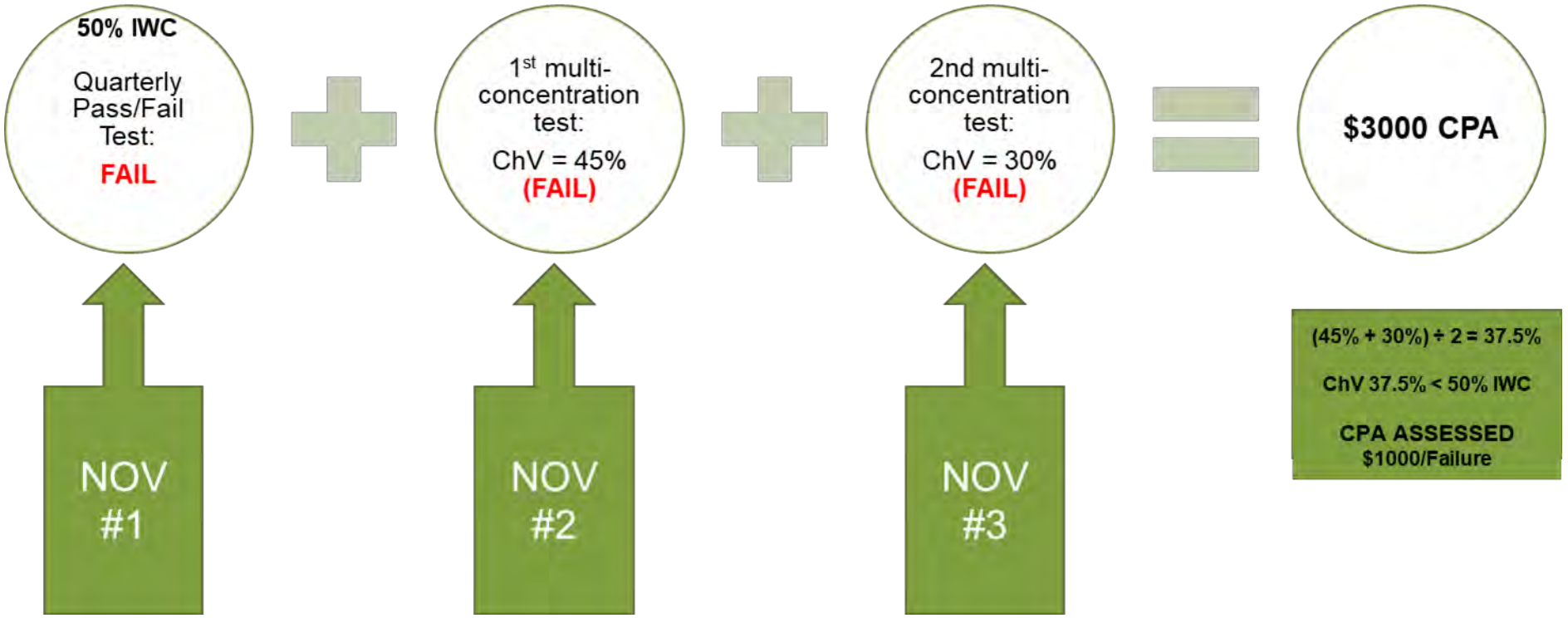
- Quarterly tests run as pass/fail at permit indicated %
- If **PASS** – nothing further is needed until next quarter
- If **FAIL** – 2 multi-concentration tests required (1 per month for 2 months)
- EACH non-complaint result = NOV
- Civil Penalty Assessment (CPA) is based off ChV average of the 2 follow-up tests
  - If ChV average is below the permit IWC, each NOV equates to $1000

If a facility has received multiple CPAs within the past year, penalty may be increased.

If testing requirement is for February:




50% IWC
Quarterly Pass/Fail Test:
FAIL
1st multi-concentration test:
ChV = 45%
(FAIL)
2nd multi-concentration test:
ChV = 90%
(PASS)
NO CPA!
NOV #1
NOV #2
No NOV & return to normal testing schedule
(45% + 90%) ÷ 2 = 67.5%
ChV 67.5% > 50% IWC
NO CPA


50% IWC
Quarterly Pass/Fail Test:
FAIL
1st multi-concentration test:
ChV = 45%
(FAIL)
2nd multi-concentration test:
ChV = 30%
(FAIL)
$3000 CPA
NOV #1
NOV #2
NOV #3
(45% + 30%) ÷ 2 = 37.5%
ChV 37.5% < 50% IWC
CPA ASSESSED
$1000/Failure

Review

- The facility is responsible for following permit requirements, NOT the contract lab.

- Dual reporting requirement - Report on eDMR **and** submit signed AT-Form:

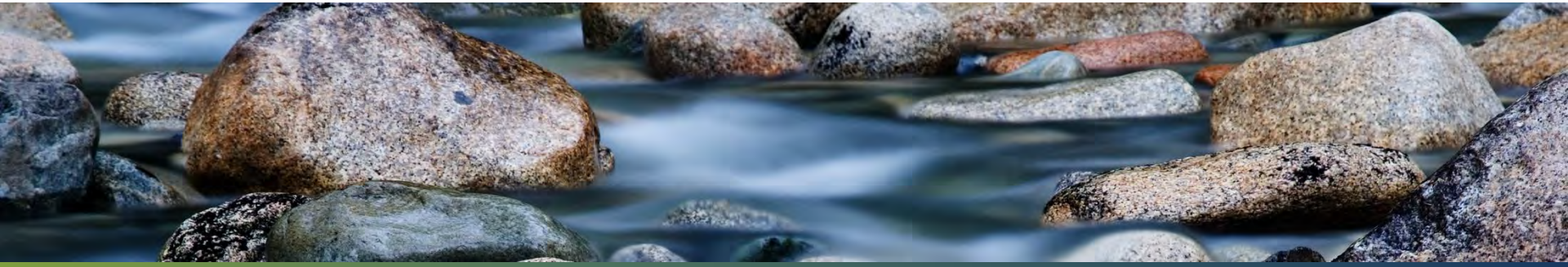
  ATForms.ATB@deq.nc.gov (we respond to confirm receipt)

  or 1621 MSC, Raleigh, NC 27699-1621

- AT forms must be signed by ORC or a delegated facility person.

- A blank AT form must be filled out and submitted for invalid/missed tests.

- The issues above, or any failure to follow permit, can result in a Notice of Violation or Civil Penalty.



Aquatic Toxicology Branch (ATB) Staff

Molly Nicholson - Supervisor
919-743-8424
molly.nicholson@deq.nc.gov

Madison Myers - QA/QC & Lab Certification
919-743-8423
madison.myers@deq.nc.gov

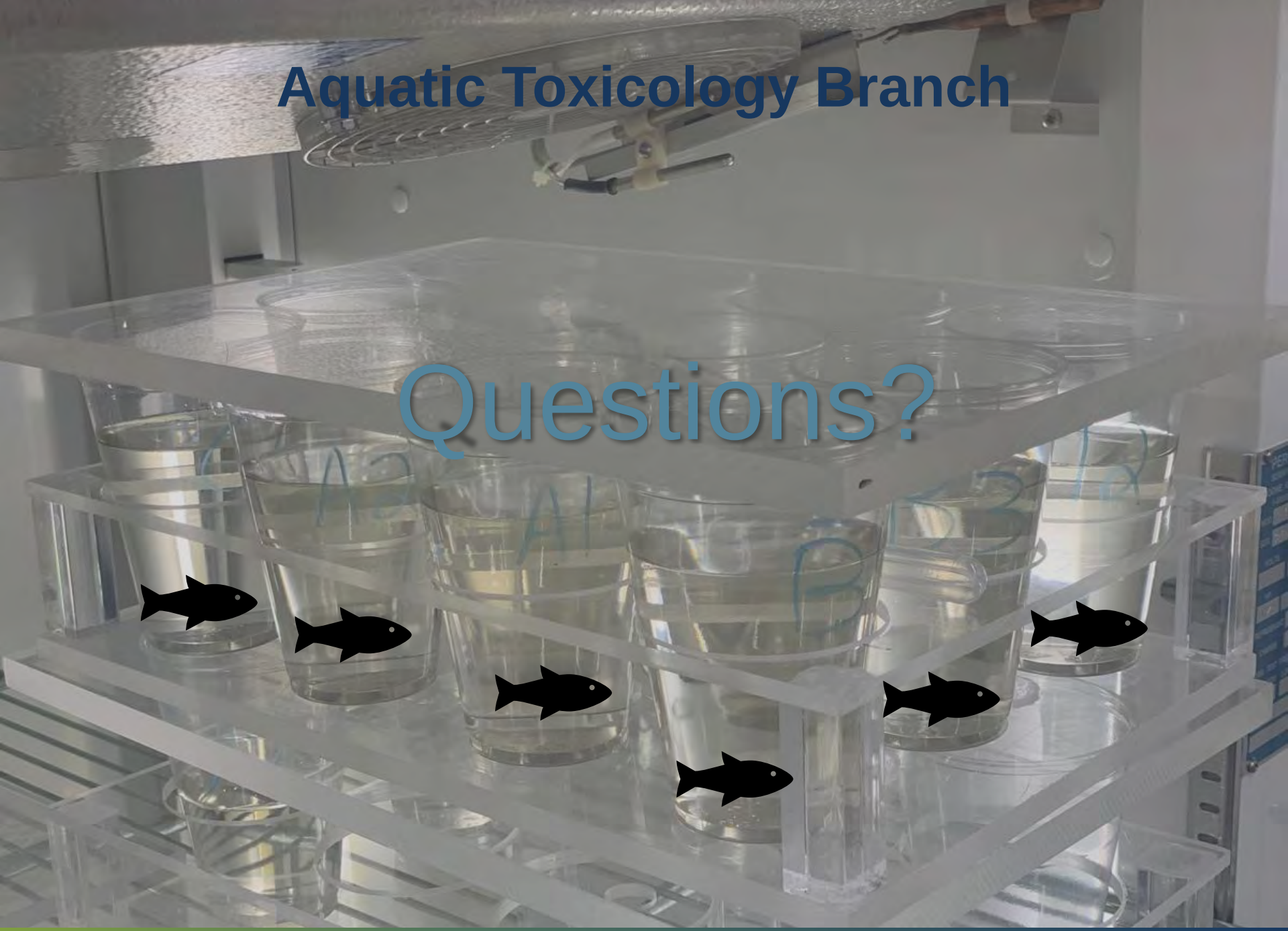
Krystyna Fender - ATB Lab Testing
919-743-8433
krystyna.fender@deq.nc.gov

Mariela Cortes – ATB Lab Culturing
919-743-8436
mariela.cortes@deq.nc.gov

Cindy Moore has retired as of February 2026

https://www.deq.nc.gov/about/divisions/water-resources/water-sciences/aquatic-toxicology-branch-atb

Aquatic Toxicology Branch



TRACKING TOXICITY FROM INDUSTRIAL WASTEWATER

Beth Thompson

BT Solutions, LLC

TOPICS TO COVER

- What happens if I fail a toxicity test (or 2)?
- What is a Refractory Toxicity Assessment?
- How can a Refractory Toxicity Assessment be used by a POTW?
- How can a Refractory Toxicity Assessment be used by industries?

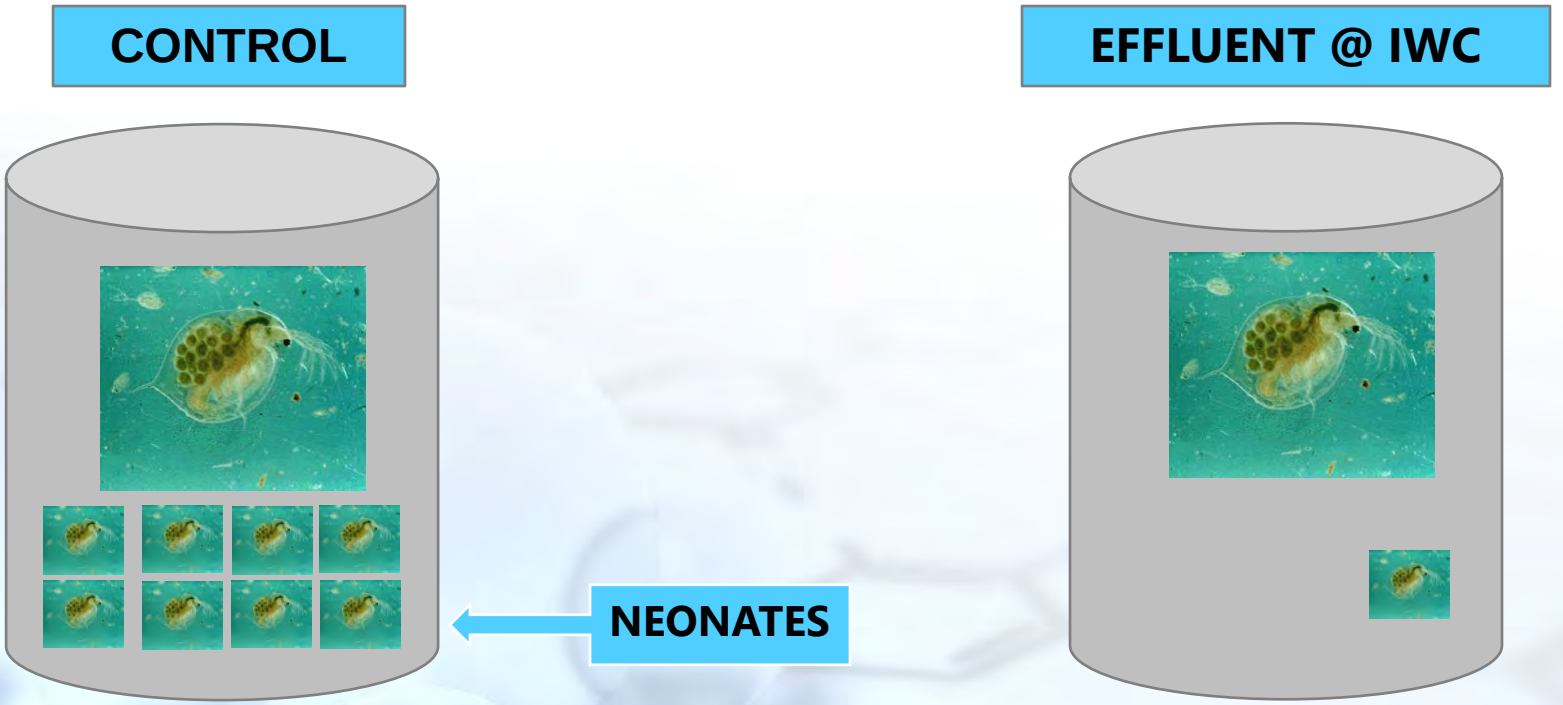
Ceriodaphnia dubia

Order: Cladocera

Common: Water Flea

Alias: Sarah Daphne

A DAY IN THE LIFE OF SARAH DAPHNE



A TRE MAY BE REQUIRED

TRE = TOXICITY REDUCTION EVALUATION

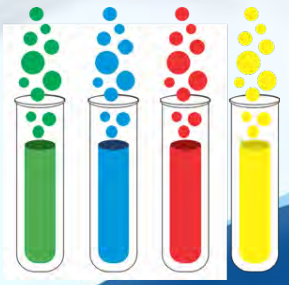
TIE = TOXICITY IDENTIFICATION EVALUATION

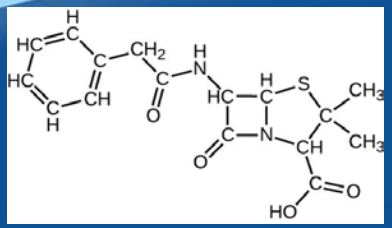
US Environmental Protection Agency (EPA). 1992. *Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I.* EPA 600-6-91-005F.

US Environmental Protection Agency (EPA) 1991. *Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures*. EPA 600-6-91-003.

WHAT IS A TOXICITY REDUCTION EVALUATION?

- **Procedures developed by the EPA to address toxicity test failures.**
- **Use a really toxic final effluent sample to conduct a *characterization study*.**
- **Characterization study allows you to narrow down the field of chemicals to identify (metal, organic, volatile, etc).**
- **Conduct targeted analytical procedures and correlate the results with toxicity.**





PROBLEMS ENCOUNTERED WITH TRADITIONAL TIE METHODS

- **Sometimes these procedures do not provide definitive results.**
- **Sometimes we identify a category of compounds but don't know how they enter the WWTP system.**
- **Sometimes we suspect an industrial user is causing pass-through toxicity but need an impartial process to determine which one.**

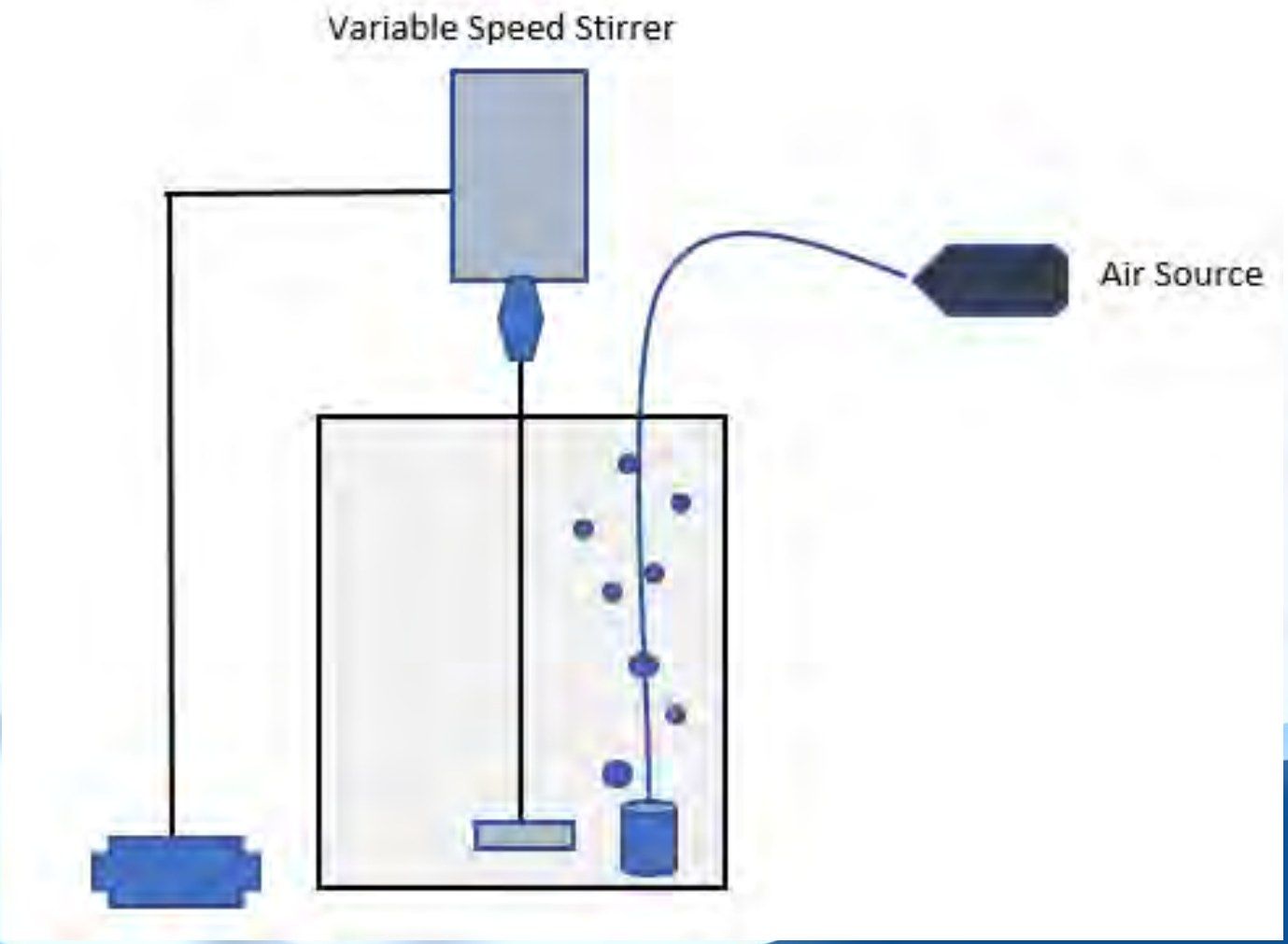
REFRACTORY TOXICITY ASSESSMENT

United States Environmental Protection Agency

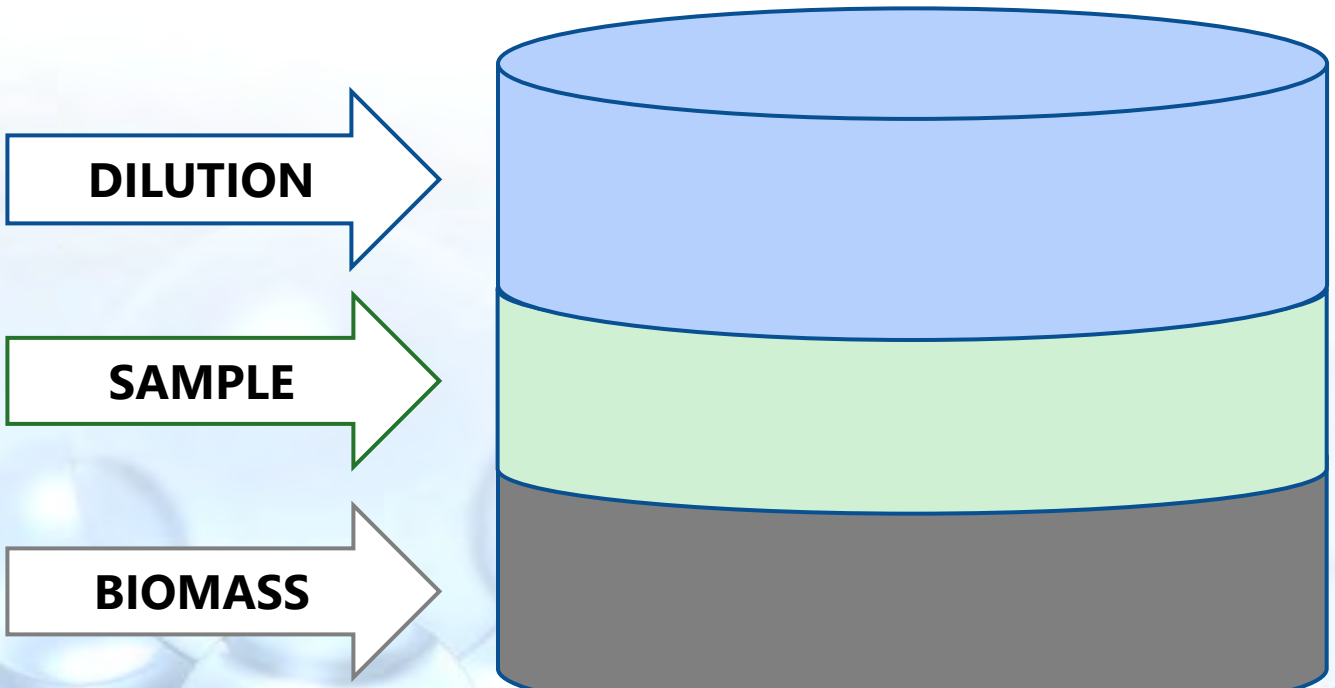
Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants

EPA-833B-99-002

WHAT IS A REFRACTORY TOXICITY ASSESSMENT?



CONTENTS OF A BIOREACTOR



TYPES OF REFRACTORY TOXICITY SIMULATIONS

Continuously fed reactors

Fill and draw

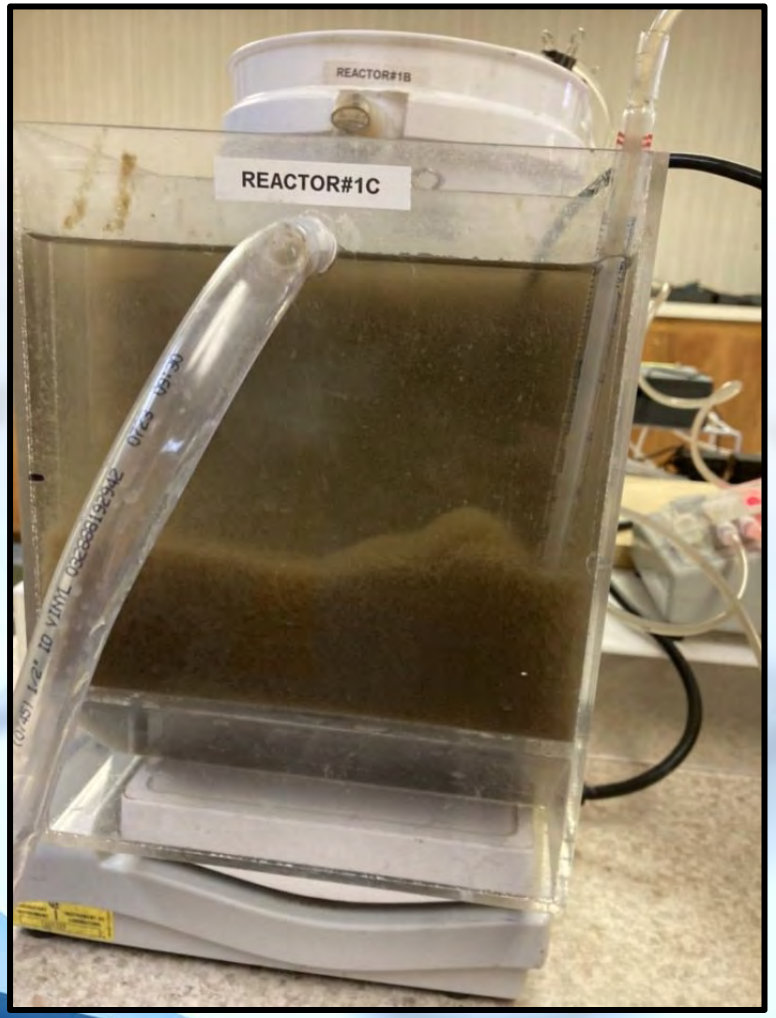
IMPORTANT FACTORS TO CONSIDER:

- Dissolved Oxygen
- pH
- F:M ratio
- MLSS concentration
- Hydraulic detention time
- Solids retention time

BIOREACTOR MONITORING

- Dissolved Oxygen
- CBOD
- Temperature
- pH
- Flow rates
- Specific Oxygen Uptake Rate
- MLSS / VSS
- Sludge Volume Index
- Micro Population

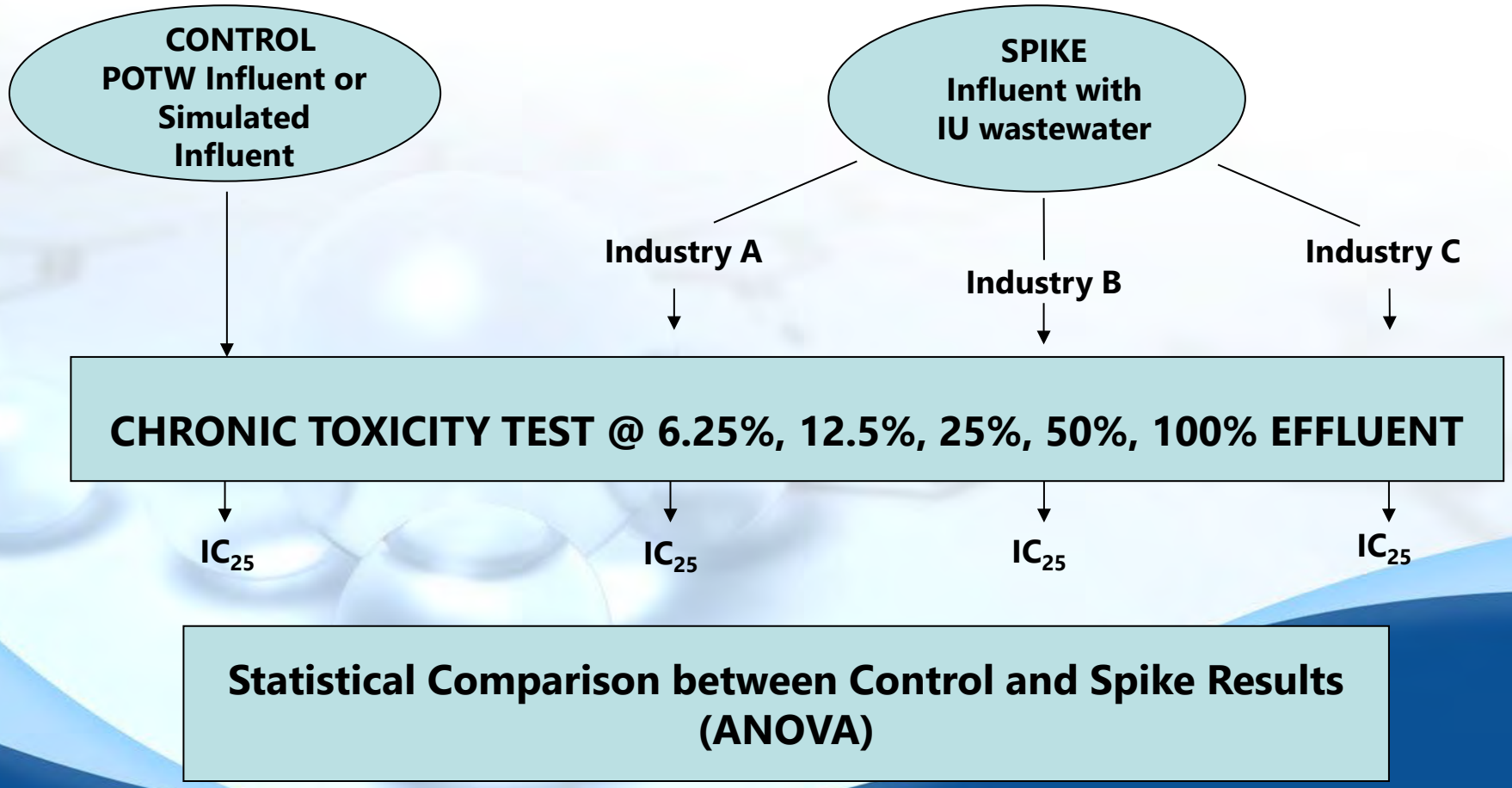
BIOREACTOR EFFLUENT TESTING



BIOREACTOR EFFLUENT ANALYSIS

- Toxicity
- BOD_5
- Ammonia
- COD
- Specific organics/inorganics
- And anything else that needs to be assessed for treatability…

TYPICAL RTA DESIGN FOR TOXICITY ASSESSMENT

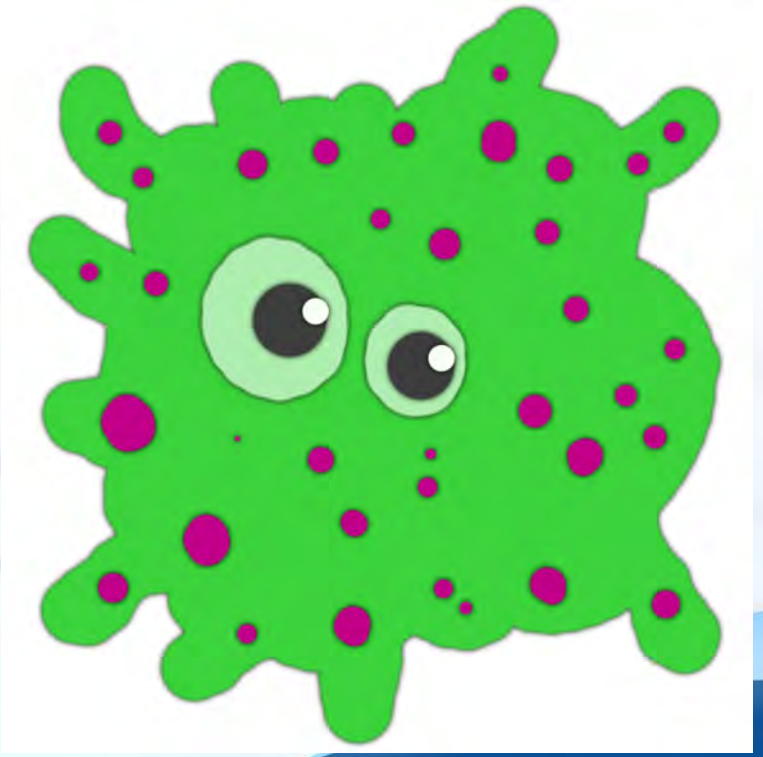


STUDY DESIGN

- What is your objective?
- What control(s) should you have?
- What are the operational parameters to simulate?
- What parameters will you test? How often?
- Does the biomass need an acclimation period?
- What are the average influent characteristics?

OTHER CONSIDERATIONS FOR BIOREACTOR DESIGN

- **Is there enough 'food' from the industrial wastewater to support the biomass?**
- **What loading rate to use?**
- **Batch or flow through?**
- **One sample or multiple?**
- **What day(s) to sample?**
- **How long to maintain bioreactors?**



WHAT CAN YOU LEARN FROM AN RTA?

- **Is there an industrial impact on the biomass, causing sub-standard treatment?**
- **Is there a toxicant that passes-through the treatment process?**
- **Is there an unexpected additive effect from multiple industries?**
- **Did anyone expect foaming?**

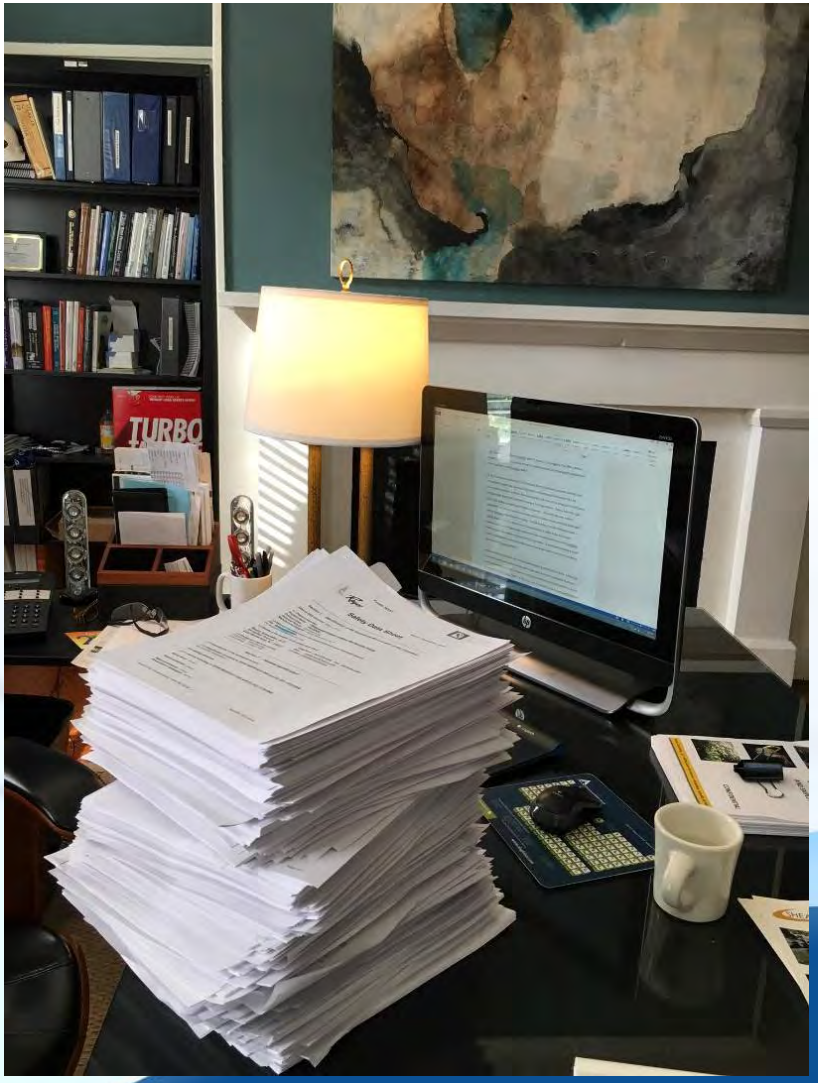
QUESTION:

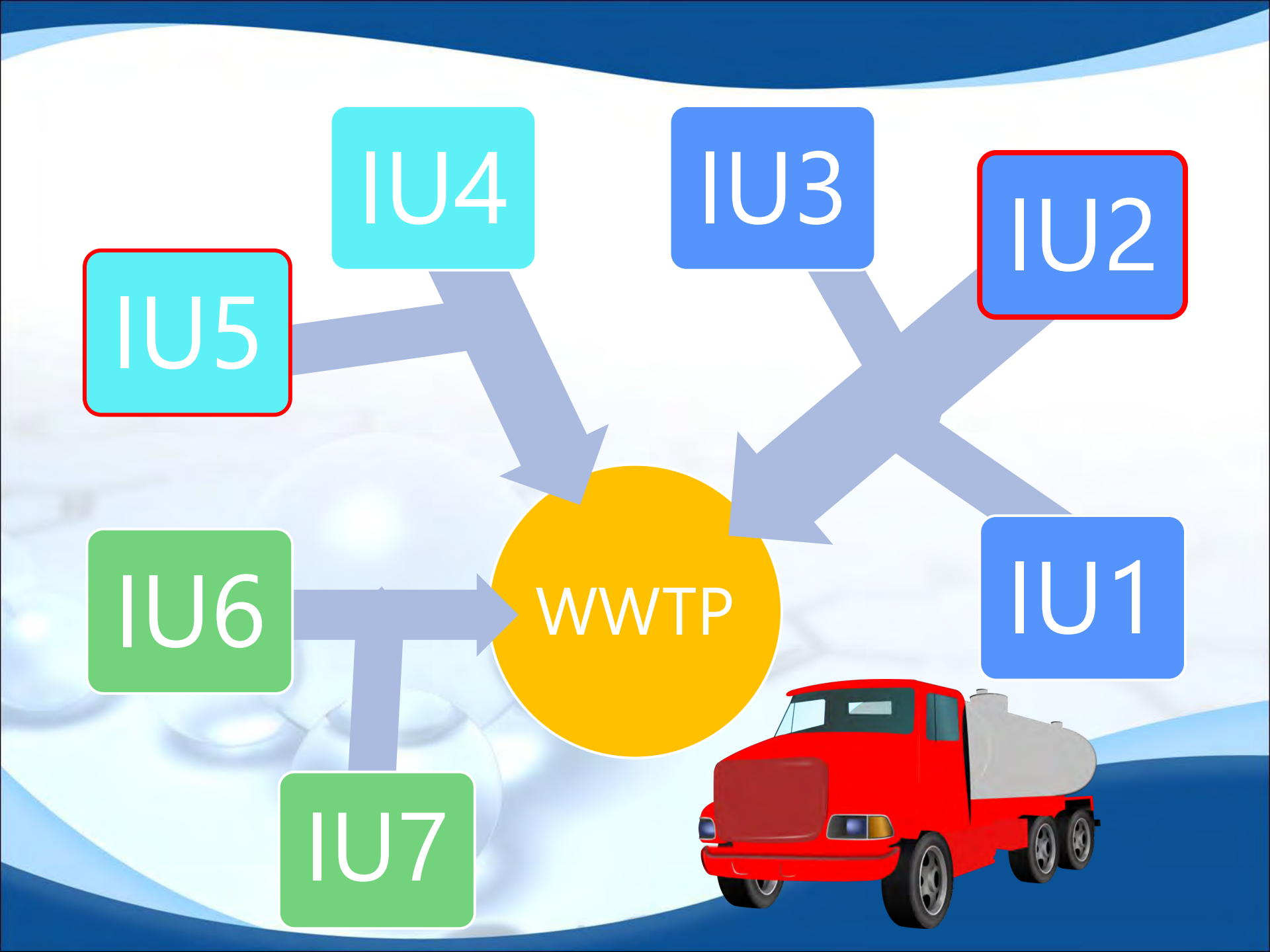
Bioreactors can be used to evaluate all of the following except:

a. Whole effluent toxicity removal
b. Treatability of specific organic compounds
c. Impact of industrial wastewater on biomass
d. Specific compound causing toxicity test failures

WHICH IUs TO SAMPLE?

- Do you really need (or want) to test all 15 industries? Prioritize!
- Which industries have potential to cause toxicity? Look at SDS.
- If you are concerned about one industry, sample two or three others as well in case it becomes a legal issue (impartial).
- Don’t make large decisions with only one data point.




IU4
IU3
IU2
IU5
IU6
WWTP
IU1
IU7

PRETREATMENT PROGRAM APPLICATIONS

- **Collect samples of IU wastewater each time POTW samples for toxicity and HOLD sample.**
- **If POTW fails toxicity, then run RTA on some or all IU samples collected.**
- **Program paid for by the IU fees.**

MAJOR BENEFIT FOR PRETREATMENT RTA PROGRAM

Industrial users become concerned about their discharges' potential to cause toxicity at the POTW.

INDUSTRIAL APPLICATIONS

- **Industrial users or direct dischargers**
- **Direct dischargers can use it to determine which process line causes pass-through toxicity.**
- **Can use it to assess how a process chemical substitution will impact the POTW or the industry's own wastewater facility.**
- **Sometimes the industrial treatment facility isn't a simple activated sludge system, so this can lead to creative simulations.**


Control
Baseline
20L
22qt.
20qt.
15L
16qt.
12qt.
10L
8qt.
5L
4qt.

QUESTION:

Bioreactors can simulate conventional wastewater treatment processes but not industrial treatment facilities:

a. True
b. False

Beth Thompson

(803) 447-8471

beth@btsolutionsSC.com

Strider

Director of Canine Services

Karlene Spencer

Pretreatment Specialist

Toxicity Testing QA/QC

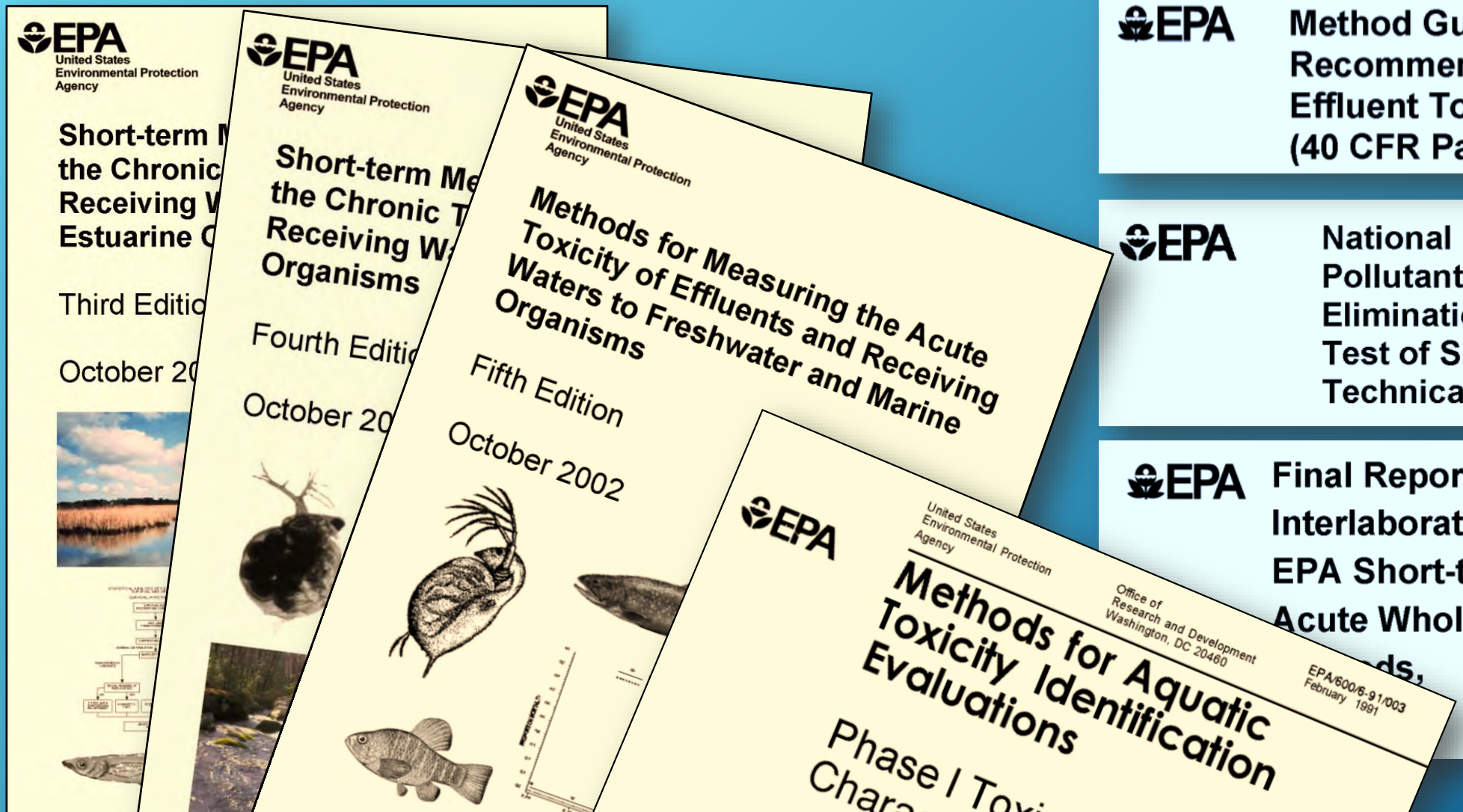
Jaydon Perez
Jim Sumner

How does quality control differ in a toxicity laboratory?

How do we address quality with organisms?

- Methods / SOPs
- General QC
- Training
- Traceability

Adherence to Approved Methods



EPA Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System

EPA Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing (40 CFR Part 136)

EPA National Pollutant Discharge Elimination System Test of Significant Toxicity Technical Document

EPA Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test

Method Update Rule

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 136

[EPA–HQ–OW–2022–0901; FRL 9346–02–OW]

RIN 2040–AG25

Clean Water Act Methods Update Rule for the Analysis of Effluent

AGENCY: Environmental Protection Agency (EPA).

while improving data quality. In addition, this update to the CWA methods will incorporate technological advances in analytical technology and make a series of minor changes and corrections to existing approved methods. As such, the EPA expects that these changes will not result in any negative economic impacts.

DATES: This final rule is
June 17, 2024. The inco
reference of certain mat
this rule and is approve

FOR FURTHER INFORMATION CONTACT: Tracy Bone, Engineering and Analysis Division, Office of Water (4303T), Environmental Protection Agency, 1200 Pennsylvania Avenue NW, Washington, DC 20460–0001; telephone number: 202–564–5257; email address: *bone.tracy@epa.gov*.

	Aquatic Toxicity	
8. Toxicity, acute, fresh water organisms, LC_{50}, percent effluent.	Water flea, *Cladoceran, Ceriodaphnia dubia* acute.	2002.0.[25]
	Water flea, *Cladocerans, Daphnia pulex* and *Daphnia magna* acute.	2021.0.[25]
	Fish, Fathead minnow, *Pimephales promelas,* and Bannerfin shiner, *Cyprinella leedsi,* acute.	2000.0.[25]
	Fish, Rainbow trout, *Oncorhynchus mykiss,* and brook trout,	2019.0.[25]

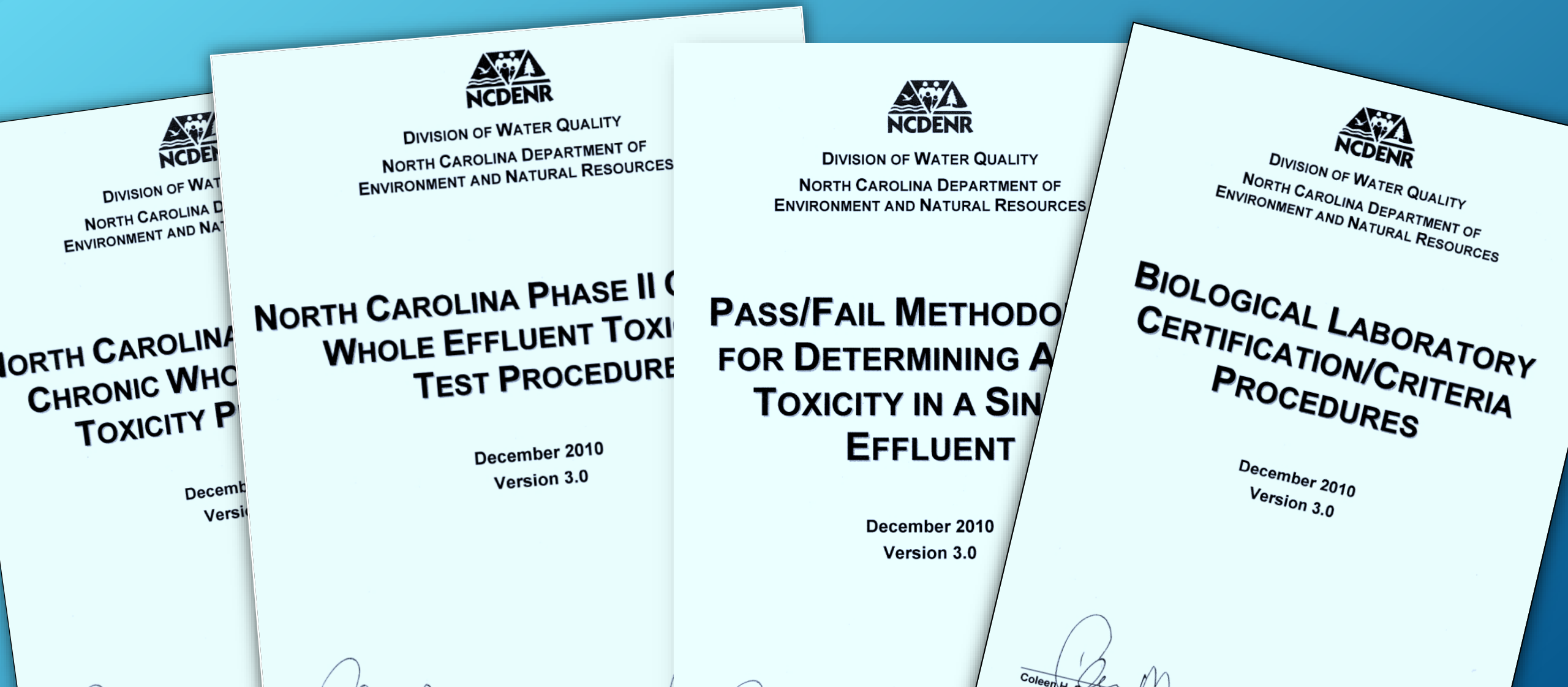
EPA United States Environmental Protection Agency

Office of Water | EPA 821-R-02-012-ES

www.epa.gov | December 2016

Whole Effluent Toxicity Methods Errata Sheet

NC Methods Provide Additional Guidance and Protocols that may Differ from EPA



Most significant changes come from client audits

ENVIRONMENTAL® STANDARDS	ON-SITE LABORATORY AUDIT
Audit Sponsor	Tennessee Valley Authority (TVA)
Laboratory Name	Environmental Testing Solutions, Inc. (ETS)
Laboratory Street Address	351 Depot Street
Laboratory City State Zip	Asheville, NC 28801
Audit Start Date	6/26/25
Audit End Date	6/26/25
Audit Team Member	Gary P. Yakub
Scope of Audit	Quality Management System
	Data Reduction and Reporting
	Project Management
	Sample Management
	Sample Preparation
	Sample Generation
	Short-Term Methods for Estimating The Chronic Toxicity of Effluents and Receiving Waters To Freshwater Organisms by US EPA-821-R-013 1000.0/1002.0
Standard	North Carolina Administrative Code (NCAC) Title 15A Section .0800
Standard	Analytical Methods
Standard	Laboratory Quality Documents: QAM and SOPs
Date Pre-Audit Requested	5/23/25
Date Pre-Audit Received	6/23/25
Date Draft Report Issued	7/8/25
Date Laboratory Requested to Reply	7/15/25
Date Laboratory Reply Received	7/11/25
Date Report Finalized	7/14/25
Date Response Requested	8/4/25
Date Response Received	
Date Response Adequacy Assessed	

SUMMARY OF FINDINGS

Number of Critical Findings	0
Number of Moderate Findings	1
Number of Minor Findings	0

This audit report has been prepared by Environmental Standards, Inc. for Tennessee Valley Authority (TVA) and Environmental Testing Solutions, Inc. (ETS) and should be considered valid for a period of 2

TENNESSEE VALLEY AUTHORITY

01 Market Street, Chattanooga, Tennessee 3740

March 13, 2023
Jim Sumner
Environmental Testing Solutions, Inc.
PO Box 7565
Asheville, NC 28802-7565

Dear Mr. Sumner,

On March 3, 2023 TVA conducted i
Solutions (ETS) located at 351 Dep
attendance as well as yourself. Pr
documents to submit to us. Thos
TVA 2022 WET compliance year
PAF 002A chronic tests you con
records by February 19, 2023.

Our findings of this audit are
deficiencies relative to testin
that you prepare a schemat
system, as well as a master
minnow and daphnid acut
documents to us by May

TVA appreciates the con
compliance testing. If y

Sincerely,

Rick M. Sherrard, Ph.
Senior Toxicologist:
Regulatory Environm
Tennessee Valley A
1101 Market St, P
Chattanooga, TN
rmsherrard@tva.

Eastman Chemical Company
Quality Audit Report

Company Audited: Environmental Testing Solutions, Inc. (Asheville, NC)

Audit Dates: March 28, 2024

Lead Auditor: Patricia Crawford, Kara Lowe

Audit Team Members: Gretchen Cookenour, Jim Maitland, Drew Nagel

Audit Participants: Jim Summer, Kelley Keenan, Jaydon Perez

Audit Type: Contract Laboratory

Summary of Findings:

This assessment covers ETS in Asheville, NC. The focus of this audit was on the ETS Quality System and the data integrity of the aquatic toxicity testing (ceriodaphnia dubia and fathead minnow). ETS sent copies of their policies, procedures, and methods prior to the audit with no errors found. A tour of the facility and testing process was observed by the audit team.

Strengths

- Personnel were courteous, professional, and very knowledgeable of aquatic toxicity testing.
- Impressive Quality System that was well maintained and organized.
- The sample receiving process seems stream-lined and efficient to ensure accurate log in and transfer of samples to the appropriate laboratory section.
- The sample receipt logs, calibration/verification records, temp logs, thermometer calibrations etc. were up to date with the appropriate information and the logs were readily accessible.
- Building has a backup generator in case of power outages as well as each incubator has its own backup battery.
- Proactively recycles consumables when possible.
- Extensive training process for employees.
- Very knowledgeable about pathogen in SFHR that can invalidate tests.

Status of Prior Audit Nonconformances: None found in during 2020 audit.

NOTE: ETS acquired the aquatic toxicity testing (ceriodaphnia dubia and fathead minnow) in April 2017.

Improvement Opportunities: Eastman personnel will send current and new NPDES permits to ETS to review to insure they are following the correct procedures stated in the permit. This came from the issue of the new 2023 permit having different sample flow-blending percentages than the previous permit. Eastman personnel changed the flow-blending percentages, but this was not communicated to ETS. ETS had to reissue all reports since the permit change (August 2023, November 2023, and February 2024). ETS also asked to receive additional sample volume due to the new test solution percentages listed in the new permit.

Non-Conformities: None were found.

Corrective Action and Preventive Action (CAPA) Plan: N/A

Report Issued: 6/6/24

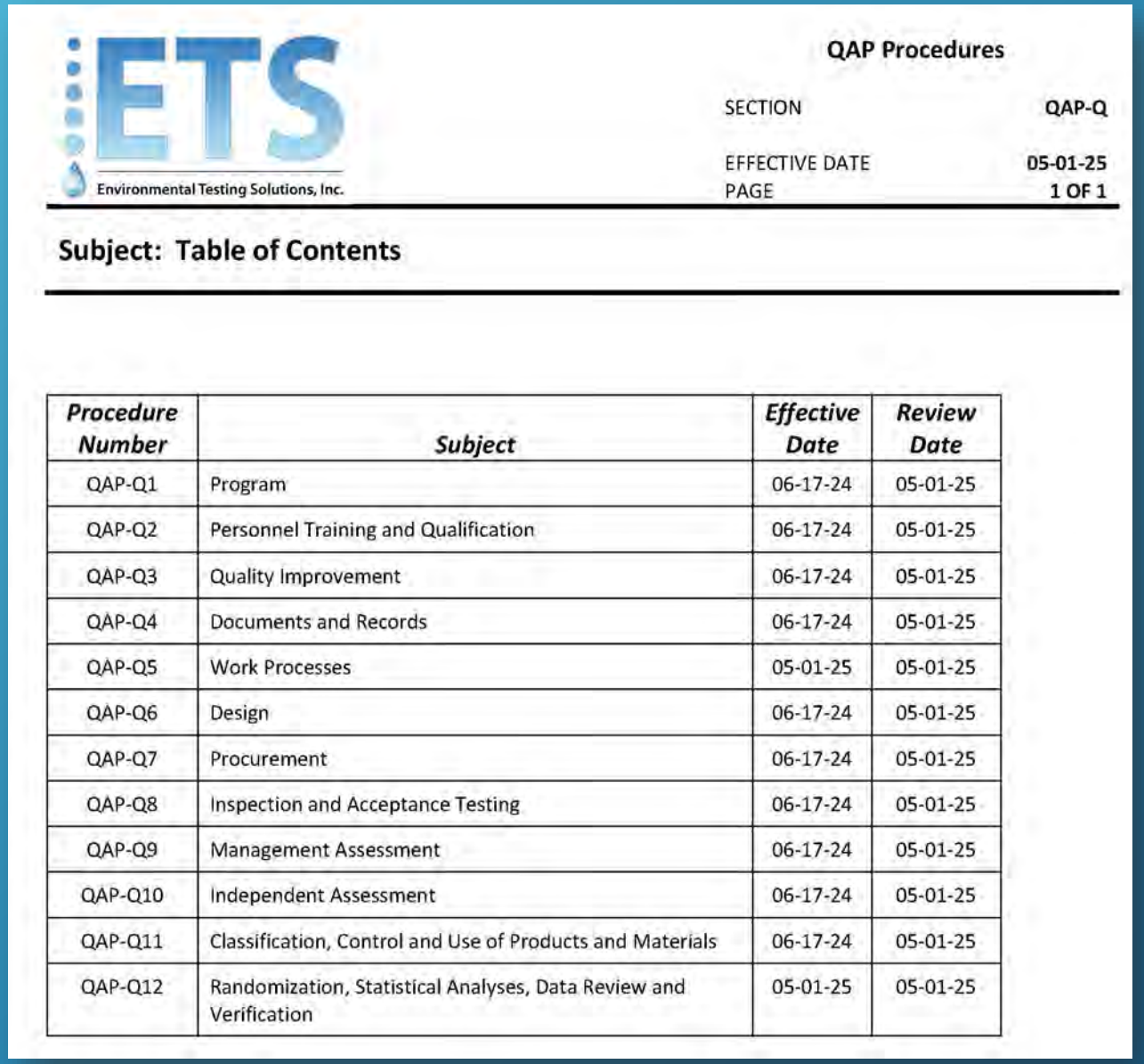
Procedures/QAP

Policies

QAP

SOP's

- Task Instructions
- General Procedures
- Bacteria Procedures
- Chemistry Procedures
- Aquatic Toxicity Procedures



ETS
Environmental Testing Solutions, Inc.

QAP Procedures

SECTION QAP-Q

EFFECTIVE DATE 05-01-25

PAGE 1 OF 1

Subject: Table of Contents

Procedure Number	*Subject*	*Effective Date*	*Review Date*
QAP-Q1	Program	06-17-24	05-01-25
QAP-Q2	Personnel Training and Qualification	06-17-24	05-01-25
QAP-Q3	Quality Improvement	06-17-24	05-01-25
QAP-Q4	Documents and Records	06-17-24	05-01-25
QAP-Q5	Work Processes	05-01-25	05-01-25
QAP-Q6	Design	06-17-24	05-01-25
QAP-Q7	Procurement	06-17-24	05-01-25
QAP-Q8	Inspection and Acceptance Testing	06-17-24	05-01-25
QAP-Q9	Management Assessment	06-17-24	05-01-25
QAP-Q10	Independent Assessment	06-17-24	05-01-25
QAP-Q11	Classification, Control and Use of Products and Materials	06-17-24	05-01-25
QAP-Q12	Randomization, Statistical Analyses, Data Review and Verification	05-01-25	05-01-25

Consistent Format

Certified Method

Signed off by both Lab Supervisor and QA Officer

Revisions and what caused revisions to take place

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Environmental Testing Solutions, Inc.

Chemistry Procedures

SECTION	SOP-C2
REVISION NUMBER	9
EFFECTIVE DATE	05-01-25
PAGE	1 OF 7

Subject: Dissolved Oxygen (SM 4500-O H-2021)

Approval

Title	Name	Signature	Date
Laboratory Supervisor	Kelley E. Keenan		05-01-25
Quality Assurance Officer	Jim Sumner		05-01-25

Document Revision History

Effective Date	Revision number	Review Type	Evaluators	Revisions
12-01-00	0	Internal	Jim Sumner (ETS)	Original document
09-01-09	1	Internal	Jim Sumner (ETS)	• Updated exhibits during document review.
06-01-11	2	Internal	Jim Sumner (ETS)	• Updated exhibits during document review.
01-01-13	3	Internal	Jim Sumner (ETS)	• Updated procedure and references to the approved analytical method identified in USEPA Method Update Rule II (MUR II), May 18, 2012.
10-01-17	4	Internal	Jim Sumner (ETS)	• Updated procedure to include NELAP requirements. • Verification of thermometers using NIST thermometers changed to annually. • Additional guidance included in SOP. • Method number revised based on 2017 MUR.
01-04-19	5	External (SC HDEC) Internal	Haley Anderson (SC DHEC) Jim Sumner (ETS)	• Updated procedure to clarify: Follow the posted chart to find the correct reading based on the temperature, altitude, and salinity table (Exhibit C2.2). If the dissolved oxygen reading is not within ± 0.2 mg/L if the theoretical value in the table, then the meter must be calibrated.
02-17-20	6	External (TVA) Internal	Rick Sherrard (TVA) Jim Sumner (ETS)	• Updated procedure and benchsheet to include the serial number of the meter used to perform dissolved oxygen.
07-01-21	7	Internal	Jim Sumner (ETS)	• Updated procedure and references to the approved analytical method identified in USEPA Method Update Rule, May 19, 2021.
05-01-24	8	Internal	Jim Sumner (ETS)	• Updated procedure and references to the approved analytical method identified in USEPA Method Update Rule, April 16, 2024.
05-01-25	9	Internal	Jim Sumner (ETS	• Changed procedure to LDO method for measuring dissolved oxygen.

Consistent Format

What is the method used for

Brief Description

QA Requirements

Interferences?

Subject: Dissolved Oxygen (SM 4500-O H-2021)

Scope and Application

This method is used to measure the dissolved oxygen of water samples used in toxicity tests, wastewater, receiving water, and drinking water.

Summary of Method

The dissolved oxygen is measured by the optical-probe method. The optical probe uses luminescence-based oxygen sensors to measure the light-emission characteristics of a luminescent reaction: oxygen quantitatively quenches the luminescence. The change in luminescence signal's lifetime correlates to the DO concentration. Measurements are recorded to the nearest 0.1 mg/L.

Dissolved oxygen measurement procedures are based on Standard Methods 4500-O H-2021.

Quality Control

Calibration: The dissolved oxygen meter must be calibrated each day **before use**. The meter internal calibration adjusts for temperature, altitude and salinity for determining dissolved oxygen solubility.

The temperature reading of the dissolved oxygen meter must be verified at least **annually** (once every calendar year) with a traceable NIST thermometer (SOP-G12).

Additional quality control guidance is provided in QAP-Q5.

Interferences

Chlorine dioxide interferes with this reaction. Biofouling due to bacteria or algal growth can prevent oxygen permeation through the window. Bacteria and algae may also generate or consume oxygen, resulting in erroneous readings; this can be minimized by rinsing the probe between readings to keep the sensor clean. Oils can close the membrane and sensor cap, prohibiting oxygen from diffusing to the sensor; frequent rinsing between measurements can minimize this problem.

Equipment, Procedure, Safety, References, and Exhibits

Equipment and Materials

HACH HQ430d Flexi meter equipped with a LDO probe
BOD bottle
Rinse bottle
Deionized water
Waste container
Solubility of Dissolved Oxygen Table (Compensation for Temperature, Altitude, and Salinity)
Daily Meter Calibration and Standardization Benchsheet

Procedure (Meter: HACH HQ430d Flexi, SN250100050300 equipped with a LDO101 probe)

A. Air Calibration.

1. Each day before analysis, calibrate the meter. The calibration is recorded on the Daily Meter Calibration and Standardization Benchsheet (Exhibit C2.1).

2. Turn the meter **ON**. Wait for the meter to connect to the LDO101 probe.

3. Remove the probe from the BOD bottle partially filled with deionized water (so that the probe is not submerged in the water), rinse the probe with deionized water, shake off any excess water, and gently dry the cap with a clean towel. Place the probe back in the BOD bottle.

4. Press the **CALIBRATE** button and then press **READ**. Wait for the meter to stabilize. The meter will calibrate to 100% saturation, adjusted for temperature, altitude and salinity (0 ppt salinity). Press **DONE** to review the calibration, then press **STORE**. Follow the posted chart to find the correct reading based on the temperature, altitude, and salinity table (Exhibit C2.2). The dissolved oxygen reading displayed must be within ± 0.5 mg/L if the theoretical value in the table. If the reading is not within the acceptance limits, then the meter must be calibrated.

5. Record the calibration readings in the Daily Meter Calibration and Standardization Benchsheet.

Corrective Action: If the meter does not calibrate properly, check the probe cap. Gently clean the cap with deionized water and dry with a clean towel. Refer to the instrument manual for instructions on the care and maintenance of the LDO probe. Maintenance activities are recorded following SOP-G9: Instrument Maintenance and Repair.

B. Measurement of Sample DO.

1. Once the meter has been calibrated, submerge the probe in the sample and stir gently. Allow the reading to stabilize and record the value on the appropriate logsheet. Rinse the probe with deionized water prior to measuring the DO of the next sample. Continue reading and recording values for all other samples.

2. Read directly in mg/L and report to the nearest 0.1 mg/L.

Corrective Action: Corrective actions for toxicity samples, which exceed dissolved oxygen tolerance limits for organisms in toxicity tests, are addressed in test specific SOPs.

Safety and Hazardous Waste Management

Safety glasses, gloves and lab coats should always be worn while handling samples. Excess samples may be flushed down the sink.

Review Policy-P6: General Safety Policy and Policy-P9: Radiation Protection Policy for additional safety requirements.

References

Standard Methods for the Examination of Water and Wastewater, 24th Edition, 2023. American Public Health Association, 800 I Street, NW, Washington DC 20001-3710.

- Method: 4500-O H-2021.

TNI Standard. Management and Technical Requ
Analysis. The NELAC Institute, PO Box 2439, We

Instrument Manual

USEPA. October 2002. Methods for Measuring
Freshwater and Marine Organisms, 5th ed. EPA-
Cincinnati, OH.

USEPA. October 2002. Short-Term Methods for
Receiving Waters to Freshwater Organisms, 4th ed. EPA-821-R-02-013. US Environmental Protection Agency, Cincinnati, OH.

USEPA. October 2002. Short-Term Methods for Estimating the Chro
Receiving Waters to Marine and Estuarine Organisms, 3rd ed. EPA-8
Protection Agency, Cincinnati, OH.

Exhibits

Exhibit C2.1: Daily Meter Calibration and Standardization Benchshe
Exhibit C2.2: Solubility of Dissolved Oxygen Table
(Compensation for Temperature, Altitude, and Salinity

Bench Sheets and Charts as Exhibits

Traceable to SOP Revision

ETS

Page _____

Daily Meter Calibration and Standardization

Analyst(s) _____ Calibration date _____

Reagent Incubator #1 (Thermometer SN 5030) temperature (°C): _____ (Standards and samples must be warmed to 25.0 ± 1.0°C before taking measurements

Conductivity (SM 2510 B-2021, Meter: Accumet Model AR20, SN 93312452) RL = 14.9 µmhos/cm

Calibration:

Reference standard	True value (µmhos/cm)	Internal Cell Constant
INSS	1000	

Standardization:

Reference standard	True value (TV) (µmhos/cm)	Conductivity corrected to µmhos/cm (C)	% RS = C / TV x 100
INSS	14.9		
INSS	146.9		
INSS	717.5		
INSS	1412		
INSS	2000		
INSS	6667		

Salinity (SM 2520 B-2021, Meter: YSI PRO30, SN 18D104324) RL = 1.0 ppt

Calibration:

Reference standard	Initial Salinity (ppt)	Correction (ppt)	Final Salinity (True value = 25.0) (ppt)
INSS			25.0

Laboratory control standards:

Reference standard	True value (TV) (ppt)	Salinity ppt (C)	% RS = C / TV x 100
INSS	0.71		
INSS	35.0		

Duplicate sample precision:

Sample ID	Conductivity / Salinity corrected to µmhos/cm or ppt	%RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%)
	S	
Duplicate	D	

Note: The duplicate sample precision should be performed on an effluent or control sample used for a toxicity test.

Dissolved Oxygen (SM 4500-O H-2021, Meter: HACH HQ430d Flexi, SN 250100050330)

Air calibration (based on laboratory DO Saturation table):

Ambient temperature (°C)	DO Saturation (mg/L)	Meter calibration reading (mg/L)	Difference (mg/L) Acceptance Limits = ± 0.5 mg/L

pH (SM 4500-H⁺ B-2021, Meter: Accumet Model AR20, SN 93312452)

Calibration:

	pH 4.00	pH 7.00	Slope (%)
Reference standard number	INR	INR	

Laboratory control standard:

Reference standard	True value (S.U.)	Measured value (S.U.)	Control Limits
INR	10.00		9.90 – 10.10

Duplicate sample precision:

Sample ID	pH S.U.	Acceptable range = ± 0.20 S.U.
	S	
Duplicate	D	

Note: The duplicate sample precision should be performed on an effluent or control sample used for a toxicity test.

Solubility of Dissolved Oxygen (mg/L) Correction for Temperature, Altitude and Salinity

(SM 4500 O H-2021, Meter: HACH HQ430d Flexi, SN250100050330)

Temperature (°C)	Dissolved Oxygen (mg/L)	
	Freshwater	Saltwater (25 ppt)
17.0	8.99	7.63
17.5	8.90	7.56
18.0	8.81	7.49
18.5	8.72	7.41
19.0	8.63	7.34
19.5	8.54	7.27
20.0	8.45	7.21
20.5	8.37	7.14
21.0	8.30	7.07
21.5	8.21	7.01
22.0	8.13	6.95
22.5	8.05	6.89
23.0	7.98	6.82
23.5	7.91	6.76
24.0	7.83	6.70
24.5	7.76	6.65
25.0	7.68	6.60
25.5	7.61	6.54
26.0	7.54	6.47
26.5	7.48	6.42
27.0	7.41	6.37
27.5	7.35	6.31
28.0	7.28	6.26
28.5	7.22	6.21
29.0	7.15	6.16
29.5	7.09	6.11
30.0	7.03	6.06

Note: Solubility corrected for elevation in Asheville, NC (1984 feet = 707 mm Hg Atmospheric Pressure (946 hPa), 0.93 correction).

Bench Sheets

Certified Method
Method Number
Reporting Limit
Meter Used and Serial Number

Bench Sheets Bound in Logbooks

Logbook Tracking System in Place

Conductivity (SM 2510 B-2021, Meter: Accumet Model AR20, SN 93312452)

RL = 14.9 µmhos/cm

Analyst	
Date analyzed	
Time analyzed	

Reviewed by	
Date reviewed	

Sample temperature (°C): _____ (Samples must be warmed to 25.0 ± 1.0°C before taking conductivity measurements.)

Calibration:

Reference standard	True value (µmhos/cm)	Internal Cell Constant
INSS	1000	

Standardization:

Reference standard	True value (TV) (µmhos/cm)	Conductivity corrected to µmhos/cm (C)	% RS = C / TV x 100
INSS	14.9		
INSS	146.9		
INSS	717.5		
INSS	1412		
INSS	2000		
INSS	6667		

Duplicate sample precision:

Sample number	Sample ID	Conductivity corrected to µmhos/cm	%RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%)
		S	
	Duplicate	D	

Sample measurements:

Sample number	Sample ID	Conductivity corrected to µmhos/cm	Reported conductivity (µmhos/cm)
TV = ND	Blank – Deionized water		

Standardization:

Reference standard	True value (TV) (µmhos/cm)	Conductivity corrected to µmhos/cm (C)	% RS = C / TV x 100
INSS			

SOP C4-Revision 9 Exhibit C4.2

Standard Operating Procedures

EPA documents provide guidance

- **Culturing organisms**

Multiple sources can be used

- **Dilution water**
- **Algae media**

Inconsistencies in manuals addressed

- **Metal criteria for food**

SOPs developed over time with refining our methods to provide better quality

Freshwater Toxicity Testing Procedures

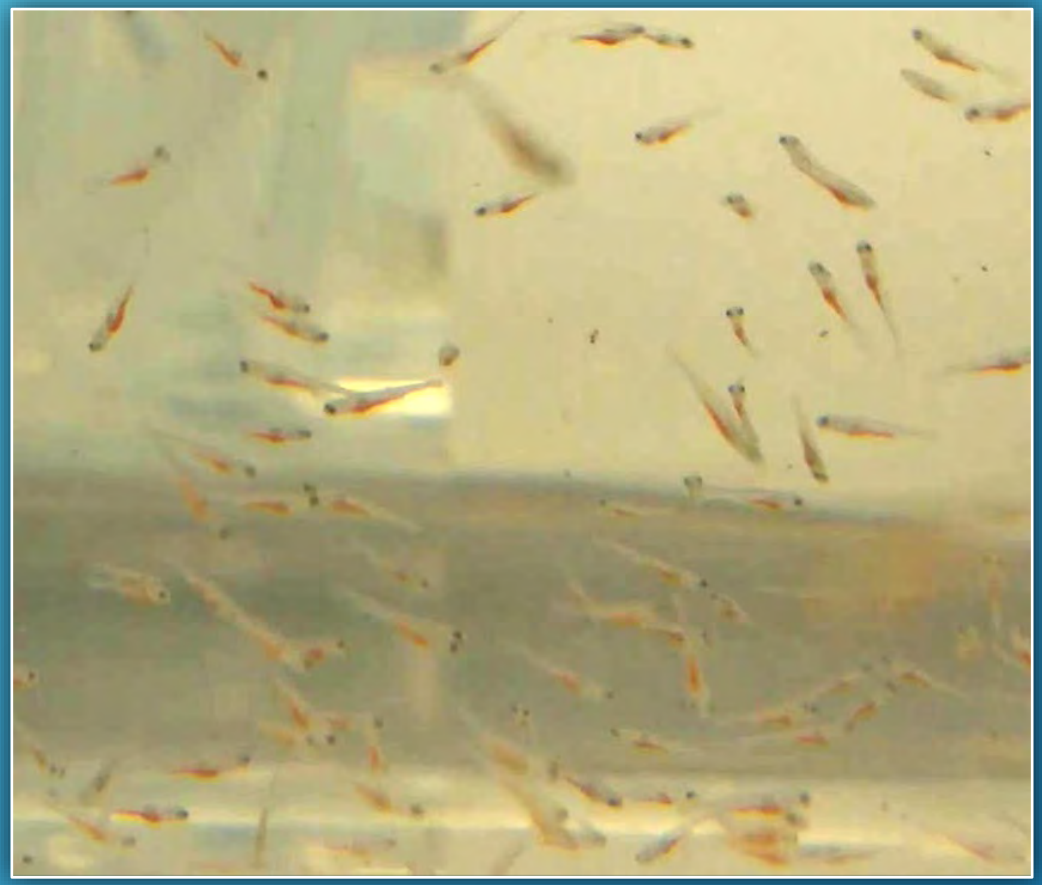
Procedure Number	*Subject*
SOP-AT1	Preparation of Synthetic Water
SOP-AT2	Subject: Maintenance of *Raphidocelis subcapitata* (formerly *Selenastrum capricornutum*) Cultures
SOP-AT3	*Selenastrum capricornutum* Chronic Toxicity Test
SOP-AT4	*Selenastrum capricornutum* Chronic Reference Toxicity Test
SOP-AT5	Taxonomic Identification of *Selenastrum capricornutum*
SOP-AT6	Preparation of YWT (Yeast, Wheat Grass, Trout Chow) Mixture
SOP-AT7	Maintenance of Daphnid Cultures
SOP-AT8	*Ceriodaphnia dubia* Neonate Collection
SOP-AT9	Daphnid Acute Toxicity Test, EPA 2002.0, EPA 2021.0
SOP-AT10	Daphnid Acute Reference Toxicity Test, EPA 2002.0, EPA 2021.0
SOP-AT11	*Ceriodaphnia dubia* Chronic Toxicity Test, EPA 1002.0
SOP-AT12	North Carolina *Ceriodaphnia dubia* Pass/Fail Chronic Toxicity Test, EPA 1002.0
SOP-AT13	North Carolina *Ceriodaphnia dubia* Phase II Chronic Toxicity Test, EPA 1002.0
SOP-AT14	*Ceriodaphnia dubia* Chronic Reference Toxicity Test, EPA 1002.0
SOP-AT15	Taxonomic Identification of *Ceriodaphnia dubia*
SOP-AT16	Preparation of Newly Hatched Brine Shrimp
SOP-AT17	Maintenance of Fathead minnow (*Pimephales promelas*) Cultures
SOP-AT18	*Pimephales promelas* Acute Toxicity Test, EPA 2000.0
SOP-AT19	*Pimephales promelas* Acute Reference Toxicity Test, EPA 2000.0
SOP-AT20	*Pimephales promelas* Chronic Toxicity Test, EPA 1000.0
SOP-AT21	*Pimephales promelas* Chronic Reference Toxicity Test, EPA 1000.0
SOP-AT22	Taxonomic Identification of *Pimephales promelas*

Ceriodaphnia Cultures

Timing of culture activities refined to provide optimal offspring production on test initiation dates

Minnow Culture

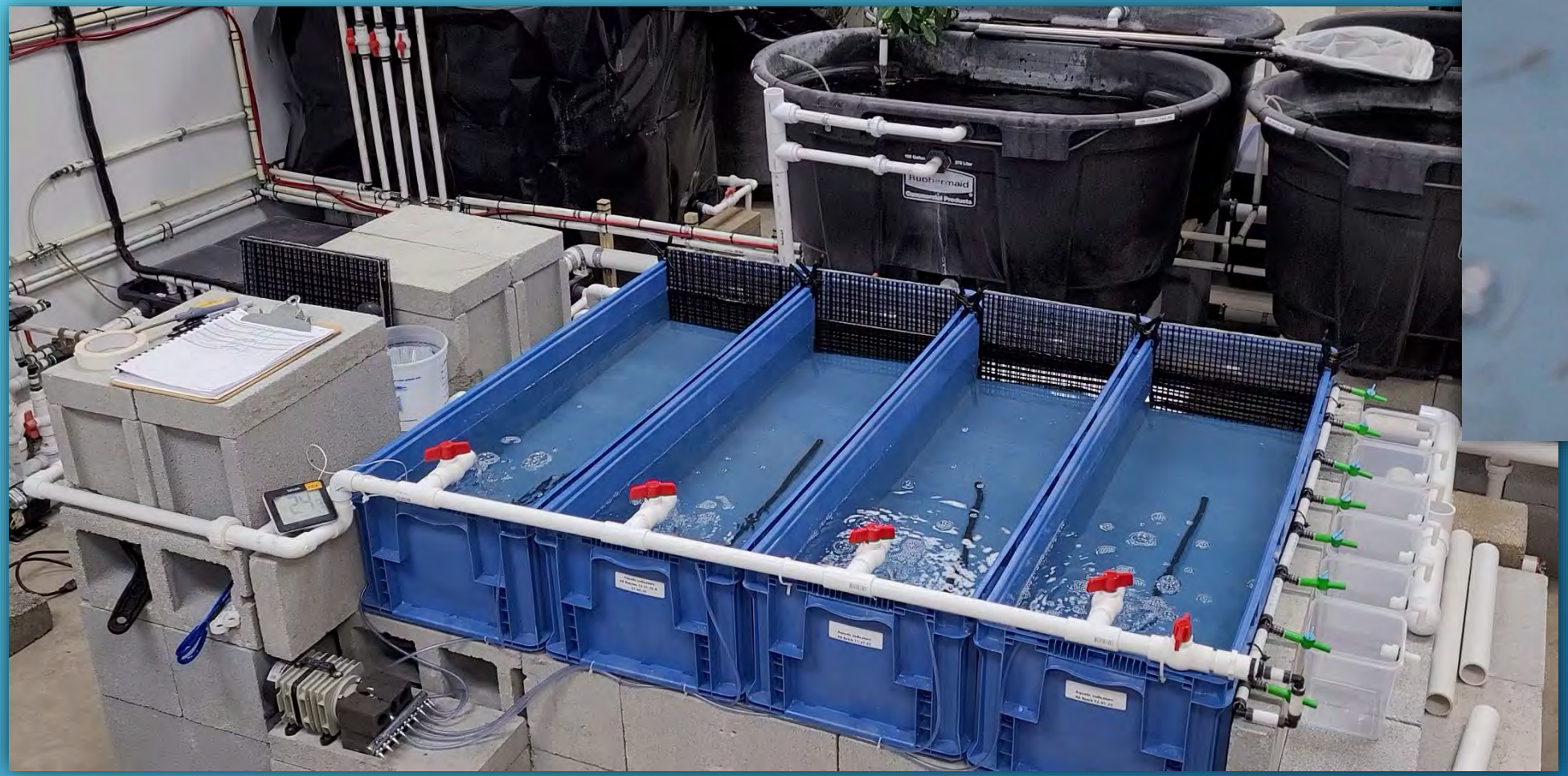
Purchased versus in-house source of minnows



Trial and error with how system operates and what provides optimal health and egg production

Mysid Culture

In development



Different Types of Dilution Water

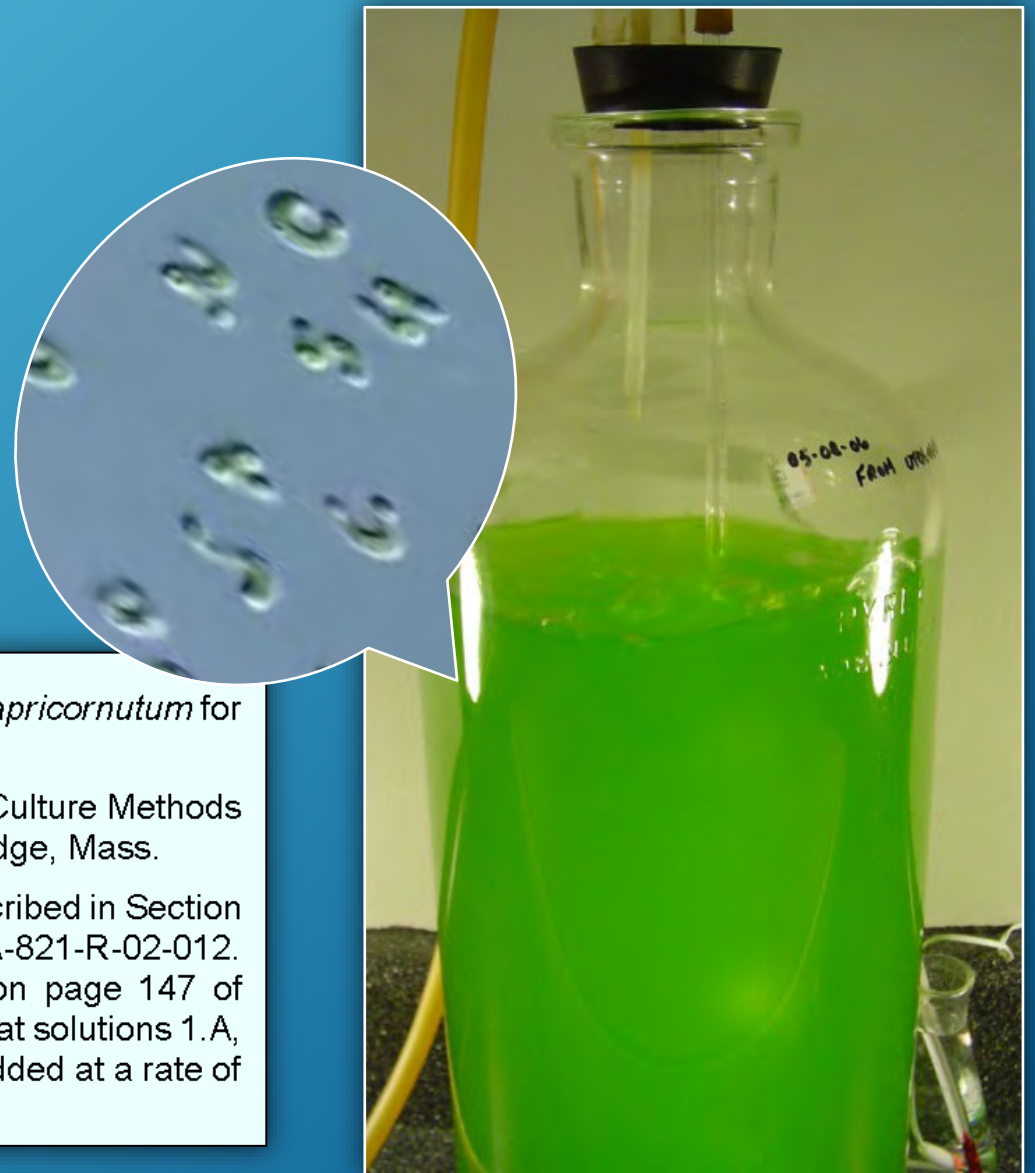
Surface, Synthetic, Mineral

Synthetic water is more consistent than the other types

Multiple Methods can be Used: Media to Grow Algae for *Ceriodaphnia* Food

Purchased algae versus in-house culture

Selected NC modification of EPA method based on algae health to provide optimal nutrition for *Ceriodaphnia*



(6) Acceptable alternative culture media utilized to culture the algae *Selenastrum capricornutum* for use as *Ceriodaphnia* food are:

(a) The MBL medium as described by Handbook of Phycological Methods: Culture Methods and Growth Measurements. 1973. J.Stein, ed. University Press, Cambridge, Mass.

(b) Additional nutrients may be used in the preparation of algae medium described in Section 13.6.15 of EPA-821-R-02-013 and Appendix A1, Section 3.10.3 of EPA-821-R-02-012. Specifically, the volume of nutrient stock solutions found in Table 1 on page 147 of EPA-821-R-02-013 or p 133 of EPA-821-R-02-012 may be adjusted so that solutions 1.A, 1.D, and 2 are added at a rate of 2 ml/L and solutions 1.B and 1.C are added at a rate of 6 ml/L.

Concentration of Metals in YWT used for *Ceriodaphnia* Food

Calculated Nutrient Composition

Amino Acid Concentration	(% of total diet)
Arginine	2.80
Lysine	3.30
Methionine	1.00
Cystine	0.40
Tryptophan	0.45
Histidine	1.00
Leucine	3.05
Isoleucine	1.95
Phenylalanine	1.65
Tyrosine	1.30
Threonine	1.65
Valine	2.10

Mineral Concentration		
Calcium	%	2.35
Phosphorous	%	1.55
Potassium	%	0.65
Sodium	%	0.55
Magnesium	%	0.10
Iron	ppm	245.00
Zinc	ppm	135.00
Manganese	ppm	35.00
Copper	ppm	10.00
Cobalt	ppm	0.05
Iodine	ppm	9.20

Vitamin Concentration		
Vitamin A	IU/g	14.50
Vitamin D_3	IU/g	0.95
Alpha-Tocopherol	IU/kg	780.00
Thiamine	ppm	83.00
Riboflavin	ppm	120.00
Niacin	ppm	540.00
Pantothenic Acid	ppm	240.00
Choline	ppm	6665.00
Pyrodoxine	ppm	72.00
Folic Acid	ppm	20.00
Biotin	ppm	1.25
Vitamin B12	Mcg/kg	100.00
Vitamin K	ppm	24.00
Ascorbic Acid.....	ppm	530.00

YWT = Yeast + Wheat Grass + Trout Chow

Concentration of individual metals exceed 1 µg/L EPA criteria (i.e. Matrix Interferences)

Identified that metals are dietary additives in the trout chow

YWT prepared at the feeding rate is analyzed (total solids = 1.7 – 1.9 g/L)

Side-by-Side reference tests of old lots to new lots of YWT

Metal concentrations of old lots to new lots of YWT are compared

Concentration of Metals in *Artemia* used for Shrimp and Fish Food

Concentration of individual metals exceed 1 µg/L EPA criteria (i.e. Matrix Interferences)

Identified that metals are mainly contained in the un-hatched cysts

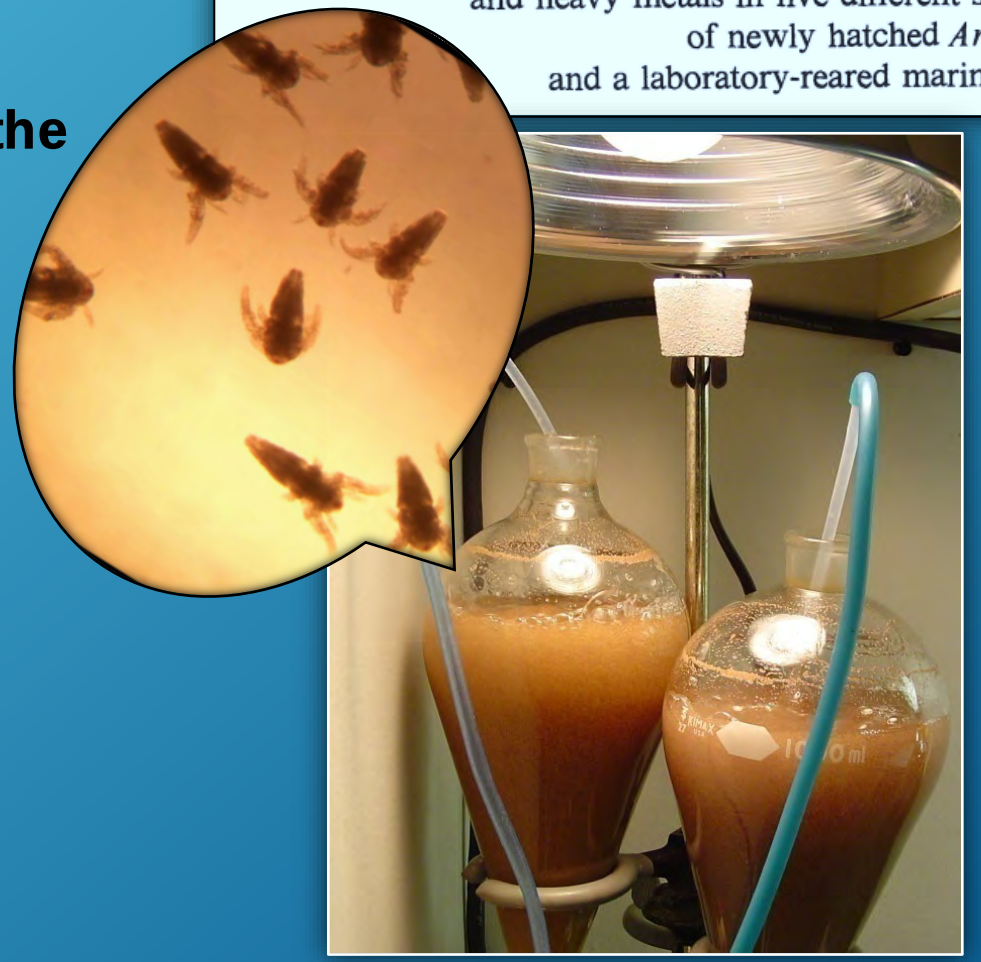
Hatched *Artemia* diluted to the feeding rate is analyzed (350 to 500 shrimp per 50 µL)

Side-by-Side reference tests of old lots to new lots of *Artemia*

Metal concentrations of old lots to new lots of Artemia are compared

The Brine Shrimp Artemia. *1980. Vol. 3. Ecology, Culturing, Use in Aquaculture*
G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 456 p.

International Study on *Artemia*[1]
VIII. Comparison of the chlorinated hydrocarbons and heavy metals in five different strains of newly hatched *Artemia* and a laboratory-reared marine fish



What types of water can be used in toxicity tests?

1. Mineral water
2. Surface water
3. Synthetic water
4. Dechlorinated tap water
5. All of the above

Method Modifications and Special Considerations

- Experimental testing required when modifications to methods requested
- Demonstrate that quality is not compromised or deviation will not alter the toxicity of the discharge
- Techniques required outside the scope of the methods

Influence of Outside Groups

EPA Method

11.10.9.2 For immediate drying and weighing, place live larvae onto a 500 µm mesh screen in a large beaker to wash away debris that might contribute to the dry weight. Each group of larvae is rinsed with deionized water to remove food particles, transferred to a tared weighing boat that has been properly labeled, and dried at 60°C, for 24 h or at 100°C for a minimum of 6 h.

ETS SOP

6. Beginning with the first replicate cup of the control.

   a. Count and record (in the appropriate section) the number of living and dead larvae in each replicate cup on the chronic benchsheet. Record comments, if applicable. Discard any dead larvae.

   b. Holding the mesh sieve over an empty beaker, pour the test water containing the larvae from one replicate cup through the sieve. The larvae will be retained on the mesh.

   c. Immediately submerge the sieve containing the larvae into the ice water. Keep the larvae in the ice water for 3 to 4 seconds.

   d. Remove the sieve from the ice water. Carefully rinse the larvae with deionized water to remove any excess food or detritus.

   e. Using forceps, remove the microweight pan from the appropriate 20-ml glass beaker or well on the spot plate. Using the forceps, transfer the larvae from the mesh to the microweight pan. In the process, to ensure the larvae are dead, sever their spinal cords with forceps. Ensure that all the larvae have been transferred to the microweight pan. Verify against the number recorded in Step 6.a. above.

   A study was performed to determine if solids are lost by this method of killing the larvae before they are placed on the microweight pans. The study determined that the amount of solids lost from larvae killed by severing the spinal cords was not significantly different than the amount of moisture lost during the weighing process (study performed using wet weights, Exhibit AT20.10).

Test Interferences

Other organisms removed to prevent competition for food or confusion with testing organisms

Algae can interfere with *Ceriodaphnia* movement, ability to filter food and reproduce

Test Interferences and Site-Specific Considerations

Techniques to control naturally occurring pathogens

Permit modifications to allow treatment prior to testing

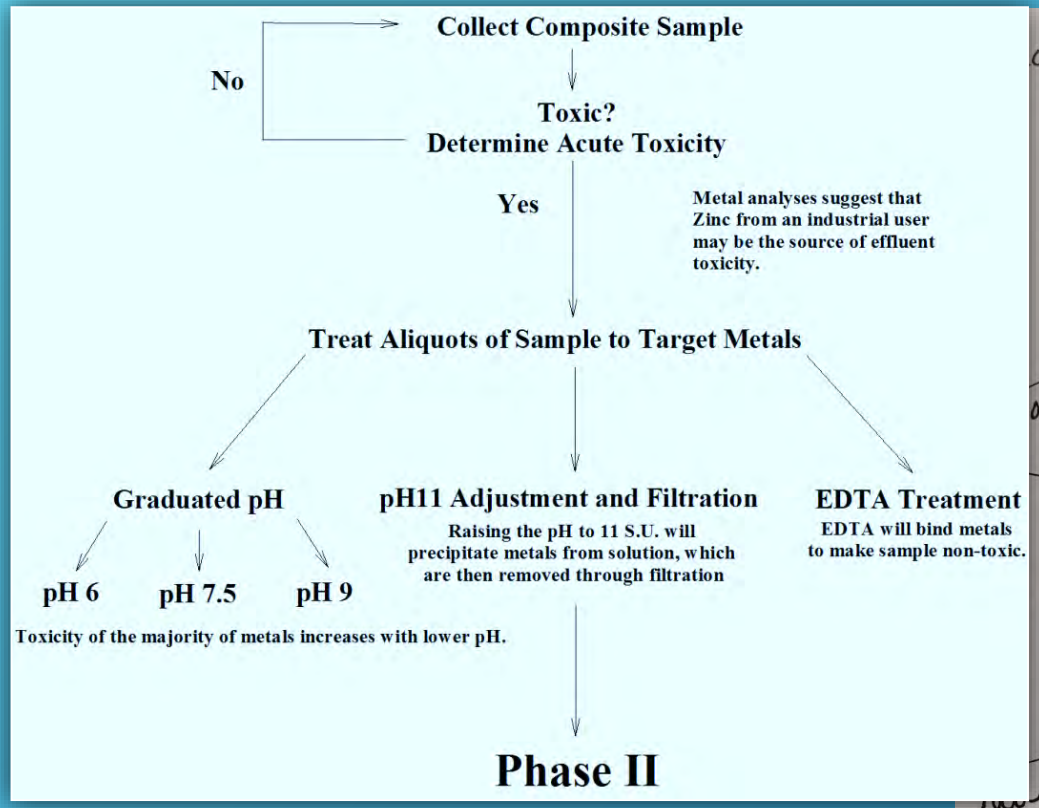
EVIDENCE THAT VARIABILITY IN AMBIENT FATHEAD MINNOW SHORT-TERM CHRONIC TESTS IS DUE TO PATHOGENIC INFECTION

LYNN ADAMS KSZOS,*† ARTHUR J. STEWART† and JAMES R. SUMNER‡
†Oak Ridge National Laboratory, P.O. Box 2008, Oak Ridge, Tennessee 37831-6351, USA
‡CKY Environmental Services, Inc., Oak Ridge, Tennessee 37830, USA

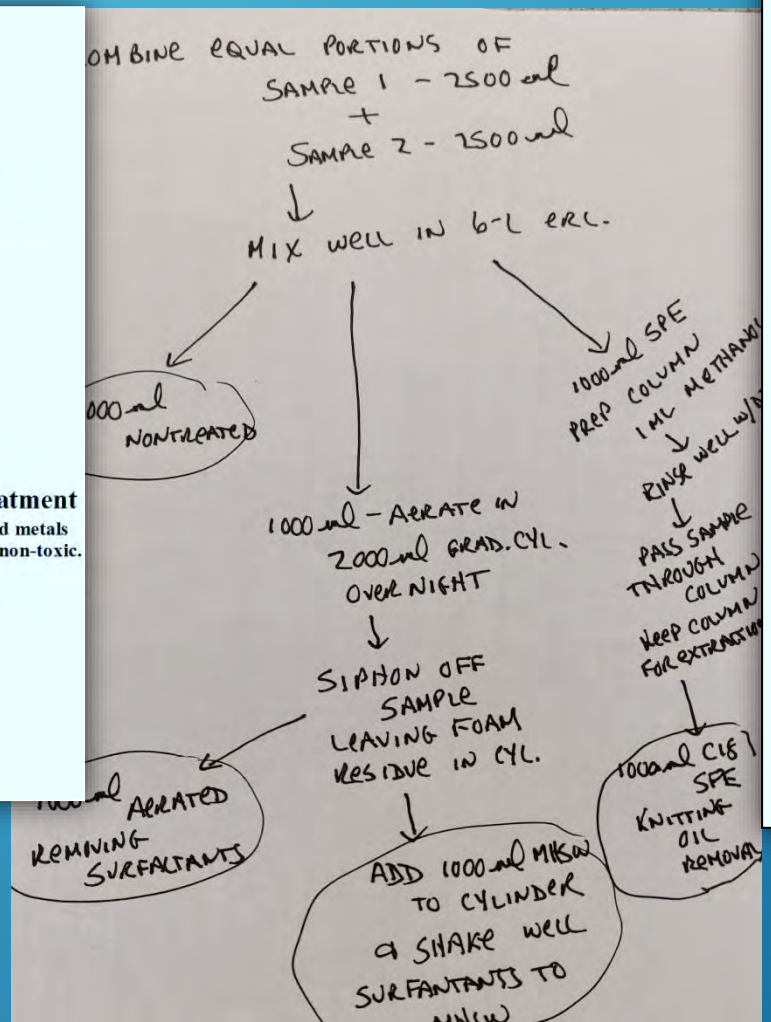
Toxicity Identification Evaluations

Outside the Scope of Methods

Flow Charts



Handwritten Notes



Project Plans

PROPOSAL FOR COMPARISON OF UV AND FILTRATION PROTOCOLS FOR PATHOGEN CONTROL IN FATHEAD MINNOW SHORT-TERM CHRONIC WET TESTS

Objectives:
This study is designed to determine the optimal UV and filtration protocols for removal of fish pathogens (i.e., effective pathogen control with least impact on sample quality) and to compare the effectiveness of the selected UV and filtration protocols during a 7-day chronic test. Results will be useful in discussions with regulators concerning appropriate treatment options at several facilities where suspected pathogen interference in WET tests has occurred.

Method development:
Pathogenic interference in WET tests has been attributed to a number of naturally occurring fish pathogens. Flexibacter (*Aeromonas hydrophilia*) is among the most commonly suspected organisms causing test interference, including in samples, and will be used as a surrogate to determine the optimal treatment for the removal of fish pathogens prior to WET test initiation.

Sterilized river water will be inoculated with *Aeromonas hydrophilia*. Treatments consisting of UV treatment and filtration will then be performed on aliquots of this inoculated water. River water will provide a better correlation to actual test conditions than culture medium since color and suspended particles present in river water may interfere with UV sterilization.

A presence/absence method will be used to determine how successful each treatment is in removing *Aeromonas*. Using this method, positive tubes containing bacteria (*Aeromonas*) will become turbid. Negative tubes, which do not contain bacteria, will remain clear.

Procedure:

1. Collect river water that has a history of pathogenic infection in fathead minnow toxicity tests and is believed to be non-toxic.
2. Sterilize river water. Mix the river water well and pour into Nalgene autoclavable bottles. Autoclave the river water at 121°C (15 lbs of pressure) for 15 minutes.
3. Allow the water to cool. Using a sterile inoculating loop, inoculate the water with *Aeromonas hydrophilia* (purchased from a bacteria culture supplier, e.g. BD Diagnostic Systems).
4. Mix the water well and maintain at room temperature for 24-hours.

QC Documentation

- General lab QC
- Testing conditions
- Analyses of water
- Consumables
- Transfer volumes
- Multiple weigh
- Taxonomy
- PE studies
- Reference testing

General Laboratory QA/ QC and Chemical Analyses

ETS falls under the requirements of NC DW, WW, ATU and Radiological certification or the regulatory agency that has the most stringent requirements

2025 Quality Assurance Activities

QA Activity	January	February	March	April	May	June	July	August	September	October	November	December
Synthetic water check (yearly)		01.29.25									11-20-25	
NIST Therm. Calibration											11.24.25	
Thermometer calibration			03.26.25			06/17.18/25			09.24.25			12.19.25 12.28.25
Balance calibration	01.14.25											
Weight calibration			Due 2028									
Pipette calibration			03.17.25			06.13.25			09.23.25			12.15.25
Graduated cylinder						06.18.25						12.18.25
Luminosity check			03.07.25 03.13.25			06.10.25			09.16.25 09.19.25			12.15.25
Americamysis taxonomy		02.11.25										
Ceriodaphnia taxonomy			03.11.25			06.03.25			09.04.25			12.08.25
Pimephales taxonomy	01.03.25			04.01.25			07.03.25			10.06.25		
Menidia taxonomy	02.07.25			04.10.25				08.05.25		10.07.25		
M. beryllina Acute/Chronic Reference		02.04.25		04.16.25			07.08.25				11.04.25	
Transfer volume	01.29.25											
Ceriodaphnia Count Verification	01.22.25											
Minnow / shrimp multiple weighs			01.23.25									
Artemia count, reference, analytical	02.03.25											
Algal Slant												11.26.25
Conductivity/Salinity ATC												COND 12.16.25 SAL 12.28.25
YWT Analytical	02.05.25											
KY Reference Submittal			03.19.25			06.23.25			09.24.25			DISSOLVED
Time Verification	01.24.25	02.17.25	03.20.25	04.22.25	05.20.25	06.19.25	07.14.25	08.25.25	09.24.25	10.23.25	11.07.25	12.11.25
Radiological Annual Review											11.25.25	

Temperature, pH, Dissolved Oxygen, Conductivity, Salinity, Alkalinity, Hardness, Chlorine, Balances, Pipettes, Autoclave, Dish Washing Soap Checks, etc.

Maintain Testing Conditions

Timer Verification Quarterly

Verify correct time and photoperiod

Luminosity Verified Quarterly

Verify light intensity on each shelf

Light meter verified against separate meter

Randomization Templates

All tests randomized to eliminate temperature/light artifacts

Templates created using a statistical program

Temperature

Min/Max thermometer recorded daily

Temperature recorded at two locations, twice a day

Thermometers verified quarterly against NIST

NIST thermometer verified annually by outside vendor

Synthetic Water Analyzed

Metals, pesticide and PCB check

- Annually
- When chemical lots change
- When culture health in question

Parameter	Result	Reporting Limit	Units	Flag
Total Metals				
Mercury	ND	0.00020	mg/L	
Aluminum	**0.005**	0.050	mg/L	J
Arsenic	ND	0.001	mg/L	
Cadmium	ND	0.0001	mg/L	
Cobalt	**0.00002**	0.001	mg/L	J
Copper	ND	0.001	mg/L	
Iron	**0.003**	0.050	mg/L	J
Lead	ND	0.001	mg/L	
Nickel	ND	0.001	mg/L	
Silver	ND	0.001	mg/L	
Zinc	ND	0.005	mg/L	

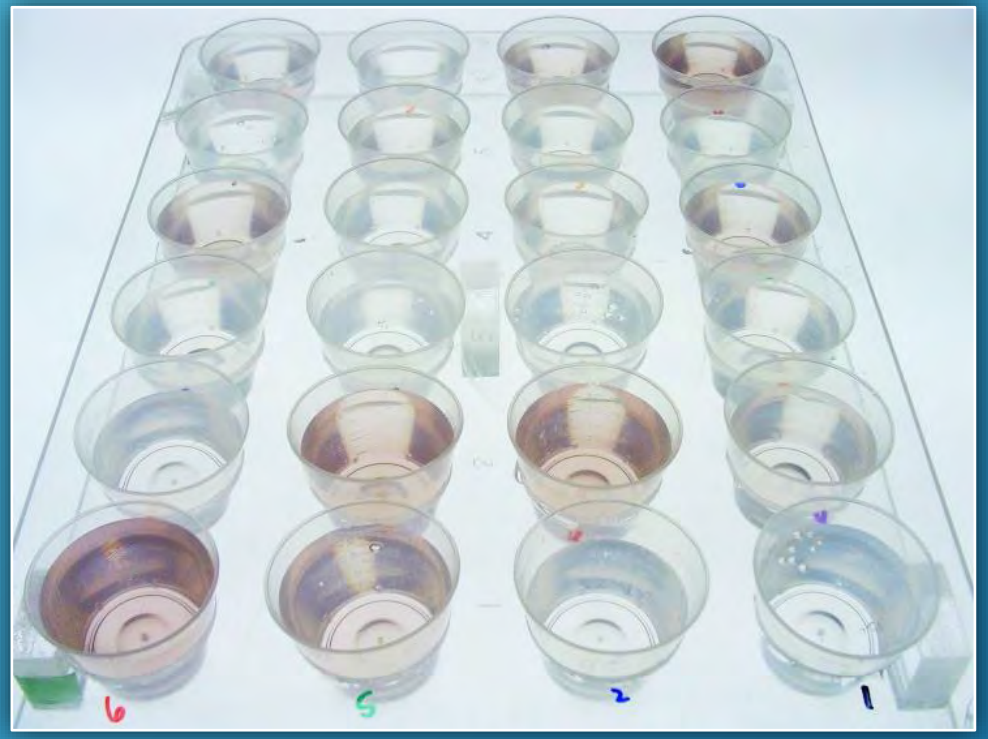
Parameter	Result	Reporting Limit	Units
Organochlorine Pesticides			
Aldrin	ND	0.050	ug/L
alpha-BHC	ND	0.050	ug/L
beta-BHC	ND	0.050	ug/L
delta-BHC	ND	0.050	ug/L
Gamma-BHC (Lindane)	ND	0.050	ug/L
Chlordane	ND	0.500	ug/L
4,4'-DDD	ND	0.050	ug/L
4,4'-DDE	ND	0.050	ug/L
4,4'-DDT	ND	0.050	ug/L
Dieldrin	ND	0.050	ug/L
Endosulfan I	ND	0.050	ug/L
Endosulfan II	ND	0.050	ug/L
Endosulfan sulfate	ND	0.050	ug/L
Endrin	ND	0.050	ug/L
Endrin aldehyde	ND	0.050	ug/L
Heptachlor	ND	0.050	ug/L
Heptachlor epoxide	ND	0.050	ug/L

Parameter	Result	Reporting Limit	Units
Organochlorine Pesticides			
Toxaphene	ND	0.500	ug/L
Methoxychlor	ND	0.050	ug/L
Surrogates			
2,4,5,6-Tetrachloro-m-xylene			
Decachlorobiphenyl			
PCBs			
PCB-1016	ND	0.500	ug/L
PCB-1221	ND	0.500	ug/L
PCB-1232	ND	0.500	ug/L
PCB-1242	ND	0.500	ug/L
PCB-1248	ND	0.500	ug/L
PCB-1254	ND	0.500	ug/L
PCB-1260	ND	0.500	ug/L

Consumables

New and old lots compared in chronic tests

Cubitainers, solo cups and medicine cups



Yearly Transfer Volume Verification

Determine volume of water transferred with test organisms into test solutions

ETS Environmental Testing Solutions, Inc.

Ceriodaphnia dubia Transfer Volume

Analyst: J. Perez

Date: 02-05-26

Ambient temperature: 25.3°C

Numerically label 10 medicine cups.
Add 15 mL MHSW to each of the 10 cups.
Measure and record the weight of each cup containing MHSW.
Transfer 5 *Ceriodaphnia* to each cup, following procedures identified in SOP-AT9 for Daphnid acute toxicity tests.
Measure and record the weight of each cup containing MHSW with 5 *Ceriodaphnia*.
Determine each transfer volume, average transfer volume, and estimated volume to transfer 1 *Ceriodaphnia*.

Replicate Number	Initial Weight Medicine cup + 15 mL MHSW (g)	Final Weight Medicine cup + 15 mL MHSW + 5 *Ceriodaphnia* transferred (g)	Transfer Volume Final - Initial Weight (g = mL)
1	17.8223	17.9681	**0.1458**
2	19.6029	19.8380	**0.2351**
3	17.7254	17.9711	**0.2457**
4	17.5981	17.7978	**0.1997**
5	19.7152	19.9480	**0.2328**
6	18.1686	18.3621	**0.1935**
7	20.6639	20.7647	**0.1008**
8	17.8196	18.0472	**0.2276**
9	21.9439	22.1333	**0.1894**
10	20.1176	20.3205	**0.2029**
		Average volume to transfer 5 organisms (mL):	**0.1973**
		Estimated volume to transfer 1 organism (mL):	**0.0395**

Yearly Multiple Weigh Verification

Verify drying time for minnow weights is sufficient

Multiple weigh of final pans for growth determinations in chronic toxicity tests.

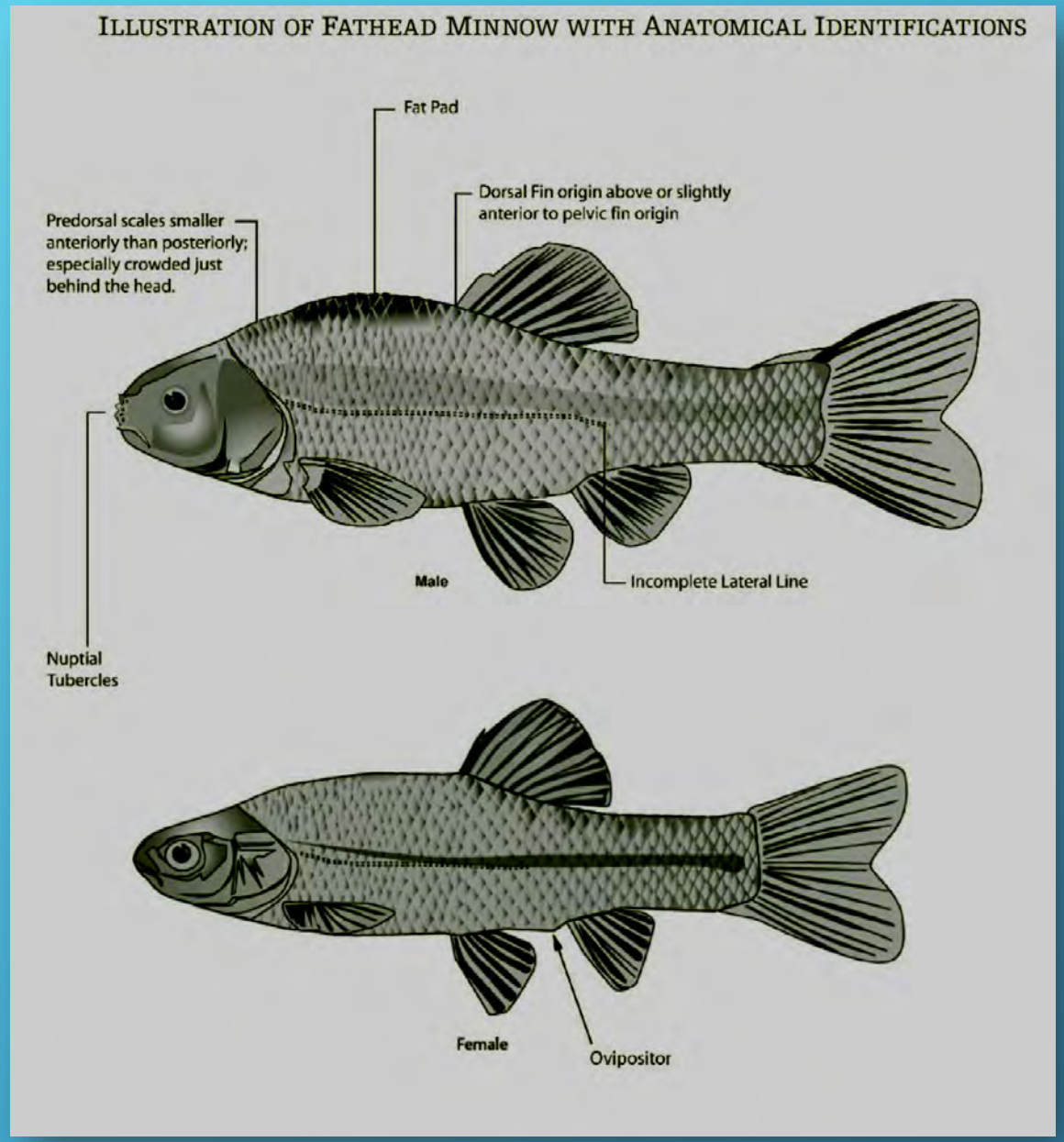
Test type:	***P. promelas* Chronic Reference Toxicant Test**
Test dates:	**January 06-13, 2026**
Associated test:	**PpKClCR # 124**

1st Weight = Pans were dried for 24-hours at 60 ± 2°C, desiccated, and weighed following standard operating procedures.

2nd Weight = The same pans used for determining the 1st weight measurements were dried for an additional 24-hours at 60 ± 2°C, desiccated, and weighed following standard operating procedures.

	1st Weight	2nd Weight		
Analyst:	JS	JP	Difference (mg)	Percent Difference from 1st Weight (%)
Tray color code:	Light Pink	Light Pink		
Date:	01-15-26	01-16-26		
A	23.58	23.59	0.01	0.04
B	21.96	21.97	0.01	0.05
C	23.23	23.24	0.01	0.04
D	20.48	20.52	0.04	0.20
E	25.07	25.08	0.01	0.04
F	23.45	23.47	0.02	0.09
G	23.84	23.86	0.02	0.09
H	22.58	22.62	0.04	0.18
I	21.65	21.67	0.02	0.09
J	22.27	22.30	0.03	0.13
K	18.80	18.82	0.02	0.11
L	21.48	21.52	0.04	0.19
M	21.08	21.11	0.03	0.14
N	25.32	25.34	0.02	0.08
O	22.00	22.02	0.02	0.09
P	23.04	23.06	0.02	0.09
Q	18.15	18.15	0.00	0.00
R	20.75	20.74	-0.01	-0.05
S	21.47	21.51	0.04	0.19
T	20.51	20.54	0.03	0.15
U	14.03	14.05	0.02	0.14
V	16.68	16.68	0.00	0.00
W	17.70	17.71	0.01	0.06
X	15.77	15.77	0.00	0.00
Y	13.06	13.06	0.00	0.00
Z	15.05	15.04	-0.01	-0.07
AA	13.34	13.35	0.01	0.07
BB	15.05	15.05	0.00	0.00
		Average	0.02	0.08

Taxonomy



Quarterly for organisms cultured in-house

Performance Evaluations

Final Report - DMRQA PT

Study: DMRQA45-WET

Opening Date: June 6, 2025 - Closing Date: August 29, 2025

Facility: Environmental Testing Solutions, Inc.
351 Depot Street
Asheville, NC 28801
USA

Contact: Ms. Kelley Keenan, President
828-350-9364

EPA Lab ID: NC01230

NPDES Permit ID:

Ceriodaphnia Method 21 (PT-21-WET) — **Lot #: 8565-21**

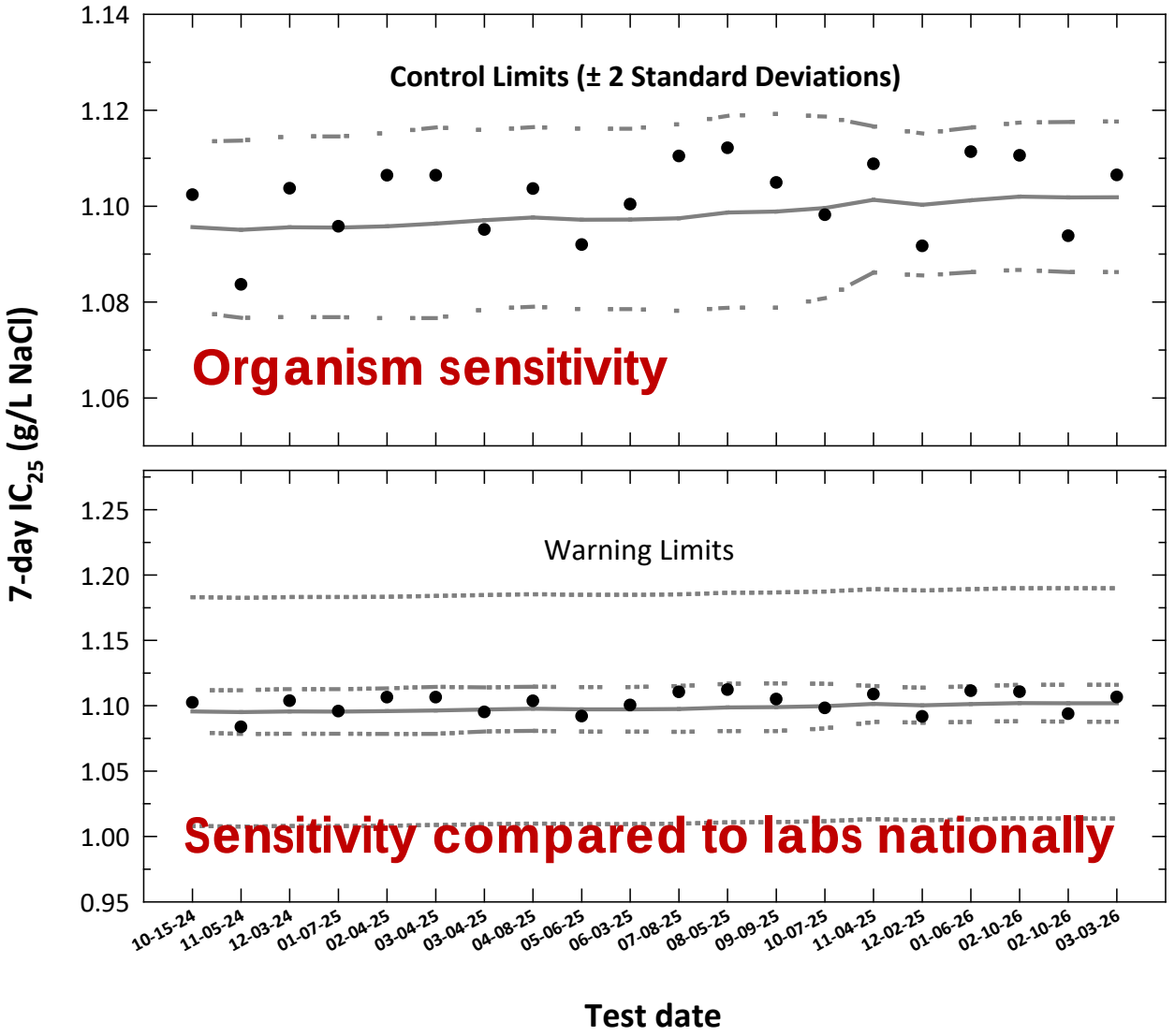
NELAC Code	Analyte	Method Code	Method Description	Units	Assigned Value	Result	Acceptance Limits	Evaluation
3315	Ceriodaphnia Chronic MHSF - Survival NOEC	10253040	EPA 1002.0 - Ceriodaphnia dubia, 3-Brood Chronic, daily renewal, MHSF, 25°C (2002)	S.U.	25	25	12.5 - 50	Acceptable
3315	Ceriodaphnia Chronic MHSF - Reproduction IC25	10253040	EPA 1002.0 - Ceriodaphnia dubia, 3-Brood Chronic, daily renewal, MHSF, 25°C (2002)	S.U.	29.4	30.5	23.1 - 35.8	Acceptable
3315	Ceriodaphnia Chronic MHSF - Reproduction NOEC	10253040	EPA 1002.0 - Ceriodaphnia dubia, 3-Brood Chronic, daily renewal, MHSF, 25°C (2002)	S.U.	25	25	12.5 - 50	Acceptable

Reference Toxicant Testing

Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

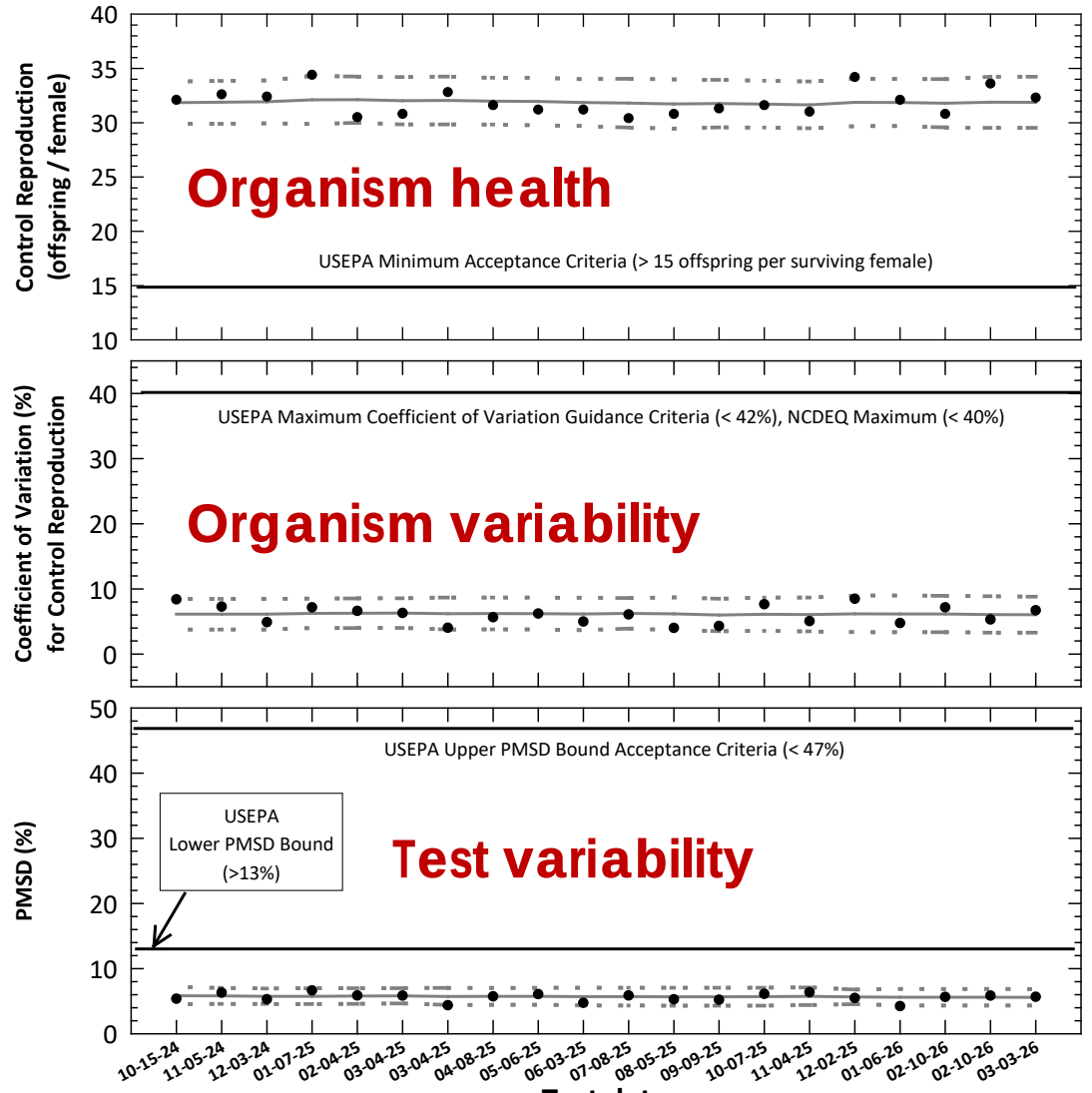
Source: In-house Culture



Ceriodaphnia dubia

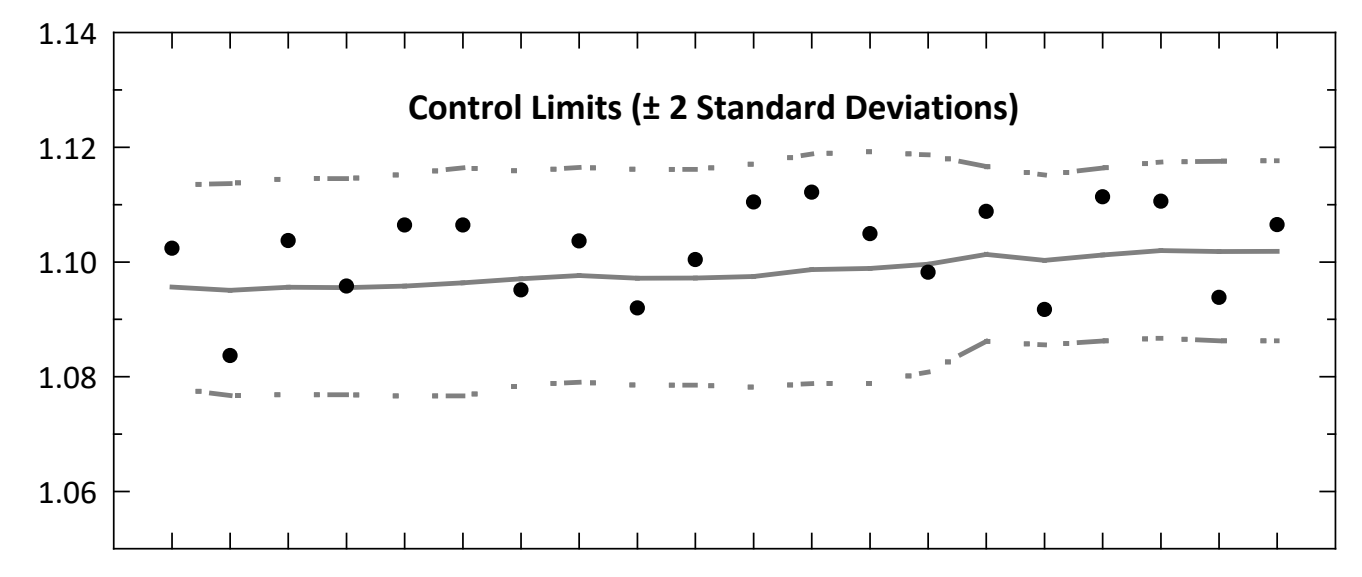
Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: In-house Culture

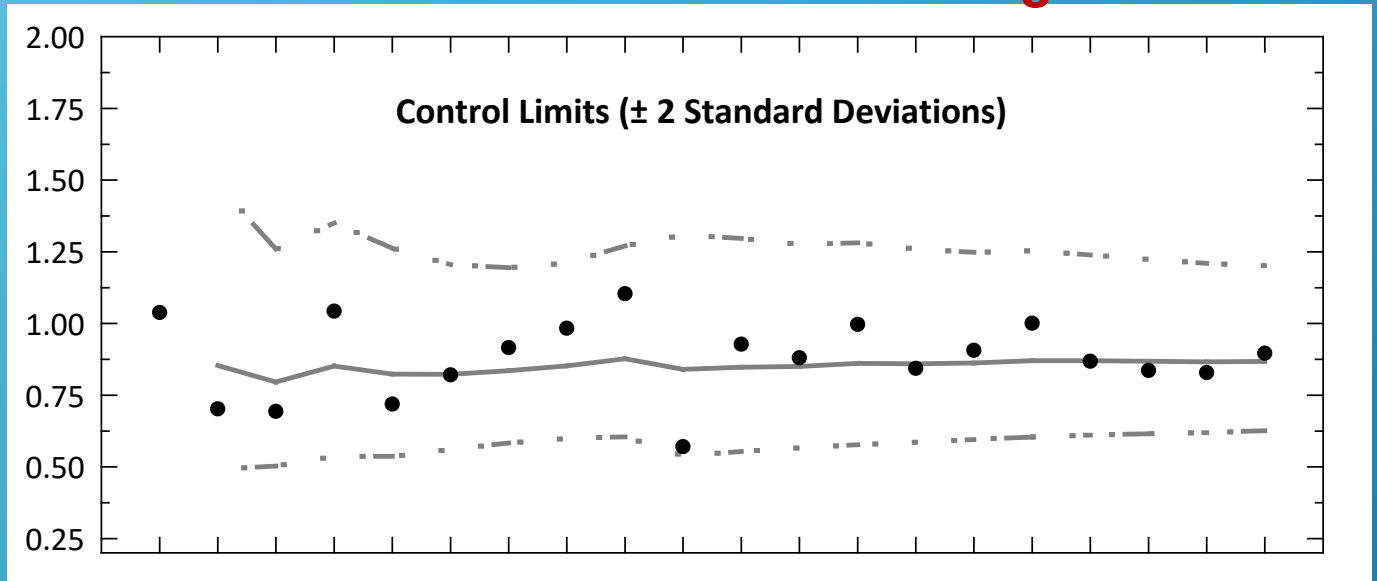


Reference Toxicant Testing

ETS: Control limits ± 15 mg/L



NC Certified lab: Control limits ± 290 mg/L



Variability between laboratories

Proficiency of laboratory and technicians

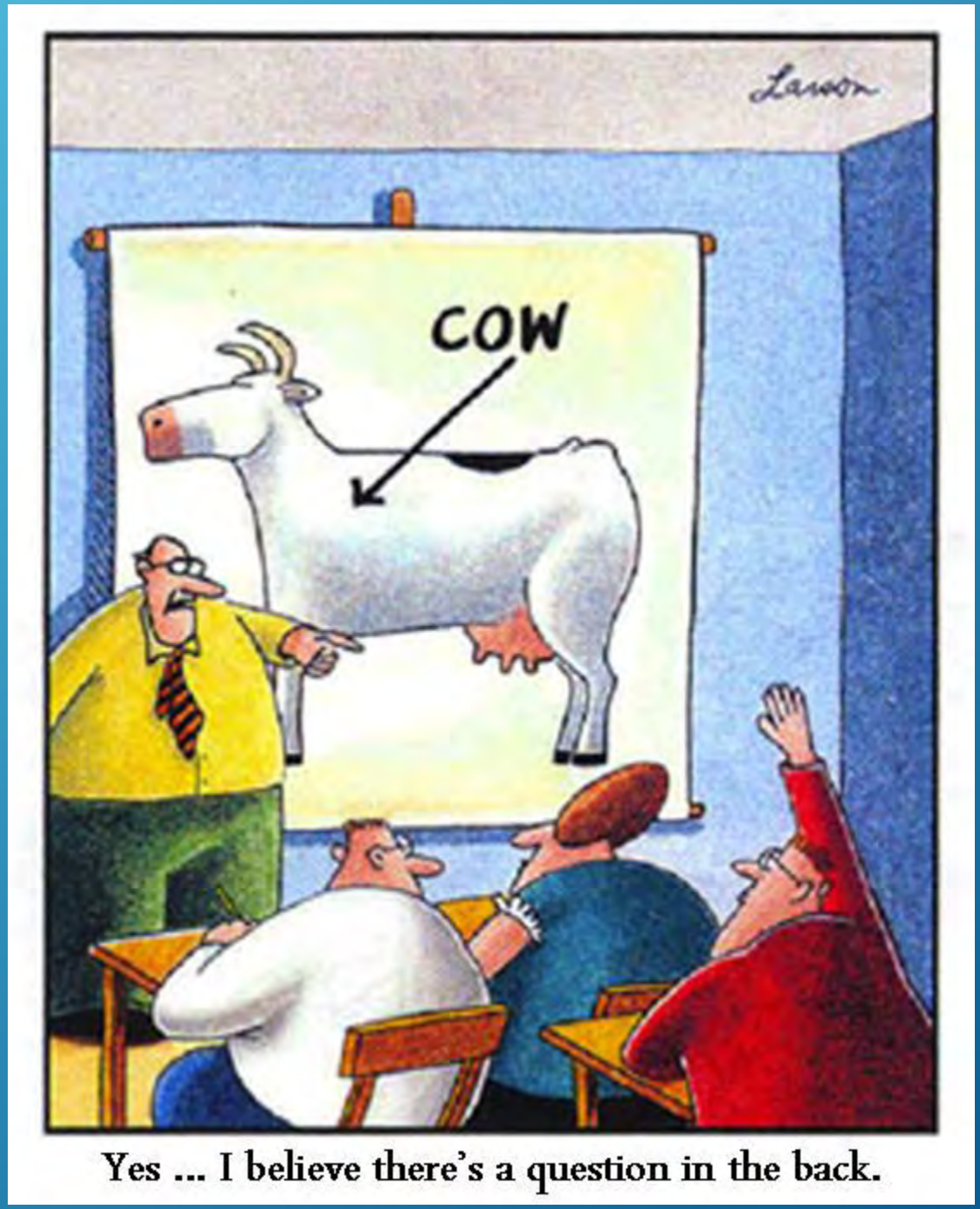
What Quality Control is required for toxicity tests?

1. Multiple weigh studies
2. Consumable testing
3. Side-by-side reference testing of new food
4. Transfer volumes
5. PE Studies
6. All of the above

Difficulties of Training

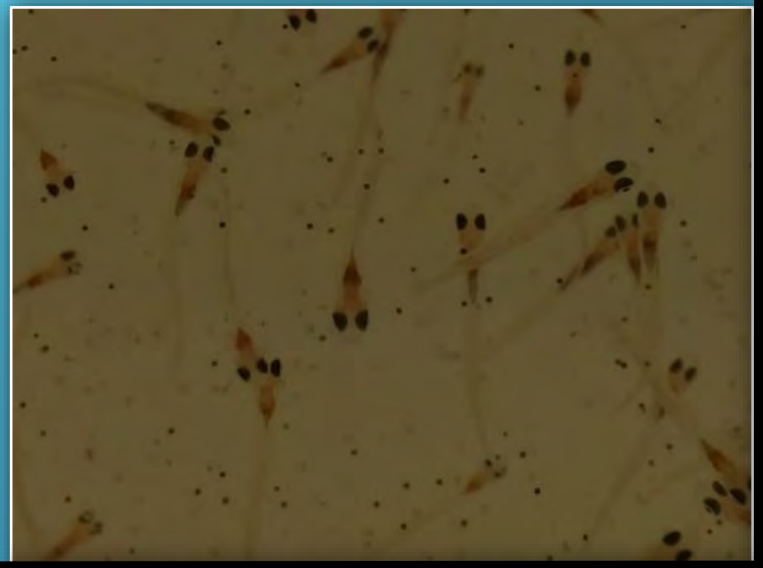
Technicians may not know simple things

Multiple analysts are involved in a single test



Difficulties of Training

- Health of organisms
- Working with multiple types of organisms
- Troubleshooting problems
- Timing!
- Maintaining a culture

	Saltwater	Freshwater
Vertebrates		
Invertebrates		

Organisms Add a Layer of Complexity To Training

Ability of analyst to identify organism's health and more importantly what may have affected their health

<u>Successfully</u>

Maintain Cultures

Use those cultures to perform reference tests

Fathead Minnow Health

Culturing vertebrates is completely different than invertebrates

Behavioral signs in addition to visual observations of health

Healthy

Dropsy – bacteria infection

Sunken Belly – parasite infection

How to Troubleshoot

Timing!

Knowing when organisms will be born and timing this with tests to be initiated

2026 Testing Schedule

Testing Week

Tiles in Tanks

Workshop?

JANUARY

S	M	T	W	T	F	S
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	31

FEBRUARY

S	M	T	W	T	F	S
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28

MARCH

S	M	T	W	T	F	S
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

APRIL

S	M	T	W	T	F	S
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30		

MAY

S	M	T	W	T	F	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30
31						

JUNE

S	M	T	W	T	F	S
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30				

JULY

S	M	T	W	T	F	S
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30	31	

AUGUST

S	M	T	W	T	F	S
						1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23	24	25	26	27	28	29
30	31					

SEPTEMBER

S	M	T	W	T	F	S
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30			

OCTOBER

S	M	T	W	T	F	S
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	31

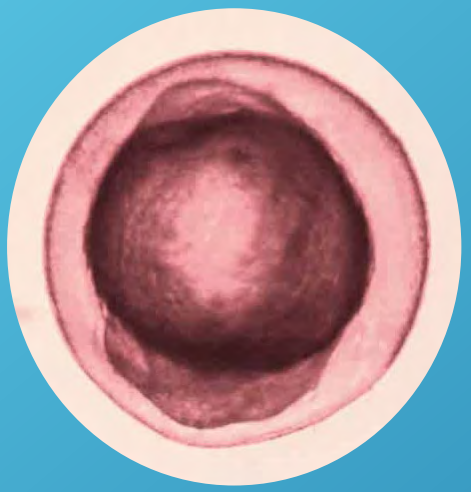
NOVEMBER

S	M	T	W	T	F	S
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30					

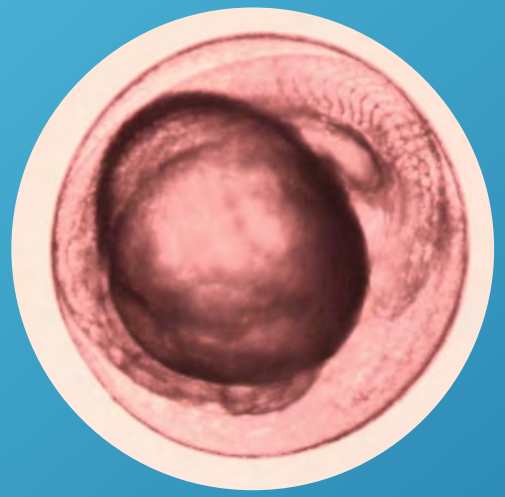
DECEMBER

S	M	T	W	T	F	S
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30	31		

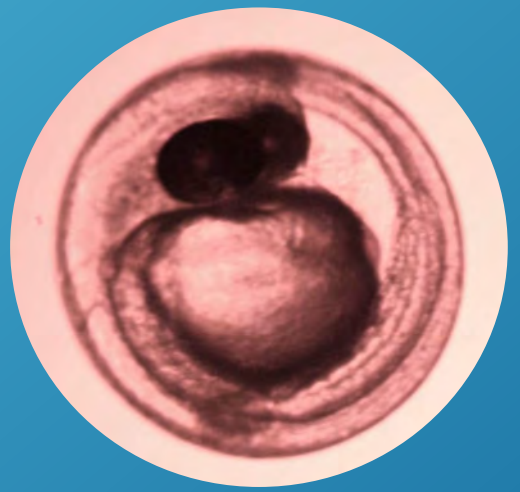
Day 1



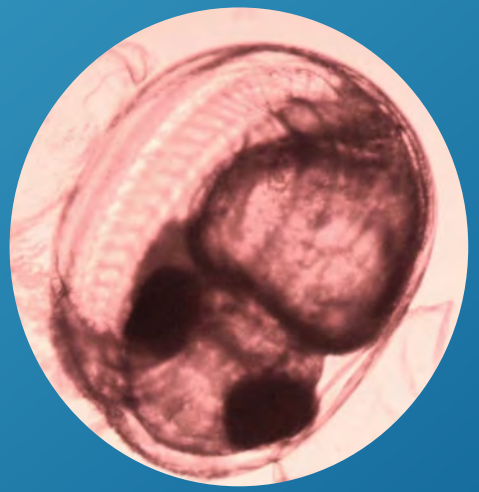
Day 2



Day 3



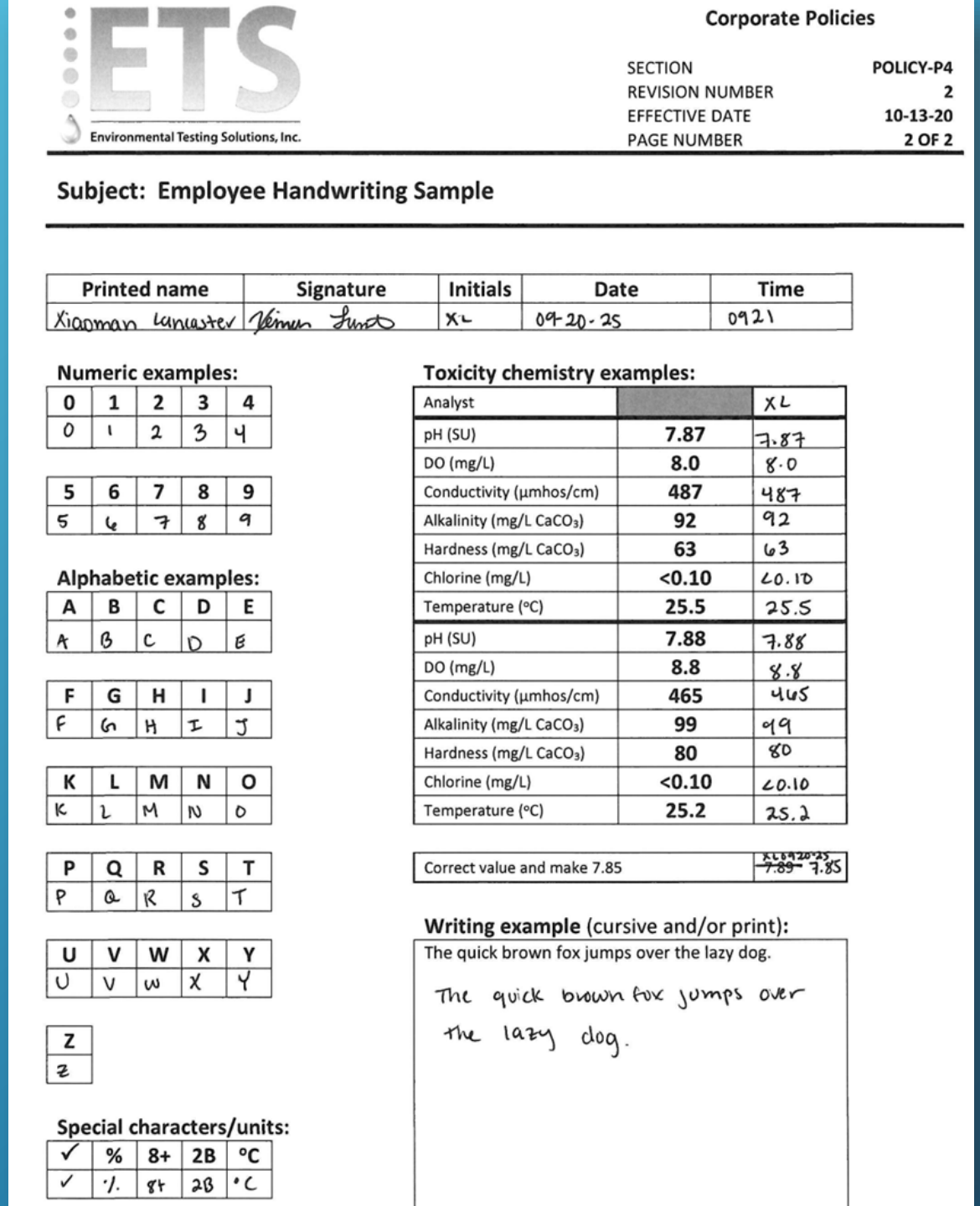
Day 5



Day 7

Policies

- Ethics
- Read NC lab certification rules
- Employee handbook
- Handwriting sample
- Safety
- What will get you fired?



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Corporate Policies

SECTION	POLICY-P4
REVISION NUMBER	2
EFFECTIVE DATE	10-13-20
PAGE NUMBER	2 OF 2

Subject: Employee Handwriting Sample

Printed name	Signature	Initials	Date	Time
Xiaoman Lancaster	Xiaoman Lancaster	XL	09-20-25	0921

Numeric examples:

0	1	2	3	4
0	1	2	3	4

5	6	7	8	9
5	6	7	8	9

Alphabetic examples:

A	B	C	D	E
A	B	C	D	E

F	G	H	I	J
F	G	H	I	J

K	L	M	N	O
K	L	M	N	O

P	Q	R	S	T
P	Q	R	S	T

U	V	W	X	Y
U	V	W	X	Y

Z
Z

Special characters/units:

✓	%	8+	2B	°C
✓	%	8+	2B	°C

Toxicity chemistry examples:

Analyst		XL
pH (SU)	7.87	7.87
DO (mg/L)	8.0	8.0
Conductivity (µmhos/cm)	487	487
Alkalinity (mg/L $CaCO_3$)	92	92
Hardness (mg/L $CaCO_3$)	63	63
Chlorine (mg/L)	<0.10	<0.10
Temperature (°C)	25.5	25.5
pH (SU)	7.88	7.88
DO (mg/L)	8.8	8.8
Conductivity (µmhos/cm)	465	465
Alkalinity (mg/L $CaCO_3$)	99	99
Hardness (mg/L $CaCO_3$)	80	80
Chlorine (mg/L)	<0.10	<0.10
Temperature (°C)	25.2	25.2

Correct value and make 7.85	~~XL 9-20-25 7.89~~ 7.85

Writing example (cursive and/or print):

The quick brown fox jumps over the lazy dog.

The quick brown fox jumps over the lazy dog.

Quality Assurance Plan

Subject: Table of Contents

Subject: Quality Improvement

Toxicity Testing: **(continued)**

Description of Activity	Minimum Frequency	Acceptance of Activity
***Artemia* nauplii (SOP-AT16)**		
Samples are submitted to a certified laboratory for inorganic and organic analyses.	Each lot	Analyses must be comparable to previous *Artemia* lots.
Verification of the number of *Artemia* nauplii fed to organisms in chronic toxicity tests.	Each lot	350 – 500 nauplii per 50 µL (1 drop)
Verification of nutritional quality.	Each lot	Side-by-side reference toxicant tests are performed with the new and old *Artemia* lots in *Pimephales promelas* chronic toxicity tests. Organism health and sensitivity using the new lot must be within established laboratory limits.
Consumables		
Medicine and Solo cups	Each lot	*Ceriodaphnia dubia* survival and reproduction or minnow survival and growth must not be significantly reduced ($\alpha = 0.05$).
Cubitainers	Each lot	*Ceriodaphnia dubia* survival and reproduction or minnow survival and growth must not be significantly reduced ($\alpha = 0.05$).
Yeast, Wheat Grass, Trout Chow (YWT) mixture (SOP-AT6)		
Samples are submitted to a certified laboratory for inorganic and organic analyses.	Each lot	Analyses must be comparable to previous YWT lots.
Verification of total solids.	Each batch	1.7 – 1.9 g/L
Verification of nutritional quality.	Each lot	Side-by-side reference toxicant tests are performed with the new and old YWT lots in *Ceriodaphnia dubia* chronic toxicity tests. Organism health and sensitivity using the new lot must be within established laboratory limits.
	Each batch	*Ceriodaphnia dubia* survival and reproduction is evaluated in cultures prior to being used in toxicity tests.
***Selenastrum capricornutum* (SOP-AT2)**		
Verification of cell count.	Each batch	$3.0 – 3.5 \times 10^7$ cells/mL (tests and cultures)

Notebook

Required to take notes!

AT 1 – Preparation of Synthetic Water

MHSW - Prep.
- Clean tank and rinse w/10% nitric.
- Fill w/ 50 L Milli-Q water.
- aerate

$NaHCO_3$	5.20 g
$CaSO_4 \cdot 2H_2O$	3.30 g
$MgSO_4$	3.30 g
KCl	0.20 g

- expires in 14 days
- continuous aeration

Alkalinity Q.C.
- 100 mL of synthetic water
- add 0.020N H_2SO_4 until titrated to 4.5 SU.
  - multiply by 10 to get alkalinity

Hardness Q.C
50 mL of synthetic water
2 mL of hardness buffer and a hint of Erichrome Black
Fill 10 mL pipette w/ ETDA.
Titrate to a blue color.
Multiply by 20 to get total hardness.

SSW

$NaHCO_3$	2.75 g
$CaSO_4 \cdot 2H_2O$	1.50 g
$MgSO_4$	1.50 g
KCl	0.10 g

Task Instructions

Simple instructions that may not require a SOP

New employees may not have basic lab skills

- **Data entry**
- **Rounding rules**

Hands-On Demonstration

- **How to use a balance**
- **How to use each type of pipette**

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Task Instructions

SECTION	SOP-T1
REVISION NUMBER	3
EFFECTIVE DATE	10-13-20
PAGE NUMBER	2 OF 2

Subject: Data Entry

Data Entry Requirements

- All entries are to be written **LEGIBLY** using an **indelible black ink pen.**
- Data entries must be traceable to the date and the person making the entry.
- Entries should be made in chronological order.
- Entries must **not** be obliterated, backdated or written over. Care must be taken not to obscure any information.
- Correction fluid and erasures are **not** permitted.
- A single line must be used to mark through errors. If you are crossing out a row of data, use one line with your **initials and date** (i.e. ~~24.3 25.3 1.3~~ KEK 10-01-17).
- The person making the alteration or amplification must initial and date the new entry.
- Data forms must be completely filled in. Spaces left blank will be filled in with "not applicable" (NA) or with a line and initialed and dated.
- Data forms must be uniquely identified (e.g., consecutively numbered).
- All data must be filled out by the analyst. This includes date, time, analyst initials, data, calculations, units or any other data that is required on the benchsheet, logbook or data entry forms. The **only** exception is sample numbers.
- Times must be military in the HHMM format (i.e. 4 pm = 1600).
- Dates must be in the following format: MM-DD-YY (i.e. October 1, 2017 = 10-01-17)
- Decimal points must be clearly visible.
- All benchsheets, logbooks or data entry forms are **legal documents** and must be able to stand alone if questioned.
- If multiple analysts are using the same benchsheet, logbook or data entry forms, each analyst must put their initials on the benchsheet, logbook or data entry forms. (i.e. Calibration logbook, toxicity data sheets).
- Care must be taken to ensure that all entries are legible.

Pipette Volume Verification

Used as a simple way to verify that a technician is pipetting correctly

Fixed-Volume Pipette Calibration

Reference: ISO 8655-6:2002 (E)

Analyst:	JP
Date of Calibration:	03-09-26
Calibration due date:	09-2026
Pipet manufacturer:	Fisherbrand HandyStep
Serial/ID number:	17E59354
Nominal Volume:	0.1
Location of use:	Toxicity lab

Test Conditions	Normal
Barometric pressure, kPa or mbar	102.0
Relative Humidity, %	47
Ambient temp., degrees C	22.4
Test liquid temp., degrees C	22.4
Z correction factor	1.0034

Leak Test	
Time begin:	1334
Time end:	1335
Pass/Fail	Pass

Balance check	
Balance manufacturer	Mettler Toledo
Serial Number	C006982938
Location of use	Chemistry lab
Weight set serial #	20410

Pipet Calibration

Dial setting:		0.1	units:	mL
Time begin:	1329			
Replicate	Mass	Units		Volume (V)
m_1	0.0990	g		0.09934
m_2	0.0991	g		0.09944
m_3	0.0986	g		0.09894
m_4	0.0992	g		0.09954
m_5	0.1001	g		0.10044
m_6	0.0987	g		0.09904
m_7	0.0993	g		0.09964
m_8	0.0991	g		0.09944
m_9	0.0989	g		0.09924
m_{10}	0.0992	g		0.09954
Mean mass	0.09912		PASS	
Time end:	1335			
Mean vol.	0.0995			
s	0.0004			
% CV	0.4141			
CV Tolerance	PASS			
PASS/FAIL	PASS			

COMMENTS:

Mean mass acceptable range: ± 1.8% or 0.0018 g

Mean mass acceptable range: 0.0982 to 0.1018 g

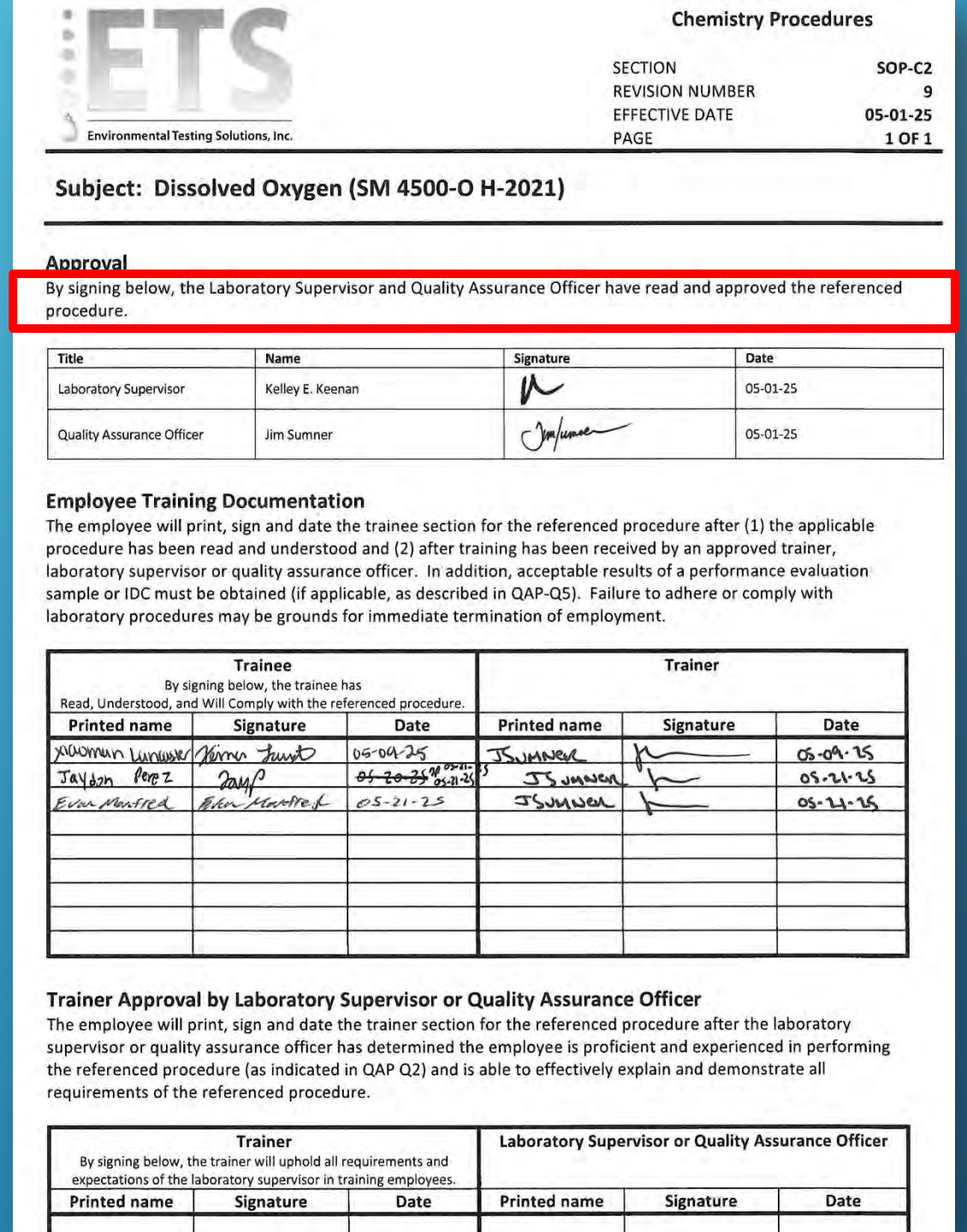
CV tolerance acceptable range: < 1.5%

A **leak test** may be performed by filling the pipette tip with distilled or deionized water to the maximum nominal volume and placing the pipette on a vibration-free stand. Observe the meniscus in the tip for 1 minute. After 1 minute, there should be no visible droplet formation at the tip. If there are no signs of droplet formation, proceed with the calibration check. If droplet formation is observed, successful maintenance and corrective action must be taken or the pipette must not be used.

Training Documentation

Start with easy procedures and then move to more complex procedures

- **Observe trainer or "shadowing"**
- **Read SOP**
- **Perform task with observation**
- **Acceptable performance of reference tests**



ETS Environmental Testing Solutions, Inc.

Chemistry Procedures

SECTION	SOP-C2
REVISION NUMBER	9
EFFECTIVE DATE	05-01-25
PAGE	1 OF 1

Subject: Dissolved Oxygen (SM 4500-O H-2021)

Approval

By signing below, the Laboratory Supervisor and Quality Assurance Officer have read and approved the referenced procedure.

Title	Name	Signature	Date
Laboratory Supervisor	Kelley E. Keenan		05-01-25
Quality Assurance Officer	Jim Sumner		05-01-25

Employee Training Documentation

The employee will print, sign and date the trainee section for the referenced procedure after (1) the applicable procedure has been read and understood and (2) after training has been received by an approved trainer, laboratory supervisor or quality assurance officer. In addition, acceptable results of a performance evaluation sample or IDC must be obtained (if applicable, as described in QAP-Q5). Failure to adhere or comply with laboratory procedures may be grounds for immediate termination of employment.

Trainee (By signing below, the trainee has Read, Understood, and Will Comply with the referenced procedure.)			Trainer		
Printed name	Signature	Date	Printed name	Signature	Date
[illegible]	[illegible]	05-09-25	JSUMNER		05-09-25
Jaydon Perez		~~05-20-25~~ 05-21-25	JSUMNER		05-21-25
Evan Manfred		05-21-25	JSUMNER		05-21-25

Trainer Approval by Laboratory Supervisor or Quality Assurance Officer

The employee will print, sign and date the trainer section for the referenced procedure after the laboratory supervisor or quality assurance officer has determined the employee is proficient and experienced in performing the referenced procedure (as indicated in QAP Q2) and is able to effectively explain and demonstrate all requirements of the referenced procedure.

Trainer (By signing below, the trainer will uphold all requirements and expectations of the laboratory supervisor in training employees.)			Laboratory Supervisor or Quality Assurance Officer		
Printed name	Signature	Date	Printed name	Signature	Date

Written Tests

Answered without the aid of SOPS or notebooks

Subject: Cultures, Food and Feeding

Analyst:

Printed name	Signature	Date

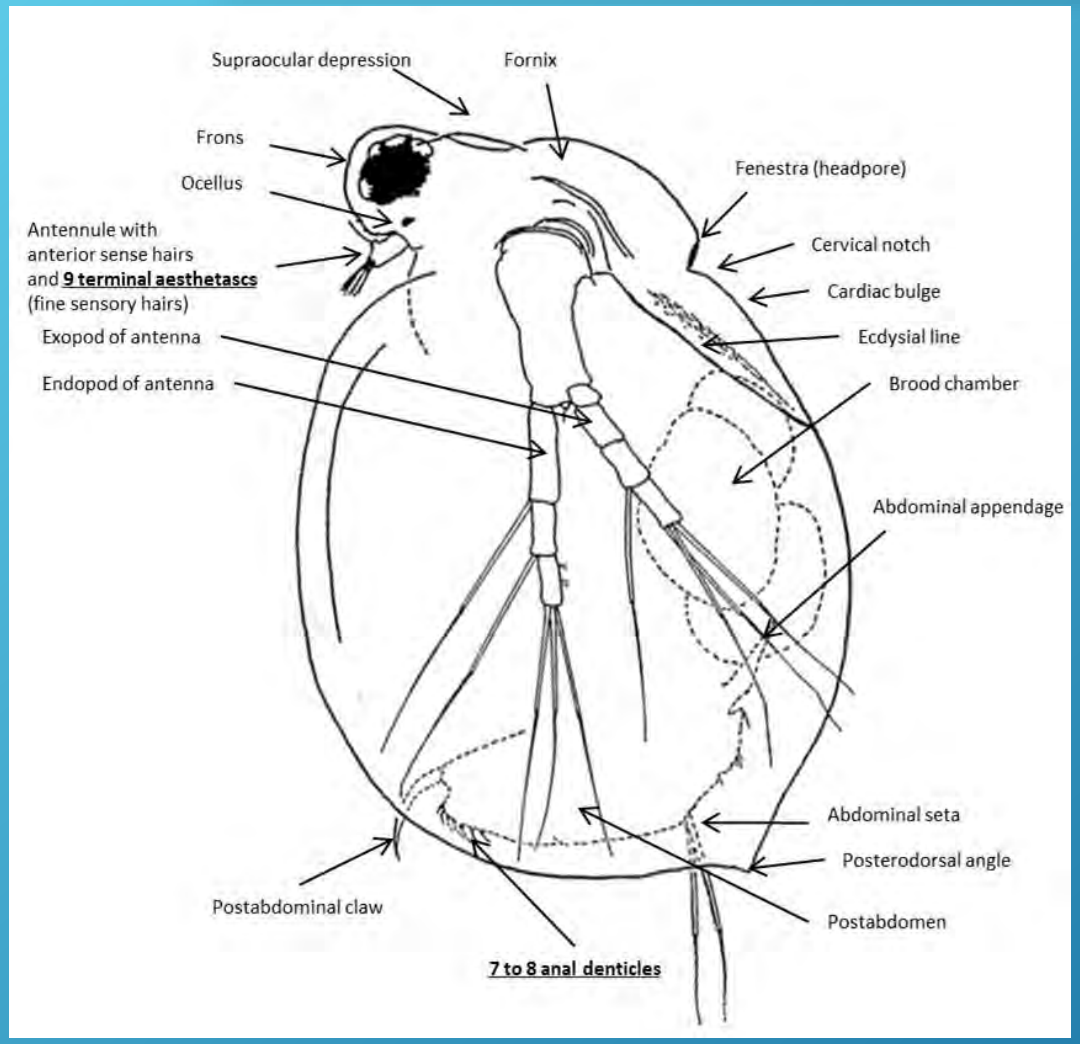
Written test:

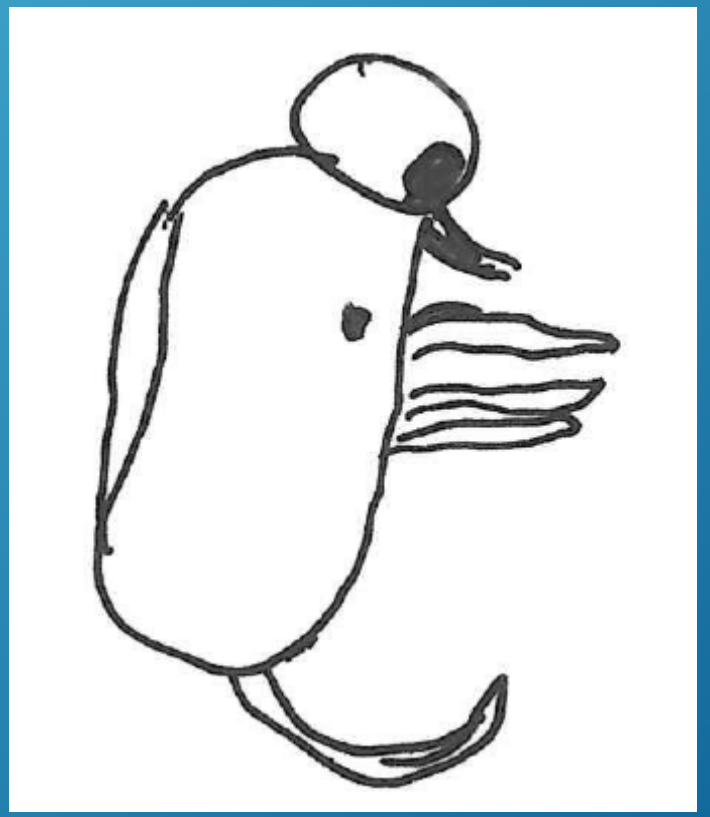
Algae:

1. What is the algae genus/species used for feeding cultures?
   NC Tests?
   Out of state tests?
2. Is the algae purchased? If it is, where is it purchased from?
3. What additional QC is required for algae and what is the frequency?
4. What is the expiration date for algae?
5. What is the cell density of algae for cultures?
   NC chronic tests?
   Out of state chronic tests?
6. How is the cell density determined?
7. What is used to resuspend algae concentrate to achieve this cell density?
8. How long is algae incubated?
9. How long is algae settled and at what temperature?
10. Where is algae stored?
11. At what temperature is algae stored and why is it stored at this temperature?
12. If additional algae is needed, how do you know what batch to use?
13. If multiple batches of algae are being used, which batch do you use?
14. Where is the batch of algae identified on culture and test bench sheets?
15. Describe how algae media is prepared:
16. What are the primary constituents of algae media?
17. What is the expiration date of algae media?
34. What volume does setting 1 correspond to on the repeater pipette?
    Setting 2?
35. How often is the food shaken during feeding?
36. Describe how you wash the tip for the repeater pipette.
37. Draw a picture of what a *Ceriodaphnia* looks like. In your diagram, identify organs and characteristics.
38. When feeding *Ceriodaphnia* cultures, is the light table on or off?
39. What activities are performed to maintain individual *Ceriodaphnia* cultures each day?
    a. Sunday
    b. Monday
    c. Tuesday
    d. Wednesday
    e. Thursday
    f. Friday
    g. Saturday
40. What activities are performed to maintain backup *Ceriodaphnia* cultures each day?
    a. Sunday
    b. Monday
    c. Tuesday
    d. Wednesday
    e. Thursday
    f. Friday

Expectations versus Reality

37. Draw a picture of what a *Ceriodaphnia* looks like. In your diagram, identify organs and characteristics.





Practical Tests

Subject: ***Pimephales promelas*** **Acute Toxicity Test, EPA 2000.0**

Analyst: _____

Reviewed performed by	Date	Reference Test # and/or Project #

Test Initiation:

Activity	Comments
Test/Sample preparation	
Glassware/plasticware prepared according to test type	
Number of replicates and test vessels correct based on test type	
Sample mixed thoroughly	
Aliquot of sample warmed to test temperature (25.0 ± 1.0°C)	
Sample diluted correctly and mixed	
Dilution water type correct and batch used recorded	
Volume prepared correct based on test type and number of replicates	
Volume of each replicate correct and measured	
Aliquot of each concentration/control poured off for chemical analyses, including full-strength sample	
Chemistry analyses demonstrated dilutions performed correctly and analyses met test acceptability criteria	
Test randomized and template used recorded	
Temperatures measured and recorded for each concentration/control and within acceptance limits (25.0 ± 1.0°C)	
Test organisms and initiation	
Larvae 1 to 14 days old	
Fed 2 to 5 hours prior to test initiation	
Temperature of larvae within acceptance limits (25.0 ± 1.0°C)	
Volume of transfer water poured off for pH analyses	
Larvae concentrated using mesh screen	
10 larvae loaded into each test cup, pipette tip cut to ensure larvae are not injured	
Larvae introduced into test solutions below water surface and allowed to swim from transfer pipette into solution to minimize dilution	
Unusual behavior of larvae recorded, if applicable	
Repeat count performed	
Visual inspection of larvae selected (health/appearance) acceptable	
Test placed into incubator and covered, location recorded	
Test initiated within 36-hours of sample collection	
All test initiation information recorded on benchsheet	

Direct observation of analyst

24-hour Check:

Activity	Comments
Temperatures measured and recorded for each concentration/control and within acceptance limits (25.0 ± 1.0°C)	
Aliquot of each concentration/control poured off for chemical analyses	
Chemistry analyses met test acceptability criteria	
Number of living/dead organisms recorded at test initiation time ± 15 minutes	
Comments of larvae health recorded, if applicable	
Dead larvae removed and discarded	
Test placed into incubator and covered	
All 24-hour check information recorded on benchsheet	

Test Termination:

Activity	Comments
Temperatures measured and recorded for each concentration/control and within acceptance limits (25.0 ± 1.0°C)	
Aliquot of each concentration/control poured off for chemical analyses	
Chemistry analyses met test acceptability criteria	
Number of living/dead organisms recorded at test initiation time ± 15 minutes	
Comments of larvae health recorded, if applicable	
All 48-hour termination information recorded on benchsheet	
Test larvae discarded, test cups rinsed with tap water and recycled (if applicable)	

Test Acceptibilty:

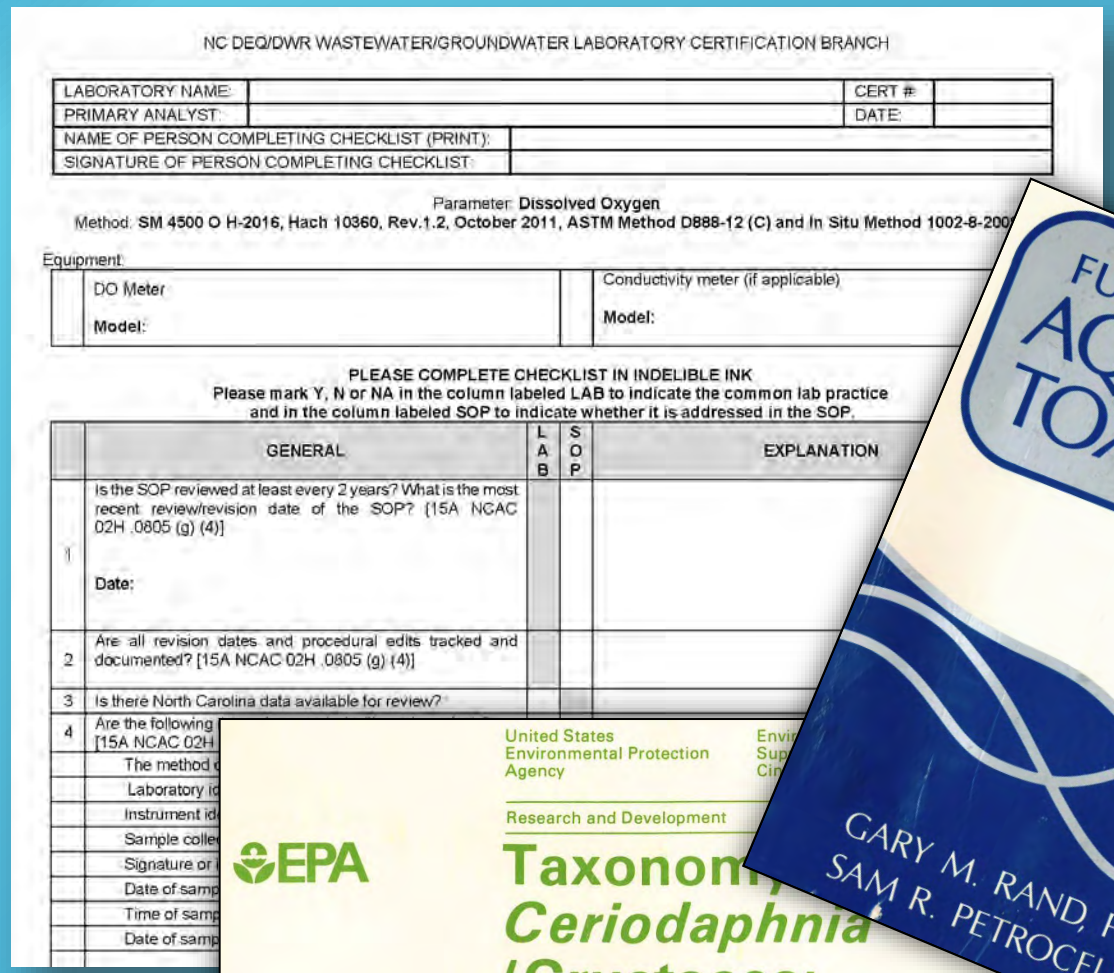
Activity	Comments
Control survival ≥ 90%	
Reference toxicant test within control limits and performed within 7 days of compliance test (if applicable)	

Advanced Training

Complete state audit checklists

Read Standard Methods
EPA manuals
other documents

Participate in audits



NC DEQ/DWR WASTEWATER/GROUNDWATER LABORATORY CERTIFICATION BRANCH

LABORATORY NAME:		CERT #:	
PRIMARY ANALYST:		DATE:	
NAME OF PERSON COMPLETING CHECKLIST (PRINT):			
SIGNATURE OF PERSON COMPLETING CHECKLIST:			

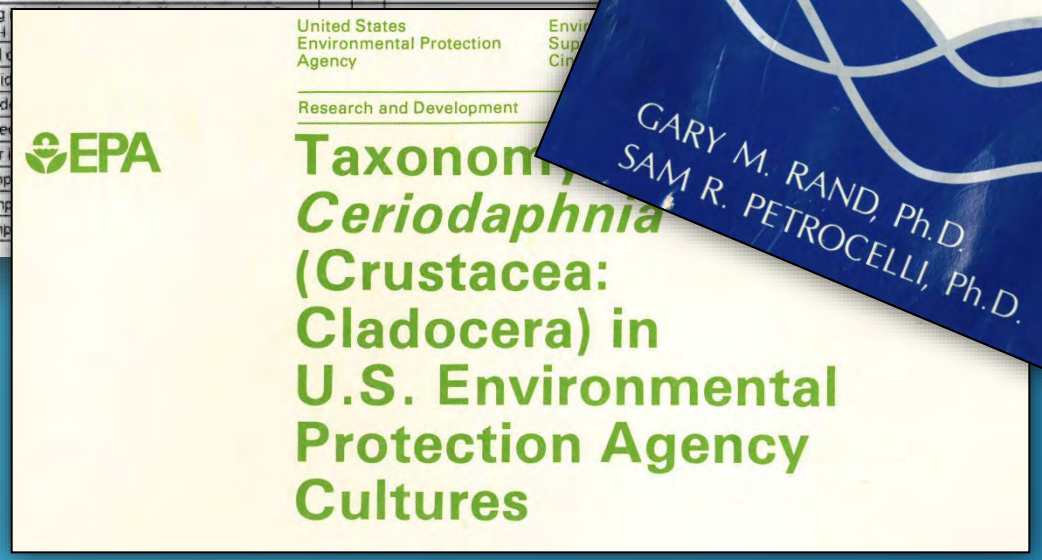
Parameter: **Dissolved Oxygen**
Method: **SM 4500 O H-2016, Hach 10360, Rev.1.2, October 2011, ASTM Method D888-12 (C) and In Situ Method 1002-8-200**

Equipment:

	DO Meter **Model:**		Conductivity meter (if applicable) **Model:**

PLEASE COMPLETE CHECKLIST IN INDELIBLE INK
Please mark Y, N or NA in the column labeled LAB to indicate the common lab practice and in the column labeled SOP to indicate whether it is addressed in the SOP.

	GENERAL	LAB	SOP	EXPLANATION
1	Is the SOP reviewed at least every 2 years? What is the most recent review/revision date of the SOP? [15A NCAC 02H .0805 (g) (4)] **Date:**			
2	Are all revision dates and procedural edits tracked and documented? [15A NCAC 02H .0805 (g) (4)]			
3	Is there North Carolina data available for review?			
4	Are the following [15A NCAC 02H			
	The method			
	Laboratory id			
	Instrument id			
	Sample colle			
	Signature or			
	Date of samp			
	Time of samp			
	Date of samp			

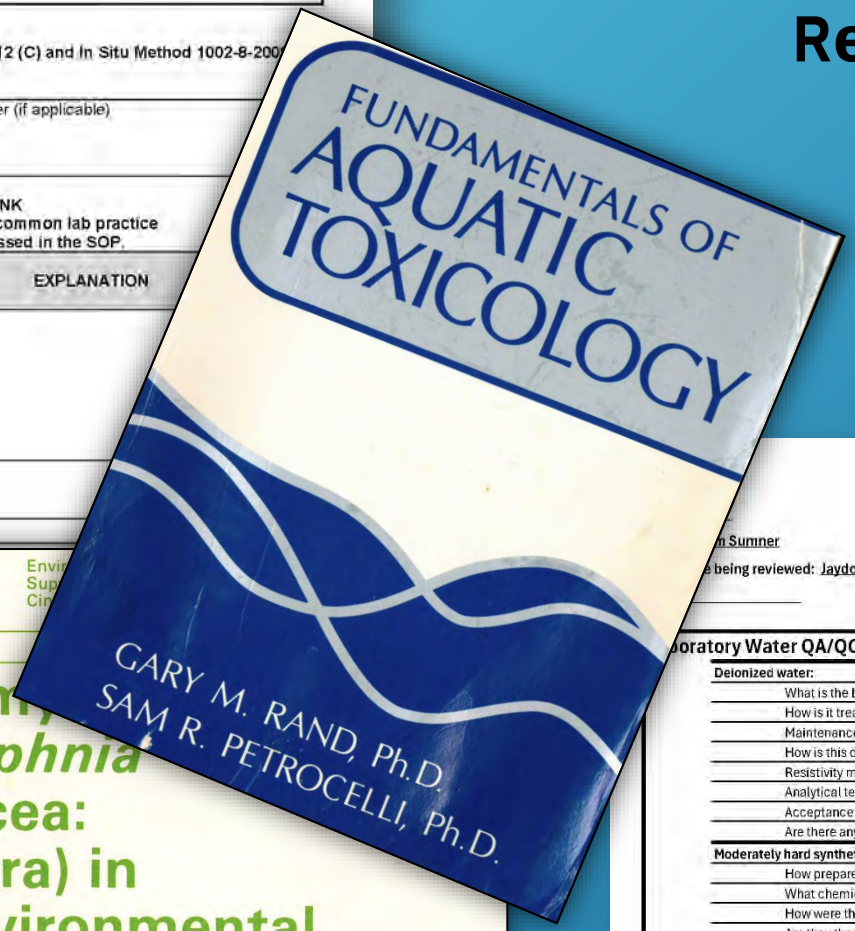


United States Environmental Protection Agency

Research and Development

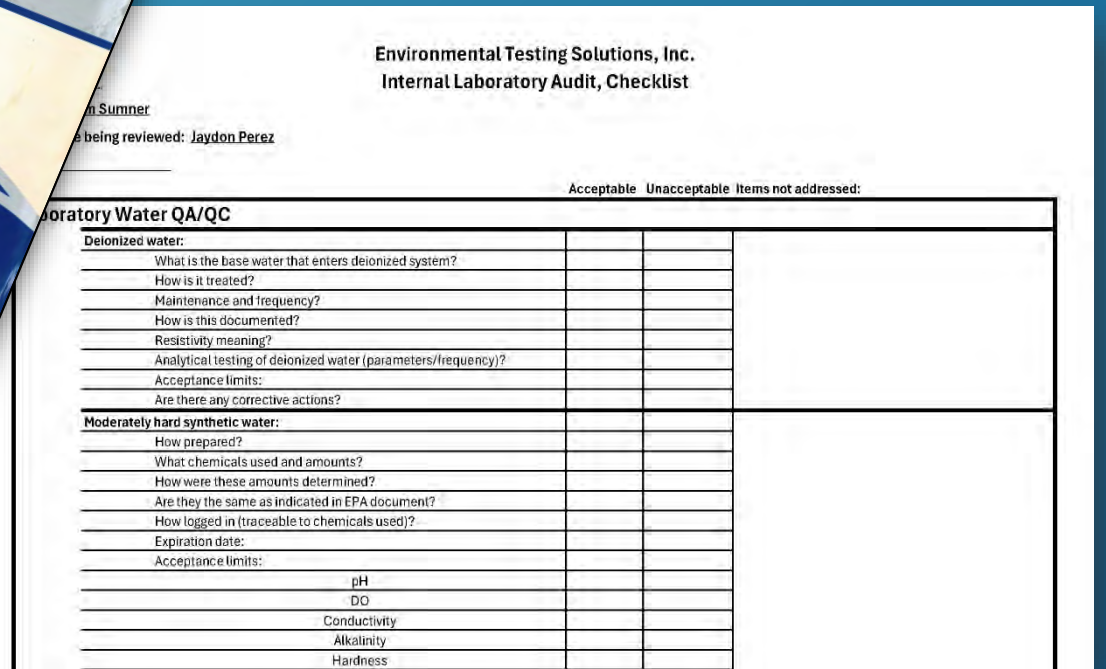
EPA

Taxonomy of *Ceriodaphnia* (Crustacea: Cladocera) in U.S. Environmental Protection Agency Cultures



FUNDAMENTALS OF AQUATIC TOXICOLOGY

GARY M. RAND, Ph.D.
SAM R. PETROCELLI, Ph.D.



Environmental Testing Solutions, Inc.
Internal Laboratory Audit, Checklist

n Sumner

e being reviewed: Jaydon Perez

	Acceptable	Unacceptable	Items not addressed:
oratory Water QA/QC			
Deionized water:			
What is the base water that enters deionized system?			
How is it treated?			
Maintenance and frequency?			
How is this documented?			
Resistivity meaning?			
Analytical testing of deionized water (parameters/frequency)?			
Acceptance limits:			
Are there any corrective actions?			
Moderately hard synthetic water:			
How prepared?			
What chemicals used and amounts?			
How were these amounts determined?			
Are they the same as indicated in EPA document?			
How logged in (traceable to chemicals used)?			
Expiration date:			
Acceptance limits:			
pH			
DO			
Conductivity			
Alkalinity			
Hardness			

SCARS or “Pink Forms”

ETS
Environmental Testing Solutions, Inc.

Surveillance and Corrective Action Report (SCAR)

Date:		Surveillance performed by:	
Logbook:		Analysis performed by:	
		Date:	

Type of corrective action needed:

☐	Date missing	☐	Not calculated correctly
☐	Time missing	☐	LCS not calculated
☐	Sample identification missing	☐	Duplicate not calculated
☐	Data “write over”	☐	Blank missing
☐	Cross-out was not initialed	☐	QC out of range (footnote)
☐	All blank lines are not crossed through and initialed		
☐	Other: _____		

Correction(s) performed by:	
Date of correction(s):	

Explanation of corrective action: _____

_____

Corrective action taken to prevent reoccurrence: _____

_____

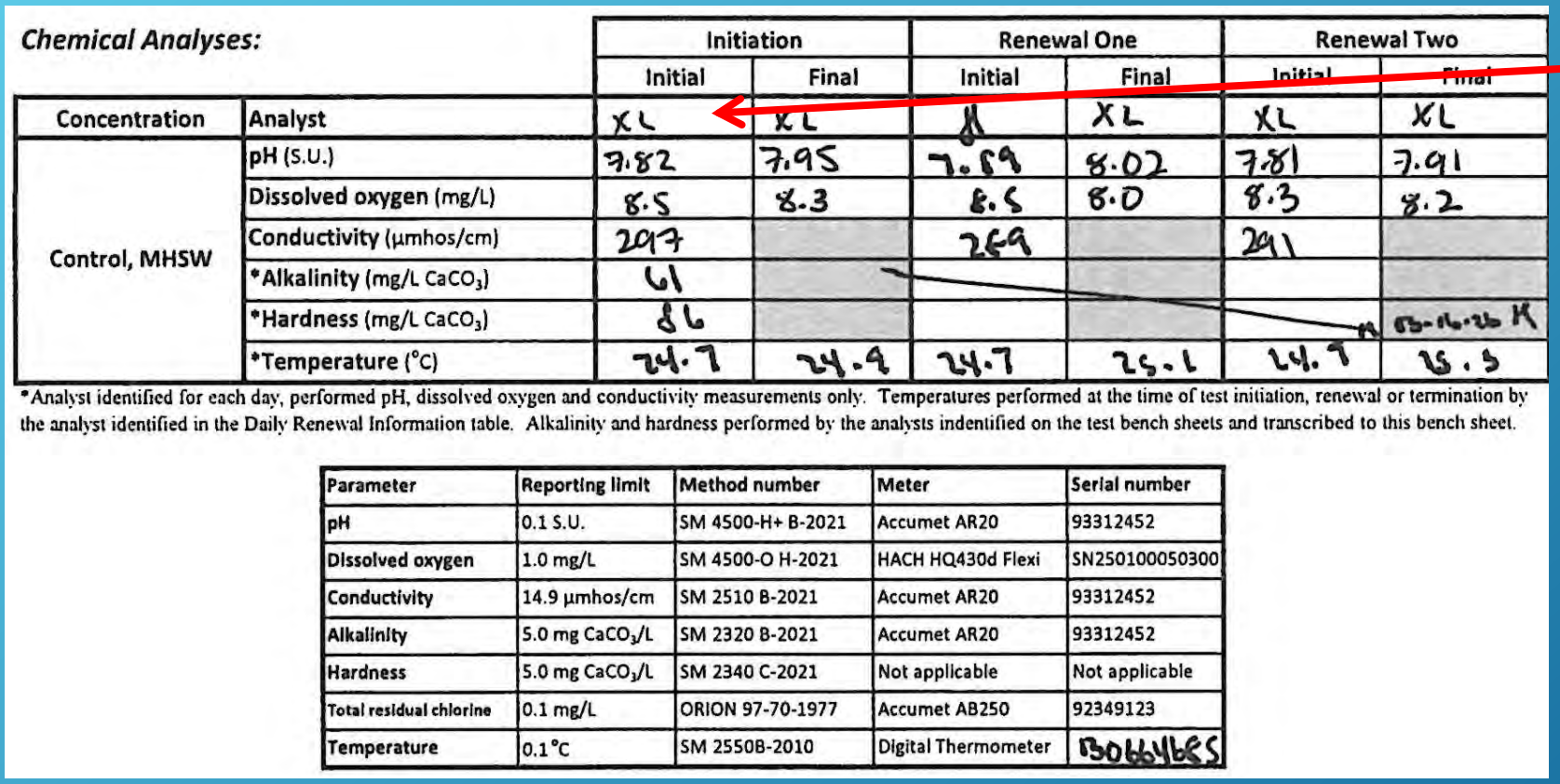
QAP Q3 – Exhibit Q3.1, revision 06-20-12

Traceability

- Everything must be traceable to QC documentation
  - chemical analyses performed
  - source of organisms used in testing
  - water used for diluting samples
  - food
- Training documentation
- Calibrations
- SOP’s

Chemical Analyses

- What analyst performed chemical analyses?
- What method used?
- What instrument was used?



Chemical Analyses:

Concentration	Analyst	Initiation Initial	Initiation Final	Renewal One Initial	Renewal One Final	Renewal Two Initial	Renewal Two Final
Control, MHSW		XL	XL	JL	XL	XL	XL
	pH (S.U.)	7.82	7.95	7.89	8.02	7.81	7.91
	Dissolved oxygen (mg/L)	8.5	8.3	8.5	8.0	8.3	8.2
	Conductivity (µmhos/cm)	297		269		291	
	*Alkalinity (mg/L $CaCO_3$)	61					
	*Hardness (mg/L $CaCO_3$)	86					03-16-26 KM
	*Temperature (°C)	24.7	24.9	24.7	25.1	24.7	25.3

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table. Alkalinity and hardness performed by the analysts indentified on the test bench sheets and transcribed to this bench sheet.

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Total residual chlorine	0.1 mg/L	ORION 97-70-1977	Accumet AB250	92349123
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	150664565

Page 1 of 1

North Carolina Chronic Pass/Fail Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*
(EPA-821-R-02-013 Method 1002.0, NC Modification – December 2010, Version 3.0) - Control Bench Sheet

Control #: 2 Date: 03-11-26

Test Grouping Information:

Facility	Project #
6	
5 WAYNESVILLE	21374
4 TRYON	21373
3 MARSHALL 002	21381
2 FRANKLIN WWTP	21378
1 - Control	

Test Organism Information:

Organism Source:	In-house Culture
Age:	< 24-hours old
Source (culture board):	03-03-26 B
Replicate #	1 2 3 4 5 6 7 8 9 10 11 12
Culture board cup #	7 7 8 9 10 11 12 15 17 18 22 23
Date and time organisms were born between:	03-11-26 0505 TO 1013
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.18
	Temperature (°C): 25.0

Daily Renewal Information:

Day	Date	Test Initiation, renewal, *feeding or termination Time	Analyst	MHSW Batch	Selenastrum Batch	YWT Batch	Location Incubator/Shelf	Randomizing Template
0	03-11-26	Initiation/Feeding 1245	JL	03-03-26B	03-06-26	03-06-26	281	GREEN
1	03-12-26	Feeding 0818	JL					
2	03-13-26	Renewal 1/Feeding 0850	JL	03-03-26B				
3	03-14-26	Feeding 0920	WN					
4	03-15-26	Feeding 0930	WN					
5	03-16-26	Renewal 2/Feeding 0834	JL	03-03-26B				
6	03-17-26	Feeding 0745	JL					
7	03-18-26	Termination 0633	JL					

*Organisms fed daily 50 µL NC *Selenastrum* and 50 µL YWT per replicate using HandyStep repeat pipettor SN 17E59354.

Chemical Analyses:

Concentration	Analyst	Initiation Initial	Initiation Final	Renewal One Initial	Renewal One Final	Renewal Two Initial	Renewal Two Final
Control, MHSW		XL	XL	JL	XL	XL	XL
	pH (S.U.)	7.82	7.95	7.89	8.02	7.81	7.91
	Dissolved oxygen (mg/L)	8.5	8.3	8.5	8.0	8.3	8.2
	Conductivity (µmhos/cm)	297		269		291	
	*Alkalinity (mg/L $CaCO_3$)	61					
	*Hardness (mg/L $CaCO_3$)	86					03-16-26 KM
	*Temperature (°C)	24.7	24.9	24.7	25.1	24.7	25.3

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table. Alkalinity and hardness performed by the analysts indentified on the test bench sheets and transcribed to this bench sheet.

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Total residual chlorine	0.1 mg/L	ORION 97-70-1977	Accumet AB250	92349123
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	150664565

Survival and Reproduction Data:

Day	Observations	Replicate number 1	2	3	4	5	6	7	8	9	10	11	12
2 Renewal One	Adult mortality (L = Live, D = dead)	L	L	L	L	L	L	L	L	L	L	L	L
5 Renewal Two	Number of broods present	2	2	2	2	2	2	2	2	2	2	2	2
	Number of young produced	17	19	17	19	14	14	19	16	17	16	16	20
	Adult mortality (L = Live, D = dead)	L	L	L	L	L	L	L	L	L	L	L	L
7 Final	Number of broods present	1	1	1	1	1	1	1	1	1	1	1	1
	Number of young produced	12	14	14	14	16	17	12	17	15	18	15	14
	Total young produced	29	33	31	33	30	31	31	33	32	34	31	34
	Final adult mortality (L = Live, D = dead)	L	L	L	L	L	L	L	L	L	L	L	L
	X for 3rd Broods	X	X	X	X	X	X	X	X	X	X	X	X

Control Acceptance Criteria:

% of Male Adults (≤ 20%)	0%
% Adults having 3rd Broods (≥ 80%)	100%
% Mortality (≤ 20%)	0%

Mean Offspring/Female (≥ 15 offspring/surviving female)	31.8
% CV (< 40%)	5.0%

SOP AT12-Revision 4-Exhibit AT12.3

Comparison of analyst's handwriting

Analyst	XL	XL
pH (S.U.)	7.82	7.95
Dissolved oxygen (mg/L)	8.5	8.3
Conductivity (µmhos/cm)	297	
*Alkalinity (mg/L $CaCO_3$)	61	
*Hardness (mg/L $CaCO_3$)	86	
*Temperature (°C)	24.7	24.9

ETS Environmental Testing Solutions, Inc.

Corporate Policies

SECTION	POLICY-P4
REVISION NUMBER	2
EFFECTIVE DATE	10-13-20
PAGE NUMBER	2 OF 2

Subject: Employee Handwriting Sample

Printed name	Signature	Initials	Date	Time
Xiaoman Lancaster	Xiaoman Lancaster	XL	09-20-25	0921

Numeric examples:

0	1	2	3	4
0	1	2	3	4

5	6	7	8	9
5	6	7	8	9

Alphabetic examples:

A	B	C	D	E
A	B	C	D	E

F	G	H	I	J
F	G	H	I	J

K	L	M	N	O
K	L	M	N	O

P	Q	R	S	T
P	Q	R	S	T

U	V	W	X	Y
U	V	W	X	Y

Z
Z

Special characters/units:

✓	%	8+	2B	°C
✓	%	8+	2B	°C

Toxicity chemistry examples:

Analyst		XL
pH (SU)	7.87	7.87
DO (mg/L)	8.0	8.0
Conductivity (µmhos/cm)	487	487
Alkalinity (mg/L $CaCO_3$)	92	92
Hardness (mg/L $CaCO_3$)	63	63
Chlorine (mg/L)	<0.10	<0.10
Temperature (°C)	25.5	25.5
pH (SU)	7.88	7.88
DO (mg/L)	8.8	8.8
Conductivity (µmhos/cm)	465	465
Alkalinity (mg/L $CaCO_3$)	99	99
Hardness (mg/L $CaCO_3$)	80	80
Chlorine (mg/L)	<0.10	<0.10
Temperature (°C)	25.2	25.2

Correct value and make 7.85	~~7.89~~ 7.85 XL 09-20-25

Writing example (cursive and/or print):

The quick brown fox jumps over the lazy dog.

The quick brown fox jumps over the lazy dog.

Did the analyst receive training prior to performing this test?

Analyst	XL	XL
pH (S.U.)	7.82	7.95
Dissolved oxygen (mg/L)	8.5	8.3
Conductivity (µmhos/cm)	297	
*Alkalinity (mg/L $CaCO_3$)	61	
*Hardness (mg/L $CaCO_3$)	86	
*Temperature (°C)	24.7	24.9

ETS
Environmental Testing Solutions, Inc.

Chemistry Procedures

SECTION	SOP-C2
REVISION NUMBER	9
EFFECTIVE DATE	05-01-25
PAGE	1 OF 1

Subject: Dissolved Oxygen (SM 4500-O H-2021)

Approval

By signing below, the Laboratory Supervisor and Quality Assurance Officer have read and approved the referenced procedure.

Title	Name	Signature	Date
Laboratory Supervisor	Kelley E. Keenan		05-01-25
Quality Assurance Officer	Jim Sumner		05-01-25

Employee Training Documentation

The employee will print, sign and date the trainee section for the referenced procedure after (1) the applicable procedure has been read and understood and (2) after training has been received by an approved trainer, laboratory supervisor or quality assurance officer. In addition, acceptable results of a performance evaluation sample or IDC must be obtained (if applicable, as described in QAP-Q5). Failure to adhere or comply with laboratory procedures may be grounds for immediate termination of employment.

Trainee (By signing below, the trainee has Read, Understood, and Will Comply with the referenced procedure.)			Trainer		
Printed name	Signature	Date	Printed name	Signature	Date
[illegible]	[signature]	05-09-25	JSUMNER		05-09-25
Jayson Perez	[signature]	~~05-20-25~~ 05-21-25	JSUMNER		05-21-25
Evan Montfort	[signature]	05-21-25	JSUMNER		05-21-25

Trainer Approval by Laboratory Supervisor or Quality Assurance Officer

The employee will print, sign and date the trainer section for the referenced procedure after the laboratory supervisor or quality assurance officer has determined the employee is proficient and experienced in performing the referenced procedure (as indicated in QAP Q2) and is able to effectively explain and demonstrate all requirements of the referenced procedure.

Trainer (By signing below, the trainer will uphold all requirements and expectations of the laboratory supervisor in training employees.)			Laboratory Supervisor or Quality Assurance Officer		
Printed name	Signature	Date	Printed name	Signature	Date

Did the analyst pass a blind study?

Analyst	XL
pH (S.U.)	7.82
Dissolved oxygen (mg/L)	8.5
Conductivity (µmhos/cm)	207
*Alkalinity (mg/L $CaCO_3$)	61
*Hardness (mg/L $CaCO_3$)	86
*Temperature (°C)	24.7

Ongoing Demonstration of Capability (ODC)

Analyte:	Conductivity, SM 2510 B-2021
Analyst:	X. Lancaster
Date analyzed:	01-09-26
Standard conc (µmhos/cm):	2000.0

Replicate number	Measured value (µmhos/cm)	%RS	Acceptance Limits
1	1990.0	**99.5**	80 - 120
2	2000.0	**100.0**	80 - 120
3	2000.0	**100.0**	80 - 120
4	2010.0	**100.5**	80 - 120

%RSD	0.41	Acceptance Limits < 15%

Note: %RS (reference standard recovery) = measured value / true value X 100
%RSD (relative standard deviation) = standard deviation / mean X 100

Did the analyst pass an audit checklist or written test?

Analyst	XL	XL
pH (S.U.)	7.82	7.95
Dissolved oxygen (mg/L)	8.5	8.3
Conductivity (µmhos/cm)	297	
*Alkalinity (mg/L $CaCO_3$)	61	
*Hardness (mg/L $CaCO_3$)	86	
*Temperature (°C)	24.7	24.9

NC DEQ/DWR WASTEWATER/GROUNDWATER LABORATORY CERTIFICATION BRANCH

LABORATORY NAME:	Environmental Testing Solutions Inc.	CERT #:	600
PRIMARY ANALYST:	XL	DATE:	030226
NAME OF PERSON COMPLETING CHECKLIST (PRINT):	Xiao man Lancaster		
SIGNATURE OF PERSON COMPLETING CHECKLIST:	Xiaoman Lancaster		

Parameter: **Dissolved Oxygen**

Method: **SM 4500 O H-2016, Hach 10360, Rev.1.2, October 2011, ASTM Method D888-12 (C) and In Situ Method 1002-8-2009**

Equipment:

	DO Meter **Model:** Hach HQ430d Flqi		Conductivity meter (if applicable) **Model:** NA

PASS

PLEASE COMPLETE CHECKLIST IN INDELIBLE INK
Please mark Y, N or NA in the column labeled LAB to indicate the common lab practice and in the column labeled SOP to indicate whether it is addressed in the SOP.

	GENERAL	LAB	SOP	EXPLANATION
1	Is the SOP reviewed at least every 2 years? What is the most recent review/revision date of the SOP? [15A NCAC 02H .0805 (g) (4)] **Date:** 05-01-25		Y	Jim revises & prints new date & everyone signs off again.
2	Are all revision dates and procedural edits tracked and documented? [15A NCAC 02H .0805 (g) (4)]		Y	In front page of SOP section?
3	Is there North Carolina data available for review?	Y		
4	Are the following items documented with each analysis? [15A NCAC 02H .0805 (g) (2)]	Y	Y	benchsheet? where to find?
	The method or SOP reference			on logbook What is method?
	Laboratory identification			on logbook
	Instrument identification			on logbook
	Sample collector			on COC
	Signature or initials of the analyst			on COC & logbook
	Date of sample collection			on COC & in logbook
	Time of sample collection			on COC
	Date of sample analysis			on logbook
	Time of sample analysis			only on daily calibration logbook if first thing calibrated. In "time" box
	Sample identification			on COC & logbook
	Proper units of measure			on logbook What are units
	Final value to be reported			on logbook
	Facility name or permit number [NC WW/GW LCB Approved Procedure for the Analysis of Dissolved Oxygen (DO)]			on COC & logbook
	Parameter analyzed [NC WW/GW LCB Approved Procedure for the Analysis of Dissolved Oxygen (DO)]			logbook or certificaton sheet
	Conductivity calibration standard and check standard values and check standard evaluation [NC WW/GW LCB Approved Procedure for the Analysis of Dissolved Oxygen (DO)]			NA
	Meter calibration and/or verification date and time(s) [NC WW/GW LCB Approved Procedure for the Analysis of Dissolved Oxygen (DO)]	Y	Y	In daily calibration logbook
	DO Meter Calibration variables (temperature, elevation or barometric pressure [in mmHg], and salinity) [NC WW/GW LCB Approved Procedure for the Analysis of Dissolved Oxygen (DO)]	Y	Y	In daily calibration book & correction factor table

Revised 11/15/2023

Serial numbers identified for balances used in weight determinations, pipettes used for feeding and meters used for chemical analyses

Method numbers identified for chemical analysis

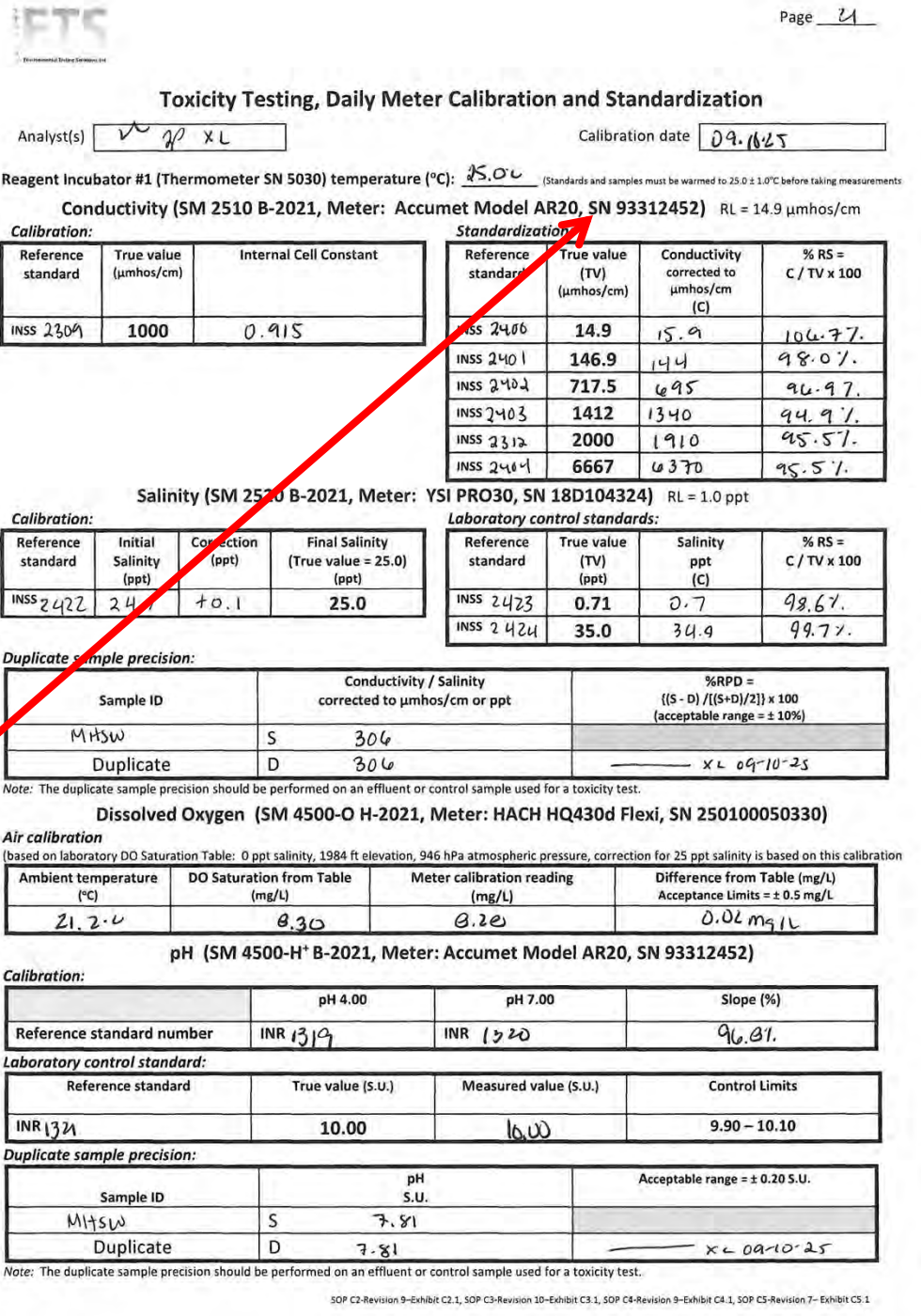
Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Total residual chlorine	0.1 mg/L	ORION 97-70-1977	Accumet AB250	92349123
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	[illegible]

Each serial number must trace back to calibrations

Standards used to calibrate within expiration dates?

How are standards logged into the laboratory?

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Total residual chlorine	0.1 mg/L	ORION 97-70-1977	Accumet AB250	92349123
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	B0664685



FTS

Page 21

Toxicity Testing, Daily Meter Calibration and Standardization

Analyst(s) XL Calibration date 09.1625

Reagent Incubator #1 (Thermometer SN 5030) temperature (°C): 25.0c (Standards and samples must be warmed to 25.0 ± 1.0°C before taking measurements

Conductivity (SM 2510 B-2021, Meter: Accumet Model AR20, SN 93312452) RL = 14.9 µmhos/cm

Calibration:

Reference standard	True value (µmhos/cm)	Internal Cell Constant
INSS 2309	1000	0.915

Standardization:

Reference standard	True value (TV) (µmhos/cm)	Conductivity corrected to µmhos/cm (C)	% RS = C / TV x 100
INSS 2406	14.9	15.9	106.77.
INSS 2401	146.9	144	98.0%.
INSS 2402	717.5	695	96.97.
INSS 2403	1412	1340	94.9%.
INSS 2312	2000	1910	95.5%.
INSS 2404	6667	6370	95.5%.

Salinity (SM 2520 B-2021, Meter: YSI PRO30, SN 18D104324) RL = 1.0 ppt

Calibration:

Reference standard	Initial Salinity (ppt)	Correction (ppt)	Final Salinity (True value = 25.0) (ppt)
INSS 2422	24.9	+0.1	25.0

Laboratory control standards:

Reference standard	True value (TV) (ppt)	Salinity ppt (C)	% RS = C / TV x 100
INSS 2423	0.71	0.7	98.6%.
INSS 2424	35.0	34.9	99.7%.

Duplicate sample precision:

Sample ID	Conductivity / Salinity corrected to µmhos/cm or ppt	%RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%)
MHSW	S 306	
Duplicate	D 306	—— XL 09-10-25

Note: The duplicate sample precision should be performed on an effluent or control sample used for a toxicity test.

Dissolved Oxygen (SM 4500-O H-2021, Meter: HACH HQ430d Flexi, SN 250100050330)

Air calibration

(based on laboratory DO Saturation Table: 0 ppt salinity, 1984 ft elevation, 946 hPa atmospheric pressure, correction for 25 ppt salinity is based on this calibration

Ambient temperature (°C)	DO Saturation from Table (mg/L)	Meter calibration reading (mg/L)	Difference from Table (mg/L) Acceptance Limits = ± 0.5 mg/L
21.2.c	8.30	8.20	0.01 mg/L

pH (SM 4500-H+ B-2021, Meter: Accumet Model AR20, SN 93312452)

Calibration:

	pH 4.00	pH 7.00	Slope (%)
Reference standard number	INR 1319	INR 1320	96.81.

Laboratory control standard:

Reference standard	True value (S.U.)	Measured value (S.U.)	Control Limits
INR 1321	10.00	10.00	9.90 – 10.10

Duplicate sample precision:

Sample ID	pH S.U.	Acceptable range = ± 0.20 S.U.
MHSW	S 7.81	
Duplicate	D 7.81	—— XL 09-10-25

Note: The duplicate sample precision should be performed on an effluent or control sample used for a toxicity test.

SOP C2-Revision 9–Exhibit C2.1, SOP C3-Revision 10–Exhibit C3.1, SOP C4-Revision 9–Exhibit C4.1, SOP C5-Revision 7– Exhibit C5.1

NC Certified Lab
Chemistry performed with Reference Test

Chemical and Physical Determinations:

		Test Start		1st Renewal		2nd Renewal	
		Start	End	Start	End	Start	End
pH	Control	8.21	8.13	8.12	7.94	8.03	8.12
	High Conc.	8.19	8.08	8.14	7.90	8.17	8.13
D.O.	Control	7.83	7.33	8.05	7.67	7.85	7.60
	High Conc.	7.88	7.46	7.85	7.43	7.89	7.52
Temp.	Control	24.7	25.6	24.3	25.5	24.6	25.2
	High Conc.	24.6	25.2	25.5	25.2	25.0	25.0

Conductivity of 1600 mg/L concentration: 3380

Analyst? Units? Meter used?
Can the data be traced?
Were test concentrations prepared accurately? Not able to properly validate data.
Minimum data analyzed

ETS: Chemistry for Same Test Type

Species: *Ceriodaphnia dubia* CdNaClCR #: 322

Daily Chemistry:

Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity and hardness performed by the analyst identified on the bench sheet specific for each analysis and transcribed to this bench sheet.

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	N	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	8.16	7.85	7.76	7.86	7.80	7.87
	Dissolved oxygen (mg/L)	8.2	8.1	8.4	8.2	8.4	8.1
	Conductivity (µmhos/cm)	293		306		298	
	Alkalinity (mg $CaCO_3$/L)	63				62	
	Hardness (mg $CaCO_3$/L)	86				88	
	Temperature (°C)	24.9	25.3	25.0	25.2	24.9	24.9
600 mg NaCl/L	pH (S.U.)	8.14	7.85	7.81	7.87	7.85	7.89
	Dissolved oxygen (mg/L)	8.2	8.1	8.4	8.2	8.4	8.1
	Conductivity (µmhos/cm)	1300		1360		1360	
	Temperature (°C)	25.0	25.0	25.0	24.9	24.9	25.1
800 mg NaCl/L	pH (S.U.)	8.13	7.86	7.84	7.87	7.86	7.89
	Dissolved oxygen (mg/L)	8.2	8.2	8.4	8.2	8.4	8.1
	Conductivity (µmhos/cm)	1650		1740		1770	
	Temperature (°C)	25.0	25.0	24.9	24.9	25.0	24.8
1000 mg NaCl/L	pH (S.U.)	8.12	7.85	7.84	7.89	7.87	7.90
	Dissolved oxygen (mg/L)	8.2	8.1	8.4	8.2	8.4	8.1
	Conductivity (µmhos/cm)	1960		2120		2100	
	Temperature (°C)	25.0	25.2	24.9	25.1	24.8	24.8
1200 mg NaCl/L	pH (S.U.)	8.11	7.86	7.85	7.89	7.89	7.90
	Dissolved oxygen (mg/L)	8.2	8.2	8.4	8.2	8.4	8.1
	Conductivity (µmhos/cm)	2300		2500		2490	
	Temperature (°C)	25.0	25.2	25.0	25.1	24.8	25.2
1400 mg NaCl/L	pH (S.U.)	8.10	7.86	7.86	7.89	7.89	7.93
	Dissolved oxygen (mg/L)	8.2	8.1	8.4	8.2	8.4	8.0
	Conductivity (µmhos/cm)	2620		2800		2810	
	Temperature (°C)	25.1	25.1	25.0	25.1	24.8	24.8
		Initial	Final	Initial	Final	Initial	Final

SOP AT14-Revision 6–Exhibit AT14.1

Species: *Ceriodaphnia dubia* CdNaClCR #: 322

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	XL	BSL	BSL	BSL	BSL	XL	XL	N
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.79	7.91	7.68	8.05	7.77	7.94	7.85	8.12
	Dissolved oxygen (mg/L)	8.4	8.2	8.6	8.1	8.5	8.2	8.4	7.7
	Conductivity (µmhos/cm)	302		290		281		298	
	Alkalinity (mg $CaCO_3$/L)			61					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	24.8	24.9	24.8	25.1	24.8	25.2	25.0	25.3
600 mg NaCl/L	pH (S.U.)	7.86	7.94	7.71	8.04	7.79	7.95	7.90	8.07
	Dissolved oxygen (mg/L)	8.4	8.3	8.6	8.1	8.4	8.2	8.4	7.7
	Conductivity (µmhos/cm)	1370		1290		1330		1350	
	Temperature (°C)	24.9	25.2	24.8	24.9	24.9	25.2	24.8	25.1
800 mg NaCl/L	pH (S.U.)	7.88	7.95	7.84	8.03	7.95	7.96	7.92	8.06
	Dissolved oxygen (mg/L)	8.4	8.2	8.5	8.1	8.4	8.2	8.4	7.7
	Conductivity (µmhos/cm)	1750		1460		1710		1770	
	Temperature (°C)	25.0	24.9	24.8	24.9	24.9	24.9	24.8	25.3
1000 mg NaCl/L	pH (S.U.)	7.89	7.95	7.84	8.02	7.98	7.97	7.94	8.06
	Dissolved oxygen (mg/L)	8.4	8.2	8.5	8.1	8.4	8.2	8.4	7.7
	Conductivity (µmhos/cm)	2080		2020		2060		2130	
	Temperature (°C)	25.0	24.9	24.9	24.9	25.0	25.1	24.9	25.4
1200 mg NaCl/L	pH (S.U.)	7.91	7.96	7.88	8.02	7.99	7.98	7.95	8.04
	Dissolved oxygen (mg/L)	8.4	8.2	8.5	8.0	8.4	8.2	8.4	7.7
	Conductivity (µmhos/cm)	2460		2340		2430		2480	
	Temperature (°C)	24.9	25.1	24.9	25.1	25.0	24.9	24.9	25.2
1400 mg NaCl/L	pH (S.U.)	7.91	7.96	7.89	8.01	8.00	7.98	7.96	8.04
	Dissolved oxygen (mg/L)	8.4	8.2	8.5	8.1	8.5	8.2	8.4	7.7
	Conductivity (µmhos/cm)	2830		2610		2770		2820	
	Temperature (°C)	24.9	24.9	24.8	25.1	25.0	24.9	24.9	25.2
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

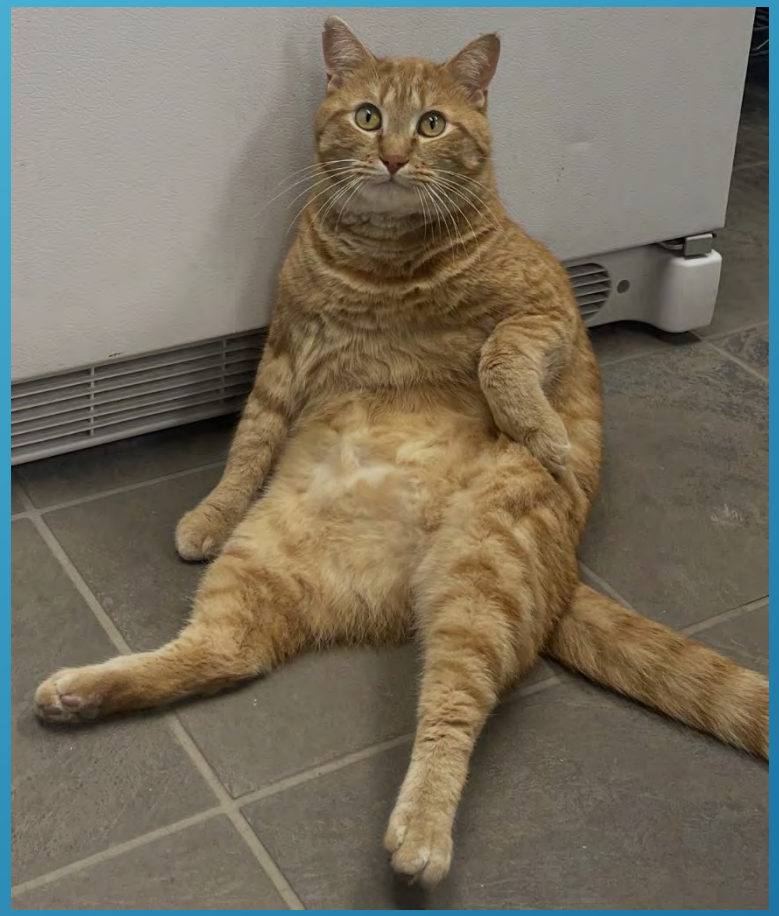
SOP AT14-Revision 6–Exhibit AT14.1

Questions?

Phone (828) 350-9364

Jim Sumner
jim@etsnclab.com

Jaydon Perez
jaydon@etsnclab.com



ETS
Environmental Testing Solutions, Inc.

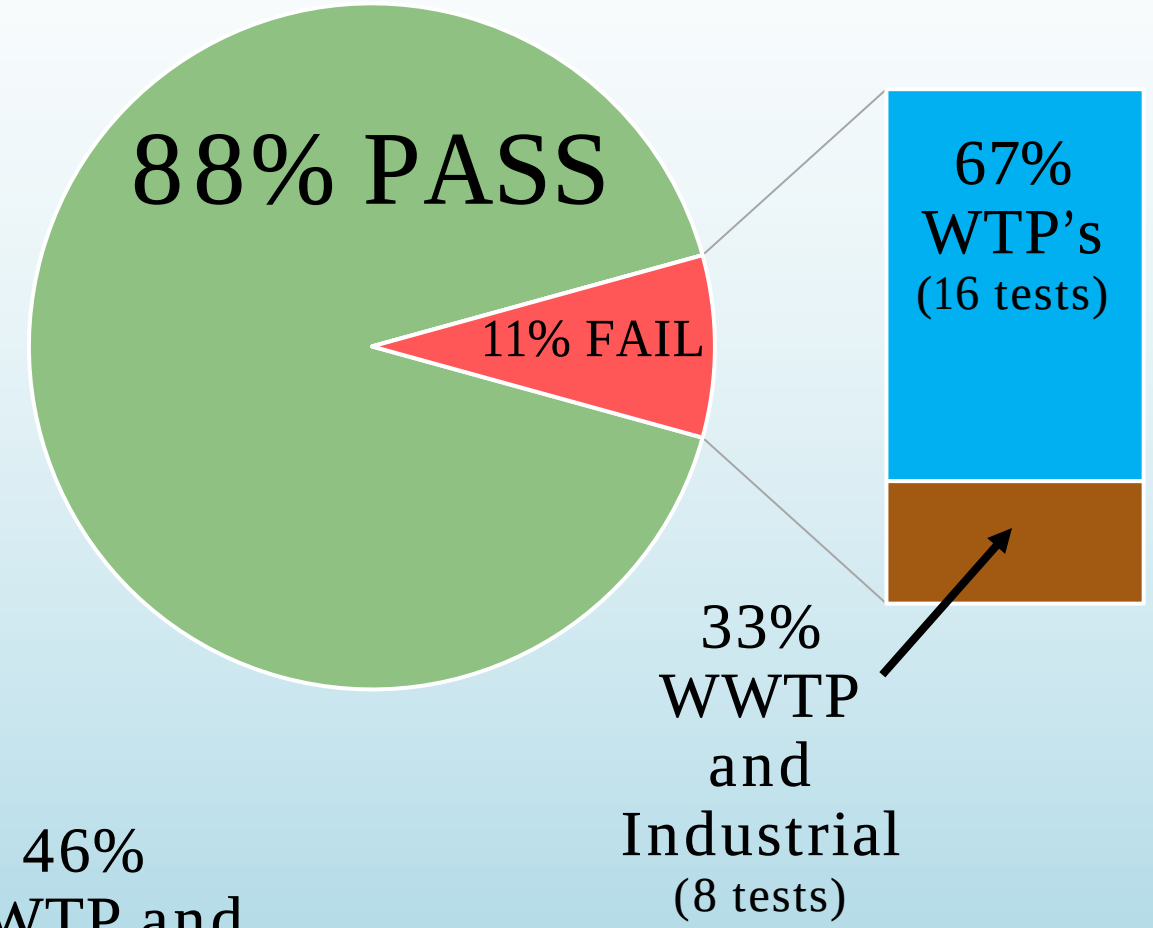
www.etsnclab.com

Test Failures

2025 Test Failures

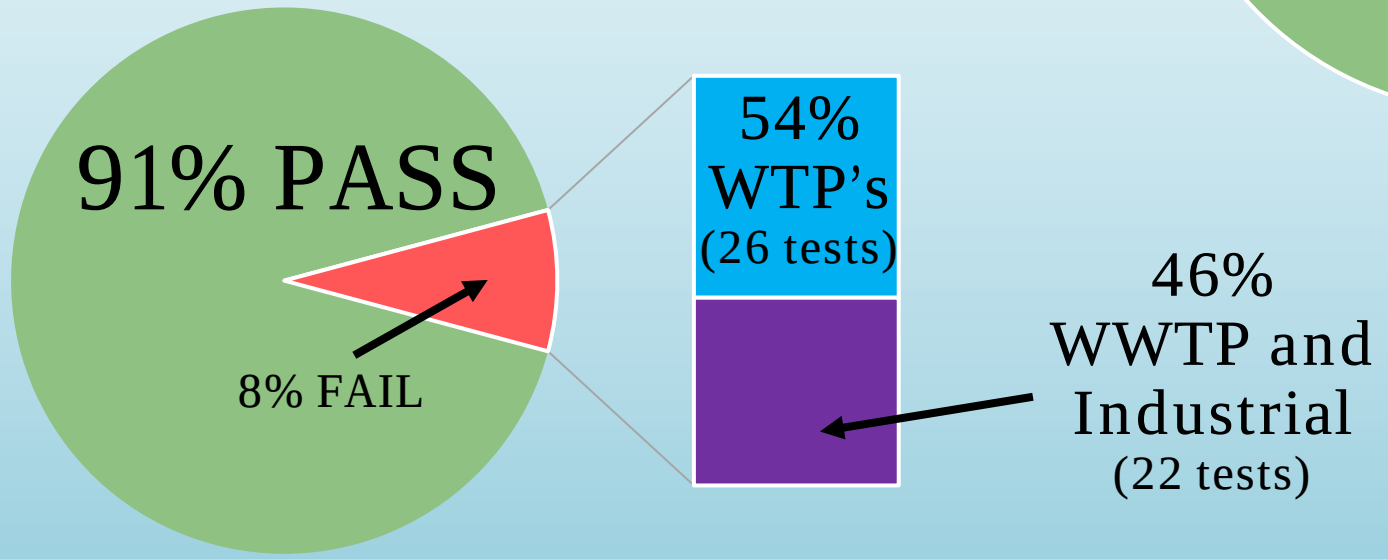
Pass/Fail Acute

(224 tests)



Ceriodaphnia Pass/Fail Chronic

(528 tests)



Conventional WTP's

Coagulation, flocculation and sedimentation followed by filtration and disinfection

Primary concerns:

- Chlorine (chloramine)
- Dechlorination
- Metals
- Polymers

Chlorine

Total residual chlorine < 100 ppb

Chloramine 12 ppb

Chemical Analyses:

Concentration	Analyst	Initial	Final
Control SSW	pH (S.U.)	7.75	7.43
	Dissolved oxygen (mg/L)	8.1	8.1
	Conductivity (µmhos/cm)	152	
	*Alkalinity (mg/L $CaCO_3$)	35	
	*Hardness (mg/L $CaCO_3$)	42	
	*Temperature (°C)	24.F	24.9
Test Concentration	pH (S.U.)	7.82	7.97
	Dissolved oxygen (mg/L)	7.8	8.1
	Conductivity (µmhos/cm)	4186	
	*Temperature (°C)	24.9	24.9
100%	pH (S.U.)	7.80	
	Dissolved oxygen (mg/L)	7.8	
	Conductivity (µmhos/cm)	530	
	*Total residual chlorine (mg/L)	0.621	

Test Organism Information:

Organism Source:	Aquatox, Inc.
Batch (ATOX Batch Pp):	07-07-14
Age (1 to 14 days old):	2 DAYS OLD
Date and time organisms were born between:	07-07-14 1600-1700
Average transfer volume:	0.2542 mL
Transfer bowl information:	pH (S.U.): 7.85
	Temperature (°C): 25.1

EPA loading requirement for freshwater species of < 0.40 g/L at 25.0°C has been documented by ETS to never be exceeded using 1 to 14 day old *P. promelas*.

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by the analyst performing the toxicity test. Alkalinity, hardness and total residual chlorine performed by the analysts identified on the test specific bench sheets and transcribed to this bench sheet.

Survival Data **(number of living organisms):**

Hours	Control				Test Concentration			
	Replicate				Replicate			
	A	B	C	D	E	F	G	H
0 Initiation	10	10	10	10	10	10	10	10
24 Termination	10	10	10	10	0 10d	0 10d	0 10d	0 10d
	Mean survival: 100%				Mean survival: 0%			

Comment codes: d = dead, u = unhealthy, bs = bent spines, s = stressed

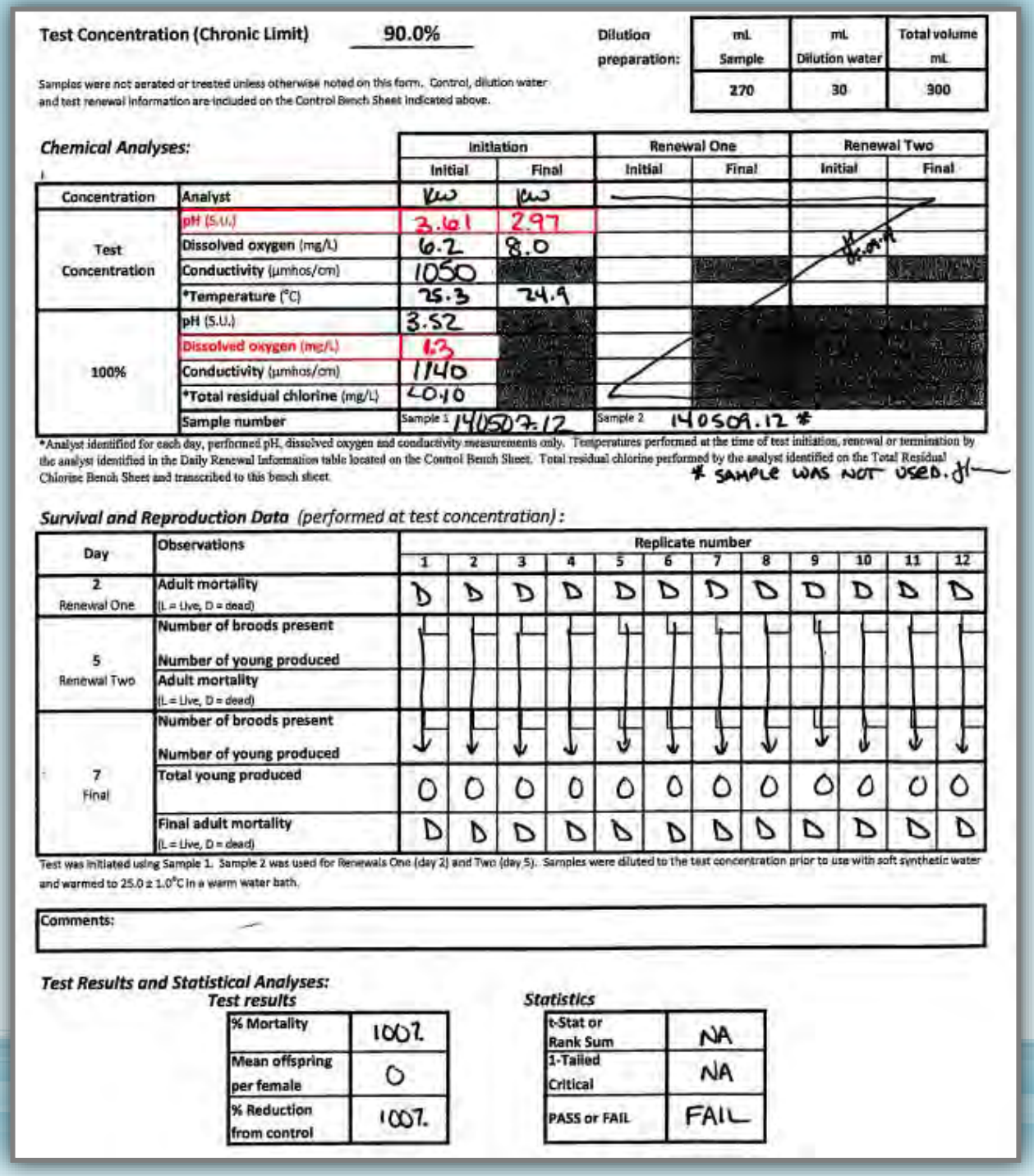
Statistics:

Method	VISUAL INSP
t-Stat or Rank Sum	NC
1-Tailed Critical	NC
PASS or FAIL	FAIL

Dechlorination

Dissolved oxygen
> 5.0 mg/L

Significant pH drop
6.0 – 9.0 S.U.



Test Concentration (Chronic Limit) 90.0%

Samples were not aerated or treated unless otherwise noted on this form. Control, dilution water and test renewal information are included on the Control Bench Sheet indicated above.

Dilution preparation:

mL Sample	mL Dilution water	Total volume mL
270	30	300

Chemical Analyses:

		Initiation		Renewal One		Renewal Two	
		Initial	Final	Initial	Final	Initial	Final
Concentration	Analyst	KW	KW				
Test Concentration	pH (S.U.)	3.61	2.97				
	Dissolved oxygen (mg/L)	6.2	8.0				
	Conductivity (µmhos/cm)	1050					
	*Temperature (°C)	25.3	24.9				
100%	pH (S.U.)	3.52					
	Dissolved oxygen (mg/L)	1.3					
	Conductivity (µmhos/cm)	1140					
	*Total residual chlorine (mg/L)	<0.10					
	Sample number	Sample 1 140507.12		Sample 2 140509.12 *			

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on the Control Bench Sheet. Total residual chlorine performed by the analyst identified on the Total Residual Chlorine Bench Sheet and transcribed to this bench sheet.

* SAMPLE WAS NOT USED. JH

***Survival and Reproduction Data** (performed at test concentration):*

Day	Observations	Replicate number											
		1	2	3	4	5	6	7	8	9	10	11	12
2 Renewal One	Adult mortality (L = Live, D = dead)	D	D	D	D	D	D	D	D	D	D	D	D
5 Renewal Two	Number of broods present	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
	Number of young produced	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
	Adult mortality (L = Live, D = dead)	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
7 Final	Number of broods present	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
	Number of young produced	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
	Total young produced	0	0	0	0	0	0	0	0	0	0	0	0
	Final adult mortality (L = Live, D = dead)	D	D	D	D	D	D	D	D	D	D	D	D

Test was initiated using Sample 1. Sample 2 was used for Renewals One (day 2) and Two (day 5). Samples were diluted to the test concentration prior to use with soft synthetic water and warmed to 25.0 ± 1.0°C in a warm water bath.

Comments:

Test Results and Statistical Analyses:

Test results

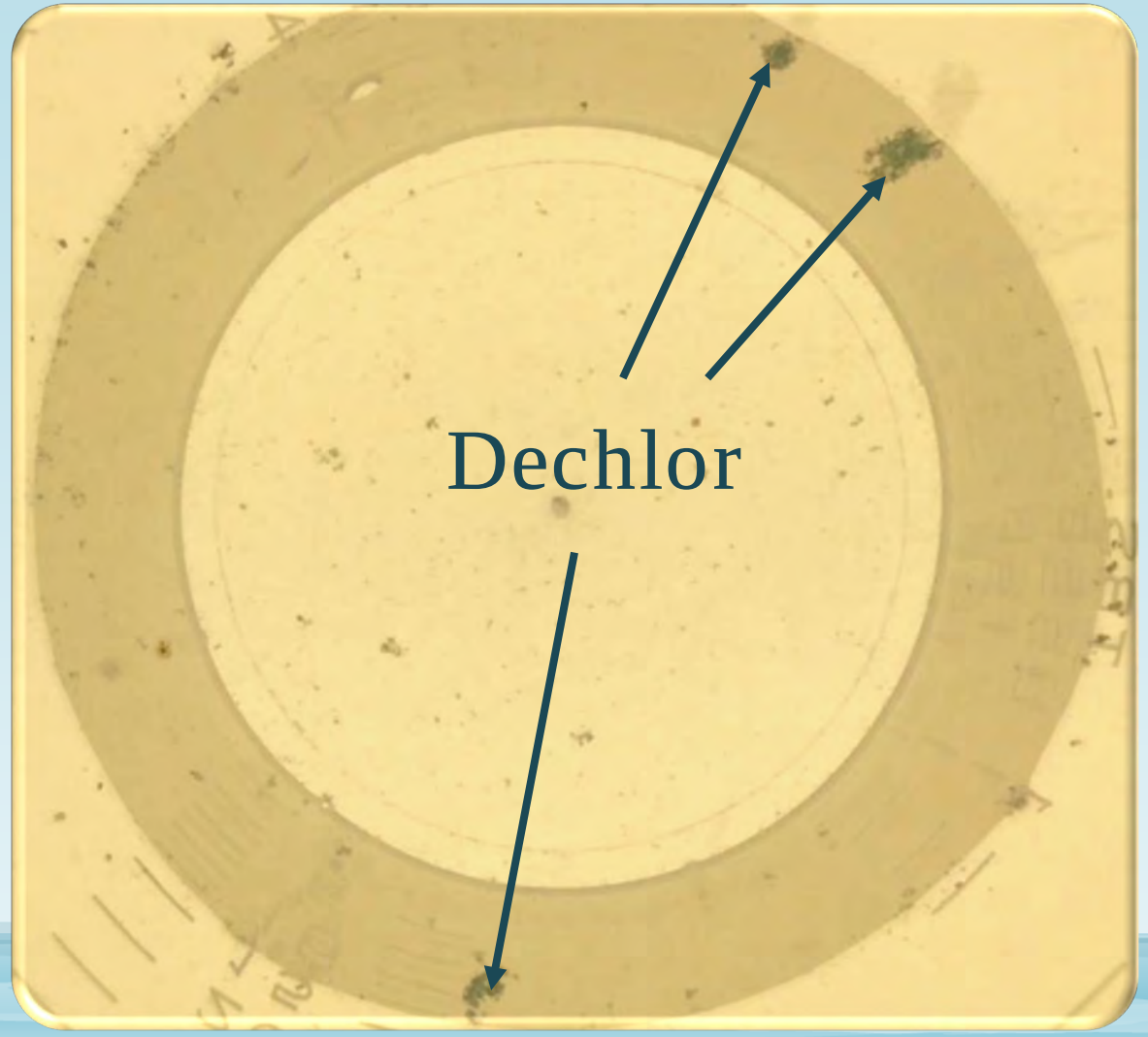
% Mortality	100%
Mean offspring per female	0
% Reduction from control	100%

Statistics

t-Stat or Rank Sum	NA
1-Tailed Critical	NA
PASS or FAIL	FAIL

Balance Chlorine to Dechlorination

Total Residual Chlorine (ppb)	Dechlor (ppb)
50	22
100	41
250	92
500	172
1000	302
1200	341



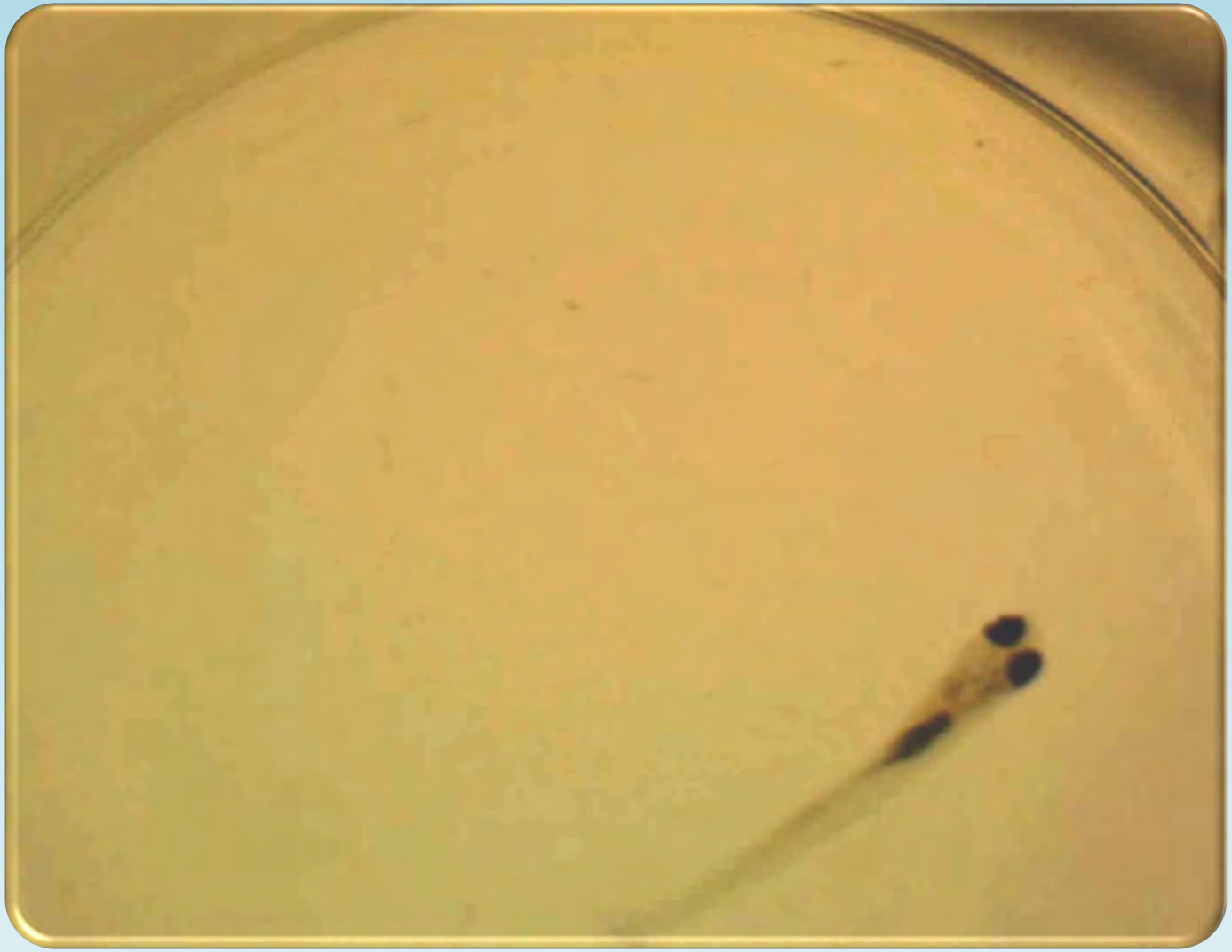
Metals

Sources:

Corrosion inhibitors from drinking water, cooling towers

Runoff from galvanized fences, metal roofs, painted surfaces

Pre-treatment dischargers to WWTP's



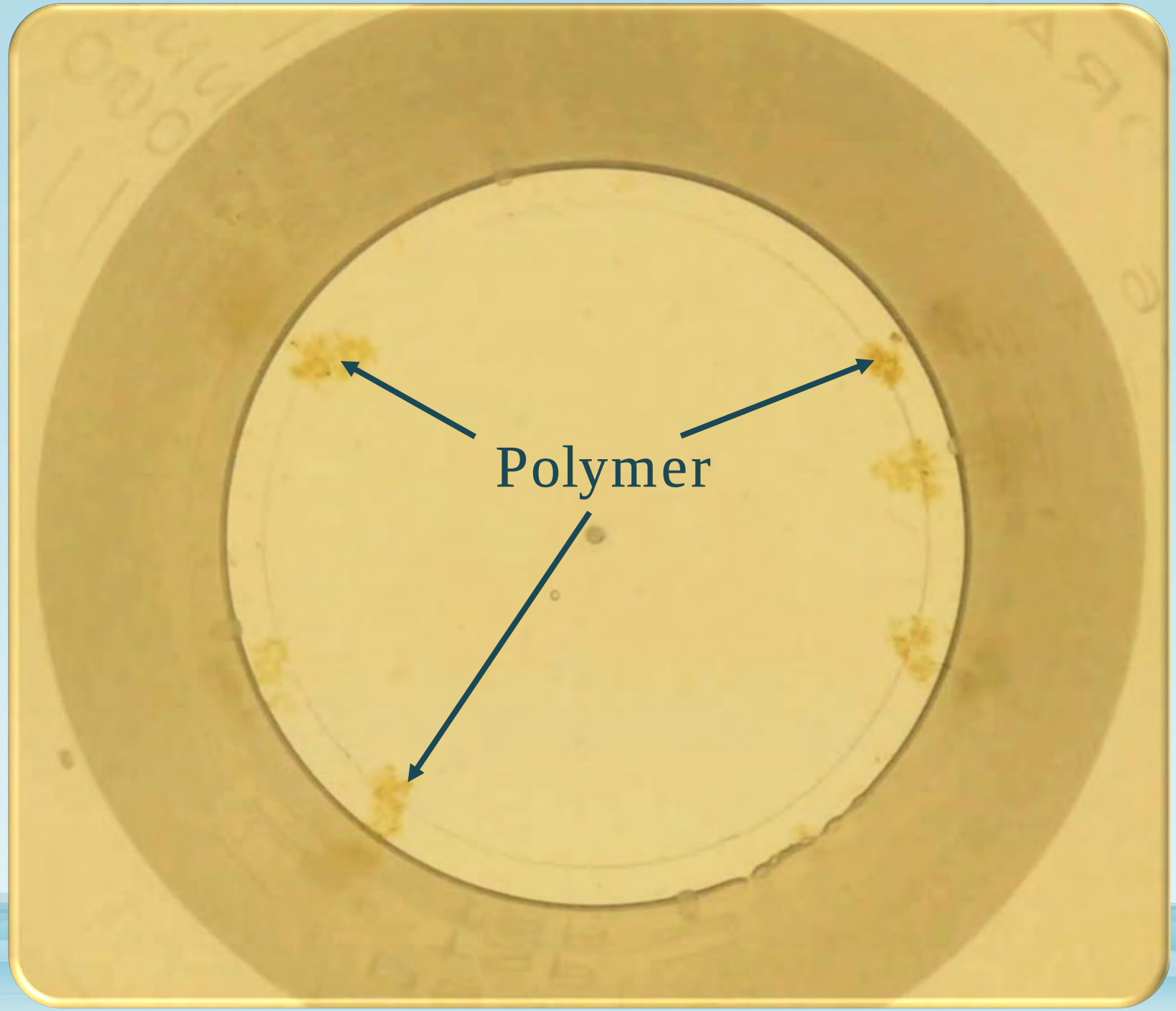
Bent spines in minnows indicates metal exposure

Polymers Differ Greatly

Effectiveness
in binding solids

Toxicity
at application rate

Unbound polymer
will precipitate
food in test cups



Ion Exchange and Membrane Technology WTP's

Ion Exchange: water softener technology
Membrane Technology: reverse osmosis and nanofiltration

Primary concerns:

- Ionic imbalances
- Chlorine
- Dechlorination

Ionic Imbalances with Mysid Shrimp

Effluent discharge differs in the composition of salts in comparison to sea water

Difference does not support the survival of Mysid shrimp

Can be determined by balancing major ions to concentrations found in sea water

Wastewater Treatment Plants

Primary concerns:

- Housekeeping
- Communication
- Industrial waste streams
- Chemical usage
- Other potential issues

Housekeeping / Communication

Know what your employees are doing!

What cleaners are they using?

Use of pesticides / herbicides around the treatment plant

Biocides for root control

External Impacts

Communicate with your town officials

Road deicing – what type?
How will elevated
chloride concentrations impact your
treatment plant?

What chemicals are the local WTP using?
Peroxides, amines, polymers,
corrosion inhibitors

Industrial Waste Streams

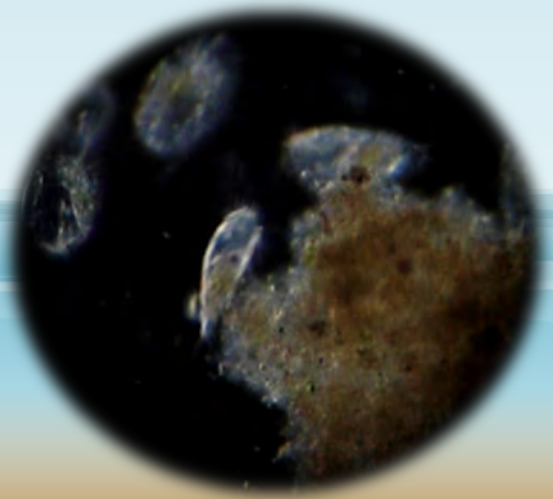
Visit pre-treatment facilities on a regular basis:

- Identify new processes
- Chemical usage and changes
- Potential contaminants in their waste stream

Enforce pre-treatment permit!

Benchtop Testing

Use benchtop tests to screen new industries or waste streams
- Change the abundance of organisms in sludge?
- Cause toxicity?
- Set limits on contaminants through pre-treatment program



Chemical Usage

Change in source or vendor may change the chemical form of the same product

Volume measurement must be adjusted

The lowest bidder may not be the best option

Purity, contamination, percent active ingredients?

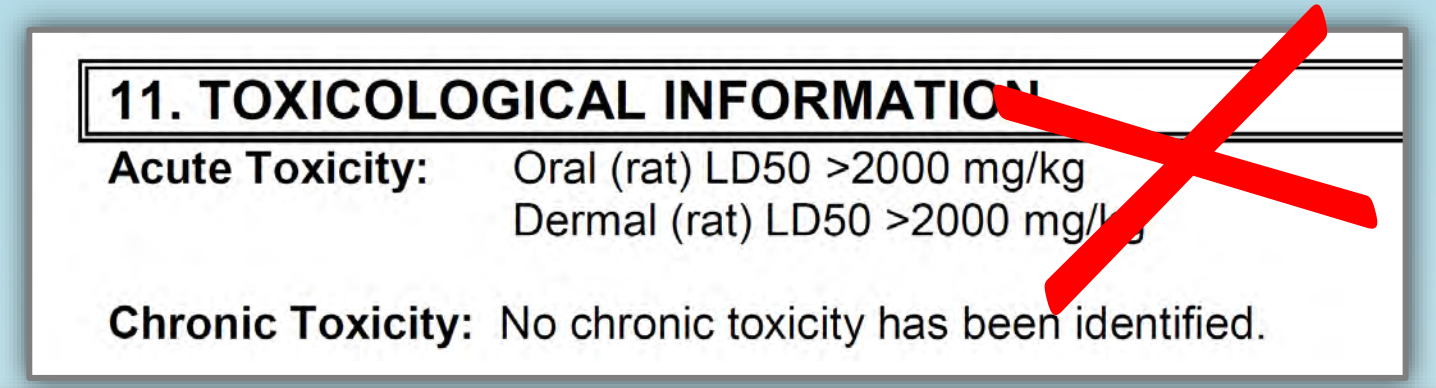
3. COMPOSITION/INFORMATION ON INGREDIENTS		
Synonyms	Polyaluminum chloride, solution	
Chemical Name	**CAS No**	**Weight-%**
Water	7732-18-5	55-85
Aluminum Chloride Hydroxide Sulfate	39290-78-3	15-45

Safety Data Sheets (SDS)

How much product can I use?

What section do I look at?

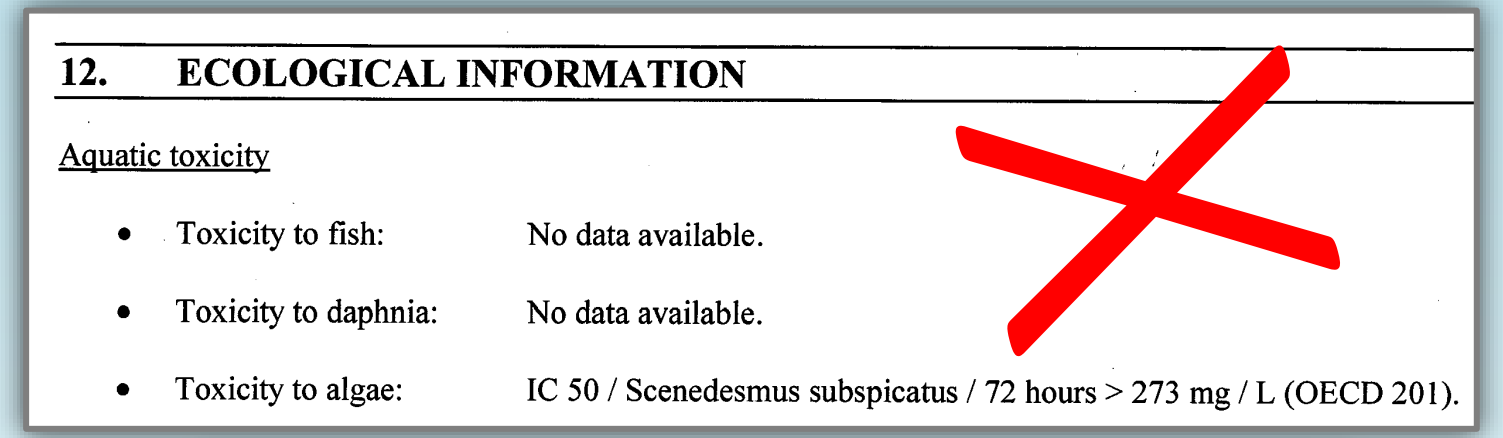
If there is no toxicity data, what do I do?



11. TOXICOLOGICAL INFORMATION

Acute Toxicity: Oral (rat) LD50 >2000 mg/kg
Dermal (rat) LD50 >2000 mg/kg

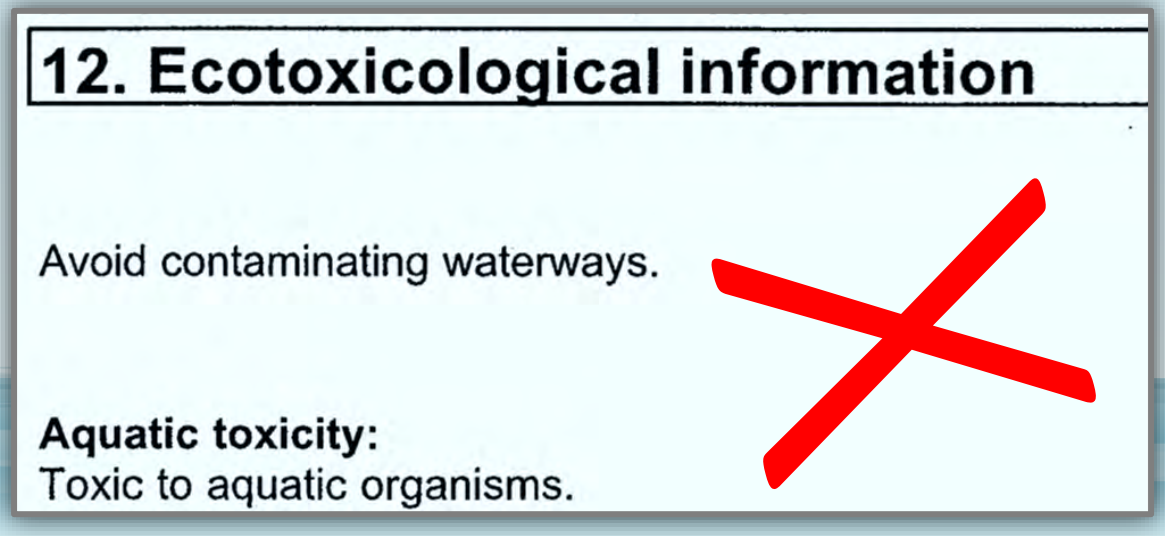
Chronic Toxicity: No chronic toxicity has been identified.



12. ECOLOGICAL INFORMATION

Aquatic toxicity

- Toxicity to fish: No data available.
- Toxicity to daphnia: No data available.
- Toxicity to algae: IC 50 / Scenedesmus subspicatus / 72 hours > 273 mg / L (OECD 201).



12. Ecotoxicological information

Avoid contaminating waterways.

Aquatic toxicity:
Toxic to aquatic organisms.

Safety Data Sheets (SDS)

12. ECOLOGICAL INFORMATION

Ecological Assessment
The ecological properties of this material have not been fully investigated.
All ecological information provided was conducted on a structurally similar product.

Duration: 48 hr. **Procedure:** Static.
Species: Fathead Minnow (Pimephales promelas)
354 ppm LC50

Duration: 96 hr
Species: Zebra Fish (Brachydanio rerio)
>1000 mg/l LC50

Duration: 24hr
Species: Coho Salmon (Oncorhynchus kisutch)
10 mg/l LC50

Duration: 48 hr **Procedure:** Static
Species: Water Flea (Ceriodaphnia dubia)
83 ppm LC50

Duration: 48 hr
Species: Water Flea (Daphnia magna)
98 mg/l EC50

12 Ecological information

AQUATIC TOXICOLOGY

Daphnia magna 48 Hour Static Acute Bioassay
LC50= 352; No Effect Level= 135 mg/L
Fathead Minnow 96 Hour Static Acute Bioassay
LC50= 465; No Effect Level= 100 mg/L

BIODEGRADATION

COD (mg/g): 1120
TOC (mg/g): 450

Other Potential Issues

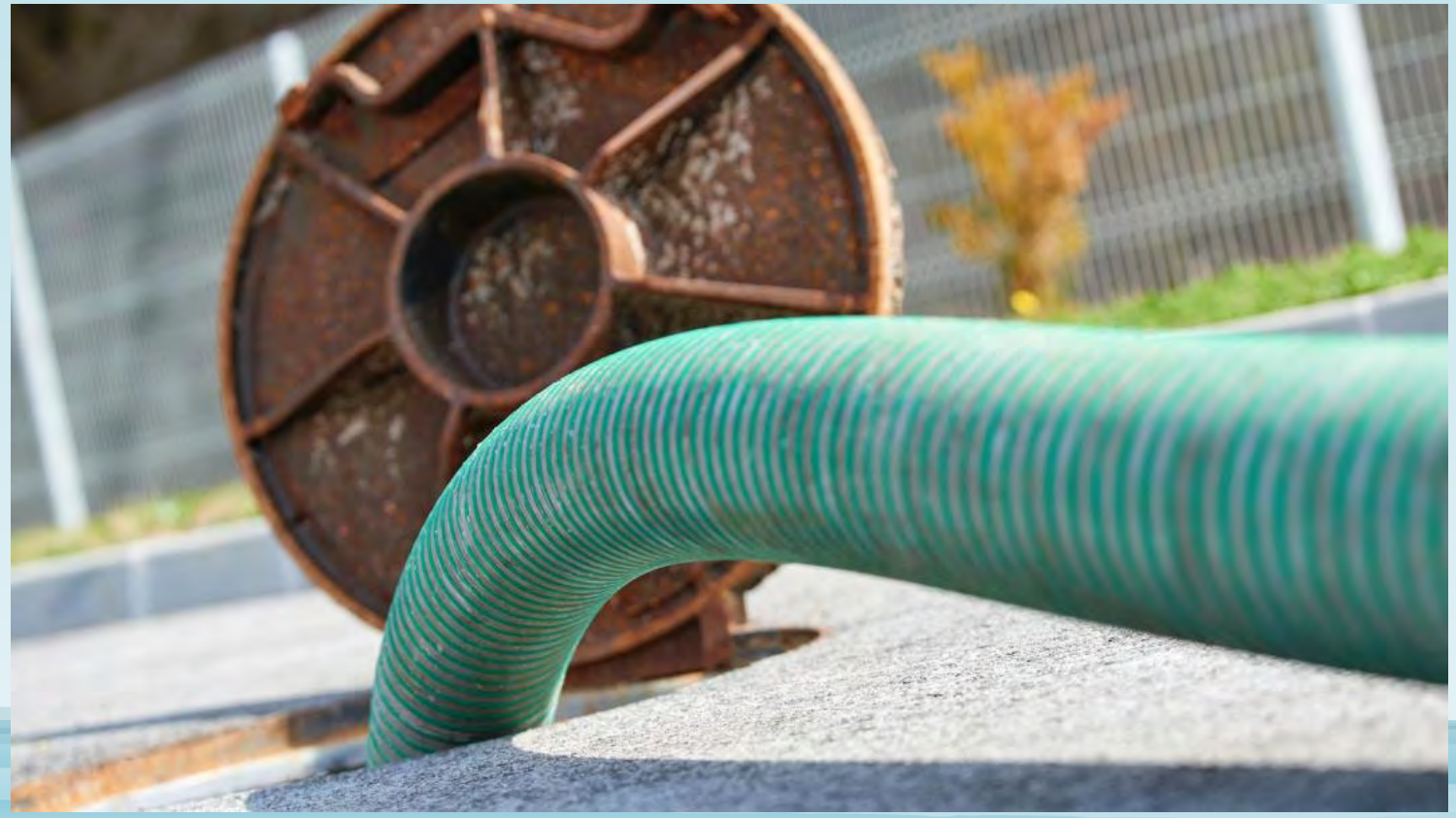
Industry closures: what are they dumping down the drains?

Waste haulers: screen the types of waste you receive

Illegal dumping

Meth Labs

Pass-through toxicity from amine compounds



Check your permit!

A. (4) TOXICITY IDENTIFICATION EVALUATION (TIE)
[15A NCAC 02B.0200]

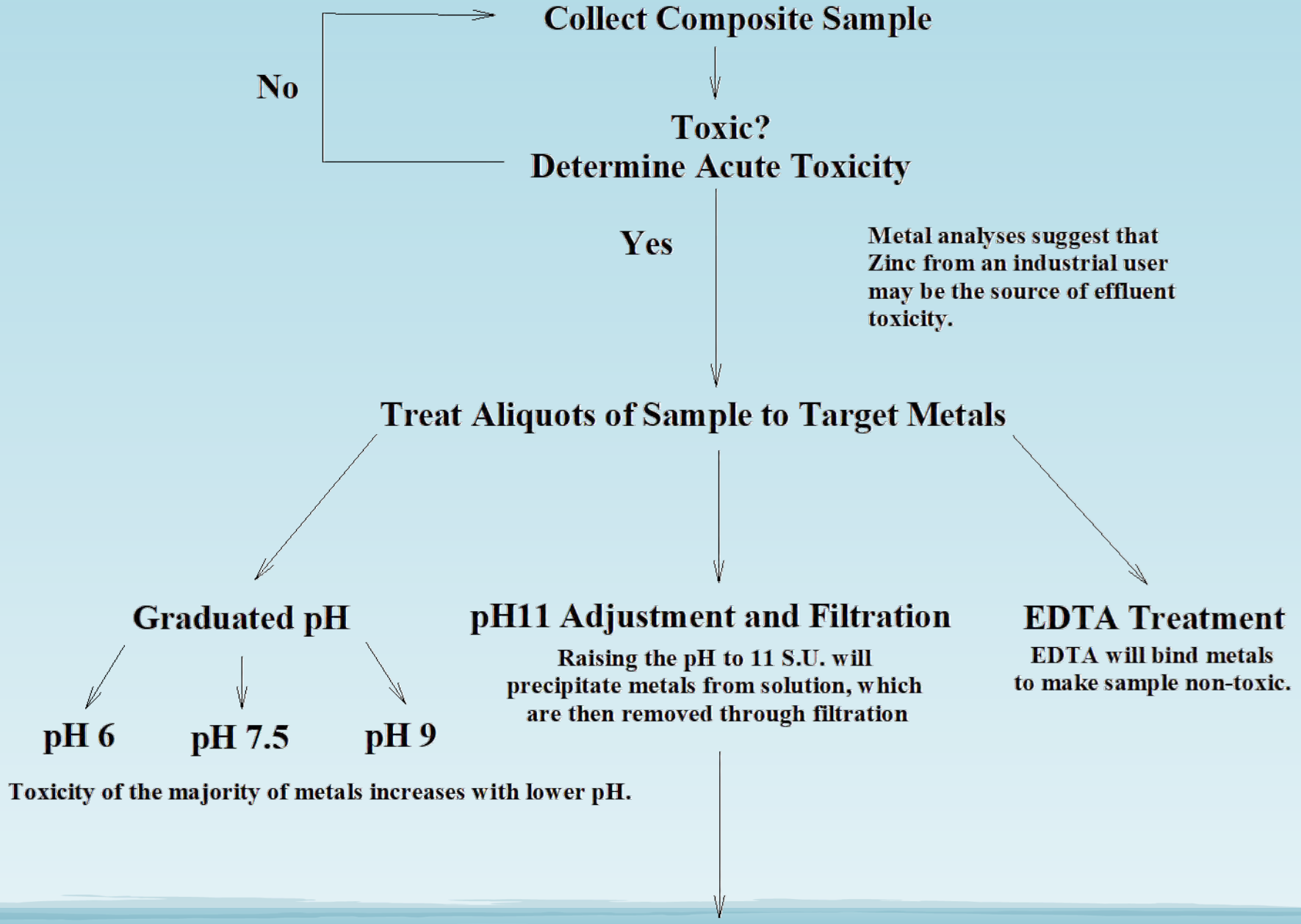
Should any whole effluent toxicity test produce a chronic value less than 12%, the permittee will undertake toxicity identification evaluations (TIEs) using the procedures described in *Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I*, EPA/600/6-91/005F, May 1992 or similar methods. A final report will be generated and submitted to the address below no later than 60 days following the initial test producing a chronic value less than 12%. The primary objective of the TIE activity will be to confirm or rule out polymer as the source of toxicity. The report detailing findings of the TIE is to be sent to the following address:

Attention: North Carolina Division of Water Resources
Water Sciences Section/Aquatic Toxicology Branch
1621 Mail Service Center
Raleigh, NC 27699-1621

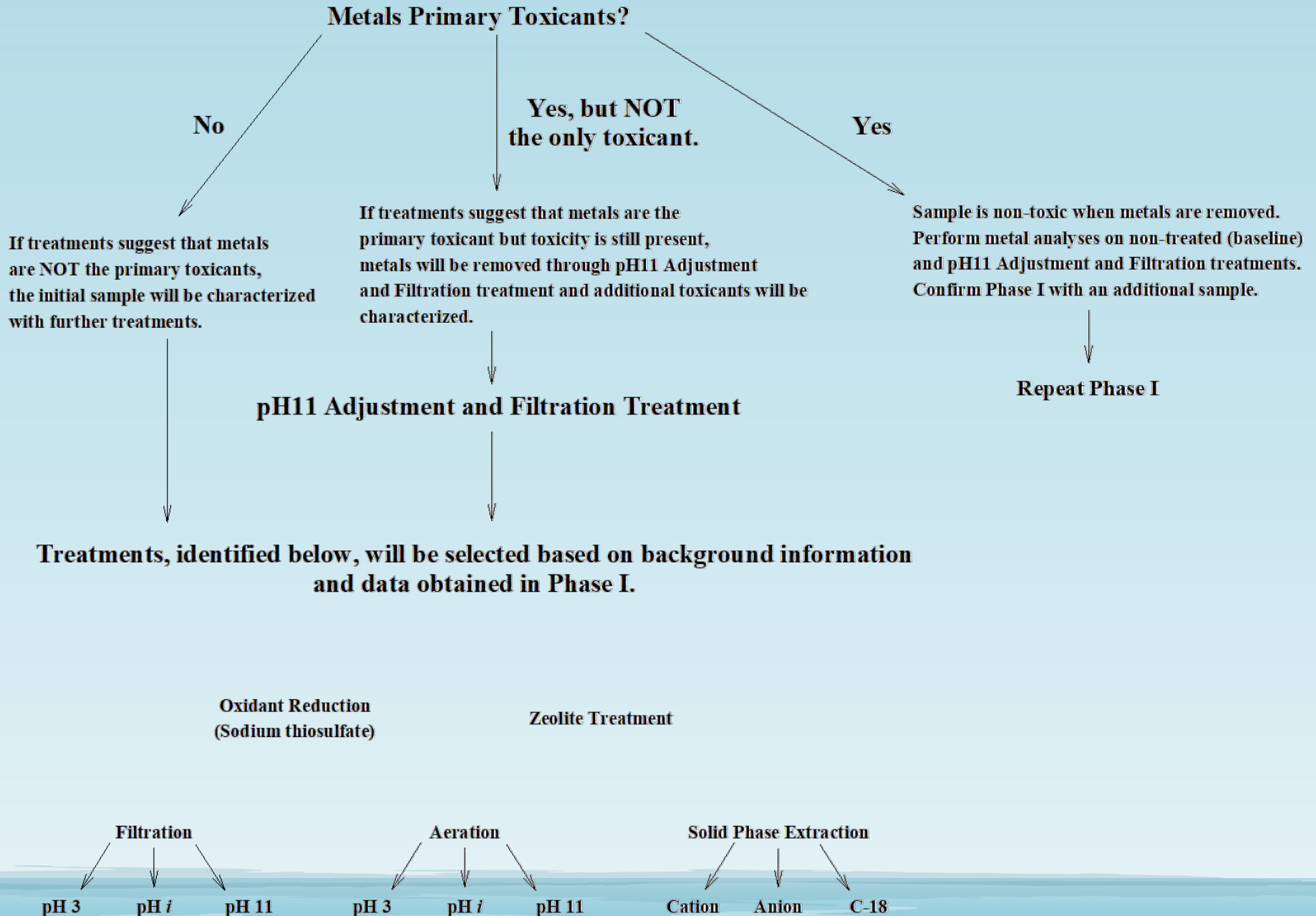
Treatments Designed to Target Suspected Toxicant

Test	Class of Compound Targeted
Baseline (Non-treated)	Determines the toxicity of a non-manipulated sample. This test is used for a comparison to the treatment tests.
Activated Carbon Absorption	Removal of metals and organic compounds.
Sodium Thiosulfate or Sodium Hydrosulfite	Reduces oxidant compounds. Chlorine, ozone, chlorine dioxide, mono- and di-chloramines, bromine, and iodine are highly neutralized by this treatment.
EDTA	Chelates dissolved metal compounds. (i.e. Aluminum, Barium, Cadmium, Cobalt, Copper, Iron, Lead, Manganese, Nickel, Zinc, Arsenic, Mercury, Silver, Magnesium, Strontium, Thallium)
Ion Exchange	Removal of cations or anions from solution.
Graduated pH	Alters the speciation of dissolved, ionic compounds. Ammonia, hydrogen sulfide, and metals are highly susceptible to changes in toxicity by this treatment.
Aeration	Removal or volatile or semi-volatile compounds from solution. This treatment also oxidizes reduced compounds through the increase of the dissolved oxygen concentration of the sample.
Filtration	Removal of colloidal and particulate material. Altering the pH to 11 S.U. will precipitate most metal compounds from solution, which can then be removed by filtration.
C18 Solid Phase Extraction	Removal of nonionic, hydrophobic organic compounds.
Silica Gel	Removal of polar organic compounds.

Phase I


Collect Composite Sample
No
Toxic?
Determine Acute Toxicity
Yes
Metal analyses suggest that Zinc from an industrial user may be the source of effluent toxicity.
Treat Aliquots of Sample to Target Metals
Graduated pH
pH 6
pH 7.5
pH 9
Toxicity of the majority of metals increases with lower pH.
pH11 Adjustment and Filtration
Raising the pH to 11 S.U. will precipitate metals from solution, which are then removed through filtration
EDTA Treatment
EDTA will bind metals to make sample non-toxic.
Phase II

Phase II


Metals Primary Toxicants?
No
Yes, but NOT the only toxicant.
Yes
If treatments suggest that metals are NOT the primary toxicants, the initial sample will be characterized with further treatments.
If treatments suggest that metals are the primary toxicant but toxicity is still present, metals will be removed through pH11 Adjustment and Filtration treatment and additional toxicants will be characterized.
Sample is non-toxic when metals are removed. Perform metal analyses on non-treated (baseline) and pH11 Adjustment and Filtration treatments. Confirm Phase I with an additional sample.
Repeat Phase I
pH11 Adjustment and Filtration Treatment
Treatments, identified below, will be selected based on background information and data obtained in Phase I.
Oxidant Reduction (Sodium thiosulfate)
Zeolite Treatment
Filtration
pH 3
pH i
pH 11
Aeration
pH 3
pH i
pH 11
Solid Phase Extraction
Cation
Anion
C-18

Questions?

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