# Training New Toxicology Analysts

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Addendum

Initial Demonstration of Capability for Analysts
Ongoing Demonstration of Capability for Analysts

Appendix of Changes



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1. SCOPE AND APPLICATION

1.1. These procedures are used to train new analysts in the Toxicology Section. The order of procedures can change based on the needs of the laboratory.

2. SUMMARY OF THE METHOD

2.1. New analysts are familiarized with the layout of the laboratory and the function and location of instruments, equipment, logbooks, record keeping (paper and electronic), etc. Standard Operating Procedures (SOPs) and Methods are assigned in the Laboratory Information Management System (LIMS). An experienced analyst demonstrates each procedure and monitors the new analyst as the new analyst performs procedures. Various proficiencies/trainings are recorded in paper logs, LIMS (SOPs, PTs, Methods), and Excel spreadsheets (QC tracking).

2.2. General Toxicology Laboratory procedures for Whole Effluent Toxicity (WET) tests can be seen in the United States Environmental Protection Agency (USEPA) WET training videos, https://www.epa.gov/npdes/npdes-training. A presentation on USEPA NPDES WET testing methods can be found at https://www.epa.gov/sites/production/files/2016-11/documents/101816_module_2.pdf.

2.3. Definitions

2.3.1. *Americamysis bahia (A. bahia)*: mysid shrimp; marine invertebrate used in saltwater acute and chronic tests; purchased from outside vendor

2.3.2. *Ceriodaphnia dubia (C. dubia)*: water flea; freshwater invertebrate; used in freshwater acute and chronic tests; cultured in-house

2.3.3. *Cyprinella leedsi (C. leedsi)*: bannerfin shiner; freshwater vertebrate fish; one of two species used in freshwater acute tests; purchased from outside vendor

2.3.4. *Dunaliella tertiolecta (Dun)*: marine algae; used for Algal Growth Potential tests with salinity greater than 7.0 ppt; cultured in-house

2.3.5. *Menidia beryllina (M. beryllina)*: inland silverside; marine vertebrate fish; used in saltwater acute and chronic tests; purchased from outside vendor

2.3.6. *Pimephales promelas (P. prom)*: fathead minnow; freshwater vertebrate fish; used in freshwater acute and chronic tests; purchased from outside vendor

2.3.7. *Raphidocelis subcapitata (R. sub)*: freshwater algae; formerly known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata*; used for Algal Growth Potential tests with salinity less than 1.0 ppt; cultured in-house



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2.3.8. *Artemia* nauplii: brine shrimp; saltwater invertebrate used to feed freshwater and saltwater organisms. These are cultured in-house for each facility or SRT test.

2.3.9. Standard Reference Toxicant (SRT) Test: Quality control test method used within the lab to verify test accuracy. This test method uses a lab made standard reference toxicant with known organism response. It must be run either monthly (*C. dubia* tests) or concurrently with facility tests (all other organism tests). SRT tests must pass test criteria and are evaluated against lab-maintained control charts.

2.3.10. Aquatic Toxicity Bioassay: A biological test that uses living aquatic organisms to determine the toxicity of a sample. Ex. WET tests.

2.3.11. Whole Effluent Toxicity (WET) Tests: Used to measure the effects of an effluent sample on living aquatic organisms at several different dilutions. They can be Acute or Chronic.

2.3.12. Acute WET test: Endpoint is Lethal Concentration 50% (LC50) after 48 or 96 hours and is based on survival; 48-hour test does not get renewed; 96-hour test is renewed at 48 hours.

2.3.13. Chronic WET test: Endpoints are No Observed Effect Concentration (NOEC) and Inhibition Concentration 25% (IC25) after 7 days (typically) and is based on survival and growth/reproduction; test is renewed daily.

2.3.14. No Observed Effect Concentration (NOEC): The highest concentration that is not significantly different than the control. Endpoint in a Chronic WET test.

2.3.15. Lowest Observed Effect Concentration (LOEC): The lowest concentration that is significantly different than the control. Endpoint in a Chronic WET test.

2.3.16. Inhibition Concentration 25% (IC25): The concentration at which there is a 25% reduction in a non-quantal biological measurement (such as reproduction and growth) compared to the control. Endpoint in a Chronic WET test.

2.3.17. Lethal Concentration 50% (LC50): The concentration at which 50% of test organisms die. Endpoint in an Acute WET test.

2.3.18. Percent Minimum Significant Difference (PMSD): Smallest percentage decrease in growth or reproduction from the control that could be determined as statistically significant in a test.

2.3.19. Control Percent Coefficient of Variation (CV) (Control % CV): This represents the test's precision and reliability based on the control group's results. A low % CV represents good test consistency, while a high % CV represents more scatter of results and lowers the confidence of the test's reliability.



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3. EQUIPMENT AND SUPPLIES

3.1. Personal protection equipment (gloves, lab coats, and safety glasses).

3.2. Computer with access to SOPs, LIMS, and other sites related to the toxicology section.

3.3. All the equipment listed within each SOP.

4. METHODS / ORDER OF TRAINING (FLEXIBLE)

4.1. The analyst is required to read each SOP before performing the procedure covered by the SOP.

4.2. **Swirling Algae**

4.2.1. When algae cultures/AGP samples are stored in incubator #7 (salt water) and #8 (freshwater) they must be hand swirled at least once daily (not required for algae on shaker racks or in Inc #9).

4.3. **Meters and Kits**

4.3.1. The meters to be calibrated are Dissolved Oxygen (DO) (SOP TA-06.44), pH (SOP TA-06.43), and the combination conductivity and salinity meter (SOP TA-06.36). Kits are for alkalinity and hardness (SOP TA-06.08), total residual chlorine (TRC) (SOP TA-06.22), and ammonia (SOP TA-06.35).

4.4. **Balances**

4.4.1. Balances are in the Tox Lab (Room B246), the Algal Lab (Room B227A), and the Micro Lab (Room B212) and should be used and maintained according to SOP TA-06.34 (403TS/00), SOP TA-06.38 (PB403-S/FACT), and SOP BB-36 (XA105DU).

4.4.1.1. Annually: All weights calibration check for each balance.

4.4.1.2. Annually: Tox's 'class 1 weights' (S01) are compared to Chemistry's 'class 1 weights' or equivalent (class 0 weights are even better, some of the Chem weights are class 0).

4.4.1.3. Annual maintenance by vendor (contracted).

4.5. **Culturing *Ceriodaphnia dubia***

4.5.1. Methods found in SOPs TA-02.00 and TA-05.02 include record keeping, setting up, transferring, counting, and maintaining *C. dubia* cultures.

4.6. **Preparing Culture Media and Test Dilution Waters (MHW, MHWCD, and ASW)**

4.6.1. All media are aerated for 48 hours after initial mixing (two-step process each taking 24 hours). Every new batch of water made is entered into LIMS Standard Prep Tracker.

4.6.1.1. Moderately hard water (MHW) is prepared for *Cyprinella leedsi* and *Pimephales promelas* facility and SRT tests. Refer to SOP TA-03.15.



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4.6.1.2. Moderately hard water with sodium selenate and vitamin B12 (MHWCD) is prepared for *C. dubia* culturing, facility and SRT *C.* dubia tests. Refer to SOP TA-03.15. The methods of preparation for the sodium selenate solution and B12 solution are in SOP TA-09.08.

4.6.1.2.1. The sodium selenate is also added to the medium used for growing the *Raphidocelis subcapitata* for *C. dubia* food. Refer to SOP TA-01.04.

4.6.1.3. Artificial salt water (ASW) is prepared for *Menidia beryllina* and *Americamysis bahia* facility and SRT tests, and *Artemia* culturing. Refer to SOP TA-03.02.

## 4.7. Measuring and Recording Water Quality Parameters

4.7.1. Water quality parameters are taken on sample waters, dilution waters, organism waters (ordered (upon arrival) and in-house (weekly)), DI water (weekly), and well water (monthly).

4.7.1.1. Parameters to measure: temperature (SOP TA-06.13), pH (SOP TA-06.43), DO (SOP TA-06.44), alkalinity and hardness (SOP TA-06.08), conductivity and salinity (SOP TA-06.36), total residual chlorine (TRC) (SOP TA-06.22), and ammonia (SOP TA-06.35).

4.7.1.1.1. Prepared waters: Parameters are taken on all prepared water after 48 hours of aeration. Parameters must be taken before the prepared water can be used. Prepared waters do not get DO measured.

4.7.1.1.2. Sample water parameters: Take initial DO reading first, if out of range aerate while sample warms; all parameters are performed on warmed water (and after aeration when necessary); record the pH, temperature, and DO (initial and final) in the *Bioassay Sample Log-in Logbook*; pH, DO, TRC, alkalinity, hardness, and ammonia are recorded in the *Parameter Logbook* and their respective logbooks in LIMS.

4.7.1.1.2.1. Freshwater parameters: No salinity; record in associated logbooks and in LIMS logbooks.

4.7.1.1.2.2. Marine water parameters: No hardness; record in associated logbooks and in LIMS logbooks.

4.7.1.1.3. Organism waters:

4.7.1.1.3.1. Ordered—No TRC; record in *Organism Log-in Logbook*, *Parameter Logbook*, and the respective logbooks in LIMS.



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4.7.1.1.3.2. In-house— pH, DO, conductivity, and temperature; record in *Daily Feeding Logbook*.

4.7.1.1.4. DI water: Test weekly for TRC, pH, ammonia, alkalinity, and conductivity; record in LIMS logbooks.

4.7.1.1.5. Well water: Test monthly for TRC, pH, ammonia, alkalinity, and conductivity; record in LIMS logbooks.

## 4.8. Culturing, Collection, and Use of *Artemia*

4.8.1. The method for *Artemia* culturing is found in SOP TA-01.03.

4.8.1.1. Set-up cysts in separatory funnels with ASW and aerate overnight.

4.8.1.2. Allow *Artemia* to settle; disperse into light box and allow hatched *Artemia* to move towards the light. Collect from light box using a siphon. Aerate the collected *Artemia* until use.

4.8.1.3. If *Artemia* cysts need disinfection, or hatch rate increased, refer to SOP TA-01.03.

## 4.9. Receiving, Logging-in, and Prepping Samples

4.9.1. Refer to SOP TA-06.14.

4.9.1.1. Samples are picked up and checked out from Receiving.

4.9.1.2. Samples warmed to 25 ± 1°C; aliquot taken for parameter measurements (refer to section 4.7.).

4.9.1.3. Salinity, DO, and pH adjustments are done as needed per SOPs TA-08.06 and TA-07.13.

4.9.1.3.1. Preparation of acid and base solutions to adjust the pH are found in SOP TA-10.10.

4.9.1.3.2. Dechlorination methods are done per SOP TA-09.18.

## 4.10. Receiving, Logging-in, and Prepping Purchased Organisms

4.10.1. Refer to SOP TA-04.20. The organisms are shipped in bags. Each bag contains organisms that are batched by species and age.

4.10.1.1. Bags are removed from shipping container and placed into plastic Carolina dishes.

4.10.1.2. Aliquots of organism waters are collected for parameters (refer to section 4.7.).

4.10.1.3. Organisms are fed *Artemia* and observed for at least two hours before use in a test. The water in the Carolina dishes is aerated.

4.10.1.4. Organism receipt, water parameters, and organism conditions are recorded in the *Organism Log-in Benchsheets* logbook.



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## 4.11. Chronic and Acute WET Testing

4.11.1. Two general types of toxicity tests: acute and chronic. Each test type will use one invertebrate organism (*C.* dubia [freshwater], *A.* bahia [marine]) and one vertebrate organism (*P. prom* [freshwater], *C. leedsi* [freshwater, typically only acute], *M.* beryllina [marine]). A corresponding standard reference toxicant test (SRT) must be run either within 30 days prior to or concurrently with a facility test.

4.11.1.1. Typically, we run *C. dubia* SRTs monthly because the number of organisms need to run them concurrently with a facility is too many. All other tests are run concurrently with the facility test.

4.11.2. Each test SOP includes test initiation, interim counts, cleaning, parameter methods, test renewals, test termination, and SRT method.

4.11.2.1. Acute *C. dubia* SOP TA-07.01

4.11.2.2. Acute *P. promelas* and *C. leedsi* SOP TA-07.02

4.11.2.3. Acute *A. bahia* SOP TA-07.03

4.11.2.4. Acute *M. beryllina* SOP TA-07.04

4.11.2.5. Chronic *C. dubia* SOP TA-07.05

4.11.2.6. Chronic *P. promelas* and *C. leedsi* SOP TA-07.09

4.11.2.7. Chronic *A. bahia* SOP TA-07.15

4.11.2.8. Chronic *M. beryllina* SOP TA-07.14

4.11.2.9. Standard reference toxicants KCl (current) and NaCl (alternative) SOP TA-09.01.

## 4.12. Weighing Chronic Tests

4.12.1. Chronic fish (*P. promelas* and *M. beryllina*) and chronic mysid shrimp (*A. bahia*) are weighed after their tests have been terminated. The methods for making weigh boats and weighing organisms are found in SOP TA-06.21.

4.12.2. Before weigh boats or organisms can be weighed, they must be dried in the oven at 60°C for 24 hours; or 100°C for 1 hour for empty weigh boats and 8 hours for weigh boats with organisms. The weigh boats are placed in a desiccator to cool. The weigh boats need to be left next to the balance for 30 minutes to acclimate to the conditions of the room before being weighed.

## 4.13. Talosys Temperature Monitoring

4.13.1. After a test has been terminated, Talosys (https://mytalosys.talosys.io/signin/email) is used to determine the range of temperatures the test was exposed to inside the incubator. The methods for using Talosys can be found in the Talosys Temperature Monitoring



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System Userguide. Temperatures in select test vessels are also recorded throughout the test in the test bench sheets logbooks.

4.14. **Periodic Lab Maintenance**

4.14.1. Weekly: Take parameters on in-house culture waters and organism waters. Also, check the chlorine, ammonia, alkalinity, pH, and conductivity of the DI water (refer to section 4.7.).

4.14.2. Monthly: Check light intensities (SOP TA-06.26), photoperiod times (SOP TA-06.30), and monthly SRTs (*C. dubia)*. Check the chlorine, ammonia, alkalinity, pH, and conductivity of the lab well water (refer to section 4.7.).

4.14.3. Quarterly: Check pipette calibrations (SOP TA-06.32), change well water filter, make new YCT and perform a suitability test.

4.14.4. Annually: Check class 1 weight calibrations against Chemistry's class 1 or class 0 weights, check balance calibrations using all calibrated class 1 weights, check thermometer calibrations using the Fluke 7130 Temperature Calibration Micro-Bath and SOPs TA-06.13 and LB-035, check organism IDs (*C. dubia* in house, all others by supplier), send in-house water/food samples out for chemistry analysis.

4.15. **Preparation of food for *C. dubia***

4.15.1. Yeast, cereal leaves, and fish flake/trout chow (YCT) is prepared per SOP TA-01.01. The freshwater algae *Raphidocelis subcapitata* is concentrated (SOP TA-01.04). Daily, the two are mixed in equal parts and used to feed the *C. dubia* cultures and tests (SOP TA-02.00). Make sure each solution is properly swirled or shaken to ensure a homogenous solution.

4.15.1.1. Before use, the YCT must be checked for suitability (SOP TA-01.07).

4.16. **Statistical Analysis of WET Tests**

4.16.1. After tests are terminated, statistical analyses are performed using the EPA WET Spreadsheet (this gets pulled from the website each time you need one).

4.16.1.1. Chronic: Endpoints are survival and reproduction/growth with results expressed as 25% Inhibition Concentration (IC25; required for NPDES permits issued in Florida), no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC); follow methods in SOP TA-07.17.

4.16.1.2. Acute: Endpoints are survival with results expressed as lethal Concentration 50 (LC50); follow methods in SOP TA-07.17.

4.16.1.3. A fact sheet about the spreadsheet can be consulted here.

4.17. **Preparation of Algal Media**



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4.17.1. The freshwater algae species *Raphidocelis subcapitata* and the marine algae species *Dunaliella tertiolecta* are cultured in the lab (SOP TA-04.08) for use in algal growth potential (AGP) tests. *R. subcapitata* is also a component of the *C. dubia* food.

4.17.1.1. Preliminary algal assay procedure (PAAP) medium (SOP TA- 03.03) is used for culturing *R. subcapitata* in flasks. PAAP is added to agar for culturing *R. subcapitata* on agar plates (SOPs TA- 03.13 and TA-04.08).

4.17.1.2. Artificial seawater (ASW) medium is used for culturing *D. tertiolecta* (SOP TA-03.04). This is not to be confused with the ASW prepared for Toxicity marine bioassays. ASW medium is added to agar for culturing *D. tertiolecta* on agar plates (SOPs TA- 03.13 and TA-04.08).

4.18. **Algal Growth Potential (AGP)**

4.18.1. Samples for AGP tests are checked out of Receiving, a prep worksheet and labels are made, samples are checked back into LIMS (SOP TA-08.09).

4.18.2. Initial AGP sample handling procedures are in SOP TA-08.01.

4.18.2.1. AGP lab blank is prepared (SOP TA-08.08).

4.18.2.2. Filters used for filtering AGP samples are washed to remove trace contaminants (SOP TA-08.01).

4.18.3. AGP test set-up, inoculation, and termination procedures are in SOPs TA-08.03 and TA-08.05.

4.18.4. Coulter Counter Model Multisizer 4e calibration and use is in SOP TA-06.41. Coulter Multisizer 4e instructions are in \\floridadep\data\DEAR\Labs\Lab5\Biology\BenchCommon\ToxCommon\Methods & Manuals\Manuals\Beckman Coulter.

4.18.5. After an AGP test has been terminated, the data is entered in LIMS, verified, then uploaded to LIMS (SOP TA-08.07).

4.19. **Documentation**

4.19.1. Electronic Documentation of SOP review and Method proficiency is found in the LIMS Training Module.

4.19.2. See the addendum on page 14 for the tasks involved with documenting the initial demonstration of capability and the ongoing demonstration of capability for analysts.

5. DATA ARCHIVAL

5.1. If a problem occurs during sample processing, a nonconformance report (NCR) describing the problem and the corrective action is created in LIMS.



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5.2. FDEP Laboratory Information Management System (LIMS)

5.3. Log sample receipt, temperature, and condition into *Bioassay Sample Log-in* logbook (per SOP TA-06.14).

6. QUALITY CONTROL

6.1. Refer to the DEP Laboratory Quality Manual for corrective action and handling out-of-control data.

6.2. Use a black, waterproof pen for all bench sheets and logbooks.

7. SAFETY/HAZARDOUS WASTE MANAGEMENT

7.1. Proper laboratory protection, including lab hood or ventilation system, lab coat, gloves and safety glasses, is required when working with chemicals and unprocessed samples.

7.2. Refer to the DEP Health and Safety Manual.

7.3. Refer to the DEP Laboratory Quality Assurance Manual for sample disposal.

7.4. Read MSDS information for reagent handling and disposal.

8. REFERENCES

8.1. DEP Laboratory Quality Manual

8.2. DEP Health and Safety Manual

8.3. EPA 821-R-02-012 *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th Edition, October 2002.*

8.4. EPA 821-R-02-013 *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, 4th Edition, October 2002*

8.5. EPA 821-R-02-014 *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, 3rd Edition, October 2002.*

8.6. EPA-600/9-78-018 *The Selenastrum capricornutum Printz Algal Assay Bottle Test*

8.7. EPA-MARINE-1974 *Marine algal Assay Procedure: Bottle Test*

8.8. EPA WET NPDES Spreadsheet

8.9. Beckman Coulter MS4e User Manual

8.10. Talosys Temperature Monitoring System Userguide

8.11. USEPA WET Training Videos

8.12. USEPA NPDES WET Testing Methods



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8.13. DEP SOP BB-36, *Calibration, Use, and Maintenance of Mettler Toledo XA105DU Balance*

8.14. DEP SOP LB-023

8.15. DEP SOP LB-035, *Calibration of lab Thermometers Using a Constant Temperature Calibration Bath*

8.16. DEP SOP TA-01.01, *YCT (Yeast, Cereal Leaves, Flake-Fish Food, or Trout Chow) Preparation*

8.17. DEP SOP TA-01.03, *Artemia nauplii Culture, Collection, and Counting*

8.18. DEP SOP TA-01.04, *Raphidocelis subcapitata Culture for Ceriodaphnia dubia Food*

8.19. DEP SOP TA-01.07, *YCT (Yeast, Cereal Leaves, Flake-Fish Food, Or Trout Chow) And Artemia Suitability Tests*

8.20. DEP SOP TA-02.00, *Feeding Test-Organism Cultures*

8.21. DEP SOP TA-03.02, *Preparation of Artificial Seawater (ASW)*

8.22. DEP SOP TA-03.03, *Preparation of Preliminary Algal Assay Procedure (PAAP) Medium for Freshwater Algae Cultures*

8.23. DEP SOP TA-03.04, *Preparation of Artificial Seawater (ASW) Medium for Culturing Dunaliella tertiolecta*

8.24. DEP SOP TA-03.13, *Preparation of Plate-Count Agar (PCA)*

8.25. DEP SOP TA-03.15, *Preparation of Synthetic Moderately Hard Freshwater*

8.26. DEP SOP TA-04.01, *Maintenance of Ceriodaphnia dubia Mass and Chronic-Bioassay Cultures*

8.27. DEP SOP TA-04.08, *Culture of Algae-Assay Inoculation Stocks*

8.28. DEP SOP TA-04.20, *Receiving and Maintaining Purchased Test Organisms*

8.29. DEP SOP TA-05.02, *Production and Maintenance of Ceriodaphnia dubia for Chronic and AcuteToxicity Testing*

8.30. DEP SOP TA-06.08, *Measuring Total Alkalinity and Hardness*

8.31. DEP SOP TA-06.13, Thermometer Accuracy Check and Use

8.32. DEP SOP TA-06.14, *Bioassay Sample Log-In Procedure*

8.33. DEP SOP TA-06.21, *Weighing Chronic Tests*

8.34. DEP SOP TA-06.22, *Measuring Total Residual Chlorine using Hach® Pocket Colorimeter™ II Test Kit*

8.35. DEP SOP TA-06.26, *Measurement of Light Intensity for AGP Cultures, Animal Cultures, Toxicity Incubators, and the Bioassay Laboratory*

8.36. DEP SOP TA-06.30, *Timer Verification: Culture Lab and Toxicity Incubators*



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8.37. DEP SOP TA-06.32, *Calibration and Verification of Mechanical and Disposable Pipettes in the Bioassay/AGP Lab*

8.38. DEP SOP TA-06.34, *Calibration and Use of the Mettler Toledo 403TS/00 Balance*

8.39. DEP SOP TA-06.35, *Measuring Total Ammonia using the Hach Ammonia Nitrogen Pocket Colorimeter II Test Kit*

8.40. DEP SOP TA-06.36, *Calibration and Use of the Fisherbrand™ Traceable™ Bench Conductivity Meter for Conductivity and Salinity*

8.41. DEP SOP TA-06.38, *Calibration and Use of Mettler Toledo PB403-S/FACT Balance*

8.42. DEP SOP TA-06.41, *Calibration and Use of the Coulter Counter Model Multisizer 4e*

8.43. DEP SOP TA-06.43, *Calibration and Use of Fisherbrand™ Accumet™ AB315 pH/mV* Meter

8.44. DEP SOP TA-06.44, *YSI Multilab 4010-1 Meter Calibration and YSI IDS ProOBOD DO Probe Maintenance*

8.45. DEP SOP TA-07.01, *Ceriodaphnia dubia Acute Toxicity Testing Method*

8.46. DEP SOP TA-07.02, *Cyprinella leedsi and Pimephales promelas Acute Toxicity Testing*

8.47. DEP SOP TA-07.03, *Americamysis (formerly Mysidopsis) bahia Acute Toxicity Testing Methods*

8.48. DEP SOP TA-07.04, *Menidia beryllina Acute Toxicity Testing Methods*

8.49. DEP SOP TA-07.05, *Ceriodaphnia dubia Chronic Toxicity Testing Methods*

8.50. DEP SOP TA-07.09, *Pimephales promelas and Cyprinella leedsi Chronic Toxicity Testing* Methods

8.51. DEP SOP TA-07.13, *Salinity Adjustment of Samples: Toxicity Bioassay*

8.52. DEP SOP TA-07.14, *Menidia beryllina Chronic Toxicity Testing Methods*

8.53. DEP SOP TA-07.15, *Americamysis bahia Chronic Toxicity Testing Methods*

8.54. DEP SOP TA-07.17, Using EPA Whole Effluent Toxicity (WET) Statistics for Acute and Chronic Bioassay Tests

8.55. DEP SOP TA-08.01, *Initial Algal Assay Sample Handling*

8.56. DEP SOP TA-08.03, *Set-up and Inoculation of Algal Assay Tests*

8.57. DEP SOP TA-08.05, *Termination of Algal Assay Tests*

8.58. DEP SOP TA-08.06, *pH Adjustment of Samples: Algal Assay and Toxicity Bioassay*

8.59. DEP SOP TA-08.07, *Data Verification and LIMS Upload for Algal Growth Potential Tests*

8.60. DEP SOP TA-08.08, *AGP Lab Blank Analysis and Blank Data Review*



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8.61. DEP SOP TA-08.09, *Sample Custody, Log Book, Labels, and Prep Worksheet Manager for Algal Growth Potential (AGP) Samples*

8.62. DEP SOP TA-09.01, *Potassium Chloride Reference Toxicant Stock Solutions*

8.63. DEP SOP TA-09.08, *Preparation of B12 and Sodium Selenate for use in 20% DMW, MHWCD, and Raphidocelis subcapitata Culture Media*

8.64. DEP SOP TA-09.09, *Preparation of PAAP-Medium Stock Solutions*

8.65. DEP SOP TA-09.10, *Artificial Seawater Basal Stock Solution for Culturing Marine Algae*

8.66. DEP SOP TA-09.18, *Dechlorination Procedure using Sodium Thiosulfate*

8.67. DEP SOP TA-10.10, *Preparation of Stock HCl (Acid) and NaOH (Base) Solutions*

8.68. MSDS Data Sheets

8.69. TNI Standard – Volume 1, Module 7 Quality Systems for Toxicity Testing

# Addendum

Bioassays showing Initial Demonstration of Capability for all WET methods for which the Biology Program is accredited have been performed multiple times since the lab's inception in the mid-1970s. SRT logbooks for the last five years are available in room B246 or A216R. SRT control charts can be found in ToxCommon. The Toxicity group functions as a *work cell* with all analysts/analyst trainees taking part in performing various aspects of the test. After training, any member of the toxicity group can perform all aspects of a WET test and/or an SRT test.

**Initial Demonstration of Capability for analysts is accomplished by**:

1) Reading appropriate SOP for a specific task.
2) Task being demonstrated by a trained analyst.
3) Task being performed by trainee under close supervision of trained analyst.
4) Task being performed by trainee and checked by trained analyst.
5) Training for toxicity tests include reading SOPs, preparing dilution water, making toxicants, making and pouring up test concentrations, loading SRTs (prior to loading facility tests), measuring test parameters, feeding test organisms, cleaning test vessels, renewing tests, terminating tests, etc.
6) Analysts are also trained in culturing tests organisms, handling ordered organisms, receiving/preparing bioassay samples, statistical analysis of test data and other aspects.
7) A valid (per EPA Methods) SRT test initiated (loaded) by the trainee.

**Ongoing Demonstration of Capability for analysts can include:**

1) Performing passing SRTs or facility tests.



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2) Participating in annual PT tests.

Records for Capability are recorded in LIMS, Test benchsheets (logbooks), and ToxCommon.

## Appendix of Changes:



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