# Training New Toxicology Analysts

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Addendum

Initial Demonstration of Capability for Analysts
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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 is based on what is most practical for the analyst to start learning first in the lab but 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 (wide ranging routine tasks), LIMS (SOPs, PTs, Methods), and Excel spreadsheets (SRT 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*: Common name is mysid shrimp referred to in lab as *A. bahia*. Marine invertebrate; used in saltwater acute and chronic tests; purchased from outside vendor.

2.3.2. *Ceriodaphnia dubia*: Common name is water flea referred to in lab as *C. dubia*. Freshwater invertebrate; used in freshwater acute and chronic tests; cultured in-house.

2.3.3. *Daphnia magna*: Referred to in lab as *D.* magna. Freshwater invertebrate; used in freshwater acute and chronic tests; purchased from outside vendor.

2.3.4. *Cyprinella leedsi*: Common name is bannerfin shiner referred to in lab as *C. leedsi*. Freshwater vertebrate; one of two species (the other being *P. promelas*) that may be used in freshwater acute tests; purchased from outside vendor.

2.3.5. *Dunaliella tertiolecta*: Referred to in lab as *Dun*. Marine algae; used for Algal Growth Potential tests with salinity greater than 7.0 ppt; may also be used as another marine organism for toxicity bioassays; cultured in-house.

2.3.6. *Menidia beryllina*: Common name is inland silverside referred to in lab as *M. beryllina*. Marine vertebrate; used in saltwater acute and chronic tests; purchased from outside vendor.



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2.3.7. *Pimephales promelas*: Common name is fathead minnow referred to in lab as *P. prom*. Freshwater vertebrate; used in freshwater acute and chronic tests; purchased from outside vendor.

2.3.8. *Pseudokirchneriella subcapitata*: Referred to in lab as *P.* sub. Freshwater algae; formerly known as *Selenastrum capricornutum*; used for Algal Growth Potential tests with salinity less than 1.0 ppt; may also be used as another freshwater organism for toxicity bioassays; cultured in-house.

2.3.9. *Artemia* nauplii*:* Referred to in lab as *Artemia*. Saltwater invertebrate that is used to feed freshwater and saltwater organisms. These are cultured in-house for each facility or SRT test.

2.3.10. 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.

2.3.11. Acute WET test: Endpoint is 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.

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

2.3.13. No Observed Effect Concentration (NOEC): The highest concentration that is not significantly different than the control.

2.3.14. Lowest Observed Effect Concentration (LOEC): The lowest concentration that is significantly different than the control.

2.3.15. Inhibition Concentration 25% (IC25): The concentration at which there is a 25% reduction in a non-quantal biological measurement (such as reproduction, growth, fecundity) compared to the control.

2.3.16. Lethal Concentration 50% (LC50): The concentration at which 50% of test organisms die.

2.3.17. 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.

3. EQUIPMENT AND SUPPLIES

3.1. Personal protection equipment (Latex or non-latex 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.



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4. METHODS / ORDER OF TRAINING (FLEXIBLE)

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

4.2. **Temperatures**

4.2.1. Daily task, read and record the temperatures from the lab in LIMS. Temperatures that need to be taken include the incubators, walk-in refrigerator, walk-in freezer, and culture room. This method is based on SOP TA-06.01.

4.3. **Meters and Kits**

4.3.1. The meters to be calibrated are DO (SOP TA-06.39), 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 Wet Lab (Room B205), and the Micro Lab (Room B212) and should be used and maintained according to SOP TA-06.38 (PB403-S/FACT) and SOP BB-36 (XA105DU).

4.4.1.1. Quarterly: All weights calibration check.

4.4.1.2. Annually: Tox ‘S’ weights compared to Chemistry ‘S’ weights or equivalent.

4.4.1.3. Annual maintenance by vendor (contracted).

4.5. **Culturing *Ceriodaphnia dubia***

4.5.1. Methods found in SOPs TA-04.01, 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, 20% DMW, and ASW)**

4.6.1. All media are aerated for 24 hours after initial mixing. 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.

4.6.1.2. Moderately hard water with sodium selenate and vitamin B12 (MHWCD) is prepared for *C. dubia* culturing, facility and SRT 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.



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4.6.1.2.1. The sodium selenate is also added to the medium used for growing the *Pseudokirchneriella subcapitata* for *C. dubia* food. Refer to SOP TA-01.05.

4.6.1.3. 20% diluted mineral water (20% DMW) may also be used for *C. dubia* culturing, facility tests, and SRT tests.Refer to SOP TA-03.01.

4.6.1.4. Artificial salt water (ASW) is prepared for *Menidia beryllina* and *Americamysis bahia* facility and SRT tests. 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 and in-house), 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-06.39), 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).

4.7.1.2. All parameters are taken on all prepared water after 24 hours of aeration. Parameters must be taken before the prepared water can be used.

4.7.1.2.1. Sample waters: All parameters are performed; record the pH, temperature, and DO in the *Sample Log-in Logbook*; pH, DO, TRC, alkalinity, hardness and ammonia are recorded in their respective logbooks in LIMS.

4.7.1.2.2. Freshwater diluents: No salinity; diluent parameters record in LIMS.

4.7.1.2.3. Marine waters: No hardness; sample parameters recorded in *Sample Log-in Logbook*; diluent parameters recorded in LIMS.

4.7.1.2.4. Organism waters:

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

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

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

4.7.1.2.6. 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 methods for *Artemia* culturing are found in SOP TA-01.03.



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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-01.05.

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

4.9.1. Refer to SOP TA-06.14.

4.9.1.1. Samples picked up and checked out from Receiving.

4.9.1.2. Sample warmed to 25 ± 1°C; aliquot taken for parameter measurements (refer to step 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 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.

4.11. **Chronic and Acute WET Testing**

4.11.1. Two general types of toxicity tests: acute and chronic. Each test type can use different organisms, including *P. promelas*, *C. dubia*, *D. magna*, *A. bahia*, *C. leedsi*, *M. beryllina*, and *P. subcapitata.* A corresponding standard reference toxicant test (SRT) must be run either within 30 days prior or concurrently with a facility test. Rarely used organisms (ex. *D. magna* tests) are run in accordance with methods found in EPA Manuals. Demonstrations of Capability consisting of SRTs are run with and around these tests using rarely used organisms.



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4.11.2. Each test SOP includes test initiation, interim counts, cleaning, parameter methods, 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. Chronic *Psuedokirchneriella subcapitata* SOP TA-07.10

4.11.2.10. Acute *D. magna* EPA Method 2021 (EPA-821-R-02-012)

4.11.2.11. Standard reference toxicants KCl (current) and NaCl (alternative) SOP TA-09.01. Cadmium for *P. subcapitata* SOP TA-09.21.

## 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 then need to be left next to the balance for 30 minutes to acclimate to the conditions of the room before being weighed.

## 4.13. CheckPoint

4.13.1. After a test has been terminated, CheckPoint is used to determine the range of temperatures the test was exposed to inside the incubator. The methods for using CheckPoint can be found in the CheckPoint manuals at \\tlhlabapp\CHEM_APPS\Tempsys-CheckPoint\Manuals. Room temperature and temperatures in select test vessels are also recorded.

## 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 balance calibrations (using a minimum of five S-weights), light intensities (SOP TA-06.26), photoperiod times (SOP TA-06.30) and



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monthly SRTs. 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) and check balance calibrations using all S-weights.

4.14.3.1. Light timers (photo-period) SOP TA-06.30 are TNI required quarterly (the FDEP Laboratory checks the calibration monthly).

4.14.4. Annually: check thermometer calibrations using the Fluke 7130 Temperature Calibration Micro-Bath and SOPs TA-06.13 and LB-035.

4.14.4.1. Light intensity – TA-06.26 is TNI required annually (the FDEP Laboratory checks the intensity monthly) NOTE: AGP tox test require measurement at the beginning of the test.

4.14.4.2. Compare Biology 'S' weights against Chemistry "ultra" weights.

4.14.4.3. Organism IDs – *C. dubia* done in house; all others by supplier.

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

4.15.1. Yeast, cereal leaves, and trout chow (YCT) is prepared per SOP TA-01.01. The FDEP Laboratory uses fish flake food in place of trout chow. The freshwater algae *Pseudokirchneriella subcapitata* is concentrated to 3.9 x $10^7$ - 4.6 x $10^7$ cells/L (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: 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: 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

4.17.1. The freshwater algae species *Psuedokirchneriella 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. They may also be used



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for chronic bioassays (SOPs TA-07.10 and TA-07.11). *P. subcapitata* is 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 *P. subcapitata* in flasks. PAAP is added to agar for culturing *P. subcapitata* on agar plates (SOPs TA- 03.13 and TA-04.19).

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 is added to agar for culturing *D. tertiolecta* on agar plates (SOPs TA- 03.13 and TA-04.19).

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-06.16).

4.18.3. AGP test set-up, inoculation, and termination procedures are in SOPs TA-08.02, 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\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.

5.2. FDEP Laboratory Information Management System (LIMS)



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

5.4. Fill in information requested at top of *Bioassay Benchsheets* as completely as possible prior to test initiation. Include full name of facility, address, contact person, date and time collected, field TRC, LIMS sample and job numbers, test chamber ID, test chamber size, test solution volume, food type, and food batch numbers. Access scanned Field Sheets in the Lab Receiving folder, \\floridadep\data\dear_scans\Labs\BizhubScans\Receiving, to obtain sampling information.

## 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.

6.3. To correct raw data entries, place a single line through the incorrect or illegible entry. Write the corrected entry near the error, date and initial the correction. Do not overwrite entries.

6.3.1. The corrected entry is written as near its appropriate space as possible or else in the comment box on the appropriate bench sheet.

6.3.2. If the correction requires an explanation, write a comment and reference the error. Initial and date the explanation.

6.3.3. Use sequential alphabetical annotation to identify errors and corresponding comments (use A for first error and use A for corresponding explanatory comment; use B for second error, etc.).

## 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 Laboratory Health and Safety Plan.

7.3. Refer to the DEP Laboratory Quality 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 Laboratory Health and Safety Manual



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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 1974 *Marine algal Assay Bottle Test*

8.8. EPA WET NPDES Spreadsheet

8.9. CheckPoint Manuals

8.10. Beckman Coulter MS4e User Manual

8.11. USEPA WET Training Videos

8.12. USEPA NPDES WET Testing Methods

8.13. DEP SOP BB-036, *Calibration, Use, and Maintenance of Mettler Toledo XA105DU Balance*

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

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

8.16. DEP SOP TA-01.03, *Artemia nauplii Culture*

8.17. DEP SOP TA-01.04, *Pseudokirchneriella subcapitata Culture for Ceriodaphnia dubia Food*

8.18. DEP SOP TA-01.05, *Decapsulation of Artemia Cysts.*

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-03.01, *Preparation of 20% Dilute Mineral Water (DMW)*

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

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

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

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

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



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

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

8.29. DEP SOP TA-04.18, *Culture Algae on Agar Plates*

8.30. DEP SOP TA-04.19, *Starting Algal Cultures in Flasks from Algal Cultures on Agar Plates*

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

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

8.33. DEP SOP TA-06.01, *Temperature Monitoring of Walk-in Refrigerator, Freezer, and Toxicity Laboratory Incubators*

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

8.35. DEP SOP TA-06.11, *Compressed-Air In-Line-Filter Maintenance*

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

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

8.38. DEP SOP TA-06.16, *Washing Filters for Algal Assay to Remove Trace Contaminants*

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

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

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

8.42. DEP SOP TA-06.30, *Timer Verification: Culture Lab and toxicity Incubators*

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

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

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

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

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

8.48. DEP SOP TA-06.39, *Thermo Scientific Orion Star A213 Benchtop RDO/DO Meter Calibration and DO Measurement*

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



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

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

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

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

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

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

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

8.57. DEP SOP TA-07.10, *Psuedokirchneriella subcapitata Chronic Toxicity Test Method*

8.58. DEP SOP TA-07.11, *Inoculation of Psuedokirchneriella subcapitata Toxicity Assay*

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

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

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

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

8.63. DEP SOP TA-08.01, *Initial Algal Assay and Limiting Nutrient Sample Handling*

8.64. DEP SOP TA-08.02, *Algal Growth Potential (AGP) and Limiting Nutrient (LN) Algal Assay Set-Up*

8.65. DEP SOP TA-08.03, *Inoculation of Algal Assay Tests*

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

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

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

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

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

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

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

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

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



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8.75. DEP SOP TA-09.11, *Preparation of Nutrient Spike Solutions for Limiting Nutrient Algal Assays*

8.76. DEP SOP TA-09.17, *Preparation of Algal Assay Nutrient Spike-Verification Solutions*

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

8.78. DEP SOP TA-09.21, *Preparation and Testing of Cadmium Chloride and Copper Chloride Reference Toxicant Stock Solutions*

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

8.80. MSDS Data Sheets

8.81. 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 labs inception in the mid-1970s. SRT logbooks for the last five years are available in room B246. SRT 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 but is not limited to and does not require all the following:**

1) Performing monthly and/or concurrent SRTs.
2) Statistical analysis of previously analyzed data.



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3) Participating in annual PT tests.
4) Scoring ≥ 90% on written exams.
5) Reviewing SOPs.
6) Demonstrating procedures to other analysts.

Records for Capability are found in proficiency logs, LIMS, Test benchsheets (logbooks), ToxCommon and other places deemed appropriate (ex. file cabinet).

## Appendix of Changes:

| | |
|---|---|
| 2/13/2020 | Updated links |
| 2/23/2021 | Updated links and instructions to reflect multisizer change from the Coulter Counter Z2 to the Coulter Counter Model Multisizer 4e. Updated link to most recent DEP Health and Safety Manual. |
| 2/17/2022 | Updated links, added table of contents, corrected minor grammatical errors, removed some outdated content. |
| 2/24/2023 | Updated the pH meter SOP, updated links, and checked for grammar. |
| 2/26/2024 | Reviewed for grammar, word choice, and clarity. Updated all links. Removed non-existent references. Removed procedures no longer in use and updated as needed. |



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