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 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/training 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. Marine invertebrate; used in acute and chronic tests; purchased from outside vendor.

2.3.2. *Ceriodaphnia dubia*: Common name is water flea. Freshwater invertebrate; used in acute and chronic tests; cultured in-house.

2.3.3. *Daphnia magna*: freshwater invertebrate; used in acute and chronic tests; purchased from outside vendor.

2.3.4. *Cyprinella leedsi*: Common name is bannerfin shiner. Freshwater vertebrate; one of two species that may be used in acute tests; purchased from outside vendor.

2.3.5. *Dunaliella tertiolecta*: 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. Marine vertebrate; used in acute and chronic tests; purchased from outside vendor.

2.3.7. *Pimephales promelas*: Common name is fathead minnow. Freshwater vertebrate; used in acute and chronic tests; purchased from outside vendor.

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2.3.8. *Pseudokirchneriella subcapitata*: 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:* 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. PMSD (percent minimum significant difference): 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.

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.

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4.2. Temperatures

4.2.1. Daily, read and record (paper logbooks and LIMS) the temperatures in the lab. 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-06.04), pH (SOP TA-06.37), and the combination conductivity and salinity meter (SOP TA-06.25). Kits are for alkalinity and hardness (SOP TA-06.08), total residual chlorine (TRC; SOP TA-06.22), and ammonia (SOP TA-06.33).

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-06.31 (PB403-S/FACT) and SOP PCR-2.1-1.3 (XA105DU).

4.4.1.1. Monthly: 5 weight calibration check.

4.4.1.2. Quarterly: All weights calibration check.

4.4.1.3. Annually: Tox 'S' weights compared to Chemistry 'S' weights or equivalent.

4.4.1.4. 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, 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 and recorded in the *Record of Preparation Logbook*.

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. The methods of preparation for the sodium selenate solution and B12 solutions are in SOP TA-09.08.

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

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

4.6.1.4. Artificial salt water (ASW) is prepared per SOP TA-03.02 and is used for *Menidia beryllina* and *Americamysis bahia* facility and SRT tests.

4.7. Measuring and Recording Water Quality Parameters

4.7.1. Water quality parameters are taken on sample waters, dilution waters, and organism waters (ordered and in-house). DI water, TRC, pH, ammonia and conductivity are checked weekly (typically on Thursdays).

4.7.1.1. Parameters to measure: temperature (SOP TA-06.13), pH (SOP TA-06.37), DO (SOP TA-06.04), alkalinity and hardness (SOP TA-06.08), conductivity and salinity (SOP TA-06.25), total residual chlorine (TRC; SOP TA-06.22), and ammonia (SOP TA-06.33).

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; record pH, temperature, DO in *Sample Log-in Logbook*; TRC, alkalinity, hardness and ammonia are recorded in their respective logbooks.

4.7.1.2.2. Freshwater diluents: no salinity; record in *Dilution Water Logbook*

4.7.1.2.3. Marine waters: no hardness; sample parameters recorded in *Sample Log-in Logbook*, diluent parameters recorded in *Dilution Water Logbook*

4.7.1.2.4. Organism waters:

4.7.1.2.4.1. Ordered—all; record in *Organism Log-in Logbook*

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

4.7.1.2.5. DI water: TRC, pH, ammonia, alkalinity and conductivity only; record in LIMS Lab Water QC Log

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

4.8.1. The methods for *Artemia* culturing are 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 *Artemia* to hatch and move towards the light. Collect from light box using a siphon. Aerate the collected *Artemia* until use.

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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 signed out from Receiving.

4.9.1.2. Sample temperatures taken immediately and recorded in *Sample Log-in Logbook.*

4.9.1.3. Sample warmed to 25 ± 1 °C; aliquot taken for parameter measurements (refer to Step 4.7.).

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

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

4.9.1.4.2. 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 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 fed *Artemia* and observed for at least two hours before use in test. Culture dishes are aerated while organisms acclimate.

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.

4.11.2. Each test SOP includes test initiation, interim counts, cleaning, and parameters methods; test termination procedure, and SRT method.

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

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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* 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-09.19.

4.12. Weighing Chronic Tests

4.12.1. Chronic fish and chronic *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 degrees Celsius for 24 hours or 100 degrees Celsius for 1 hour for 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 in \\tlhlabapp\CHEM_APPS\Tempsys-CheckPoint\Manuals. Thermometer temperatures and temperature 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 TRC, 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; SOP TA-06.31), light intensities, (SOP TA-06.26), photoperiod times (SOP TA-06.30) and monthly SRTs.

4.14.3. Quarterly: check pipette calibrations (SOP TA-06.32) and check balance calibrations using all S-weights (SOP TA-06.31).

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4.14.3.1. Light timers (photo-period) SOP TA-06.30 TNI required quarterly (we do monthly)

4.14.4. Annually: check thermometer calibrations using the Fluke 7130 Temperature Calibration Micro-Bath and SOPs TA-06.13 and LB-035-1.3.

4.14.4.1. Light intensity – TA-06.26 TNI required annually (we do 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 (SOP TA-01.01). We use fish flake food in place of trout chow. The freshwater algae *Pseudokirchneriella subcapitata* is concentrated to 3.9×10^7-4.5×10^7 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 (2012), with TOXCALC® used as backup.

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-07.16.

4.16.1.2. Acute: Lethal concentration 50 (LC50); follow methods in SOP TA-07.07.

4.16.1.3. The EPA WET Spreadsheet can be downloaded by clicking the link above. Under "WET Guidance," open the "Programmatic" drop down menu. The download link will appear. 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 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

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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 Z2 calibration and use is in (SOP TA-06.19). Coulter Multisizer 4e instructions are in X:\Labs\Lab5\Biology\Bench Common\ToxCommon\Beckman Coulter.

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

4.19. **Documentation**

4.19.1. Document every task the trainee becomes proficient in. Hard copy documentation should be written in the New Analysts' training log. 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).

5.3. Log sample receipt, temperature and condition into *Bioassay Sample Log-in* book (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,

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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, X:\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, http://www.dep.state.fl.us/labs/library/lab_sops.htm

8.2. DEP Laboratory Health and Safety Plan, https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

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*

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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 Spreadsheet (2012)

8.9. \\tlhlabapp\CHEM_APPS\Tempsys-CheckPoint\Manuals

8.10. \\fldep1\DEAR\Labs\Lab5\Biology\Bench Common\ToxCommon\Beckman Coulter

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

8.12. DEP SOP TA-01.03, *Artemia Nauplii Culture*

8.13. DEP SOP TA-01.04, *Pseudokirchneriella subcapitata Culture for Ceriodaphnia dubia food*

8.14. DEP SOP TA-01.05, *Decapsulation of Artemia cysts.*

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

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

8.17. DEP SOP TA-03.01, *Preparation of 20% Dilute Mineral Water (DMW)*

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

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

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

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

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

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

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

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

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

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

8.28. DEP SOP TA-05.02, *Production of Ceriodaphnia dubia for Chronic Toxicity Testing*

8.29. DEP SOP TA-06.01, *Temperature monitoring of Walk-in Refrigerator, Freezer and Toxicity Lab Incubators*

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8.30. DEP SOP TA-06.04, *YSI Model 58 Dissolved Oxygen Meter Calibration and Measurement*

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

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

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

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

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

8.36. DEP SOP TA-06.17, *Measuring Total Ammonia*

8.37. DEP SOP TA-06.18, *AMSCO Renaissance Series 3021 Autoclave Calibration and Use*

8.38. DEP SOP TA-06.19, *Calibration and Use of the Coulter Counter Model Z2*

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 Kits*

8.41. DEP SOP TA-06.23, *Calibration and Use of Accumet Model AR10 pH* Meter

8.42. DEP SOP TA-06.25, *Calibration and Use of Mettler Toledo SevenMulti for Measuring Conductivity, Salinity and TDS*

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

8.44. DEP SOP TA-06.28, *Using Data Loggers to Record Temperatures*

8.45. DEP SOP TA-06.30, *TIMER VERIFICATION: Culture Lab and toxicity Incubators*

8.46. DEP SOP TA-06.31, *Calibration and Use of Mettler Toledo PB403-S/FACT Balance*

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

8.48. DEP SOP TA-06.33, *Measuring Ammonia Using Hach Ammonia Nitrogen Test Kits for Freshwater and Saltwater samples*

8.49. DEP SOP TA-07.01, *Ceriodaphnia dubia Acute Toxicity Testing Methods*

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

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

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

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

8.54. DEP SOP TA-07.07, *Statistical Analysis of Acute Toxicity Testing Methods*

8.55. DEP SOP TA-07.09, *Pimephales promelas and Cyprinella Chronic Toxicity Testing*

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

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

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

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

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

8.61. DEP SOP TA-07.16, *Using ToxCalc for Statistical Analysis of Chronic Bioassays*

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

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

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

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

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

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

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

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

8.70. DEP SOP TA-09.01, *Sodium Chloride Reference Toxicant Stock Solutions*

8.71. DEP SOP TA-09.08, *Preparation of Selenium Solution for use in 20% DMW, MHWCD and Pseudokirchneriella subcapitata Culture media*

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

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

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

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

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

8.77. DEP SOP TA-09.19, *Preparation and testing of Cadmium Chloride and Copper Chloride Reference Toxicant Stock Solutions*

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

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

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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 1970's. SRT logbooks dating from 2009-2018 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, 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.
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 (log books), ToxCommon and other places deemed appropriate (ex. file cabinet).

Appendix of Changes:

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