Menidia beryllina Chronic Toxicity Testing Methods

1. SCOPE AND APPLICATION

This method is based on 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, Test Method 1006.0. It describes the procedures used to estimate the chronic toxicity of whole effluent and receiving water (marine or salinity adjusted aqueous samples) on 7 to 11-day old *Menidia beryllina* (inland silverside) during a 7-day, static renewal exposure. Tests covered by this procedure are BSY-CS-MFS and BSY-CD-MFS.

2. SUMMARY OF METHOD

2.1. Samples are received and prepared in the laboratory. Sample physical-chemistry (phys-chem) parameters are measured and samples are adjusted as needed. Sample and control waters are placed into test jars. Test organisms are received, fed and held for observation prior to loading into test jars. Organisms are then loaded into test jars. Tests are incubated in laboratory incubators bath for seven days. While tests are in progress, survival/mortality and phys-chem parameters are measured and recorded daily before and after renewal. The test endpoints are survival and growth (measured as dry weight) of the organisms. Results are entered into the Laboratory Information Management System (LIMS).

2.2. Test acceptability criteria: 80% or greater survival in the controls and an average dry weight of ≥0.50 per surviving control organism, dried immediately after test termination.

2.3. For this test, it is presumed that the salinity requirement of 5‰-32‰ (±2‰ of the selected test salinity) has been met and all effluents and control water have been salinity adjusted as needed (Refer to SOP TA-07.13). The required test salinity is determined per the facility's NPDES permit, by consultation with FDEP Regional Operation Center (ROC) Personnel, or by SOP TA-07.13. Filter sample through a 60µm screen to remove debris and/or indigenous organisms if needed. Also, adjust pH (TA-08.06) and dissolved oxygen concentration; dechlorinate (TA-09.18), etc.

2.4. Interferences: Refer to EPA-821-R-02-014 Section 13.3. Includes toxic substances introduced by contaminants in dilution water or on hands, glassware, sample hardware and testing equipment, dissolved oxygen <4.0 mg/L, pH <6.0 or >9.0 SU; salinity < 5 or > 35 parts per thousand; ammonia, chlorine, debris, and indigenous organisms that may attack or be confused with the test organisms.

3. EQUIPMENT AND SUPPLIES

3.1. Four autoclaved, glass bioassay jars (1-L) for each test concentration

3.1.1. A test with 6 test concentrations and a control, using 4 replicates per concentration (required minimum) would require 24 jars. Tests are generally run using 1L glass jars and 500 mL test solution/replicate.

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3.2. Autoclaved graduated cylinders, 100, 250, 500, and 2,000 mL for making test solutions.

3.3. Two autoclaved 6000mL Erlenmeyer flask for mixing test dilutions and to hold effluent sample.

3.4. Serological pipettes—1 ml and 10 mL, graduated

3.5. Pipetter for serological pipettes

3.6. Magnetic stir bars and stirrer

3.7. Large plastic Carolina dish to hold fish for acclimation and loading into test jars

3.8. 60µm mesh to filter sample

3.9. Droppers, and glass tubing with fire polished edges, 4 mm ID, for transferring test organisms

Note: To avoid potential injury to the *M. beryllina* test organisms, nets or screens should not be used for transfers.

3.10. Effluent samples to be tested (recommended to be warmed to 26 ± 1ºC)

3.11. Meters to measure:

3.11.1. pH calibrated per SOP TA-06.23

3.11.2. Dissolved oxygen (DO) calibrated per SOP TA-06.04

3.11.3. Temperature using digital thermometer calibrated per SOP TA-06.13

3.11.4. Conductivity/salinity/ calibrated per SOP TA-06.25

3.12. Hach® kits to measure:

3.12.1. Total residual chlorine (TRC) per SOP TA-06.22

3.12.2. Alkalinity per SOP TA-06.08

3.12.3. Hardness per SOP TA-06.08; usually not measured when conductivity is > 5,000 µmhos/cm

3.12.4. Ammonia per SOP TA-06.33

3.13. Thermo Scientific Incubator (incubator # 1, 3, or 5)

3.13.1. Incubator should maintain test temperature at 25 ± 1 °C and a photoperiod of 16h light: 8h dark. Light intensity should be 50-100 ft. candles. Sample temperatures in jars must not deviate by more than ± 3 °C for the duration of the test.

3.13.2. Other incubators or a water bath can be used to regulate test temperature.

3.14. 30 mL cups contained in parameter tray

3.14.1. Two cups per test concentration. One is for the “old” solution parameters; the other is for the “new” solution parameters. Rinse cups daily.

3.15. Waste bucket

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3.16. *Artemia* nauplii (<24 hours old)

3.16.1. Used to feed test organisms at the rate of 0.10 g wet weight per replicate on test days 0-2; at the rate of 0.15 g wet weight per replicate on days 3-6. Half the listed rate when 50% mortality occurs in a replicate. Refer to SOP TA-01.03 to culture *Artermia*.

3.17. Drying oven. Capable of sustaining 105 °C for up to eight hours or 60 °C for 24 hours. Refer to SOP TA-06.21.

3.18. Weigh boats (prepared per SOP TA-06.21)

3.19. Laboratory "S" weights

3.19.1. Two weights that span the weight range of the empty weigh boats, and then two weights that span the weights of the weigh boats plus the dried fish.

3.20. Mettler Toledo balance XA105DU (calibrate per SOP PCR-2.2), to weigh weigh-boats and dried fish.

3.21. *Menidia beryllina* test organisms: 7–11 days old, 40 per test concentration, 5 per replicate for a total of 240 organisms.

3.21.1. Order from supplier. Arrange shipping so as not to exceed required organism age and to allow time for organism acclimation as needed. Refer to SOP TA-04.20.

3.22. *Chronic Bioassay Bench Sheet.*

3.23. *Daily Physical-Chemistry Calibration Log.*

4. REAGENTS AND STANDARDS

4.1. Artificial Sea Water (ASW) prepared with laboratory grade artificial sea salts (SOP TA-03.02); or, Natural Sea Water (NSW).

5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

5.1. See SOP FS 1000 for sample collection and preservation.

5.1.1. For a valid test: a) the sample should be held at 0-6 °C prior to test initiation, and b) sample holding time should not exceed 36-hrs from time of collection (last sample time when 4 grab samples are collected over a 24-hour period, or the end of the 24-hour collection period for a composite sample) to test initiation.

5.1.2. A series of three samples (designated as samples 1, 2, and 3) are collected and used upon receipt. Sample 1 is used for 2 days, sample 2 is used for 2 days, and sample 3 is used for 3 days.

5.1.2.1. The remainder of each sample is stored in the walk-in cooler for solution renewal the following day or days.

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5.2. See SOP TA-06.14 for bioassay sample log-in procedure.

6. TEST INITIATION

6.1. Place clean 1-L test jars onto laboratory cart. Label each jar to identify the replicate (A–D), test concentration and facility sampled.

6.1.1. Place tape across front of jar, beneath jar #, and use waterproof marker to identify. Four jars for each set of control replicates needed for each sample tested, plus four jars per concentration for each sample tested.

6.2. Calibrate meters and record readings in *Daily Physical-Chemistry Calibration Log* daily during test. Also, check meters at end of day and record values.

6.3. Filter effluent sample through 60µm mesh into 6000mL Erlenmeyer flask

6.4. Fill a disposable, 30 ml cup with sample and take initial pH measurement.

6.4.1. If pH is outside acceptable range of 6.0–9.0, then an additional test with a pH-adjusted aliquot will be run. The test with the original sample need now only be a screening test, regardless of whether the pH-adjusted aliquot is used for a screening or definitive test. Refer to SOP TA-08.06 for sample pH adjustment.

6.5. In same plastic cup with sample, take initial DO measurement.

6.5.1. If DO is <4.0 mg/L (low DO) or >9.0 mg/L (super saturated DO), aerate entire sample to ~ 90% saturation prior to test initiation. Samples with a potential DO problem generally show a downward trend in DO during the first few hours of the test. Monitor the DO closely. If DO begins to fall, set up gentle aeration at <100 bubbles/min to ALL test solutions for duration of test using micropipettes and the aeration manifold.

6.6. In same plastic cup with sample, measure the conductivity, temperature and salinity (by direct measurement or by conversion) of the sample.

6.6.1. If conductivity is less than 2,000 µmhos/cm (corrected to 25 °C) or the salinity is less than 1 ppth, freshwater organisms probably should be used rather than *Menidia beryllina*. Review NPDES permit test requirements and/or contact appropriate Regional Operation Center (ROC) personnel.

6.6.2. The following web site: http://www.fivecreeks.org/monitor/sal.html can be used for salinity conversions from conductivity and temperature measurements.

6.7. Measure total residual chlorine (TRC) in the sample per SOP TA-06.22. Check field data sheet supplied by sampler for field TRC and atypical field measurements.

6.7.1. If TRC >0.1 mg/L and non-chlorine toxicity is to be measured, dechlorinate a portion of the sample using sodium thiosulfate per SOP TA-09.18. Test dechlorinated replicates of all chosen test concentrations concurrently with additional chlorinated (100% sample ONLY) replicates.

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6.8. Measure alkalinity, hardness (if appropriate), and total ammonia of sample per SOP TA-06.08 and TA-06.17 or TA-06.33.

6.9. Using a graduated cylinder, pour a minimum of500 mL of diluent into each control replicate jars.

6.10. Pour a minimum of 500mL of 100% sample into each of the 100% sample concentration jars.

6.11. Measure the desired volumes of diluent and sample into a 6,000-mL Erlenmeyer flask using two graduated cylinders. Shake/swirl flask by hand to mix or place flask on stir table and stir until homogenized. Measure 2000 mL of the mixture back into a 2000-mL cylinder. Transfer 500 mL from the 2,000-mL cylinder to a 500 mL cylinder. Empty the 500 mL cylinder into test jar A; repeat for jars B, C and D for each concentration. Rinse cylinders with deionized water between concentrations. Repeat for each test concentration.

6.11.1. For each sample tested, use four replicates per concentration in a dilution series based on the Facility's NPDES permit, instruction from the appropriate FDEP ROC or, if applicable, previous knowledge of toxicity level. Usually a minimum of 5 concentrations plus the control are used.

6.11.2. Once concentrations are poured, immediately record temperatures to prevent loss or gain of sample temperatures as best as possible

6.11.3. Example of sample volumes (0.5 dilution) for four 500 mL replicates of each concentration of a *chronic* toxicity test:

Concentration	Diluent	Sample	Total Volume
Control	2000 mL	0 mL	2000 mL
6.25%	2000 mL	2000 mL of 12.5%	4000 mL
12.5%	2000 mL	2000 mL of 25%	4000 mL
25%	2000 mL	2000 mL of 50%	4000 mL
50%	2000 mL	2000 mL of 100%	4000 mL
l00%	0 mL	2000 mL of 100%	2000 mL

6.12. Perform QA standard reference toxicant (SRT) tests monthly, using copper chloride (other toxicants can be used) for the SRT, unless chronic tests are not performed on a regular basis (at least one per month). SRTs must be performed concurrently with the facility test.

6.12.1. Example of KCl concentrations (toxicant) and diluent volumes for *chronic* SRT test. SRTs are performed at 20‰ salinity: Toxicant and concentrations can change as needed by the lab.

Concentration	Diluent	Toxicant	Total Volume
Control	2000 mL	0 mL	2000 mL
0.0625 g/L	2000 mL	2000 mL of 0.125 g/L	4000 mL
0.125 g/L	2000 mL	2000 mL of 0.25 g/L	4000 mL
0.25 g/L	2000 mL	2000 mL of 0.5 g/L	4000 mL
0.5 g/L	2000 mL	2000 mL of 1 g/L	4000 mL

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Concentration	Diluent	Toxicant	Total Volume
1 g/L	0 mL	4000 mL of 1 g/L KCl	4000 mL

6.13. Pour aliquots from all "A" replicates into the appropriate 30 mL cups in the parameter tray. Measure pH, DO, temperature, conductivity, and salinity of the solutions from the aliquots. Record all readings in appropriate columns on the *Chronic Bioassay Bench Sheet*. Record any comments pertaining to the condition or appearance of the sample. On the following seven days, rotate through all of the replicates and take parameters before and after renewing the sample.

6.13.1. Conductivity measurements (µmhos-cm) and salinity measurements (ppth) are reported to 3 significant figures. pH, DO, and temperature measurements should be read to one decimal place. Take measurements from lowest to highest concentration.

6.13.2. Day 0 "A" replicates; Day 1 "B" replicates, etc. If a replicate is not available (removed from rotation due to 100% mortality), measure aliquot from the next alphabetically sequential replicate of the same concentration.

6.14. Test initiation (0-hour and 0-Day):

6.14.1. Using a wide bore pipette, load **two** (20% of test organisms per replicate) fish into each test chamber. Repeat the loading process until **ten** fish have been loaded into each chamber. Verify count over a light table. Record counts in appropriate columns on the *Chronic Bioassay Bench Sheet.*

6.14.2. (10 fish per replicate) x (4 replicates per test concentration) = 40 fish per concentration tested.

6.14.3. A person other than the loader should verify the number of fish in each replicate.

6.15. Prepare *Artemia nauplii* and feed at a rate per steps 5.11.1, 5.11.2, and 5.11.3. Feed after initial loading and after daily renewal. Do not feed on test day 7 (see step 6.3). Use a calibrated pipet to feed fish 2 mL *Artemia* per replicate. Without contacting test liquid or chamber, gently dispense the food along the inner wall of the chamber to minimize disturbance to the fish and prevent loss or cross contamination.

6.15.1. See Step 3.16.1 for feeding instructions. Adjust amount fed as necessary to compensate for mortality and/or dissolved oxygen issues..

6.15.2. For days 0-2, concentrate <24-hour old *Artemia* (SOP TA-01.03) and place 4 mL of concentrate in a 100 mL beaker containing 20-30 mL of ASW. Bring the volume to 80 mL. Stir to mix, then pipet 2 mL to each replicate; OR feed ~0.1 ml of concentrated *Artemia* per replicate. Adjust as needed for mortality or DO problems.

6.15.3. For days 3-6, concentrate <24-hour old *Artemia* (SOP TA-01.03) and place 6 mL of concentrate in a 100 mL beaker containing 20-30 mL of ASW. Bring the volume to 80 ml. Stir to mix, then pipet 2 mL to each replicate; or, feed ~0.15 ml of concentrated *Artemia*per replicate. Adjust as needed for mortality or DO problems.

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6.16. . Use the test randomizer located on the Laboratory whiteboard to randomly place the test chambers in the proper incubator.

Note: Use covers to prevent accidental contamination and minimize evaporation.

6.13. Measure alkalinity and hardness if appropriate (SOP TA-06.08), total residual chlorine (SOP TA-06.22) and total ammonia (SOP TA-06.33 or TA-06.17) of sample, salinity adjusted sample (as needed) and control water. Record each in the corresponding log book along with control water parameters in *Dilution Water Logbook*.

Note: Hardness is not usually measured if conductivity is greater than 5,000 µmhos/cm.

7. INTERIM READINGS AND RENEWALS

7.1. At 24 (± 2-3 hours) intervals, pour aliquots of the appropriate replicates into 30 mL cups for parameters. Count the fish in each test replicate. Measure pH, temperature, DO, conductivity and salinity in the aliquots before renewing the test solutions and record the readings in appropriate columns in the *Chronic Bioassay Bench Sheet*. Clean test jars by siphoning the "old" test solutions to remove debris, dead *Artemia* and any dead test organisms. Using a tube with a fine mesh capping one end, carefully siphon all but 10%-15% of the solution from each jar into a waste bucket. Try to keep the fish within the jar. Replace the contents of each chamber with respective fresh concentrations of the sample. Count the fish in each replicate. Record live, dead, or missing fish on test bench sheet. Pour aliquots of renewed solutions into 30 ml cups. Measure pH, temperature, DO, conductivity and salinity in the aliquots and record the readings in appropriate columns in the *Chronic Bioassay Bench Sheet*.

7.1.1. Generally, Day 0 aliquots are taken from the "A" replicates; Day 1 aliquots are taken from the "B" replicates; Day 2 from "C", etc. However, before renewal and after renewal aliquots should be from the same replicate for each concentration; EXCEPT, if all organisms are dead in a replicate, the after-renewal aliquot is taken from a replicate with living organisms (if applicable for a concentration).

Note: The remaining amount of solution after siphoning should measure a half-inch to an inch from the bottom of the test jar. All counting is done over a light table. Fish are recorded as live if they move when lightly prodded or if any external or internal organs of the organism are still active.

Note: If a fish becomes stuck on the siphoning mesh, immediately return the fish to the jar. To return the fish, dip siphoning hose tip with net attached back into correct jar and allow fish to swim off.

7.2. Pour aliquots from the appropriate replicate(s) after renewal into the appropriate 30 mL cup. Measure pH, temperature, DO, conductivity and salinity (appropriate replicate for each concentration) and record readings in the appropriate columns in *Chronic Bioassay Bench Sheet*.

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7.3. Feed fish with *Artemia nauplii* concentrate daily after the renewal. Prepare *Artemia* and adjust volume per step 5.11. Return chambers to incubator and cover to reduce evaporation.

8. TEST TERMINATION

8.1. Make weigh boats two days before test termination. The day of test termination, weigh the empty weigh boats. Refer to SOP TA-06.21.

8.2. On day 7 (t = 168 hr ± 6 hr), count and record number of surviving and dead fish. Remove and discard dead fish. Pour aliquots and measure parameters of the solutions. Record all counts and readings along with test termination date and time in appropriate columns in *Chronic Bioassay Bench Sheets*. Record any comments pertaining to the appearance or condition of the fish.

Note: Counting is done over a light table. Physical-chemical readings are taken from the aliquot to minimize disturbance to the fish.

8.3. Pour the contents of Control replicate "A" onto a fine mesh net held over the waste bucket. Immediately euthanize the fish by immersing them in ice water in the Carolina dish. Keep the fish immersed for two minutes to ensure they are fully euthanized.

8.4. When the fish are dead, gently rinse them with DI water. Use forceps and place each fish into the respective weigh boat. Return weigh boat to the Carolina dish. Repeat Steps 7.3 and 7.4 for each replicate.

8.5. Dry the weigh boats containing the fish for 6 hours (minimum) at 105℃ or 24 hours at 60℃ in the sediment drying oven the day of test termination. Store the Carolina dish in the desiccator before and after they are dried.

8.5.1. Refer to SOP TA-06.21.

8.5.2. Dry for 8 hours (minimum of 6 hours) at 105 °C.

8.5.3. Fish can also be dried for 24 hours at 60 °C.

8.6. Remove the dish after cooling and weigh the weigh-boats containing the fish (per SOP TA-06.21). Record the weights in the appropriate *Chronic Bioassay Bench Sheet.*

9. STATISTICAL ANALYSIS

9.1. Complete the weight page in the *Chronic Bioassay Bench Sheets Pimephales promelas and Menidia beryllina* book.

9.2. Enter test data into statistical program to obtain LC_{50}, NOEC, LOEC, and IC25 as per SOPs TA-07.07 and SOP TA-07.16.

9.2.1. Refer to data analysis in EPA-821-R-02-014. TSK, Dunnetts, and ICP programs can be used to analyze data. *TOXCALC®, CETIS® or EPA WET Spreadsheet* statistical programs can also be used to calculate endpoints.

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9.3. Enter test results into LIMS after two-person data verification. Refer to SOP BG-01.

10. DATA ARCHIVAL

10.1. Log sample receipt, temperature and condition into *Bioassay Sample Log-in* bool per SOP TA-06.14, including LIMS numbers from Chemistry.

10.2. FDEP Laboratory Information Management System (LIMS)

10.3. Record information in appropriate logbooks (*Chronic Bioassay Bench Sheets* and *Daily Physical-Chemistry Calibration Log Book*).

10.4. Fill in information requested at top of bench sheets 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 via the Receiving folder, \\fldep1DEAR\Labs\Lab3\Receiving to obtain sampling information.

11. QUALITY CONTROL

11.1. Refer to FDEP Laboratory Quality Manual for corrective action and handling out-of-control data. Refer to EPA-821-R-02-014 Test Method 1006.0 for test conditions and acceptability criteria.

11.2. Sample should be held at 0-6ºC prior to test initiation and between renewals.

11.3. Sample holding time should not exceed 36 hours from time of collection until initial use of sample.

11.4. Incubator maintained at the selected test temperature of 25±1ºC.

11.5. Test temperature must not deviate more than 3ºC in the test jars for the duration of the test.

11.6. If a problem occurs during sample processing or testing, the following steps are performed: 1) lab supervisor is notified, 2) client is notified (client makes decision for additional testing), 3) Program Administrator is notified, and 4) a nonconformance report (NCR) describing the problem and the corrective action is created in LIMS.

11.6.1. To create NCR, open LIMS and go to Laboratory/Administrative/Non-Conformance Report.

11.7. Perform QA standard reference toxicant (SRT) tests monthly or concurrently as needed (See step 5.8).

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

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

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

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

11.9.3. 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.).

12. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

12.2. Refer to the FDEP Laboratory Quality Manual for sample disposal

12.3. Refer to the Laboratory Health and Safety Plan.

12.4. Read MSDS information for reagent handling and disposal.

13. REFERENCES

13.1. DEP Laboratory Quality Manual.

13.2. DEP Laboratory Health and Safety Plan.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, Test Method 1006.0. October 2002.

13.3. DEP SOP FS 1000, General Sampling (Document download Link).

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

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

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

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

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

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

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

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

13.12. DEP SOP TA-06.22, *Measuring Total Residual Chlorine using the Hach Pocket Colorimeter Test Kit*

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

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

13.15. DEP SOP TA-06.33, *Measuring Ammonia using HACH Ammonia Nitrogen Test Kits for Freshwater and Saltwater Samples*.

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13.16. DEP SOP PCR-2.2, *Calibration, use and maintenance of Mettler Toledo XA105DU balance*

13.17. DEP SOP TA-07.07, *Statistical Analysis of Acute Toxicity Test Results*

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

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

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

13.21. DEP SOP TA-09.01, *Sodium Chloride and Potassium Chloride Reference Toxicant Stock Solutions*

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

APPENDIX OF CHANGES

08/28/2008	Moved *Scope and Application*, *Summary of the Method* and *Interferences* from comments to body of SOP. Added *References* and *Appendix of Changes*. Removed SOP *TA-06.06* from Materials # 10 comment. Deleted calibration reference in Test Initiation # 2 comment.
07/13/2009	Updated Quality manual reference.
08/14/2009	Replaced reference to SOP TA-07.08 with reference to SOP TA-07.16.
07/21/2010	Updated reference to Biology Quality Manual. Removed reference to vinyl gloves in the Safety section comment and added a Caution statement.
03/17/2011	Updated references to Biology Quality Manual, now Bureau Quality Manual. Changed 105ºC to 100ºC throughout the SOP.
02/27/2012	Updated references to Bureau Quality Manual. Updated test bench sheet book name. Corrected 2011 Appendix of Changes date.
01/17/2014	Reformatted SOP.
02/13/2015	Formatted for 508 compliance.
03/03/2016	Updated links. Updated names of and moved the FDEP Laboratory Quality Manual and Laboratory Health and Safety Plan links to the reference section. In the reference section, listed SOP identifier before the SOP title. Italicized SOP titles. Format for Toxicity SOP consistency.
03/03/2016	Accepted format edits.
02/24/2017	Updated links and SOPs.
02/28/2018	Added hyperlinks for SOPs and manuals.

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