Salinity Adjustment of Samples: Toxicity Bioassay

1. SCOPE AND APPLICATION

1.1. This method is based on EPA-821-R-02-012 *Method for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*. It is used to adjust the salinity of bioassay diluents and test solutions as needed to meet salinity requirements of test methods and NPDES permits. Tests covered by this SOP are BSY-AS-MMY, BSY-AD-MMY. BSY-CS-MMY, BSY-CD-MMY, BSY-AS-MFS, BSY-AD-MFS, BSY-AS-MMY, BSY-CS-MFS, BSY-CDS-MFS, BSY-CS-MAR and BSY-CD-MAR.

2. SUMMARY OF THE METHOD

2.1. Sample salinity is determined. Required test salinity is determined. Artificial sea salts or hypersaline brine is added to the test solution(s) to bring the salinity to the required test salinity.

2.1.1. For NPDES compliance see Quality Control (Step 8.4).

2.2. When a salinity adjustment is necessary for a test solution, a salinity-adjusted control must be tested along with the salinity-adjusted test solution and the regular control, in order to check for any toxicity due to the addition of artificial salts. The salinity-adjusted control is adjusted using the same steps as for the bioassay test solution salinity. The salinity-adjusted control can be Natural Seawater (NSW) or aged artificial seawater (ASW) diluted to the salinity of the original test solution then salinity adjusted in the same manner as the sample. If a freshwater effluent is being salinity adjusted, well water can be salinity adjusted and used as the salinity-adjusted control. ASW or NSW can be used for the salinity adjustment of test solutions that are tested at less than 100% concentration. (Ex. tested at 25% concentration at 20 (± 1) ppth (part per thousand) salinity. Original test solution 1 ppth dilute to 25% with 26.25 ppth salinity ASW (3 parts ASW to 1 part test solution = 25% at 19.9 ppth salinity).

3. EQUIPMENT AND SUPPLIES

3.1. Magnetic stirrer

3.2. Magnetic stir-bar, 50mm, 1 per bioassay sample

3.3. Magnetic stir-bar retriever

3.4. Erlenmeyer flask, 4000mL, 1 per screening bioassay sample

3.5. Erlenmeyer flask, 6000mL, 1 per definitive bioassay sample

3.6. Squeeze bottle with DI water (used to rinse probes)

3.7. Rinse water cup, 200mL

3.8. Waste container

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3.9. YSI Model 58 DO meter – Calibrate and use per SOP TA-06.04

3.10. Conductivity to salinity conversion tables

3.11. Mettler SevenMulti® meter – Calibrate and use per SOP TA-06.25

3.12. Latex or non-latex gloves

4. REAGENTS AND STANDARDS

4.1. Bioassay laboratory grade Forty Fathoms ® Artificial Sea salts, Crystal Sea ® Marine Artificial Sea salts or hypersaline brine (HSB). HSB is prepared per SOP TA-03.09.

Note: Brands of artificial sea salts other than the one listed can be used. Use of a product does not constitute an endorsement of the product by the FDEP Biology Program.

4.2. Deionized water

4.3. Bioassay sample(s) (also called 'test solution')

5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

6. METHODS

6.1. Refer to the *Bioassay Sample Log-in* book to determine which test solutions may need to have their salinity adjusted. Refer to NPDES permits and/or consult with district personal for "required" test salinity.

- If the test solution salinity is less than 1 ppth, then freshwater test species should be used unless NPDES permit requirements or tests that are requested by district personnel dictate otherwise.
- If the test solution salinity is between 1 and 6 ppth and the source of salinity is not known, use artificial sea salts to increase the original apparent salinity by 6 ppth for mysid and 1 ppth for *Menidia*.
- If the test solution salinity is between 1 and 6 ppth and the source of salinity is known to be seawater ions and ion imbalance is not suspected (i.e. saltwater vs. groundwater ions), use artificial sea salts to increase the salinity to 6 ppth for the mysid test. No adjustment is necessary for the *Menidia* test.
- If the test solution salinity is greater than 6 ppth, no salinity adjustments are necessary unless specified in the facility's NPDES permit, the EPA test method, or requested by District personnel.

Note: Some samples, such as those from paper mills, often have high conductivity readings. Professional judgment must be used with these samples as to whether the tests should use freshwater or saltwater test species (If not specified by permit).

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6.2. Determine test solution salinity: **A**) measure the salinity directly with the SevenMulti® meter; or, **B**) measure the test solution temperature and conductivity. Calculate the conductivity (must know sample temperature) needed to obtain the salinity desired for the test solution. Use the conversion tables to calculate the salinity for the test solution.

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

6.3. If using artificial sea salts: Pour 2200 mL of sample into the 4000-mL flask for a screening bioassay. Pour 4000 mL of sample into the 6000-mL flask for a definitive (0.5 dilution) bioassay. Proceed to Step 6.4.

If using HSB, calculate the proportion of HSB to add to the sample using Steps 6.3.1 to 6.3.4. Blend calculated volumes of HSB (from Step 6.3.3) and sample (from Step 6.3.4). Skip to Step 6.7.

6.3.1. Determine the desired test salinity:

- For a test solution having an initial salinity < 1ppth, the initial test solution salinity is the desired test salinity in the calculation.
- For test solutions having an initial salinity ≥ 1ppth, determine the difference between the initial test solution salinity and the desired test solution salinity. Substitute this result value for the desired test salinity in the calculation.

6.3.2. Determine the salinity ratio of HSB to sample ((HSB salinity - desired test salinity) : (desired test salinity) then REVERSE to get the ratio.

Example:
(HSB salinity 100 - desired test salinity 20) : desired test salinity 20
(100–20) : 20 = 80 : 20 = 4 : 1 Reverse, so use 1 part HSB to 4 parts sample.

6.3.3. Determine volume of HSB needed. Divide total desired test volume by total parts (example: 1000 mL/ (1 + 4) = 1000mL/5 = 200 mL HSB).

6.3.4. Determine volume of test solution to combine with HSB to get total desired test solution volume. Subtract volume of HSB needed from total desired test volume. (example: 1000mL – 200 mL HSB = 800 mL test solution).

6.4. Place the 50mm stir-bar in the flask. Place the flask on the stirrer. Adjust stirrer speed to gently stir the test solution.

6.5. Suspend the conductivity meter probe (pre-rinsed with DI water) in the flask ensuring that the tip of the probe is in the test solution.

6.6. Place a small amount of Forty Fathoms ® bioassay grade artificial sea salts into the flask. Allow the salt to mix/dissolve in the test solution.

6.6.1. The amount of salt to be added can be calculated by subtracting the calculated salinity from the desired salinity, and multiplying the difference by the test solution volume in liters. Weigh this amount of sea salts (in grams)

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and add the salts to the appropriate volume of the test solution. Allow salts to dissolve. Make minor adjustments per Steps 6.6, 6.7, and 6.8.

6.7. Measure salinity per Step 6.2 (A). If the adjusted salinity is more than 0.5 ppt greater than the desired salinity, add unsalted sample to decrease the salinity to the desired range (± 0.5 ppt of desired salinity). To increase salinity, repeat Steps 6.6 for sea salts or Step 6.3 for HSB until the salinity of the test solution is in the appropriate range.

6.8. Continue test set up per the appropriate SOP in Biology Program SOP Category "Toxicity Testing" (SOP's begin with TA-07).

7. DATA ARCHIVAL

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

7.2. FDEP Laboratory Information Management System (LIMS)

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

7.4. 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, \\fldep1\DEAR\Labs\Lab3\Receiving, to obtain sampling information.

8. QUALITY CONTROL

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

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

8.3. 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.

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

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

8.3.3. 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.).

8.4 For NPDES compliance testing, do not use hypersaline brine (HSB) to adjust the salinity of test solutions or diluents because using HSB precludes the testing of 100% test solution. Use artificial sea salts unless otherwise stipulated (in NPDES permit).

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9. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

9.2. Refer to the DEP Laboratory Health and Safety Plan.

9.3. Refer to the DEP Laboratory Quality Manual for sample disposal.

9.4. Read MSDS information for reagent handling and disposal.

10. REFERENCES

10.1. DEP Laboratory Quality Manual, http://www.dep.state.fl.us/labs/library/lab_sops.htm

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

10.3. EPA 821-R-02-012 *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th Edition, Test Method 2002.0.*

10.4. DEP SOP FS 1000, *General Sampling*

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

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

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

10.8. DEP SOP TA-03.09, *Hypersaline Brine Production*

Appendix of Changes:

05/14/2008 Added Scope and Application, Summary of the Method and Appendix of Changes. Added Brands of artificial sea salts other than the one listed can be used. Use of a product does not constitute an endorsement of the product by the FDEP biology laboratory to **Materials** comment. Added Refer to NPDES permits and/or consult with district personal for “required” test salinity to **Method** Step 1.

03/24/2009 Added References section. Removed reference to YSI 35 meter and SOP TA-06.06 from Materials section.

02/25/2010 Replaced ‘Vinyl gloves can be substituted for individuals allergic to latex.” with ‘CAUTION Individuals allergic to latex should not use latex gloves. A suitable substitute should be found and used.’ in Safety Section. Updated reference to Biology Quality Manual.

03/07/2011 Updated reference to Biology Quality Manual. Renamed as Bureau Quality Manual. Deleted comment referencing the YSI ® Model 35 conductivity meter.

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02/22/2012	Updated reference to Bureau Quality Manual. Updated link to SOPs, and the Receiving Folder.
01/18/2014	Revised SOP.
02/13/2015	Formatted for 508 compliance.
01/07/2016	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. Added reference to SOP FS 1000. Reorganized step 6.3.
02/15/2016	Changed *sample* to *test solution*
02/27/2017	Moved NPDES Compliance Note from “Summary of Method” and “Reagents and Standards” to new point in “Quality Control” (8.4)
02/23/2018	Updated the brands of Bioassay Marine Salts in the Reagents and Standards section (4.1)

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