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 conducted by this procedure 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, 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 whole 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. 4 L Erlenmeyer flask, 1 per screening bioassay sample

3.5. 6 L Erlenmeyer flask, 1 per definitive bioassay sample

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

3.7. 200 mL rinse water cup

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3.8. Waste container

3.9. Thermo Scientific Orion Star A213 Benchtop RDO/DO Meter – Calibrate and use per SOP TA-06.39.

3.10. Conductivity to salinity conversion tables

3.11. Fisherbrand Traceable Bench Conductivity Meter – Calibrate and use per SOP TA-06.36

3.12. Latex or non-latex gloves

3.13. *Bioassay Sample Log-in* book

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 EPA-821-R-02-012 Section 7.3.7.

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 Laboratory.

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 ppt, 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 ppt and the source of salinity is not known, use artificial sea salts to increase the original apparent salinity by 6 ppt for mysid and 1 ppt for *Menidia*.
- If the test solution salinity is between 1 and 6 ppt 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 ppt for the mysid test. No adjustment is necessary for the *Menidia* test.

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- If the test solution salinity is greater than 6 ppt, 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).

6.2. Determine test solution salinity: **A**) measure the salinity directly with the Fisherbrand Traceable Bench Conductivity 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: https://www.hamzasreef.com/Contents/Calculators/SalinityConversion.php can be used for salinity conversions from conductivity and temperature measurements.

6.3. If using HSB, calculate the proportion of HSB to add to the sample using steps 6.3.1 to 6.3.5. If using artificial sea salts, proceed to step 6.4.

6.3.1. Determine the desired test salinity:

- For a test solution having an initial salinity < 1ppt, the initial test solution salinity is the desired test salinity in the calculation.
- For test solutions having an initial salinity ≥ 1ppt, 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).

6.3.5. Mix calculated volumes of HSB (from step 6.3.3) and sample (from step 6.3.4). Proceed to step 6.5.

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6.4. If using artificial sea salts, pour 2200 mL of sample into the 4 L Erlenmeyer flask for a screening bioassay. Pour 4000 mL of sample into the 6 L Erlenmeyer flask for a definitive (0.5 dilution) bioassay.

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

6.4.2. 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.

6.4.3. Place a small amount of Crystal Sea Marine Mix ® bioassay grade artificial sea salts into the flask. Allow the salt to mix/dissolve in the test solution.

6.4.3.1. 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) and add the salts to the appropriate volume of the test solution. Allow salts to dissolve. Make minor adjustments per steps 6.4.3, 6.5, and 6.6. **Conversion note: 1 g of artificial sea salt ≈ 1 ppth.**

6.4.3.1.1. First, calculate grams per liter: (Desired salinity) – (*ppth of sample*) = *a*

6.4.3.1.2. Convert volume of sample needed into liters

6.4.3.1.3. Multiply the result (of *a*) by the volume to get grams of salt needed

6.4.3.1.4. **Example**: Most permits require mysid tests be run at 20 ppth and *Menidia* tests run at 5 ppth if the sample is <5 ppth. If a sample is 3 ppth, two different sample mixtures are made;

1) Mysid: (20 ppth – 3 ppth)(4 L)= 68 g of sea salt required.

2) *Menidia:* (5 ppth – 3 ppth)(4 L)= 8 g of sea salt required.

6.5. 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 step 6.4.3 for sea salts or step 6.3 for HSB until the salinity of the test solution is in the appropriate range.

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

7. DATA ARCHIVAL

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

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7.2. 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.

7.2.1.1. The corrected entry is written as near as possible to the error or in the comment box on the appropriate bench sheet.

7.2.1.2. If the correction requires an explanation, write a comment and reference the error with a letter. Initial and date the explanation. Do not attempt to put all information (correction and comments) into a small space

7.2.1.3. 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.).

7.3. FDEP Laboratory Information Management System (LIMS)

7.4. Bioassay Sample Log-In logbook. Record per SOP TA-06.14.

7.4.1. Log sample receipt, temperature, and condition into Acute Bioassay Bench Sheets logbook. 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 \\fldep1\dear\Labs\BizhubScans\Receiving to obtain sampling information.

8. QUALITY CONTROL

8.1. Refer to DEP Laboratory Quality Manual for corrective action and handling out-of-control data. Refer to EPA 821-R-02-012 for test conditions and acceptability criteria.

8.2. 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).

8.3. If a problem occurs during the 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) Section administrator is notified. 4) A non-conformance report (NCR) is submitted describing the problem and the corrective action.

8.4. To create NCR: open LIMS and go to Laboratory/Administrative/Non-Conformance Report. Refer to DEP SOPs | Florida Department of Environmental Protection for sample collection and preservation, and the Laboratory Quality Manual for corrective action and handling out-of-control data.

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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, https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

10.2. DEP Laboratory Health and Safety Plan, https://www.floridadep.net/resources/health-safety-manual

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-06.14, *Bioassay Sample Log-In Procedure*

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

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

Appendix of Significant 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.

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03/07/2011	Updated reference to Biology Quality Manual. Renamed as Bureau Quality Manual. Deleted comment referencing the YSI ® Model 35 conductivity meter.
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)
02/21/2019	Updated links and referenced SOPs.
02/21/2020	Update links and referenced SOPs.
02/03/2021	Updated links and incorrect meter reference and edited for clarity.
01/31/2022	Updated links.
02/08/2023	Clarified salinity adjustment with artificial sea salts. Removed hyperlinks from constantly updated SOPs.

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