WEIGHING CHRONIC TESTS

1. SCOPE AND APPLICATION

1.1. This procedure is used to determine the dry weight of organisms used in chronic bioassays described in test methods found in EPA 821-R-02-013 and EPA 821-R-02-014. Tests that use this procedure are BSY-CD-FFS, BSY-CS-FFS, BSY-CS-MFS, BSY-CD-MFS, BSY-CS-MMY, and BSY-CD-MMY.

2. SUMMARY OF METHOD

2.1. Labeled weigh boats are prepared in advance of test termination. They are dried in a drying oven and stored in a desiccator. Empty weights of the weigh boats are determined and recorded. Organisms to be weighed are placed in labeled weigh boats. Weigh boats containing organisms are dried in a drying oven and then stored in a desiccator. Final weights are measured and recorded in the appropriate logbook.

2.2. Prior to weight measurements, the user must weigh and record in the appropriate logbook the weights of two Class 1 weights (or other certified weights, such as Class 2 weights) that bracket the expected weight of the weigh boats to be weighed.

2.3. Interferences include airflow and vibrations which interfere with the balance, weigh boats contaminated by moisture or body-oils, an improperly calibrated balance, as well as, contaminated Class 1 or Class 2 weights.

3. EQUIPMENT AND SUPPLIES

3.1. Weigh Boats

- 3.1.1. Aluminum foil
- 3.1.2. Fine point, black permanent marker
- 3.1.3. 4-inch Carolina dish
- 3.1.4. Scissors
- 3.1.5. Volumetric flask stopper
- 3.1.6. Forceps
- 3.1.7. Ruler, 30 cm
- 3.1.8. Weigh-boat form block (located on shelf above the MHWCD carboys in Room B246)
  - 3.1.8.1. The weigh-boat form block is a block of wood approximately 1.85 cm × 7 cm × 2 cm with 12 circular holes drilled to an approximate depth of 6 mm.
- 3.1.9. Desiccator with desiccant

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3.1.9.1. Desiccant must be blue or white in color. A pink color indicates that the desiccant is hydrated. Dry desiccant in oven before use until blue or white color returns. Vacuum source with attached tubing (if using desiccator with vacuum attachment).

3.1.9.2. Keep the desiccator depressurized even when not in use.

3.2. Weighing

3.2.1. Weigh boats

3.2.2. Filter forceps

3.2.3. Autoclave gloves

3.2.4. Organisms to be weighed

3.2.5. Mettler Toledo XA105DU balance (SN B314195659) (located in the micro lab), calibrated and used per SOP BB-36.

3.2.6. Drying oven maintained at either 100 ˚C (dry time >6 but <8 hours during business hours only) or 60 ˚C (dry time ~ 24 hours when drying overnight)

3.2.7. Certified Class 1 weights (in B246 Cabinet A2) or Class 2 weights (beside XA105DU balance)

3.2.8. *Weights for Chronic Tests* logbook and the appropriate *Chronic Bioassay Bench Sheet* for the test being conducted

4. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

5. PREPARATION OF WEIGH BOATS

5.1. Wearing gloves, lay a (~30 cm X 30 cm) sheet of aluminum foil on a flat surface. Using a ruler, mark 2 cm intervals along opposite ends of the foil. Use the ruler to connect the marks. Do the same on the other two sides of the sheet. The foil should now be marked off into 2 cm squares.

5.2. Carefully cut along one set of 2 cm lines to form 2 cm × 30 cm long aluminum foil strips, with lines every 2 cm. This should form 16 squares per strip.

5.3. As needed, lay the appropriate number of strips shiny side down on a flat surface. (Number of strips is dependent upon the test/tests the weigh boats are to be used for). Use the permanent marker to clearly number both sides of the strips (same number and same square). Underline the single numerals 2, 6, 5 and 9 to avoid confusion. The numbers should be written as close to the center of each square as practical. Begin with number 1 and continue to 24 (more or less) for fish tests or 48 (more or less) for mysid tests.

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5.4. After numbering, cut the squares from the strips. If the squares are not needed right away, clip them with a binder clip and store them in a glass Carolina dish until needed.

5.5. Using forceps, find the first square in series and center over one of the holes in the weigh-boat form block. Carefully form the aluminum foil boat by placing the bottom of the plastic flask stopper on top of the foil square and gently pressing into the hole of the form block. Remove the stopper and carefully remove the boat from the hole using the forceps.

5.5.1. After the boat is formed, make sure the number is still legible. If not, discard the boat and make a new one.

5.6. Place formed boats in a small Carolina dish in a logical order, such as a spiral, so they can be found in numeric order when needed.

5.7. Repeat step 5.5 - 5.7 until all weigh boats have been made. Cover the dish with foil to prevent dust and dirt particles from falling into the weigh boats.

5.8. Open the door of the dessicator and place Carolina dish on any shelf. Empty weigh boats should go in the older desiccator on the left and weigh boats with organisms should go in the newer desiccator on the right located next to the balance. Make sure the desiccant is blue before placing weigh boats in. Desiccant can be dried in the oven at 100°C for one hour if the color is not blue.

6. WEIGHING CHRONIC TESTS

6.1. Day 6 of the 7-day chronic test

6.1.1. NOTE: Weigh boats can be weighed any day prior to test termination day (day 7).

6.1.2. Remove the Carolina dish containing the weigh boats from the desiccator and place it in the drying oven. The foil should be folded or removed to allow air exchange. Set the oven temperature to 100 ˚C and allow the weigh boats to dry for at least one hour.

6.1.3. After at least one hour at 100 ˚C, remove the Carolina dish containing the weigh boats from the drying oven and return it to the desiccator to cool.

6.1.4. When cool, remove the Carolina dish containing the weigh boats from the desiccator and place it next to the Mettler XA105 balance in the micro lab. Fold back the foil and allow the Carolina dish to set for 15-20 minutes to acclimate the weigh boats to the room conditions.

6.1.5. Weigh the weigh boats. Use the forceps to avoid touching the weigh boats with bare flesh. Weigh the weigh boats one at a time in ascending alphanumeric order. After the fifth weigh boat and each subsequent 5 weigh boats, reweigh the first weigh boat. Continue until all weigh boats have been weighed and are within ± 0.05 mg of the first boat. If any of the first

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boat weights are > ±0.5 mg of the first time it was weighed , start over by weight boat 1, 2, 3, etc.

6.1.6. Record the weights on the *Weights for Chronic Tests* logbook and the appropriate *Chronic Bioassay Bench Sheet*. Enter weights into the 'organism final weight' folder located here: X:\Labs\Lab5\Biology\Bench Common\ToxCommon\EPA WET Spreadsheet\Organism Final Weights

6.2. Day 7 of the 7-day chronic test

6.2.1. Take the weigh boats to the test termination area in B246. Load the boats per the *Test Termination* procedure in the appropriate chronic bioassay SOP (TA-07.09, TA-07.14, TA-07.15).

6.2.2. Place the Carolina dish containing the loaded weigh boats immediately into the drying oven.

6.2.3. Weigh boats **with organisms** should be dried at **100 ˚C** for a minimum of **6 hours.** If dried at **60 ˚C**, the drying time should be a minimum of **24 hours**.

6.2.3.1. If an oven fails, temperatures between 60 ˚C and 100 ˚C can be used for drying, if the laboratory can demonstrate that the organisms are dried to a constant weight.

6.3. Day 8 (day after termination of the 7-day Chronic test)

6.3.1. Remove the Carolina dish containing the weigh boats from the drying oven. Weigh the boats as in Steps 6.1.4 to 6.1.5 and record the weights on the *Weights for Chronic Tests* logbook, the appropriate *Chronic Bioassay Bench Sheet* and in the Excel sheet in the 'organism final weight folder'.

7. DATA ARCHIVAL

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

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

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

7.2.3. 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. Record weights on the *Weights for Chronic Tests* logbook, the appropriate *Chronic Bioassay Bench Sheet*, and the "final organism weight" Excel sheet.

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8. QUALITY CONTROL

8.1. The balance is checked before use to ensure it is reading accurately. Two certified Class 1 or Class 2 weights are weighed such that they bracket the expected weights of the objects being weighed.

8.1.1. The weights are not handled with bare hands to prevent contamination from body oils. They are also kept in a closed box to prevent dirt and dust from contaminating them.

8.2. Weights are measured using a balance (Mettler XA105DU) set on a padded marble slab to minimize vibrations which could interfere with the balance.

8.3. The balance is covered to minimize air flow.

8.4. Weigh boats are desiccated to eliminate moisture as an interference in the weighing process.

8.5. Transcription of weights into bench sheets is verified by two people. Weights that are entered in an EXCEL spreadsheet or a WORD document can be verified by one person using the 'speak' function available with each program.

8.6. Forceps are used to handle the weigh boats to prevent body oils from contaminating them and affecting the weight.

8.7. If a problem occurs during the sample processing or testing, the following steps are performed:

8.7.1. Lab supervisor is notified.

8.7.2. Client is notified (client makes decision for additional testing).

8.7.3. Section administrator is notified.

8.7.4. A non-conformance report (NCR) is submitted describing the problem and the corrective action.

8.7.4.1. To create an NCR: open LIMS and go to Laboratory/Administrative/Non-Conformance Report

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

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 Laboratory Quality Assurance Manual for sample disposal.

9.3. Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management and read MSDS sheets for reagent handling and disposal.

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10. REFERENCES

10.1. EPA 821-R-02-013 Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, 4th Ed. October 2002.

10.2. EPA 821-R-02-014 Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, 3rd Ed. October 2002.

10.3. DEP SOP BB-36, Calibration, Use and Maintenance of Mettler Toledo XA105DU Balance.

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

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

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

10.7. Laboratory Quality Assurance Manual https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

10.8. Laboratory Health and Safety Manual https://www.floridadep.net/resources/health-safety-manual

10.9. MSDS Information Sheets

APPENDIX OF CHANGES

08/16/2013 Combined SOPs TA-06.20-1.15 and TA-06.21-2.0. Reformatted the combined SOP to produce TA-06.21-3.0.

02/09/2015 Formatted for 508 compliance.

08/14/2015 Updated links. Weight location. Drying time clarification.

09/14/2016 Updated links. Added *Weights for Chronic Tests* logbook to method. Added verification information.

08/28/2018 Updated links and changed font to Calibri.

08/27/2019 Updated sections 5.2-5.5.

09/01/2020 Updated order of method, added missing information about desiccators, removed irrelevant information.

08/02/2021 Updated links, checked grammar, and updated SOP numbers.

08/09/2022 Updated links and weight names, removed irrelevant information.

08/14/2023 Checked for readability and grammar.

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