Sodium Chloride and Potassium Chloride Reference Toxicant Stock Solutions

1. SCOPE AND APPLICATION

    1.1. This method is used by Toxicity Laboratory personnel to make standard reference toxicants (SRT) for *Ceriodaphnia dubia* and *Pimephales promelas* acute and chronic bioassays, and *Cyprinella leedsi*, *Menidia beryllina* and *Americamysis bahia* acute bioassays.

2. SUMMARY OF METHOD

    2.1. Sodium chloride and/or potassium chloride are weighed and added to appropriate water types to produce standard reference toxicant stock solutions of various concentrations as needed for specific test types.

    2.2. Interferences include contaminated glassware, contaminated sodium chloride or potassium chloride, contaminated dilution water(s) and weighing errors.

3. EQUIPMENT AND SUPPLIES

    3.1. Mettler PB403-S/FACT balance, calibrated per SOP TA-06.31.

    3.2. 4 x 4-inch weigh boat

    3.3. Model 1600 HAFO or Fisher Scientific® model 630F drying oven, maintained at 105 ˚C; Room B205

    3.4. 2.5 cm Magnetic stir-bar

    3.5. Magnetic stir-bar retriever

    3.6. Magnetic stirrer

    3.7. Stirring rod

    3.8. Desiccator with desiccant

        3.8.1. Desiccant must be blue or white in color. A pink color indicates that the desiccant is hydrated and will not keep the salt dry. Dry desiccant in oven before use until blue or white color returns.

    3.9. 20 L Carboy, 3

    3.10. 200 mL Beaker

    3.11. 6 L Erlenmeyer flask

    3.12. 2 L Graduated cylinder

    3.13. Parafilm®

    3.14. Labeling tape

    3.15. Black permanent marker

    3.16. Walk-in refrigerator, maintained at 4 ˚C

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4. REAGENTS AND STANDARDS

4.1. Sodium chloride (NaCl), ACS grade

4.2. Potassium chloride (KCl), ACS grade

4.3. 20% DMW (if used), prepared per SOP TA-03.01

4.4. Synthetic moderately hard water (MHW (fish) and MHWCD (C. dubia)), prepared per SOP TA-03.15

4.5. Artificial Seawater, prepared per SOP TA-03.02

5. PREPARATION OF NACL AND KCL REFERENCE TOXICANT STOCK SOLUTIONS

5.1. Use the balance (Mettler Toledo, S/N 1123052001) located in the Tox lab to carefully measure the appropriate amounts of NaCl or KCl. The amount of NaCl or KCl, volume of toxicant prepared and size of the containers ***can be changed as needed to meet the needs of the laboratory***. Please refer to the table below for quantities commonly used to make toxicants.

Test type	Organism	Toxicant (salts)	Concentration (g/L)	NaCl or KCl needed	Media	Volume of Media
Acute/Chronic	*C.dubia*	NaCl	3 g/L	12g ± 1	MHWCD or 20% DMW	4L
Acute	*C.leedsi* and *P.promelas*	NaCl	10 g/L	100g ± 1	MHW	10L
Chronic	*P.promelas*	KCl	1 g/L	20g ± 1	MHW	20L
Acute/Chronic	*A.bahia* and *M.beryllina*	KCl	2 g/L	20g ± 1	ASW	10L

5.2. Use the label tape and marker to label the 6 L flask with "NaCl 3 g/L in MHWCD or 20% DMW for *C. dubia*." Label one carboy "NaCl 10 g/L in MHW for *C. leedsi* and *P. promelas*." Label one carboy "KCl 1 g/L in MHW for *P. promelas*." Label one carboy "KCl 2 g/L in ASW 20ppt for *M. beryllina* and *Americamysis bahia*. Place the batch number (preparation date), analyst's initials, and an expiration date of 30 days from the preparation date on each label.

5.2.1. Change concentration on labels as needed to meet laboratory requirements.

5.2.2. Format for batch # is mm/dd/yy.

5.3. Using the 2 L graduated cylinder, measure **4 L of 20% DMW** or **MHWCD** into the 6 L flask labeled "NaCl 3 g/L in MHWCD for *C. dubia*;" **10 L of MHW** into the carboy labeled "NaCl 10 g/L for *C. leedsi* and *P. promelas*", **10 L of MHW** into the

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carboy labeled "KCl 1 g/L for *P. promelas* and **10 L of ASW 20 ppt** into the carboy labeled " 2 g/L KCl in ASW 20 ppt for *M. beryllina* and *Americamysis bahia*." The volumes and salt concentrations can be changed based on the needs of the laboratory.

5.4. Place the stir bar in the flask and place the flask on the magnetic stirrer. Turn the stirrer on and adjust the speed to medium.

5.5. Gradually add the NaCl to the flask. When the NaCl has completely dissolved, remove the flask from the stirrer. Use the stir-bar retriever to remove the stir bar.

5.6. Repeat Steps 5.7.-5.8., substituting the appropriate carboy for the flask; then, proceed to Step 5.10.

5.7. Record the NaCl and KCl batch information in the *Tox Record of Preparation* logbook.

5.8. Seal the flask with two layers of Parafilm® to minimize evaporation. Seal the carboys with the carboy lids.

5.9. Solutions can be stored at room temperature for the duration of the bioassay tests. If not expended during the test period, store the containers at > 0 and ≤ 6 ˚C in the walk-in refrigerator. The solutions can be stored for up to 30 days, but are normally expended during the test period(s).

6. DATA ARCHIVAL

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

6.2. Record preparation in the *Tox Record of Preparation* logbook.

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. QUALITY CONTROL

7.1. Refer to FDEP Laboratory Quality Assurance manual for corrective action and handling out-of-control data.

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8. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

8.2. Refer to FDEP Laboratory Quality Assurance Manual for sample disposal.

8.3. Refer to the FDEP Laboratory Health and Safety Plan and Hazardous Waste Management.

8.4. Read MSDS information for reagent handling and disposal.

9. REFERENCES

9.1. FDEP Laboratory Quality Manual (http://www.dep.state.fl.us/labs/library/lab_sops.htm)

9.2. FDEP Laboratory Health and Safety Plan (http://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents)

9.3. DEP SOP TA-06.15, *Calibration and Use of Mettler PC 440 Balance*

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

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

9.6. DEP SOP TA-03.02, *Preparation of Artificail Seawater (ASW)*

9.7. MSDS Information Sheets

APPENDIX OF CHANGES

12/20/2007 Added Scope and application, Summary of Method, and Appendix of Changes.

11/17/2008 Added Interference and Reference section.

08/13/2013 Reformatted. Added references to 20% DMW and Moderately Hard Water SOPs.

06/12/2014 Corrected spelling error.

02/04/2015 Added preparation of KCl toxicant to the SOP and formatted for 508 compliance.

07/20/2016 Update links. Added table, clarified concentrations.

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