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*, *Pimephales promelas*, *Menidia beryllina*, *Americamysis bahia* acute and chronic standard reference toxicity bioassays, and *Cyprinella leedsi*, and acute standard reference toxicity 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. 6 L Erlenmeyer flask

    3.2. 2 L plastic graduated cylinder

    3.3. Mettler 403TS/00 balance calibrated per SOP TA-06.34

    3.4. 4 x 4-inch weigh boat

    3.5. 2.5 cm Magnetic stir-bar

    3.6. Magnetic stir-bar retriever

    3.7. Magnetic stirrer

    3.8. Stirring rod

    3.9. 20 L Carboy

        3.9.1. One for each type of toxicant.

    3.10. Parafilm®

    3.11. Labeling tape

    3.12. Black permanent marker

    3.13. Walk-in refrigerator, maintained at 4°C

    3.14. Computer with access to the Laboratory Information Management System (LIMS)

4. REAGENTS AND STANDARDS

    4.1. Sodium chloride (NaCl), ACS grade

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

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*	KCl	1 g/L	6 g ± 0.1 g	MHWCD or 20% DMW	6 L
Acute	*C. leedsi* and *P. promelas*	KCl	2 g/L	40 g ± 0.1 g	MHW	20 L
Chronic	*P. promelas*	KCl	2 g/L	40 g ± 0.1 g	MHW	20 L
Acute/Chronic	*A. bahia* and *M. beryllina*	KCl	2 g/L	40 g ± 0.1 g	ASW	20 L

5.2. Use the label tape and marker to label the 6 L flask with "1 g/L KCl in MHWCD (or 20% DMW) for *C. dubia*." The toxicant for *C. dubia* stays in the 6 L flask and is not transferred to a carboy. Label one carboy "2 g/L KCl in MHW for *C. leedsi* and *P. promelas*." Label one carboy "KCl 2 g/L in MHW for *P. promelas*." The Toxicology lab typically makes 20 L of 2 g/L KCl in MHW and dilute the toxicant to the appropriate concentration for the freshwater fish species SRTs. Sometimes less is made due to number of SRT test being conducted, refer to schedule and the lab manager. Label one carboy "2 g/L KCl in 20ppt ASW 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. Be sure to create a LIMS serial number in Standard Preparation Tracker for the toxicant that includes all necessary information.

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. 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.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

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

5.5. Repeat Steps 5.3.-5.4., substituting the appropriate carboy for the flask; then, proceed to Step 5.6.

5.6. Record the LIMS # for the toxicant (KCl) and batch information in the Standard Preparation Tracker section of LIMS. Print out a LIMS label for the toxicant and place it on the flask or carboy.

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

5.8. 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). Expired or unused toxicant can be dumped down the sink.

6. DATA ARCHIVAL

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

6.2. Record preparation in the Standard Preparation Tracker section of LIMS.

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 as possible to the error or 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 with a letter. Initial and date the explanation. Do not attempt to put all information (correction and comments) into a small space.

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.)

6.4. Incorrect LIMS entries are corrected, and an explanation is written in the comment box along with initials and date.

7. QUALITY CONTROL

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

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

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

9.2. DEP Laboratory Health and Safety Manual https://www.fldepnet.org/system/files/Administrative-Services/governing-documents/Health%20and%20Safety%20Manual%20December%202019.pdf

9.3. DEP SOP TA-06.34, *Calibration and Use of Mettler Toledo 403TS/00 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 Artificial 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.
08/24/2018	Updated links. Corrected balance information. Updated Toxicant table.
09/13/2018	Added SOP TA-06.34 to references (M. Faircloth 09/13/2018).
09/11/2020	Updated toxicant volumes created. Updated equipment section. Updated data archival section with universal language. Updated links.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.