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 acute and chronic *Ceriodaphnia dubia*, *Pimephales promelas*, *Menidia beryllina*, *Americamysis bahia* standard reference toxicity bioassays, and acute *Cyprinella leedsi* standard reference toxicity bioassays.

2. SUMMARY OF METHOD

    2.1. Potassium chloride is 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

        2.2.1. Contaminated glassware, contaminated potassium chloride, contaminated dilution water(s) and weighing errors.

3. EQUIPMENT AND SUPPLIES

    3.1. 6 L Erlenmeyer flask (used to spin up all toxicant; used to hold toxicant for *Ceriodaphnia dubia* test)

    3.2. 1 L Class A graduated cylinder (for *Ceriodaphnia dubia* test toxicant)

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

    3.4. Plastic weigh boat

    3.5. Magnetic stir-bar

    3.6. Magnetic stir-bar retriever

    3.7. Magnetic stir plate

    3.8. 20 L Carboy

        3.8.1. One for each type of toxicant (*Pimephales promelas* toxicant and both saltwater species toxicants)

    3.9. Parafilm®

    3.10. Labeling tape

    3.11. Black permanent marker

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

4. REAGENTS AND STANDARDS

    4.1. Potassium chloride (KCl), ACS grade

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

4.3. Artificial seawater; prepared per SOP TA-03.02

5. PREPARATION OF 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 KCl. The amount of 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)	KCl needed	Media	Volume of Media
Acute/Chronic	*C. dubia*	KCl	1 g/L	6 g ± 0.02 g	MHWCD	6 L
Acute	*C. leedsi* and *P. promelas*	KCl	2 g/L	40 g ± 0.02 g	MHW	20 L
Chronic	*P. promelas*	KCl	2 g/L	40 g ± 0.02 g	MHW	20 L
Acute/Chronic	*A. bahia* and *M. beryllina*	KCl	2 g/L	40 g ± 0.02 g	ASW	20 L

5.2. Depending on what toxicant is being prepared, label the tape with marker to either:

5.2.1. Label the 6 L flask for the *C. dubia* test with "1 g/L KCl in MHWCD."

5.2.2. Label one carboy "2 g/L KCl in MHW" if making freshwater toxicant for the *C. leedsi* and *P. promelas*. The Toxicology lab typically makes 20 L of 2 g/L KCl in MHW and then dilutes the toxicant to the appropriate concentration. Sometimes less is made due to the number of SRT tests being conducted. (Refer to schedule and the lab manager.)

5.2.3. Label one carboy "2 g/L KCl in 20ppt ASW" for the *A. bahia* and *M. beryllina* tests. Place the batch number (preparation date), analyst's initials, and an expiration date of 30 days from the preparation date on each label.

5.3. Create a LIMS serial number in Standard Preparation Tracker for the toxicant that includes all necessary information.

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

5.3.2. The format for batch number is mm/dd/yy.

5.4. Add the media and total amount of KCl to your preparation flask.

5.5. Place a stir-bar in the flask and place the flask on the magnetic stir plate. Turn the stir plate on and adjust the speed so that a vortex becomes present. Let it stir for 1.5-2 hours.

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5.5.1. This is the final step for the MHWCD toxicant. However, for the ASW and MHW toxicants, continue to step 5.6.

5.6. Transfer the KCl solution into the appropriate carboy, and using the now empty flask, dilute the solution in the carboy by adding more media to the flask and transferring it to the carboy.

5.7. Print out the LIMS label for the toxicant and place it on the flask (MHWCD) or carboy (MHW/ ASW).

5.8. Seal the flask with one to 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. 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 the 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.

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.

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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 Assurance Manual and SOPs | Florida Department of Environmental Protection

9.2. https://www.floridadep.net/resources/health-safety-manual

9.3. DEP SOP TA-06.34, *Calibration and Use of Mettler Toledo 403TS/00 Balance*

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

9.5. DEP SOP TA-03.02, *Preparation of Artificial Seawater (ASW)*

9.6. 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.
05/11/2021	Updated Links. Updated to remove Sodium chloride as a toxicant.
09/12/2022	Updated links, checked for grammar, updated preparation section to reflect laboratory practices, and added notes to the equipment section to reduce wordiness of preparation section.
09/07/2023	Updated links, checked grammar and made sure font was all in Calibri.

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