Potassium Chloride Reference Toxicant Stock Solutions

1. SCOPE AND APPLICATION

1.1. This method is used by Toxicology 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

3.2. 1 L Class A graduated cylinder

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. Stir plate

3.8. 20 L Carboy

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 (ASW); prepared per SOP TA-03.02

5. PREPARATION OF KCL REFERENCE TOXICANT STOCK SOLUTIONS

5.1. Use a calibrated 3 decimal place balance (the one in the Tox lab is Mettler Toledo, S/N 1123052001) to 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. Label tape with the appropriate toxicant being prepared (containers may vary based on total volume being made, the below will reference the standard volumes):

5.2.1. All labels will include the type of toxicant (see below), LIMS number, batch number (preparation date, MM.DD.YY), expiration date (30 days from preparation date, MM.DD.YY), and analyst's initials.

5.2.1.1. Freshwater toxicant for C. dubia: label a 6 L flask with, "1 g/L KCl in MHWCD".

5.2.1.2. Freshwater toxicant for C. leedsi and/or P. promelas: label a 20 L carboy with, "2 g/L KCl in MHW".

5.2.1.3. Marine water toxicant for A. bahia and/or M. beryllina, label a 20 L carboy with, "2 g/L KCl in 20ppt ASW".

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

5.3. Add 6 L of the media (MHW, MHWCD, or ASW) and total amount of KCl to your 6 L preparation flask.

5.3.1. If making a non-standard volume, adjust amounts and containers as needed. All directions are for standard volumes based on the Table from 5.1.

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5.4. Place a stir-bar in the preparation flask and place the flask on the stir plate. Turn the stir plate on and adjust the speed so that a vortex becomes present. Let it stir until completely dissolved.

5.4.1. This is the final step for the MHWCD toxicant, continue to step 5.6. However, for the ASW and MHW toxicants, continue to step 5.5.

5.5. Transfer the KCl solution into the appropriate 20 L 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 until a total volume of 20 L is achieved.

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

5.7. Seal the flask with Parafilm®. Seal the carboys with the carboy lid.

5.8. Toxicants can be stored at room temperature for the duration of the bioassay tests. Expired or unused toxicant can be dumped down the drain.

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

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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 Assurance Manual

9.2. DEP Health and 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 SIGNIFICANT CHANGES

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

05/11/2021 Updated to remove Sodium chloride as a toxicant.

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