Preparation of Selenium Solution and B12 Solution for use in 20% DMW and MHWCD; and Selenium Solution for use in *Pseudokirchneriella subcapitata* Culture Media

1. SCOPE AND APPLICATION

1.1. This method is based on EPA 821-R-02-012, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th Edition*; and, Environment Canada Report EPS 1/RM/21: Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia* Selenium and B12 are used for 20% Dilute Mineral Water (DMW) and Synthetic Moderately Hard Water for *Ceriodaphnia dubia* (MHWCD). Selenium is used in media for culturing *Pseudokirchneriella subcapitata* for *Ceriodaphnia dubia* food.

2. SUMMARY OF METHOD

2.1. Sodium selenate is weighed and added to deionized water to produce a stock solution of sodium selenate. An aliquot of stock solution is added to deionized water to produce a sodium selenate working solution. The contents of a vial containing 1 g of B12 is added to deionized water to produce a stock solution of B12. An aliquot of stock solution is added to deionized water to produce a B12 working solution.

2.2. Definitions:

2.2.1. Sodium selenate - a nutrient for *Ceriodaphnia dubia* (*C. dubia*) that is added to *C. dubia* culture media (20% DMW or MHWCD) and *Pseudokirchneriella subcapitata* for *Ceriodaphnia dubia* food at the rate of 1 mL (2 µg/mLselenium) working solution per 1 L of media (2 µg/L selenium).

2.2.2. B12 – a necessary nutrient for culturing C. dubia that is added to *C. dubia* culture media (20% DMW or MHWCD) at the rate of 1 mL (2 µg/mLB12) working solution per 1 L of media (2 µg/L B12).

3. INTERFERENCES

3.1. Contaminated glassware, contaminated sodium selenate, contaminated B12, contaminated deionized water, and weighing errors.

3.2. Annually, a sample of stock solution is submitted for chemical analyses.

4. EQUIPMENT AND SUPPLIES

4.1. Mettler PB403-S/FACT Balance S/N 1123052001, calibrated per SOP TA-06.31

4.2. Weigh boat, plastic

4.3. Volumetric flasks with stoppers, 1 L, 2 each for Se and 2 each for B12

4.4. Metal spatula

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4.5. Magnetic stirrer

4.6. Stir bar, 2.5 cm

4.7. Stir-bar retriever

4.8. Volumetric pipet, 2 mL

4.9. Squeeze bottle for deionized (DI) water, 500 mL

5. REAGENTS AND STANDARDS

5.1. Sodium selenate (Na_2SeO_2), ACS grade
CAUTION! Sodium selenate is toxic, handle with great care (wear Personal Protective Equipment)

5.2. Cyanocobalamin (B12) Fisher Bio-Reagent

5.3. DI water (distilled water can be substituted)

6. METHOD

6.1. Sodium Selenate Solution

6.1.1. Label one flask with the following; "Sodium selenate stock, 2.39 g/L," use 2 mL/L to prepare working solution, the batch number, expiration date and analyst's initials. Label the second flask with the following; "Sodium selenate working solution, 2,000 µg/L, use 1mL/L to prepare *C. dubia* media," the batch number, an expiration date of one year from the preparation date (batch number), and analyst's initials.

6.1.1.1. The "batch number" is the date that the solution(s) is prepared. Format is mm/dd/yy, ex: 01/11/12. Add an expiration date to the label of one year after preparation date (batch number), ex: 01/11/13 for the 01/11/12 batch.

6.1.2. Half fill the flasks with DI water.

6.1.3. Weigh out 2.39 ±0.01 g of sodium selenate per SOP TA-06.31, and then place it into the flask labeled "Sodium selenate stock." Use the DI wash bottle to rinse any remaining salt from the weigh boat into the flask.

6.1.4. Swirl the flask several times to help dissolve the salt.

6.1.5. Finish filling the selenium stock solution flask to the 1,000-mL mark with DI water.

6.1.6. If the flask contains evidence of undissolved salt, place a clean stir bar in the flask. Place the flask on the magnetic stirrer and turn the stirrer on. Stir until the salt is dissolved.

6.1.7. Remove the stir bar with the stir-bar retriever. Rinse the stir bar and the stir bar retriever thoroughly with DI water.

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6.1.8. Use the 2-mL volumetric pipet to take 2 mL of selenium stock solution and add to the flask labeled "Sodium selenate working solution."

6.1.9. Swirl the flask several times to help mix the solution. Fill the flask to the 1,000-mL mark with DI water.

6.1.10. Stopper both flasks with parafilm.

6.1.11. Record the selenium stock and the selenium working solution preparation batch information in the *Tox Record of Preparation* logbook and into LIMS. Print out a LIMS label with a serial number and put on each flask.

6.1.12. Store the selenium stock and working solutions in the walk-in refrigerator.

6.1.12.1. The selenium stock and working solutions may be stored for one year (at temperature >0 and ≤ 6.0 ºC).

6.2. **B12 Soultion**

6.2.1. Label one volumetric flask with the following; "B12 Stock Solution, 1 g/L," use 2ml/L to prepare the working solution, the batch number and analyst's initials. Label the second flask with the following; "B12 working solution, 2µg/L, use 1 ml/L to prepare *C. dubia* media, the batch number, expiration date (one year from the date of preparation), and analyst's initials.

6.2.1.1. The "batch number" is the date that the solution(s) is prepared. Format is mm/dd/yy, ex: 01/11/12. Add an expiration date to the label of one year after preparation date (batch number), ex: 01/11/13 for the 01/11/12 batch.

6.2.2. Half fill the flasks with DI water.

6.2.3. Empty all the 1 g vial of Cyanocobalamin (B12) Fisher Bio-Reagent into the flask labeled B12 Stock Solution.

6.2.4. Swirl the flask until all the crystals are dissolved.

6.2.5. Finish filling the flask with DI water to the 1,000-ml line.

6.2.6. If the crystals do not dissolve, place a clean stir bar in the flask and place the flask on a magnetic stir plate until crystals completely dissolve.

6.2.7. Remove stir bar with a stir bar retriever. Rinse the stir bar and stir bar retriever thoroughly with DI water.

6.2.8. Use the 2-mL volumetric pipet to take 2 mL of the B12 stock solution and add to the flask labeled "B12 working solution."

6.2.9. Swirl the flask several times to help mix the solution. Fill the flask to the 1,00-mL mark with DI water.

6.2.10. Stopper both flasks with parafilm.

6.2.11. Record the B12 stock and the B12 working solution preparation batch information in the *Tox Record of Preparation* logbook and into LIMS. Print out a LIMS label with a serial number and put on each flask.

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6.2.12. Store the B12 stock and working solutions in the walk-in refrigerator

6.2.12.1. The B12 stock and working solutions may be stored for one year (at temperature >0 and ≤ 6.0 ºC).

7. DATA ARCHIVAL

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

7.2. Record information in *Tox Record of Preparation* logbook.

7.3. To correct raw data entries, place a single line through the incorrect entry, write the corrected entry near the error, date and initial the correction. Do not overwrite entries.

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

7.3.2. If the correction requires an explanation, write a comment and reference the error. Initial and date the explanation.

7.3.3. 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.).

8. QUALITY CONTROL

8.1. Refer to QA manual for corrective action and handling out-of-control data.

9. SAFETY/HAZARDOUS WASTE MANAGEMENT

9.1. ***Warning!*** Sodium selenate is poisonous, handle with great care. Read the warning label on the container.

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

9.3. Refer to Laboratory Quality Manual for sample disposal

9.4. Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management.

10. REFERENCES

10.1. EPA 821-R-02-012 Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th Ed. October 2002.

10.2. FDEP SOP TA-06.31 Calibration and Use of Mettler PB403-S/FACT Balance.

10.3. Laboratory Quality Manual http://www.dep.state.fl.us/labs/library/lab_sops.htm

10.4 Laboratory Health and Safety Manual and Hazardous Waste Management. https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

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10.5 Environment Canada Report EPS 1/RM/21: Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia.*

APPENDIX OF CHANGES

12/20/2007	Added Scope and Application; Summary of Method, and Appendix of Changes. Repaginated document. Deleted two blank rows between Pollution Prevention and Waste Management and Methods.
11/17/2008	Added Interference and Reference sections.
08/13/2013	Reformatted SOP.
02/10/2015	Formatted for 508 compliance.
08/17/2015	Updated links.
09/14/2016	Updated links. Updated Title of SOP.
09/15/2017	Updated links.
11/28/2017	Included B12 information

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