96-HOUR STATIC TOXICITY TEST USING SELENASTRUM CAPRICORNUTUM

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96-HOUR STATIC TOXICITY TEST USING SELENASTRUM CAPRICORNUTUM

1.0 SCOPE AND APPLICATION

The procedure for conducting a 96-hour static toxicity test using Selenastrum capricornutum is described below. The endpoint of this test is growth, measured by increase in cell count, chlorophyll content, biomass, or absorbance (turbidity). This test may be conducted on effluents, leachates or liquid phase of sediments. This test will also identify a test media that is biostimulatory (Horning and Weber, 1985).

These are standard (i.e., typically applicable) operating procedures which may be varied or changed as required, dependent on site conditions, equipment limitations or limitations imposed by the procedure or other procedure limitations. In all instances, the ultimate procedures employed should be documented and associated with the final report.

Mention of trade names or commercial products does not constitute U.S. EPA endorsement or recommendation for use.

2.0 METHOD SUMMARY

Selenastrum capricornutum is exposed to various concentrations of a test media over a 96-hour period and growth is measured at the end of the test.

3.0 SAMPLE PRESERVATION, CONTAINERS, HANDLING, AND STORAGE

The selected environmental matrix will be sampled utilizing the methodology detailed in ERT/SERAS SOPs #2012, Soil Sampling; #2013, Surface Water Sampling; #2016, Sediment Sampling, and any other procedure applicable for the media sampled.

Once collected, samples will be placed in containers constructed from materials suitable for the suspected contaminants. Because surrogate test species will be exposed to varying concentrations of the sample material, no chemical preservative are to be used. The preservation and storage protocol is therefore limited to holding the samples on ice at 4 °C for the holding time specified by the analytical method. Prior to shipping, the laboratory performing the toxicity tests will be notified of any potential hazards that may be associated with the samples.

4.0 INTERFERENCES AND POTENTIAL PROBLEMS

1. The results of a static toxicity test do not reflect temporal changes in effluent toxicity.

2. The detection limits of the toxicity of a test media are organism dependent (Horning and Weber, 1985).

3. Non-target chemicals (i.e. residual chlorine) cause adverse effects to the organisms giving false results.

4. Loss of a toxicant through volatilization and adsorption to exposure chambers may occur (Peltier and Weber, 1985).

5. The concentrations of natural nutrients in the test media may affect the results (Horning and Weber, 1985).

5.0 EQUIPMENT

5.1 Apparatus

- Selenastrum capricornutum culture
- 18 Erlenmeyer flasks - 250 mL
- Dilution water - 1.5L
- Test media - 1L
- Stock nutrient solutions
- Centrifuge - 15 - 100 mL capacity
- Graduated cylinders - 10 and 100 mL
- Erlenmeyer flask - 500 mL
- Microscope

Depending on the method used to calculate growth, other equipment may be necessary.

5.2 Washing Procedure

1. Wash with warm tap water and non-phosphate detergent.
2. Rinse with tap water.
3. Rinse with 10% Hal.
4. Rinse with deionized water.
5. Rinse with acetone.
6. Rinse with deionized water.
7. Final rinse with dilution water.

5.3 Test Organisms

Selenastrum capricornutum may be raised in house or received from an outside source. Positive identification of the species is required before beginning the test. A stock culture that is 4-7 days old is required for this test. Horning and Weber (1985) provide detailed information on the preparation of culture media and stock culture.

5.4 Equipment for Chemical Analysis

Meters are needed to measure dissolved oxygen, temperature, pH, and conductivity. Calibrate the meters according to the manufacturers specifications. Measure and record alkalinity and hardness according to a standard method (APHA, 1985).

6.0 REAGENTS

1. Dilution Water

Dilution water is moderately hard, reconstituted deionized water unless otherwise specified. The dilution water for the test is the same water used to culture Selenastrum capricornutum. See Horning and Weber (1985) for the preparation of synthetic fresh water.

2. Test Media

If the test media is a liquid, dilutions may be made directly for the required concentrations. If the test media is a liquid phase of a sediment, preliminary filtration and dilutions are required. To eliminate false negative results due to low nutrient concentrations, add 1 mL of stock culture solution (except EDTA) per liter of test media prior to preparing test concentrations.

3. Stock Culture Solution

The methods needed to prepare the stock culture solution and the amounts of chemicals needed to prepare the solution are found in Horning and Weber, 1985. One liter of test media will provide three replicates of 100 mL each for six concentrations and 400 mL for chemical analysis (Horning and Weber, 1985).

7.0 PROCEDURES

1. Maintain a stock culture of algae at 24 ± 2°C under continuous lighting.

2. Transfer 1 - 2 mL aseptically to new test media once a week in order to maintain an uncontaminated and healthy culture.

3. To prepare the inoculum, follow the steps below (Horning and Weber, 1985).

4. An inoculum is prepared from the stock solution 2 - 3 hours prior to the beginning of the test. Each milliliter of inoculum must contain enough cells to provide an initial cell density of 10,000 cells/mL in the exposure chamber. Therefore, each milliliter of inoculum must contain 1 million cells if using 100 mL test volume. Use the formula below to determine the amount of stock solution required for the test.

5. Volume of stock solution required (mL) = (# of flasks) (vol. of test soln./flask) x 10,000 cells/mL cell density in stock culture.

   a. Determine the density of cells in the stock solution.
   b. Calculate the required volume of stock solution (from the equation above).
   c. Centrifuge 50% more than the calculated value of stock solution at 1000 x g for 5 minutes.
   d. Decant the supernatant and resuspend in 15 mL of deionized water.
   e. Repeat steps c and d.
   f. Mix and determine the cell count and dilute as necessary to obtain a cell density of 106 cells/mL.

6. If possible, choose a range of concentrations that will span those with no effect to that which will cause complete mortality. The table below is used as an example and the concentrations may be

adjusted to meet the specific needs of the test.

Example 1. Test Dilutions

Test media (%)	Dilution water (mL)	Test media (mL)
0.0	300.0	0.0
1.0	297.0	3.0
3.0	291.0	9.0
10.0	270.0	30.0
30.0	210.0	90.0
100.0	0.0	300.0

7. Measure 100 mL of dilution water into each of the three (3) control flasks.

8. Mix 3 mL of test media with 297 mL of dilution water into a mixing bucket.

9. Pour 100 mL into each 1% test media flasks.

10. Continue with these dilutions until all concentrations are mixed.

11. Add 1 mL of test inoculum to each flask and begin the test.

12. At 1-2 hours, check the cell density of the controls to ensure sufficient test organisms. There are no renewals of test solutions for the duration of the test and the test is complete at 96 hours.

13. Measure and record temperature, dissolved oxygen, pH, conductivity, alkalinity, and hardness on all test solutions.

14. Growth is measured at the end of the test by cell counts, chlorophyll content or turbidity (light absorbance), or biomass. Cell counts may be determined by using a automatic particle counter or manually under a microscope. Chlorophyll content may be measured using in-vivo or in-vitro fluorescence or in-vitro spectrophotometry. Turbidity may be measured by spectrophotometry at 750 nm. Biomass is measured by multiplying the cell count by the mean cell volume or by direct gravimetric dry weight analysis. Horning and Weber (1985) provide details of the methodologies for these measurements.

15. At the completion of the test, samples should be checked under a microscope to detect any abnormal cell growth or other deviations.

16. It also may be necessary to check algal growth on a daily basis depending on the test media.

8.0 CALCULATIONS

The No Observable Effect Concentration (NOEC), the Lowest Observable Effect Concentration (LOEC), and the chronic value (CHV) are measured and recorded at the end of 96 hours. Dunnetts procedure or the

Probit Method may be used to calculate the NOEC and LOEC. When the assumptions for normality and homogeneity of variance are not met, Steel's Many - One Rank Test may be used. Other methods may be used if justified and the appropriate method is cited. Calculate the percent stimulation (%S) if growth in the concentrations exceeds the growth in the controls.

9.0 QUALITY ASSURANCE/QUALITY CONTROL

Quality control should encompass the following parameters to ensure a valid test. The guidelines in this text and in Table 1 (Appendix A) should be followed to insure adequate QA/QC.

1. Test organisms
2. Facilities/equipment
3. Test media preparation
4. Dilution water
5. Test conditions

10.0 DATA VALIDATION

Test data is invalidated for the following reasons.

1. Cell density in the controls are less than 106 cell/mL at the end of the test and the number does not vary by more than 10% between control replicates.

2. Parameters in Table 1 (Appendix A) not met.

11.0 HEALTH AND SAFETY

When working with potentially hazardous materials, follow U.S. EPA, OSHA and corporate health and safety guidelines.

12.0 REFERENCES

APHA. 1985. Standard Methods for the Examination of Water and Wastewater. 16th Ed. American Public Health Association, Washington, D.C.

Horning, W.B. and C. Weber. 1985. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-85/014. Environmental Monitoring and Support Laboratory, Cincinnati, OH. 162 pp.

Houston, Mark, SOP H. 96-Hour Static Toxicity Test Using Selenastrum Capricornutum, U.S. EPA Environmental Response Team - Technical Assistance Team TDD: 11871206.

Peltier, William H. and Cornelius Weber. 1985. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. EPA/600/4-85/013. Environmental Monitoring and Support Laboratory, Cincinnati, OH. 216 pp.

APPENDIX A
Table
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96-HOUR STATIC TOXICITY TEST USING SELENASTRUM CAPRICORNUTUM

TABLE 1. Summary of Test Conditions for Selenastrum Capricornutum 96-Hour Static Toxicity Test (Horning and Weber, 1985)

1.	Test type:	Static, non renewal
2.	Temperature:	25 ± 2°C
3.	Light intensity:	400 ± 40 foot-candles
4.	Photoperiod:	Continuous
5.	Exposure chamber size:	250-mL
6.	Test volume:	100-mL
7.	Stock culture:	4 - 7 days old
8.	Cell density:	10,000 cells/mL
9.	Replicates:	Three per concentration
10.	Shaking rate:	Twice daily by hand or 100 cpm
11.	Dilution water:	Reconstituted deionized water unless otherwise specified, also the same as the culture water without the EDTA.
12.	Test duration:	96 hours
13.	Effect measured:	Growth