GREAT SHIPS INITIATIVE

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STANDARD OPERATING PROCEDURE
Bench-Scale Procedure for Measuring Residual Toxicity Using the Alga *Selenastrum capricornutum*

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BACKGROUND

The Great Ships Initiative (GSI) is a collaborative effort to end the problem of ship-mediated invasive species in the Great Lakes-St. Lawrence Seaway System through independent research and demonstration of environmental technology, financial incentives and consistent basin-wide harbor monitoring. To that end, the GSI has established a shore-based high-flow Research, Development and Technology Evaluation (RDTE) facility in Superior, Wisconsin to provide intensive testing services to vendors of ballast treatment prospects suitable to Seaway-sized vessels. Laboratory space within the University of Wisconsin-Superior (UW-S) and University of Minnesota-Duluth is utilized to meet GSI bench-scale test objectives, as well as for non-time sensitive analysis of samples from the shore-based and shipboard scale tests. The UW-S has space in several of their research labs dedicated to the GSI project. Bench-scale experiments are conducted in the university's Aquatic Toxicity Laboratory which maintains active cultures of zooplankton, phytoplankton, and aquatic invertebrates. The laboratory contains a series of mini-diluters for water-only acute and chronic toxicity tests and is equipped to run static, intermittent renewal, and flow-through tests. A variety of meters are available for monitoring water quality including conductivity, salinity, pH, dissolved oxygen, temperature, and select ions.

INTRODUCTION

This bench-scale procedure measures the residual toxicity of water treated by a ballast water treatment method to organisms in receiving systems using the small biconcave disk-shaped algal species, *Selenastrum capricornutum*, during a 48 hour static test. During the test, organisms are continuously exposed to selected concentrations of water treated by a ballast water treatment method, with survival recorded daily for the duration of the test.

EQUIPMENT LIST

- Treated water
- Aeration source
- 125 ml or 250 mL Erlenmeyer flasks
- Inoculum of algae
- Light source
- Mechanical shaker
- Light meter
- Thermometer
- Fluorometer
- Test tube

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PROCEDURE

1. Conduct procedure in a vented work area, taking appropriate health and safety measures.

2. Prepare exposure solutions in the appropriate water type (harbor water or filtered harbor water), with the highest exposure concentration equal to the lowest concentration that resulted in 100 % mortality based on dose effectiveness testing (GSI/SOP/BS/DE/4). Make additional solutions using a 0.5 dilution scheme.

3. Age solutions in the dark at 25.0° C for 24 hours before beginning residual toxicity exposures. For any exposure solutions where survival is significantly different (α = 0.05) from the controls at 24 hours, start a new set of exposures with solutions that have been aged 24 hours in the light at approximately 1000 lumens/m^2, 150 µW/cm^2 UVA and 10 µW/cm^2 UVB.

4. If the exposure water has been treated with a chemical (i.e., hydrogen peroxide), measure the concentrations of the chemical if they are detectable. It is not necessary to chemically analyze the dilution series; however the additional information may be useful if further testing is needed.

5. Prepare the inoculum no more than two to three hours prior to beginning the experiment. Use *S. capricornutum* harvested from a four to seven day old stock culture. Ensure that each mL of inoculum contains enough cells to provide an initial cell density of approximately 200,000 cells/mL in the exposure solutions.

6. Randomize the location of each test chamber prior to algal inoculation. Begin the test following addition of the algae to the test chambers.

7. Incubate the test chambers under a 16 hour light/8 hour dark cycle at an illumination of approximately 962 lumens/m2, 151.9 µW/cm2 UVA and 8.4 µW/cm2 UVB at 25 ± 1 °C. Shake chambers continuously at 100 rpm on a mechanical shaker. These light levels represent a depth of approximately 3.0 meters under cloudy and clear conditions during the months of June and August in Ashland Harbor.

8. Randomly rotate flask positions each day to minimize possible spatial differences in illumination and temperature on growth rate. Measure light intensity in testing areas by placing a light meter at the level of test chambers. Record levels at the start of each test. Plug flasks with foam plugs to prevent contamination and to allow for gas exchange.

9. Measure and record temperature at the end of each 24 hour exposure period in at least one test blank flask. At the end of the test, measure and record temperature in at least one solution at each concentration. Also measure and record pH, conductivity,

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alkalinity and hardness in stock solutions at the beginning of the test. Attempts should be made to measure pH, conductivity, alkalinity and hardness at the end of the test also.

10. Terminate the test 48 hours after initiation. Store test solutions at less than or equal to 4 °C in the dark for later measurement. Measure algal viability in each flask using one of the following methods: 1) cell count or 2) chlorophyll content 3) viability stain (Sytox green).

11. Determine cell counts using a Sedwick-Rafter, Palmer-Maloney, hemocytometer, inverted microscope, or similar method. Count at least 400 cells per replicate to obtain ± 10 % precision at the 95 % confidence level.

12. Determine in vivo chlorophyll-a concentrations by diluting 1.0 mL sample with 4.0 ml of deionized water in a culture test tube. Mix the sample thoroughly using a vortex mixing apparatus. Insert the test tube into the analysis chamber and replace the light. Record the reading after the 8 second count down reaches zero.

13. Determine viability by adding 10 μL of Sytox Green stock solution (GSI/SOP/RDTE/SA/P/1) to each 1 mL of concentrated algae stock using a micropipetter. Lightly agitate the sample to distribute the stain. Allow the sample to incubate in the dark for 10 minutes at room temperature. Following, agitate the concentrated algae sample so that it is well mixed and immediately collect a subsample (the volume needed to fill the counting chamber) using a mechanical pipetter. Flood the counting cell with the subsample and seal the strewn material with a coverslip. Using 400X magnification, count and identify specimens along transects (or in grid cells). Cells characterized as "live" show no evidence of green fluorescence coming from nuclei and/or contents. Keep accurate records of transect lengths and widths (or grid cells counted) so that quantitative calculations may be conducted later.

14. All data should be recorded on the data recording sheet. Data should include the date that cultures were started, renewed, counted, and harvested as well as information on preparation of medium, signs of contamination, cell counts and/or chlorophyll concentrations, etc. Use a significant (95% confidence) reduction in viability of the treatments when compared to the controls to determine effects levels.

QUALITY ASSURANCE/QUALITY CONTROL

Concurrent toxicity tests of the same type as described above with a reference toxicant (KCl) must be performed. This test will document organism sensitivity.

Lab performance is demonstrated by performing at least one reference toxicant test per month if a concurrent test is not conducted as described above.

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A control chart is prepared for each combination of reference toxicants, test species, test conditions, and endpoints. The chart consists of a running plot for the 20 most recent values (LC50). End points are determined to see if they are with in acceptable limits. The control chart depicts the central tendency of the mean value and the upper and lower control values are set as two standard deviations from the mean.

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