GREAT SHIPS INITIATIVE

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STANDARD OPERATING PROCEDURE
Bench-Scale Procedure for Measuring Residual Toxicity Using Fathead Minnows (*Pimephales promelas*)

Compiled By:

Signed: _____

Title: _____

Date: _____

Approved By:

Signed: _____

Title: _____

Date: _____

Cleared For Issue By:

Signed: _____

Title: _____

Date: _____

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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 fathead minnows (*Pimephales promelas*) 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
- 500 mL beakers
- Partial immersion mercury thermometer
- Water source
- Test chemical
- Dissolved oxygen meter
- Alkalinity/hardness reagents
- pH Meter
- Ruler
- Siphon

- Temperature controlled water bath or constant temperature chamber
- Glass covers
- Analytical instrumentation to measure treatment of concern

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/2). 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 $\mu W/cm^2$ UVA and 10 $\mu W/cm^2$ UVB. 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.[1]

4. Set up a 48 hour static test with a known concentration of aged water and one control. Replicate each treatment and control three times. Fill each test beaker to 200 mL and cover with a glass cover. If the exposure water has been treated with a chemical (i.e., hydrogen peroxide), measure the concentrations of the chemical if they are detectable.

5. Add approximately 200 mL of an aged exposure solution to each of three 300 mL glass beakers and label.

6. Using juvenile fathead minnows (less than 24 hours old) obtained from Environmental Consulting and Testing, Superior Wisconsin that were fed for 24 hr prior to testing, place a minimum of 15 fish randomly in each replicate vessel, five at a time. Ensure that each treatment has three replicates with 15 fish in each replicate. If more than one lot (tank) of fish is used, combine the tanks and select fish from the pooled organisms. The length of the longest fish should be no more than 2 times the length of the shortest fish; fish loading should not exceed 0.5 g/L. .

7. Place beakers in a 25.0 +1 °C temperature-controlled water bath with 16 hour light/8 hour dark ambient laboratory light cycle.

[1] The aging process and the setting up of toxicity tests maybe altered slightly as this will likely be treatment dependant.

8. Count and record fish survival at a minimum of 24 hour intervals, with dead fish removed as soon as they are observed.

9. Measure and record temperature, dissolved oxygen, pH at test initiation and every 24 hr thereafter on the control treatment exposures. In the test chambers, temperatures should be 25 + 1ºC, DO should be >60% saturation, and pH should be between 6.5 and 8.2 during the assay. Also measure and record alkalinity and hardness on each treatment stock solution at the beginning of the test.

10. Calculate LC50 value based on the Trimmed Spearman-Karber Method (Hamilton et al., 1977) using the measured analytical value if available or the nominal concentration.

QUALITY ASSURANCE/QUALITY CONTROL

Control survival must be at least 90 % for the test to be acceptable. 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.

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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