

STANDARD OPERATING PROCEDURE
Bench-Scale Procedure for Measuring Residual Toxicity
Using *Ceriodaphnia dubia*

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RECORD OF AMENDMENTS:

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STANDARD OPERATING PROCEDURE

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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 less than 24 hour old *Ceriodaphnia dubia* neonates 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
- pH meter
- Dilution water
- Dissolved oxygen meter
- Thermometer
- Glass beakers
- Lighted magnifying lens
- Conductivity meter
- Temperature controlled chamber

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 lowest 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 ($\alpha = 0.05$) 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/m², 150 μ W/cm² UVA and 10 μ W/cm² 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. 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. Use neonates of *Ceriodaphnia* less than 24 hours old obtained from individual cultures using brood boards. Take only neonates from adults that are in their third or subsequent broods. Combine animals from multiple boards in to a single container to allow for complete randomization prior to the start of the test.
6. Add approximately 50 mL of an aged exposure solution to each of three 300 mL glass beakers and label. Repeat for each exposure and control solution. Subsequently analyze an aliquot of each exposure solution for the following parameters: temperature, dissolved oxygen, pH, conductivity, alkalinity, and hardness.
7. Add ten test organisms to each beaker and cover beakers with a glass plate. Place beakers in a 25.0 °C temperature-controlled environment with 16 hour light/8 hour dark ambient laboratory light cycle. Ensure light levels are at approximately 962 lumens/m², 151.9 μ W/cm² UVA and 8.4. μ W/cm² 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.
8. Count and record neonate survival at a minimum of 24 hour intervals. Note: If the goal of the experiment is too measure a “one-time” discharge event of treated ballast

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

- water into a receiving system the bioassay becomes a static test employing daily counts of adult survival. If the goal of the experiment is to measure a “repeated” discharge event of treated ballast water into a receiving system the bioassay becomes a static renewal employing counting of the adult survival with a transfer of the adults to new exposure solution. Control survival must be at least 90 % for the test to be acceptable. Death is defined as lack of any movement when viewing an organism with a microscope.
9. Measure and record temperature, dissolved oxygen, pH at the end of each 24 hour exposure period in control, low, middle, and high treatment. Measure and record temperature, dissolved oxygen, pH at the end of the test in at least one solution at each test concentration. Conductivity, alkalinity, and hardness should also be measured and recorded in at least one solution of each test concentration. It may be necessary to composite samples to obtain enough sample volume for analysis.
 8. Calculate LC50 and EC50 values based on the Trimmed Spearman-Kärber 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 within 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.

REFERENCES

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