GREAT SHIPS INITIATIVE
BENCH-SCALE TEST FINDINGS
Technical Report

Electrolytic Cell Component of the SiCURE™ Ballast Water Management System

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ABSTRACT

The Great Ships Initiative (GSI) conducts bench-scale (i.e., laboratory scale) status testing services to aid developers of innovative technologies which could have application as ballast water management systems (BWMSs). This report describes findings from GSI bench-scale evaluations of the SiCURE™ BWMS proposed by Siemens Water Technologies. The BWMS creates chlorine, in the form of sodium hypochlorite (NaOCl), by passing either seawater or brine through an electrolytic cell. Ultimately the SiCURE™ BWMS combines filtration, electrolytic chlorination, and proprietary system control logic to eliminate unwanted aquatic species in ballast water. GSI tested the electrolytic cell component of the SiCURE™ BWMS to assess dose effectiveness and residual toxicity in a variety of water qualities. Please see www.greatshipsinitiative.org for more information about GSI's bench-scale testing program.

GSI dose effectiveness tests of the electrolytic cell component of the SiCURE™ BWMS measured the effects of chlorine on freshwater organisms known to be relatively resilient to stressors in several water qualities including dechlorinated laboratory water (LW), filtered Duluth-Superior Harbor (DSH) water (FHW), a 50/50 mixture of LW and FHW water (FHW50) and the above water types with added salt. Test organisms included the green alga *Selenastrum capricornutum*, resting eggs of the rotifer *Brachionus calciflorus*, newly hatched rotifers *B. calciflorus*, adult copepods *Eucyclops* spp., and < 24 hour old daphnids *Daphnia magna*. The dose effectiveness test results show that the BWMS produces a chlorine solution that is significantly toxic to *Eucyclops* spp., *D. magna* and adult *B. calyciflorus* at the 4 mg/L, 6 mg/L and 10 mg/L concentrations tested in both FHW water FHW50. With the *B. calyciflorus* eggs, hatch rates were significantly impacted in FHW at 4 mg/L and 6 mg/L chlorine concentrations. However, in FHW at 10 mg/L chlorine and in FHW50 at 4 mg/L, 6 mg/L and 10 mg/L chlorine there was no significant effect on hatch rates of *B. calyciflorus* eggs. While all concentrations of chlorine tested on *S. capricornutum* caused a significant decrease in survival, 91 % of the *S. capricornutum* was still alive after 48 hours in FHW with a concentration of 10 mg/L chlorine. This lower effect at the 10 mg/L chlorine concentration in FHW was evident in *S.capricornutum*, rotifer resting eggs and *Eucyclops* spp..

GSI bench-scale residual toxicity tests were undertaken to determine the effect that the above water types and a dose of 6 mg/L chlorine, produced by the Siemens SiCURE™ system, had on residual toxicity to the fathead minnow *Pimephales promelas*, the daphnid *Ceriodaphnia dubia* and the amphipod *Hyalella azteca*. The residual toxicity test results show that there is no residual toxicity to either of these organisms at 6 mg/L chlorine in either the FHW or FHW50. However, toxicity to all organisms was seen at that concentration of chlorine in LW.

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INTRODUCTION

This Great Ships Initiative (GSI) technical report presents quantitative and measured findings from GSI bench-scale evaluations of a ballast water management system (BWMS) proposed by Siemens Water Technologies, with possible application to the Great Lakes. The SiCURE™ BWMS utilizes chlorine in the form of sodium hypochlorite (NaOCl) that is generated by passing either seawater or brine through an electrolytic cell. Ultimately this system combines filtration, electrolytic chlorination, and proprietary system control logic to eliminate unwanted aquatic species in ballast water. At the bench-scale level only the chlorination process was tested. GSI undertook these bench-scale tests during June through July of 2009 at the Lake Superior Research Institute (LSRI) of the University of Wisconsin-Superior (UWS) in Superior, Wisconsin, USA. Tests included range-finding evaluations of dose effectiveness and residual toxicity.

BACKGROUND

Great Ships Initiative (GSI)

GSI is a regional effort managed by the Northeast-Midwest Institute (NEMWI) devoted to ending the problem of ship-mediated invasive species in the Great Lakes-St. Lawrence Seaway System and globally. Since its establishment in 2006, GSI has provided independent performance/verification testing services to developers of BWMSs at the bench, land-based and shipboard scales. GSI performs informal "status testing" for systems that are in the research and development stage, and formal certification/verification tests appropriate to market-ready BWMSs.

Organization

GSI is a project of NEMWI—a Washington, D.C-based private, non-profit, and non-partisan research organization dedicated to the economic vitality, environmental quality, and regional equity of Northeast and Midwest states. The project is carried out collaboratively with contracting entities including the UWS, AMI Consulting Engineers, Broadreach Services, and the University of Minnesota-Duluth (UMD).

Ms. Allegra Cangelosi of NEMWI is GSI's Principal Investigator and Director (GSI PI). A GSI Advisory Committee comprising top-level officials of key stakeholder groups provides direct input to Ms. Cangelosi, advising on GSI award decisions, program direction, finances and fund-raising. The American Great Lakes Ports Association advises the project, assuring that the GSI is meeting the needs of the maritime industry, and coordinating maritime industry and supply chain outreach. Researchers from UWS's LSRI and the UMD's Natural Resources Research Institute (NRRI), among others, provide critical scientific and technical expertise and implementation services to the GSI PI. Dr. Mary Balcer of LSRI is the project's lead zooplankton ecologist. She is also the team leader for LSRI staff engaged in GSI research activities. Dr. Euan Reavie of NRRI leads all protist analysis and is the team leader for NRRI staff engaged in GSI research activities. Mr. Matthew TenEyck of LSRI leads all bench-testing and Whole Effluent Toxicity

(WET) tests. Ms. Deanna Regan of LSRI is responsible for GSI chemical analysis. Ms. Heidi Saillard of LSRI is responsible for GSI microbial analysis. Ms. Nicole Mays of NEMWI is GSI's Senior Quality Systems Officer and Ms. Kelsey Prihoda of LSRI is GSI's Senior Quality Assurance/Quality Control (QAQC) Officer.

GSI BENCH-SCALE TESTS

Overview

GSI bench-scale tests involve informal "status testing" to provide BWMS developers insight into the performance of BWMS processes and configurations at early stages of development relative to specific challenge conditions and scenarios. Findings are strictly the performance outcomes of the tests. That is, to maintain its independence as a testing facility, GSI does not engage in discussions with the BWMS developer on, or produce recommendations for, ways to improve the BWMS process subject to testing. Developers apply directly to GSI for these services, and GSI awards "status testing" services at no cost except for shipping the subject BWMS to and from the GSI testing facility, and costs involved in the installation and removal of the system from the GSI testing facility.

GSI bench-scale status tests take place year-round at the LSRI. The LSRI is amply equipped with staff expertise and resources to conduct the tests, and has a long history of successfully undertaking similar tests. The overarching goal of GSI bench-scale status testing is to explore dose effectiveness, chemical degradation, residual toxicity, and/or sensitivity to challenge conditions of a proposed BWMS or component thereof.

GSI Active Substance Degradation Tests

GSI bench-scale active substance degradation tests determine the effect that various water quality or environmental parameters may have on the rate of active substance degradation and/or the rate of formation of disinfection by-products of a BWMS involving active substances. No organisms are used in association with these analytical assays; instead test solutions are analyzed for their concentration of active substance (or active component of the substance). Test results are typically expressed as the percent change in active ingredient concentration.

If a BWMS does not utilize an active substance, this stage of testing does not apply. Instead, preliminary water chemistry testing may be conducted, as needed, and include measurement of basic water quality parameters such as temperature, pH, conductivity, and dissolved oxygen.

GSI Dose Effectiveness Tests

GSI bench-scale dose effectiveness tests help determine the range of concentrations of an active substance that is toxic to a variety of robust freshwater zooplankton, algae and bacteria known to be relatively resilient to stressors. Dose effectiveness test results for zooplankton and algae are typically expressed as percent survival and/or percent mortality. In the case of ephippia (i.e., resting eggs) results are expressed as percent hatch. Where applicable, results may also be

expressed in terms of a series of absolute quantifications: LC_{99}, i.e., the experimentally derived concentration of an active substance estimated to kill 99 % of the test population following 24 or 48 hours of continuous exposure; No Observed Effect Concentration (NOEC), i.e., the highest concentration of an active substance shown to have no significantly adverse effect on the test population compared to controls; and/or Lowest Observed Effect Concentration (LOEC), i.e., the lowest concentration of an active substance known to have a significantly adverse effect on the test population compared to controls.

GSI Residual Toxicity Tests

GSI bench-scale residual toxicity tests help estimate the effect that treated water (following neutralization of the active substance, a degradation period, and/or a dilution step) may have on non-target organisms in the receiving system. These test results are expressed in a manner similar to those for dose effectiveness assays. The principal difference between these tests and dose effectiveness tests is that the concentration of the active substance is adjusted to be consistent with potential discharge levels. The tests are also performed on sensitive organisms rather than robust species.

METHODS

Treatment Application, Facility and Test Apparatus

The electrolytic cell component of the Siemens SiCURE™ BWMS, as a small scale working model, consisted of passing saline water through a small electrolytic cell to generate chlorinated water. Saline water solution used to generate chlorine through the electrolytic chlorination process was prepared by dissolving 28.0 g of sodium chloride and 50.0 mg of sodium bromide in one liter of distilled water. Electrolysis conditions were adjusted so that the chlorine concentration of the product water was approximately 1000 mg/L chlorine. This chlorinated saline water was used to spike the exposure waters to generate the desired chlorine concentrations.

The biological exposure test apparatus consisted of 300 mL borosilicate high-form beakers or 12-well tissue culture plates housed within environmental chambers with controlled temperature and light regime. The environmental chambers are located within a laboratory equipped with adequate ventilation, electrical connections, and climate control located at the LSRI testing facility.

General Methods

Sample Water Preparation

Several experimental water types were prepared in the laboratory as follows:

- Duluth-Superior Harbor Water (HW): Harbor water collected from a depth of approximately three meters in the Duluth-Superior Harbor (DSH). Alkalinity ranges from 65-69 mg/L as

$CaCO_3$. Total dissolved organic carbon range is 10-22 mg/L. The water is stained with tannins.

- Laboratory Water (LW): Treated Lake Superior water from the City of Superior, Wisconsin, USA, that is passed through an activated carbon column. Alkalinity ranges from 45-50 mg/L as $CaCO_3$. Total organic carbon range is 0.5-3.0 mg/L. The water is transparent.
- LW with Saline Water (SLW): Saline LW controls prepared by adding the same amount of saline water solution to the exposure water as chlorinated saline water added to generate the exposures with the highest chlorine concentration. The highest concentration of saline water in any of the exposures was 1 %.
- Filtered HW (FHW): HW as described above and passed in sequence through a Whatman 934-AH filters followed by a Millipore 0.45 μm membrane filter prior to use.
- 50 % Filtered HW (FHW50): HW as described above for FHW but diluted by a ratio of 0.5 with LW.
- FHW with Saline Water (SFHW): Saline FHW controls prepared by adding the same amount of saline water solution to the exposure water as chlorinated saline water added to generate the exposures with the highest chlorine concentration. The highest concentration of saline water in any of the exposures was 1 %.
- 50 % FHW with Saline Water (SFHW50): FHW50 %, as described above, with the addition of saline water solution. The highest concentration of saline water in any of the exposures was 1 %.

Experimental water temperature was adjusted to the desired level by placing the beaker containing the appropriate amount of water in an incubator set to the desired test temperature.

Analytical Method of Chlorine Determination

Samples for chlorine analysis were collected from the test system apparatus using a 1000 -5000 μL pipettor set at 5000 μL. 20 mL of sample were transferred from the test system containers into a 30 mL beaker. 200 µL of potassium iodide reagent and 200 μL of acetate buffer reagent were then added to the samples. Following, the samples were analyzed for total residual chlorine concentration as soon as possible after collection. Analysis was conducted using a Thermo Orion Model 97-70 Residual Chlorine Electrode connected to an Orion Model 290A pH/mV/ISE meter.

A 100 mg/L chlorine stock solution was prepared daily by diluting 0.175 mL of a 6.0 % sodium hypochlorite solution (i.e., Clorox™ bleach) to 100 mL with deionized water. Analytical standards, ranging in concentration from 5 to 3000 μg/L, were prepared in deoxygenated deionized water by making dilutions of the 100 mg/L chlorine stock. Potassium iodide reagent and acetate buffer were added to the chlorine containing analytical standards. Chlorine present in the standards/samples oxidizes iodide to iodine in an acidic solution. The iodine concentration after the reaction is equal to the chlorine concentration present before the reaction. A calibration curve plotting log of the chlorine concentration (x-axis) versus the mV response from the Residual Chlorine Electrode (y-axis) was used to determine total residual chlorine concentrations in the samples.

Oxidation Reduction Potential (ORP) Probe Readings

Oxidation Reduction Potential (ORP) measurements were made using a Hach sc100 ORP Probe Model DRD1R5 Digital ORP differential sensor. The ORP probe was calibrated using a one point calibration daily prior to use on samples. A 600 mV calibration solution was checked with an ORP reading of 580-620 mV considered acceptable. ORP measurements on samples were taken be placing the probe into an aliquot of sample solution.

Chlorine Versus ORP Readings Over Time

The purpose of this experiment was to examine the response of the ORP probe as concentrations of chlorine varied. The test water (FHW50) consisted of equal volumes of DSH water and dechlorinated LW. The exposure concentrations were prepared by adding varying volumes of chlorinated saline solution that had been prepared using the Siemens SiCURE™ bench-scale system. Measurements of total residual chlorine concentrations and ORP readings were made at 0, 24, and 48 hours.

Experimental Methods

Dose Effectiveness Tests

GSI dose effectiveness tests measured the effects of the electrolytic cell component of the Siemens SiCURE™ BWMS on freshwater organisms known to be relatively resilient to stressors in several different water qualities including LW, FHW, FHW50, SFHW and SFHW50. Test organisms included the green alga *Selenastrum capricornutum,* resting eggs of the rotifer *Brachionus calyciflorus,* newly hatched rotifers of the species *B. calyciflorus,* adult copepods of the species *Eucyclops* spp., and < 24 hour old daphnids of the species *Daphnia magna.* The sample ID, codes, water types and chlorine concentrations of the exposure solutions for the test organisms are shown in Table 1. In all dose effectiveness tests, the Siemens SiCURE™ system was used to prepare an approximately 1000 mg/L chlorine stock solution which was then spiked into the various water types to prepare the desired exposure concentrations.

Table 1. Sample ID code and sample conditions for dose effectiveness tests involving the green alga *Selenastrum capricornutum*, resting eggs of the rotifer *Brachionus calciflorus*, adults of the rotifer *B. calciflorus*, adult copepods (*Eucyclops* spp.) and < 24 hour old daphnids (*Daphnia magna*).

Sample ID Code	Experimental Water Type	Chlorine Concentration
LW-0 mg/L	Lab Water (LW)	No Chlorine
FHW-0mg/L	Filtered Harbor Water (FHW)	
FHW50-0mg/L	50 % FHW:50 % LW (FHW50)	
SFHW-0mg/L	Filtered Harbor Water and Salt (SFHW)	
SFHW50-0mg/L	50 % FHW:50 % LW and Salt (SFHW50)	
FHW-4 mg/L	FHW	4 mg/L Chlorine
FHW50–4 mg/L	FHW50	
FHW–6 mg/L	FHW	6 mg/L Chlorine
FHW50–6 mg/L	FHW50	
FHW-10 mg/L	FHW -	10 mg/L Chlorine
FHW50-10 mg/L	FHW50	

The *S. capricornutum* dose effectiveness test was conducted according to *GSI/SOP/BS/RA/EF/5 - Procedure for Assessing Dose Effectiveness of a Ballast Treatment System Using Selenastrum capricornutum.* Briefly, 100 µL of *S. capricornutum* inoculum at a concentration of 1.0 x 10^8 cells/mL was added to 50 mL of test water in 125 mL Erlenmeyer flasks to achieve a desired concentration of approximately 200,000 cells/mL. Triplicate samples were prepared. In addition, a fourth replicate was utilized as a "chemistry" replicate, from which water chemistry samples and were collected. Once the *S. capricornutum* had been added to the flasks, the treatment exposures were spiked with an aliquot of the chlorine stock solution in saline water to prepare 4 mg/L, 6 mg/L or 10 mg/L chlorine solutions. The chlorine stock solution in saline water was prepared by running a prepared saline water solution through the electrolytic cell to prepare an approximately 1000 mg/L chlorine solution. The SFHW and SFHW50 solutions had saline water added to ensure they had the same saline water concentration as the 10 mg/L chlorine exposures.

The *S. capricornutum* exposure flasks were maintained in the dark at 25 °C on a shaker table for the duration of the test. Samples were collected from the three biological replicates at 0 hours, 24 hours and 48 hours and analyzed for live and dead cells.

The dose effectiveness test involving the rotifer resting eggs (*B. calyciflorus*) was conducted according to *GSI/SOP/BS/EF/4 - Procedure for Assessing Dose Effectiveness of a Ballast Treatment System Using Cysts of the Freshwater Rotifer Brachionus calyciflorus.* 2 mL of each appropriate water type was pipetted into eight well plate cells. The well plates served as biological replicates. Ten resting eggs were then added to each well. Following the addition of the resting eggs, the treatments were spiked with the appropriate volumes of an approximately 1000 mg/L chlorine solution produced by the electrolytic cell component of the Siemens SiCURE™ BWMS. The well plates were then placed in a 25 °C incubator with 24 hour light. Hatch rates were evaluated at 24 and 48 hours after treatment. In addition, at the time the

biological replicates were set up, chemistry beakers were set up for each water type and exposure concentration. 200 mL of exposure solution were placed in a beaker and spiked with the chlorine solution (no rotifer cysts were added to the chemistry beakers). The chemistry beakers were then placed in the same incubator as the well plates and they were used to measure temperature, dissolved oxygen, pH, conductivity, hardness and alkalinity at 48 hours.

The adult *B. calyciflorus* dose effectiveness test was conducted according to *GSI/SOP/BS/EF/3 - Bench-Scale Procedure for Assessing Dose Effectiveness of a Ballast Water Treatment System Using the Freshwater Rotifer Brachionus calyciflorus.* The test was set up in the same manner as the test with rotifer cysts, though the test involving adults was only 24 hours in duration and examined survival as the endpoint rather than hatch rate.

The dose effectiveness test involving the copepod *Eucyclops* spp. was conducted according to *GSI/SOP/BS/EF/1 - Procedure for Assessing Dose Effectiveness of a Ballast Treatment System Using a Copepod.* Three replicates per test water type were prepared in 300 mL high form beakers. Ten *Eucyclops* spp. were then added to each beaker and the beakers spiked with 1000 mg/L chlorine solution produced by the electrolytic cell component of the Siemens SiCURE™ BWMS. Mortality was noted at 24 and 48 hours after test initiation. One chemistry replicate per treatment was also set up. Here, 200 mL of exposure solution was placed in a beaker and spiked with the chlorine solution. The chemistry beakers were used to measure temperature, dissolved oxygen, pH at 24 hours and temperature, dissolved oxygen, pH, conductivity, hardness and alkalinity at 48 hours. Both the biology and chemistry replicates were placed in an incubator at 25 °C and in the dark.

To determine dose effectiveness of the electrolytic cell component of the Siemens SiCURE™ BWMS to microbes, static water only tests were performed using the following GSI standard operating procedures (SOPS)*: GSI/SOP/BS/RA/MA/1 - Procedure for Quantifying Heterotrophic Plate Counts (HPCs) using IDEXX's SimPlate® for HPC Method, GSI/SOP/BS/RA/MA/3 - Procedure for the Detection and Enumeration of Enterococcus using Enterolert™, and GSI/SOP/BS/RA/MA/4 - Procedure for the Detection and Enumeration of Total Coliforms and E.coli using IDEXX's Colilert®*. Dose effectiveness tests were conducted to determine if the proposed concentrations of chlorine produced via the electrolytic cell component are harmful to *Escherichia coli*, *Enterococcus faecalis*, and heterotrophic bacteria in the following water types: LW, FHW, sterile FHW (St.FHW), FHW50, SFHW and SFHW50. Table 2 provides the sample ID codes, water types and chlorine concentrations for the microbial dose effectiveness tests.

Table 2. Sample ID code and sample conditions for dose effectiveness tests involving the microbes *Escherichia coli, Enterococcus faecalis,* and heterotrophic bacteria.

Sample ID Code	Experimental Water Type	Chlorine Concentration
LW-0 mg/L	Lab Water (LW)	No Chlorine
FHW-0mg/L	Filtered Harbor Water (FHW)	
St.FHW-0 mg/L	Sterile FHW (St.FHW)	
FHW50-0mg/L	50 % FHW:50 % LW (FHW50)	
SFHW-0mg/L	Filtered Harbor Water and Salt (SFHW)	
SFHW50-0mg/L	50 % FHW:50 % LW and Salt (SFHW50)	
FHW-4 mg/L	FHW	4 mg/L Chlorine
FHW50–4 mg/L	FHW50	
FHW–6 mg/L	FHW	6 mg/L Chlorine
FHW50–6 mg/L	FHW50	
FHW-10 mg/L	FHW	10 mg/L Chlorine
FHW50-10 mg/L	FHW50	

At exposure periods of 0 (non-salt controls only), 24, and 48 hours, samples were neutralized with sodium thiosulfate and test organism density was determined. In the first test, ambient heterotrophic bacteria in test water were exposed as described above and were analyzed using SimPlates for Heterotrophic Plate Count (HPC) which uses IDEXX's Multiple Enzyme Technology™ (MET™) to detect as little as two viable heterotrophic bacteria per mL of water. The second test involved spiking the test water with *E. coli* and *E. faecalis*, and analyzing samples according to Colilert® and Enterolert™ tests, Quanti-Tray/2000 and the Quanti-Tray sealer from IDEXX laboratories. The Colilert® test can detect *E. coli* and at 1 colony forming unit (cfu) per 100mL and the Enterolert™ test can detect enterococci at 1 cfu/100mL. Both tests use Defined Substrate Technology® (DST) in which the bacteria metabolize the enzymes in the specific media causing the sample to fluoresce. Results for heterotrophic bacteria are reported as Most Probable Number (MPN) per 1 mL, while *E. coli* and *Enterococcus* are expressed as MPN per 100 mL. MPN is a directly related to cfu.

Residual Toxicity Tests

GSI residual toxicity tests measured the effects of the electrolytic cell component of the Siemens SiCURE™ BWMS to determine whether treated water held for a period of five days at 25 °C in complete darkness was toxic to a variety of species. The five day hold time was designed to simulate the amount of time water might be held in a ballast tank following treatment and prior to discharge. The test species included the daphnid (*Ceriodaphnia dubia*) that were less than 24 hours old, fathead minnows (*Pimephales promelas*) less than 24 hours old and amphipods (*Hyalella azteca*) approximately 7 to 8 days of age. Residual toxicity testing followed the GSI SOPs: *GSI/SOP/BS/RA/WET/1- Bench-Scale Procedure for Measuring Residual Toxicity using Ceriodaphnia dubia, GSI/SOP/BS/RA/WET/2 - Bench-Scale Procedure for Measuring Residual Toxicity using Fathead Minnows (Pimephales promelas) and GSI/SOP/BS/RA/RT/4-Bench-Scale*

Procedure for Measuring Residual Toxicity Using the Amphipod Hyalella azteca. Table 3 provides the sample ID codes, water types and chlorine concentrations used for residual toxicity tests. Note: The 6 mg/L chlorine dosage was tested at the request of the developer, as GSI land-based tests of the Siemens SiCURE™ BWMS, conducted during September 2009, were proposed to use this dosage.

Table 3. Sample ID code and sample conditions for residual toxicity tests involving the daphnid *Ceriodaphnia dubia*, the fathead minnow *Pimephales promelas* and the amphipod *Hyalella azteca*.

Sample ID Code	Experimental Water Type	Chlorine Concentration
LW-0 mg/L	Laboratory Water (LW)	No Chlorine
FHW-0mg/L	Filtered Harbor Water (FHW)	
SLW-0mg/L	LW and Salt (SLW)	
SFHW-0mg/L	Filtered Harbor Water and Salt (SFHW)	
LW-6 mg/L	LW	6 mg/L Chlorine
FHW-6 mg/L	FHW	
FHW50–6 mg/L	FHW50	

Exposure solutions (1 L of each listed in Table 3) were prepared five days prior to test initiation and the total residual chlorine concentration of each of the solutions was measured prior to placing the solutions in an incubator set at 25 °C and in the dark.

Just prior to test initiation, total residual chlorine was measured in all exposure solutions. In addition, temperature, pH, dissolved oxygen, conductivity, alkalinity and hardness were measured in each of the solutions. Once chemical measurements were made, 50 mL aliquots of each exposure solution were placed in appropriately labeled 300 mL high form beakers. Three biology replicates and three chemistry replicates were prepared for each exposure and test organism. Ten organisms of each species were placed into the exposure beakers which were then placed into an incubator set at 25 °C in 16H light:8H dark light cycle. Mortality was examined at approximately 3 hours, 24 hours and 48 hours after the organisms were added.

FINDINGS

Chlorine Versus Oxidation Reduction Potential (ORP) Readings

Figures 1-3 describe the relationship between the chlorine concentrations at 0, 24 and 48 hours, respectively, versus ORP readings. At higher chlorine concentrations, the ORP readings increased as chlorine concentrations also increased (Figure 1). This relationship however does not appear to be linear (Figure 1). At any given chlorine concentration point there appears to be some variability in ORP response (Figure 1). While at the lower levels of chlorine concentration, the ORP values remain very similar as chlorine concentration increased (Figures 2-3).

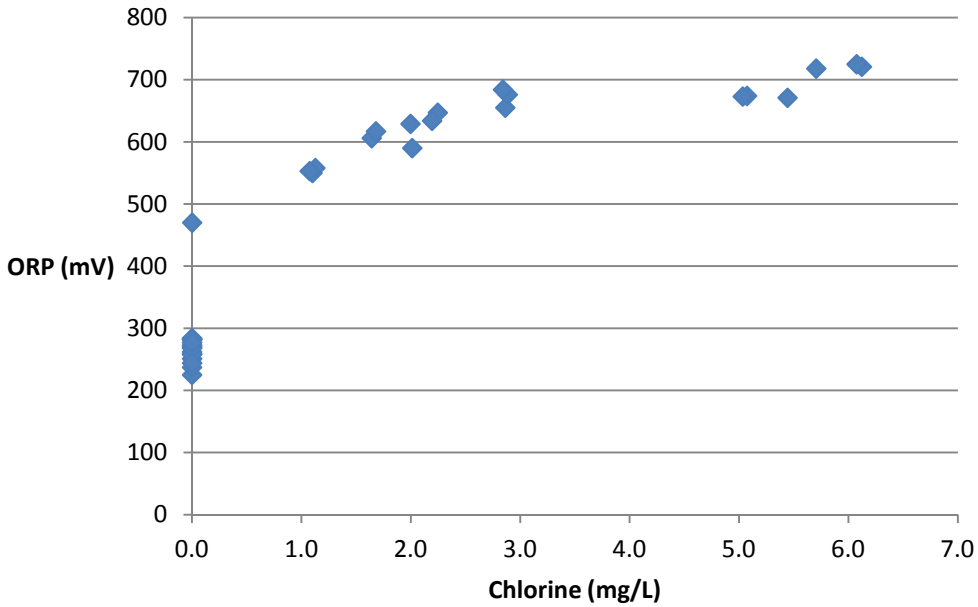

Figure 1. Concentrations (mg/L) of chlorine versus oxidation reduction potential (ORP; mV) in 50 % filtered harbor water (FHW50) at 0 hours.

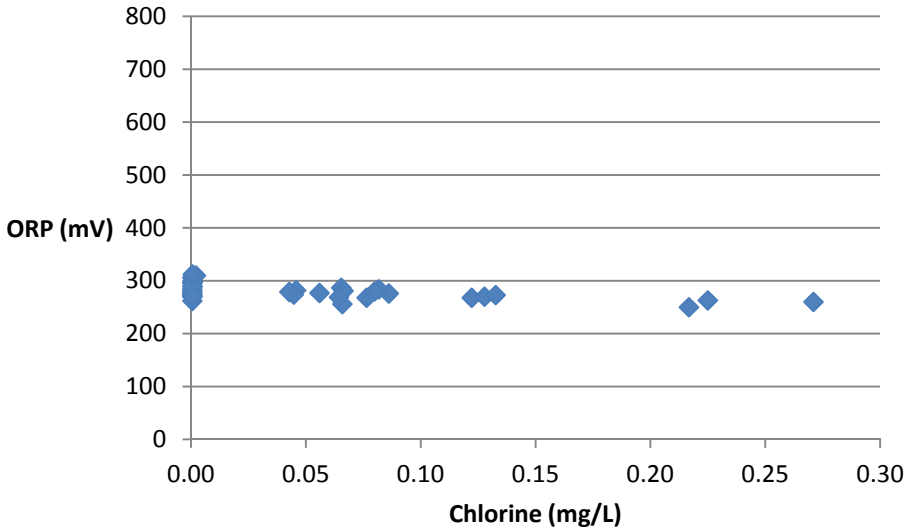

Figure 2. Concentrations (mg/L) of chlorine versus oxidation reduction potential (ORP; mV) in 50 % filtered harbor water (FHW50) at 24 hours.

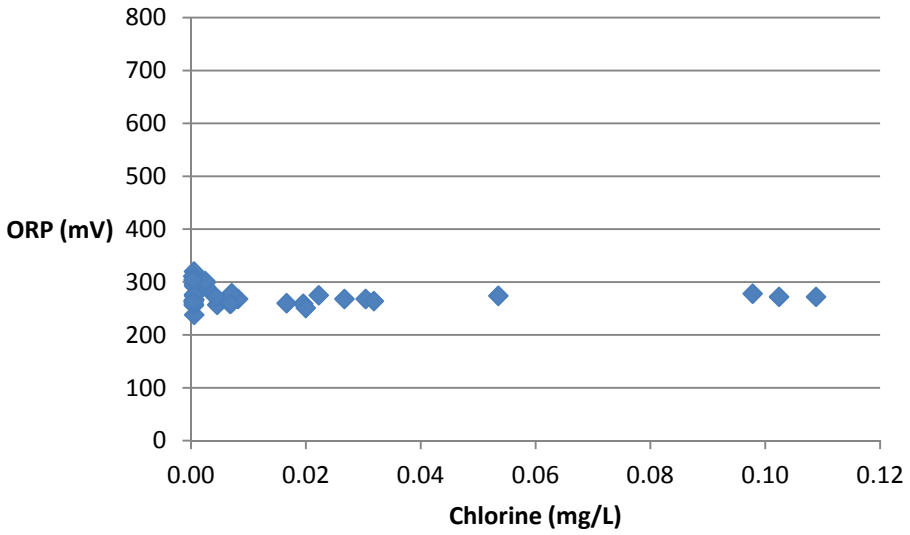

Figure 3. Concentrations (mg/L) of chlorine versus oxidation reduction potential (ORP; mV) in 50 % filtered harbor water (FHW50) at 48 hours.

Dose Effectiveness Tests

Green alga: *Selenastrum capricornutum*

Table 4 describes chlorine concentrations and ORP readings measured during the 48 hour dose effectiveness test involving the green alga *S. capricornutum*. In general, the chlorine concentration was below detection in all of the controls (Table 4). Initial chlorine concentrations in FHW and FHW50 were reduced when compared to the intended spiking concentrations (Table 4). However, measurable levels (i.e., 0.013 - 0.149 mg/L) of chlorine were still present at 48 hours (Table 4). Algae survival was 100 % in all controls tested (Table 5). In FHW, survival was significantly reduced in the 4 and 6 mg/L treatments at both the 24 and 48 hour time periods (Table 5). However, it should be noted that survival in the 10 mg/L FHW treatment was not significantly reduced at the 24 hour time point and was slightly reduced at the 48 hour time point, although this slight reduction was significant (Table 5). The FHW50 treatment results followed a typical dose-response curve with the greatest effect in the 10 mg/L treatment and the least effect in the 4 mg/L treatment. There was 0 % survival in the 10 mg/L treatment in FHW50 at 24 and 48 hours (Table 5). Table 6 summarizes the water chemistry parameters that were measured during the 48 hour dose effectiveness tests involving *S. capricornutum*.

Table 4. Total residual chlorine (mg/L) and oxidation reduction potential (ORP) results in *Selenastrum capricornutum* (green algae) dose effectiveness tests.

Sample ID	Total Residual Chlorine (mg/L)				Oxidation Reduction Potential (mV)				
	0 hr (N=1)	2 hr (N=1)	24hr (N=1)	48 hr (N=4)	0 hr (N=1)	0.25 hr (N=1)	1 hr (N=1)	24 h (N=1)	48 hr (N=4)
LW-0 mg/L	<0.003	<0.003	<0.003	<0.003	-	310	242	305	313
FHW-0 mg/L	<0.003	<0.003	<0.003	<0.003	211	241	213	187	278
FHW50-0 mg/L	<0.003	<0.003	<0.003	<0.003	240	249	217	275	291
SFHW-0 mg/L	<0.003	<0.003	<0.003	<0.003	198	205	287	265	263
SFHW50-0 mg/L	<0.003	<0.003	<0.003	<0.003	227	209	332	260	270
FHW-4 mg/L	1.79	0.27	0.10	0.013	483	344	400	268	270
FHW-6 mg/L	4.23	0.46	0.095	0.020	574	438	420	404	260
FHW-10 mg/L	7.36	0.50	0.13	0.074	633	629	436	333	274
FHW50-4 mg/L	2.30	0.24	0.074	0.046	573	497	417	272	271
FHW50-6 mg/L	4.53	0.45	0.11	0.065	615	608	577	370	279
FHW50-10 mg/L	7.82	1.96	0.22	0.149	648	680	658	382	270

Table 5. Average (N=3) percent survival (standard deviation) of *Selenastrum capricornutum* (green algae) in GSI dose effectiveness tests involving the electrolytic cell component of the Siemens SiCURE™ ballast water management system (BWMS).

Sample ID	Survival (%)		
	0 hr	24 hr	48 hr
LW-0 mg/L	100 (0)	100 (0)	100 (0)
FHW-0 mg/L	100 (0)	100 (0)	100 (0)
FHW50-0 mg/L	100 (0)	100 (0)	100 (0)
SFHW50-0 mg/L	100 (0)	100 (0)	100 (0)
FHW-4 mg/L	100 (1)	74 (8)*	3 (0)*
FHW-6 mg/L	100 (1)	74 (2)*	18 (2)*
FHW-10 mg/L	100 (0)	97 (1)	91 (5)*
FHW50-4 mg/L	100 (0)	90 (6)*	61(12)*
FHW50-6 mg/L	99 (1)	61 (4)*	59 (8)*
FHW50-10 mg/L	100 (0)	0 (0)*	0 (0)*

* Statistically significant (P value <0.05) reduced survival when compared to respective control.

Table 6. Water chemistry measurements for GSI dose effectiveness tests involving the green alga *Selenastrum capricornutum* exposed to water treated with the electrolytic cell component of the Siemens SiCURE™ ballast water management system (BWMS).

Sample ID Code	Temperature (°C) (N=6)	Dissolved Oxygen (mg/L) (N=6)	pH (N=6)	Conductivity (µS/cm) (N=5)	Hardness (mg/L $CaCO_3$) (N=3)	Alkalinity (mg/L $CaCO_3$) (N=1)
LW-0 mg/L	23.9 (23.1, 24.5)	4.2 (3.5, 6.8)	7.66 (7.14, 7.98)	137.6 (132.6,142.6)	50.0 (46.0, 54.0)	52.0
FHW-0 mg/L	23.6 (23.3, 24.0)	4.7 (4.0, 6.7)	7.98 (7.79, 8.06)	168.9 (167.7, 171.4)	66.7 (66.0, 68.0)	60.0
FHW50–0 mg/L	24.2 (23.5, 24.9)	4.7 (3.9, 7.6)	7.91 (7.54, 8.06)	164.3 (152.8, 198.4)	59.3 (58.0, 60.0)	62.0
SFHW-0 mg/L	23.3 (23.0, 23.6)	4.6 (4.3, 5.5)	8.01 (7.85, 8.07)	730 (697, 750)	70.0 (70.0, 70.0)	62.0
SFHW50–0 mg/L	23.6 (23.1, 24.0)	4.7 (4.1, 6.3)	7.89 (7.62, 8.03)	714 (689, 748)	58.7 (58.0, 60.0)	56.0
FHW-4 mg/L	23.5 (22.7, 23.9)	4.4 (4.0, 5.1)	8.03 (7.98, 8.06)	386 (382, 391)	66.0 (64.0, 68.0)	60.0
FHW-6mg/L	23.6 (22.2, 24.4)	4.5 (3.9, 4.8)	8.07 (7.97, 8.18)	495 (480, 516)	66.7 (64.0, 68.0)	60.0
FHW-10 mg/L	23.2 (22.2, 24.2)	4.9 (4.0, 5.2)	8.06 (8.00, 8.15)	731 (719, 746)	66.0 (64.0, 68.0)	64.0
FHW50–4 mg/L	23.5 (22.6, 23.8)	4.6 (4.3, 5.5)	7.92 (7.60, 8.04)	375 (363, 385)	58.7 (58.0, 60.0)	60.0
FHW50–6 mg/L	23.6 (22.2, 24.4)	4.8 (4.5, 5.0)	7.98 (7.89, 8.06)	496 (481, 504)	59.0 (58.0, 60.0)	63.2
FHW50–10 mg/L	23.4 (22.1, 23.8)	4.9 (4.4, 5.1)	8.00 (7.97, 8.06)	(720 (699, 736)	57.3 (56.0, 58.0)	56.0

Rotifer Resting Eggs: *Brachionus calyciflorus*

Table 7 describes the chlorine concentration and ORP readings measured during the 48 hour dose effectiveness test involving resting eggs of the rotifer *B. calyciflorus*. No measurable levels of chlorine were detected in the controls (Table 7). Chlorine concentrations ranged from 0.012-0.13 mg/L in FHW50 at 48 hours while the chlorine concentration ranged from 0.008-0.068 mg/L in FHW (Table 7). Hatching was significantly reduced in the 4 and 6 mg/L chlorine concentration treatments involving FHW (Table 8). No reduction in hatching was observed in the FHW50 treatments (Table 8) when compared to the controls. Table 9 summarizes the water chemistry parameters measured during this 48 hour dose effectiveness test.

Table 7. Total residual chlorine (mg/L) and oxidation reduction potential (ORP) results in *Brachionus calyciflorus* resting egg dose effectiveness tests.

Sample ID	Total Residual Chlorine (mg/L)		Oxidation Reduction Potential (mV)				
	0 hr (N=1)	48 hr (N=1)	0 hr (N=1)	0.25 hr (N=1)	1 hr (N=1)	24 hr (N=1)	48 hr (N=1)
LW-0 mg/L	<0.003	<0.003	290	292	283	278	287
FHW-0 mg/L	<0.003	<0.003	280	276	274	285	286
FHW50-0 mg/L	<0.003	<0.003	294	286	279	283	286
SFHW-0 mg/L	<0.003	<0.003	275	274	258	280	289
SFHW50-0 mg/L	<0.003	<0.003	277	274	362	280	290
FHW-4 mg/L	1.83	0.008	489	440	407	292	268
FHW-6 mg/L	3.36	0.026	594	490	389	300	272
FHW-10 mg/L	6.68	0.068	644	630	563	277	273
FHW50-4 mg/L	2.93	0.012	572	560	430	282	282
FHW50-6 mg/L	4.47	0.041	642	652	594	281	275
FHW50-10 mg/L	8.25	0.13	676	692	696	295	281

Table 8. Average (N=8) percent hatching (standard deviation) of *Brachionus calyciflorus* resting eggs in GSI dose effectiveness tests involving the electrolytic cell component of the Siemens SiCURE™ ballast water management system (BWMS).

Sample ID	Hatching (%) at 48 Hours
LW-0 mg/L	60.6 (14.8)
FHW-0 mg/L	71.1 (18.5)
FHW50-0 mg/L	65.9 (18.5)
SFHW-0 mg/L	74.1 (20.3)
SFHW50-0 mg/L	65.1 (6.5)
FHW-4 mg/L	5.0 (7.6)*
FHW-6 mg/L	45.9 (14.3)*
FHW-10 mg/L	59.4 (19.7)
FHW50-4 mg/L	66.9 (15.1)
FHW50-6 mg/L	65.9 (21.4)
FHW50-10 mg/L	62.0 (10.3)

* Statistically significant (P value <0.05) reduced hatching when compared to respective control.

Table 9. Water chemistry measurements for GSI dose effectiveness tests involving the resting eggs of the rotifer *Brachionus calyciflorus* exposed to water treated with the electrolytic cell component of the Siemens SiCURE™ ballast water management system (BWMS).

Sample ID Code	Temperature (°C) (N=2)	Dissolved Oxygen (mg/L) (N=2)	pH (N=2)	Conductivity (µS/cm) (N=2)	Hardness (mg/L $CaCO_3$) (N=2)	Alkalinity (mg/L $CaCO_3$) (N=2)
LW-0 mg/L	22.2 (21.4, 23.0)	3.8 (3.2, 4.3)	7.66 (7.47, 8.00)	143.9 (139.5, 148.3)	56.2 (54.8, 57.6)	55.6 (54.8, 56.4)
FHW-0 mg/L	22.6 (22.2, 22.9)	4.0 (3.2, 4.7)	7.97 (7.91, 8.05)	174.7 (168.7, 180.7)	70.6 (68.8, 72.4)	60.6 (58.4, 62.8)
FHW50–0 mg/L	22.3 (21.4, 23.2)	3.9 (3.2, 4.5)	7.81 (7.67, 8.02)	158.3 (154.5, 162.0)	64.6 (63.2, 66.0)	56.6 (54.8, 58.4)
SFHW-0 mg/L	22.7 (22.4, 23.0)	4.1 (3.4, 4.8)	7.96 (7.89, 8.05)	644 (635, 653)	70.4 (70.0, 70.8)	60.6 (58.8, 62.4)
SFHW50 – 0 mg/L	22.6 (22.2, 23.0)	4.1 (3.4, 4.8)	7.77 (7.61, 8.01)	742 (728, 756)	59.2 (58.8, 59.6)	56.4 (54.8, 58.0)
FHW-4 mg/L	22.8 (22.5, 23.0)	4.3 (3.6, 4.9)	8.08 (8.08, 8.09)	413 (398, 427)	72.2 (70.8, 73.6)	61.4 (60.4, 62.4)
FHW-6mg/L	22.8 (22.6, 22.9)	4.4 (3.8, 5.0)	8.11 (8.08, 8.14)	521 (509, 532)	72.0 (68.8, 75.2)	62.0 (60.4, 63.6)
FHW-10 mg/L	22.7 (22.4, 22.9)	4.5 (4.0, 5.0)	8.14 (8.06, 8.24)	748 (717, 779)	69.6 (68.8, 70.4)	63.2 (61.2, 65.2)
FHW50–4 mg/L	23.1 (23.1, 23.1)	4.2 (3.6, 4.8)	7.90 (7.79, 8.04)	381 (379, 382)	61.2 (60.4, 62.0)	57.2 (56.4, 58.0)
FHW50–6 mg/L	22.8 (22.5, 23.0)	4.3 (3.5, 5.1)	7.92 (7.81, 8.07)	513 (505, 520)	61.2 (60.8, 61.6)	59.6 (59.2, 60.0)
FHW50–10 mg/L	22.8 (22.6, 22.9)	4.6 (4.0, 5.1)	8.01 (7.98, 8.05)	734 (731, 737)	63.2 (61.2, 65.2)	59.4 (59.2, 59.6)

Adult Copepods: *Eucyclops* spp.

Table 10 describes the chlorine concentration and ORP readings measured during the 48 hour dose effectiveness test involving the adult copepod *Eucyclops* spp. No measurable levels of chlorine were detected in the controls (Table 10). Chlorine concentrations ranged from 1.93-.6.05 mg/L in FHW at 0 hours while the chlorine concentration ranged from 2.12-7.20 mg/L in FHW50 at 0 hours (Table 10). By 48 hours the chlorine concentration was substantially reduced in both water types (Table 10). Table 11 describes results for copepod survival. Survival was greater than the acceptable limit of 90 % in the controls (Table 11). Survival in both water types at all chlorine levels tested was significantly reduced when compared to the controls (Table 11). Again, FHW treated with 10 mg/L chlorine had the highest survival of 43 % (Table 11). Table 12 provides a summary of the water chemistry parameters measured during this test.

Table 10. Total residual chlorine (mg/L) and oxidation reduction potential (ORP) results in *Eucyclops* spp. (adult copepod) dose effectiveness tests.

Sample ID	Total Residual Chlorine (mg/L)			Oxidation Reduction Potential (mV)				
	0 hr (N=1)	24 hr (N=1)	48 hr (N=4)	0 hr (N=1)	0.25 hr (N=1)	1 hr (N=1)	24 hr (N=1)	48 hr (N=4)
LW-0 mg/L	<0.003	<0.003	<0.003	443	359	306	280	309
FHW-0 mg/L	<0.003	<0.003	<0.003	354	315	385	265	300
FHW50-0 mg/L	<0.003	<0.003	<0.003	389	345	416	263	282
SFHW-0 mg/L	<0.003	<0.003	<0.003	347	323	375	260	296
SFHW50-0 mg/L	<0.003	<0.003	<0.003	379	342	392	256	291
FHW-4 mg/L	1.93	0.05	0.004	567	389	384	300	279
FHW-6 mg/L	2.76	0.063	0.007	627	487	422	270	272
FHW-10 mg/L	6.05	0.09	0.038	661	670	533	280	281
FHW50-4 mg/L	2.12	0.037	0.015	632	611	490	283	272
FHW50-6 mg/L	3.87	0.06	0.029	670	683	638	275	279
FHW50-10 mg/L	7.20	0.20	0.084	696	714	713	276	283

Table 11. Average (N=3) percent survival (standard deviation) of the copepod *Eucyclops* spp. in GSI dose effectiveness tests involving the electrolytic cell component of the Siemens SiCURE™ ballast water management system (BWMS).

Sample ID	Survival (%) at 48 hours
LW-0 mg/L	93.3 (11.5)
FHW-0 mg/L	100 (0)
FHW50-0 mg/L	96.7 (5.8)
SFHW-0 mg/L	96.7 (5.8)
SFHW50-0 mg/L	100 (0)
FHW-4 mg/L	6.7 (11.5)*
FHW-6 mg/L	13.3 (11.5)*
FHW-10 mg/L	43.3 (40.4)*
FHW50-4 mg/L	30.0 (17.3)*
FHW50-6 mg/L	0 (0)*
FHW50-10 mg/L	0 (0)*

* Statistically significant (P value <0.05) reduced survival when compared to respective control.

Table 12. Water Chemistry Measurements for GSI dose effectiveness tests involving the resting eggs of the copepod *Eucyclops* spp. exposed to water treated with the electrolytic cell component of the Siemens SiCURE™ ballast water management system (BWMS).

Sample ID Code	Temperature (°C) (N=4)	Dissolved Oxygen (mg/L) (N=4)	pH (N=4)	Conductivity (µS/cm) (N=3)	Hardness (mg/L $CaCO_3$) (N=3)	Alkalinity (mg/L $CaCO_3$) (N=3)
LW-0 mg/L	24.3 (23.1, 25.5)	3.7 (3.2, 4.2)	7.61 (7.38, 7.81)	158.9 (145.3, 170.5)	57.2 (50.8, 63.6)	56.5 (51.2, 62.8)
FHW-0 mg/L	24.3 (23.0, 25.4)	4.3 (3.3, 4.9)	8.00 (7.89, 8.07)	179.1 (167.4, 193.3)	68.6 (68.4, 68.8)	56.1 (50.8, 60.4)
FHW50–0 mg/L	24.4 (22.8, 25.7)	4.1 (3.2, 4.6)	7.91 (7.71, 8.06)	163.1 (148.8, 177.2)	63.2 (60.4, 67.6)	56.1 (55.2, 58.0)
SFHW-0 mg/L	24.6 (23.5, 25.8)	4.3 (3.5, 5.0)	7.96 (7.84, 8.06)	758 (738, 797)	72.1 (68.0, 78.4)	58.9 (57.2, 61.6)
SFHW50–0 mg/L	24.4 (23.0, 25.9)	4.4 (3.4, 4.9)	7.88 (7.65, 8.04)	742 (715, 794)	65.3 (62.0, 70.4)	57.2 (55.6, 59.6)
FHW-4 mg/L	25.0 (23.4, 27.6)	3.8 (3.4, 4.4)	8.00 (7.95, 8.07)	407 (398, 416)	70.4 (68.8, 73.6)	60.1 (59.2, 60.8)
FHW-6mg/L	25.1 (23.5, 27.2)	3.9 (3.3, 4.6)	8.01 (7.96, 8.06)	508 (496, 516)	68.3 (64.8, 71.6)	58.5 (55.2, 60.4)
FHW-10 mg/L	25.0 (23.5, 27.3)	4.0 (2.8, 4.7)	8.02 (7.95, 8.07)	706 (695, 723)	72.8 (64.4, 86.8)	59.1 (57.2, 61.6)
FHW50–4 mg/L	25.1 (23.4, 27.7)	4.1 (3.6, 4.6)	7.94 (7.80, 8.06)	382 (365, 391)	61.3 (58.8, 64.8)	57.2 (53.2, 62.8)
FHW50–6 mg/L	25.0 (23.4, 27.3)	3.9 (3.5, 4.7)	7.95 (7.86, 8.05)	511 (496, 527)	60.7 (59.6, 62.4)	54.9 (54.8, 55.2)
FHW50-10 mg/L	25.2 (23.5, 27.5)	3.8 (3.0, 4.7)	8.01 (7.91, 8.09)	721 (680, 776)	60.9 (58.8, 64.8)	55.5 (52.4, 58.0)

Adult Rotifers: *Brachionus calyciflorus*

Table 13 describes the chlorine concentration and ORP readings measured in the 24 hour adult rotifer survival test. No measurable levels of chlorine were detected in the controls (Table 13). Chlorine concentrations ranged from 2.07 - 6.11 mg/L at 0 hours in FHW while the chlorine concentration ranged from 3.06 - 7.82 mg/L in FHW50 at the same time period (Table 13). 24 hours later the chlorine levels dropped to 0.051 - 0.069 mg/L in FHW while the chlorine concentrations ranged from 0.040 - 0.185 in FHW50 (Table 13). Table 14 describes the survival of the adult rotifer at 24 hours. All control survival was greater than 98.8 % (Table 14). Survival was 0 % in all water and treatment test combinations (Table 14). Table 15 summarizes the water chemistry parameters measured during this 24 hour dose effectiveness test.

Table 13. Total residual chlorine (mg/L) and oxidation reduction potential (ORP) results in adult rotifer (*Brachionus calyciflorus*) dose effectiveness tests.

Sample ID	Total Residual Chlorine (mg/L)		Oxidation Reduction Potential (mV)			
	0 hr (N=1)	24 hr (N=2)	0 hr (N=1)	0.25 hr (N=1)	1 hr (N=1)	24 hr (N=1)
LW-0 mg/L	<0.003	<0.003	301	-	-	306
FHW-0 mg/L	<0.003	<0.003	297	-	-	300
FHW50-0 mg/L	<0.003	<0.003	299	-	-	286
SFHW-0 mg/L	<0.003	<0.003	294	-	-	288
SFHW50-0 mg/L	<0.003	<0.003	295	-	-	293
FHW-4 mg/L	2.07	0.057	528	459	437	296
FHW-6 mg/L	2.80	0.051	598	536	451	288
FHW-10 mg/L	6.11	0.069	645	656	626	292
FHW50-4 mg/L	3.06	0.040	604	586	486	291
FHW50-6 mg/L	4.12	0.065	651	665	655	294
FHW50-10 mg/L	7.82	0.185	687	696	712	300

Table 14. Average (N=8) percent survival (standard deviation) of the adult rotifer *Brachionus calyciflorus* in GSI dose effectiveness tests involving the electrolytic cell component of the Siemens SiCURE™ ballast water management system (BWMS).

Sample ID	*Survival (%) at 24 hours
LW-0 mg/L	100 (0)
FHW-0 mg/L	100 (0)
FHW50-0 mg/L	100 (0)
SFHW-0 mg/L	98.8 (3.5)
SFHW50-0 mg/L	100 (0)
FHW-4 mg/L	0 (0)
FHW-6 mg/L	0 (0)
FHW-10 mg/L	0 (0)
FHW50-4 mg/L	0 (0)
FHW50-6 mg/L	0 (0)
FHW50-10 mg/L	0 (0)

* Due to a large number of ties in treatments (all zero percent mortality) cannot conduct statistical analysis due to zero variability.

Table 15. Water chemistry measurements for GSI dose effectiveness tests involving adult rotifers (*Brachionus calyciflorus*) exposed to water treated with the electrolytic cell component of the Siemens SiCURE™ ballast water management system (BWMS).

Sample ID Code	Temperature (°C) (N=2)	Dissolved Oxygen (mg/L) (N=2)	pH (N=2)	Conductivity (µS/cm) (N=2)	Hardness (mg/L $CaCO_3$) (N=2)	Alkalinity (mg/L $CaCO_3$) (N=2)
LW-0 mg/L	25.0 (24.5, 25.4)	3.5 (3.0, 3.9)	7.50 (7.31, 7.86)	152.7 (146.6, 158.7)	52.4 (52.0, 52.8)	49.8 (47.6, 52.0)
FHW-0 mg/L	24.4 (24.0, 24.7)	4.0 (3.9, 4.0)	7.94 (7.90, 7.99)	171.4 (165.8, 176.9)	74.4 (70.8, 78.0)	59.4 (56.8, 62.0)
FHW50–0 mg/L	24.8 (24.6, 24.9)	3.8 (3.6, 3.9)	7.76 (7.62, 7.98)	160.2 (151.6, 168.8)	55.6 (54.0, 57.2)	54.6 (52.8, 56.4)
SFHW-0 mg/L	24.3 (23.9, 24.6)	4.4 (4.1, 4.7)	7.93 (7.89, 7.98)	712 (703, 720)	69.0 (66.0, 72.0)	58.4 (55.2, 61.6)
SFHW50–0 mg/L	24.6 (24.5, 24.7)	4.3 (4.1, 4.4)	7.74 (7.59, 7.98)	667 (640, 694)	58.8 (58.0, 59.6)	53.8 (50.0, 57.6)
FHW-4 mg/L	23.7 (23.3, 24.0)	4.2 (4.1, 4.3)	8.03 (8.03, 8.04)	391 (383, 399)	66.6 (66.0, 67.2)	58.8 (56.0, 61.6)
FHW-6mg/L	23.9 (23.5, 24.3)	4.8 (4.4, 5.1)	8.02 (8.01, 8.03)	519 (513, 524)	68.0 (66.0, 70.0)	58.0 (56.0, 60.0)
FHW-10 mg/L	24.0 (23.4, 24.5)	4.8 (4.6, 4.9)	8.08 (8.02, 8.15)	733 (712, 753)	66.8 (66.0, 67.6)	59.0 (56.0, 62.0)
FHW50–4 mg/L	24.0 (23.4, 24.5)	3.8 (3.6, 3.9)	7.80 (7.67, 7.98)	377 (370, 383)	60.0 (60.0, 60.0)	54.2 (52.0, 56.4)
FHW50–6 mg/L	23.9 (23.5, 24.2)	4.5 (4.3, 4.7)	7.91 (7.81, 8.03)	495 (486, 503)	57.8 (57.6, 58.0)	55.8 (54.0, 57.6)
FHW50–10 mg/L	24.0 (23.5, 24.4)	4.7 (4.5, 4.9)	7.97 (7.96, 7.98)	709 (706, 711)	60.8 (60.0, 61.6)	55.2 (54.0, 56.4)

Adult Daphnids: *Daphnia magna*

Table 16 describes the chlorine concentration and ORP readings measured during the 48 hours dose effectiveness test involving the adult *Daphnia magna*. No measurable levels of chlorine were detected in the controls (Table 16). Chlorine concentrations ranged from 2.04 - 5.29 mg/L at 0 hours in FHW while the chlorine concentration ranged from 2.93 - 6.59 mg/L in FHW50 at the same time period (Table 16). Two hours later the chlorine levels dropped to 0.32 - 0.49 mg/L in FHW while the chlorine concentrations ranged from 0.22 - 2.82 in FHW50 (Table 16). Where there was still greater than 0 % survival beyond two hours, chlorine concentrations were measured and are reported in Table 16. Table 17 describes the survival of the adult daphnia at 2, 4, and 24 hours. Control survival was 100 % at 24 hours (Table 17). Survival was greatly reduced at 2 hours and ranged from 0 - 33 % in all water types and treatment combinations tested (Table 17). The FHW with a treatment of 10 mg/L of chlorine had the highest survival (33 %) when compared to any other treatment (Table 17). The water chemistry parameters measured during the 48 hour dose effectiveness test with *Daphnia magna* are summarized in Table 18.

Table 16. Total residual chlorine (mg/L) and oxidation reduction potential (ORP) results in *Daphnia magna* (adult daphnid) dose effectiveness tests.

Sample ID	Total Residual Chlorine (mg/L)				Oxidation Reduction Potential (mV)				
	0 hr (N=1)	2 hr (N=1)	4 hr (N=4)	24 hr (N=4)	0 hr (N=1)	0.25 hr (N=1)	1 hr (N=1)	4 hr (N=1)	24 hr (N=1)
LW-0 mg/L	<0.003	<0.003	-	<0.003	301	-	-	-	305
FHW-0 mg/L	<0.003	<0.003	-	<0.003	284	-	-	-	297
FHW50-0 mg/L	<0.003	<0.003	-	<0.003	277	-	-	-	303
SFHW-0 mg/L	<0.003	<0.003	-	<0.003	274	-	-	-	270
SFHW50-0 mg/L	<0.003	<0.003	-	<0.003	273	-	-	-	290
FHW-4 mg/L	2.04	0.32	0.21	-	569	451	392	318	-
FHW-6 mg/L	2.62	0.35	0.23	-	622	530	424	309	-
FHW-10 mg/L	5.29	0.49	-	0.079	646	647	566	-	269
FHW50-4 mg/L	2.93	0.22	0.13	-	580	563	392	291	-
FHW50-6 mg/L	3.62	0.51	0.25	-	645	656	592	293	-
FHW50-10 mg/L	6.59	2.82	1.47	-	671	683	673	623	-

Table 17. Average (N=3) percent survival (standard deviation) of the daphnid *Daphnia magna* in GSI dose effectiveness tests involving the electrolytic cell component of the Siemens SiCURE™ ballast water management system (BWMS).

Sample ID	Survival (%) 2 hours	Survival (%) 4 hours	Survival (%) 24 hours
LW-0 mg/L	100 (0)	100 (0)	96.7 (5.8)
FHW-0 mg/L	100 (0)	100 (0)	100 (0)
FHW50-0 mg/L	100 (0)	100 (0)	100 (0)
SFHW-0 mg/L	100 (0)	100 (0)	100 (0)
SFHW50-0 mg/L	100 (0)	100 (0)	100 (0)
FHW-4 mg/L	6.7 (5.8)*	0 (0)*	-
FHW-6 mg/L	6.7 (5.8)*	0 (0)*	-
FHW-10 mg/L	33.3 (11.5)*	26.7 (11.5)*	0 (0)*
FHW50-4 mg/L	3.3 (5.8)*	0 (0)*	-
FHW50-6 mg/L	0 (0)*	-	-
FHW50-10 mg/L	0 (0)*	-	-

* Statistically significant (P value <0.05) reduced survival when compared to respective control

Table 18. Water Chemistry Measurements for GSI dose effectiveness tests involving adult daphnids (*Daphnia magna)* exposed to water treated with the electrolytic cell component of the Siemens SiCURE™ ballast water management system (BWMS).

Sample ID Code	Temperature (°C) (N=5)	Dissolved Oxygen (mg/L) (N=5)	pH (N=5)	Conductivity (µS/cm) (N=1)	Hardness (mg/L $CaCO_3$) (N=1)	Alkalinity (mg/L $CaCO_3$) (N=1)
LW-0 mg/L	23.0 (22.6, 24.3)	6.4 (6.1, 7.0)	7.64 (7.14, 8.08)	144.7	-	-
FHW-0 mg/L	23.3 (22.8, 24.8)	6.2 (6.0, 6.6)	8.08 (7.91, 8.20)	165.4	50.0	52.0
FHW50-0 mg/L	23.4 (23.0, 24.8)	6.4 (6.2, 6.8)	7.58 (7.06, 8.18)	157.6	-	-
SFHW-0 mg/L	23.2 (22.8, 24.6)	6.2 (6.0, 6.6)	8.07 (7.95, 8.14)	780	-	-
SFHW50–0 mg/L	23.2 (22.7, 24.6)	6.2 (6.0, 6.6)	8.01 (7.75, 8.13)	787	-	-
FHW-4 mg/L	22.7 (22.4, 23.0)	6.4 (6.3, 6.5)	8.00 (7.91, 8.16)	393	72.0	62.0
FHW-6mg/L	23.1 (22.8, 23.7)	6.6 (6.5, 6.9)	7.96 (7.85, 8.29)	509	-	-
FHW-10 mg/L	23.3 (22.7, 23.8)	6.3 (6.0, 6.5)	8.07 (7.76, 8.34)	710	72.0	152.0
FHW50–4 mg/L	22.8 (22.5, 23.2)	6.5 (6.3, 6.7)	7.87 (7.79, 7.92)	364	-	-
FHW50–6 mg/L	22.8 (22.6, 23.4)	6.6 (6.4, 6.7)	7.73 (7.58, 7.95)	467	-	-
FHW50–10 mg/L	22.7 (22.4, 23.1)	6.3 (6.2, 6.4)	7.91 (7.77, 8.10)	677	-	-

Microbes: *Escherichia coli, Enterococcus faecalis, and heterotrophic bacteria*

Table 19 describes the chlorine concentration and ORP readings measured in the microbial dose effectiveness tests. No measurable levels of chlorine were detected in the controls (Table 19). Chlorine concentrations ranged from 2.22 - 7.25 mg/L at 0 hours in FHW while the chlorine concentration ranged from 2.51 - 7.92 mg/L in FHW50 at the same time period (Table 19). 24 hours later the chlorine levels dropped to 0.170 - 0.233 mg/L in FHW while the chlorine concentrations ranged from 0.123 - 0.443 in FHW50 (Table 19). Chlorine concentrations continued to drop 48 hours later from their starting levels to 0.079 - 0.107 in FHW and 0.055-2.03 mg/L in FHW50 water (Table 19). Table 20 - 22 describes the survival of the *E. coli*, *E. faecalis*, and heterotrophic bacteria, respectively. In all treatments, water combinations, and time periods tested *E. coli* was significantly reduced when compared to the controls (Table 20). Again, FHW treated with 10 mg/L of chlorine had the highest counts of *E. coli* at 2.89E+03 at 24 hours and 3.85E+04 at 48 hours (Table 20). A similar pattern was observed for the *E. faecalis* (Table 21). While, the total heterotrophic bacteria were less affected by the treatments tested (Table 22) when compared to the other two species tested. By 48 hours the heterotrophic bacteria were outside the upper level of the countable range (Table 22).

Table 19. Mean (n=3) Total residual chlorine (mg/L) and oxidation reduction potential (ORP) results for dose effectiveness tests involving *Escherichia coli, Enterococcus faecalis* and heterotrophic bacteria.

Sample ID	Total Residual Chlorine (mg/L)			Oxidation Reduction Potential (mV)				
	0 hr	24 hr	48 hr	0 hr	0.25 hr	1 hr	24 hr	48 hr
LW-0 mg/L	<0.003	<0.003	<0.003	272	-	-	299	336
FHW-0 mg/L	<0.003	<0.003	<0.003	280	-	-	297	305
FHW50-0 mg/L	<0.003	<0.003	<0.003	264	-	-	293	307
St.FHW-0 mg/L	<0.003	<0.003	<0.003	276	-	-	302	298
SFHW50-0 mg/L	<0.003	<0.003	<0.003	276	-	-	292	301
FHW-4 mg/L	2.22	0.213	0.079	528	416	466	284	281
FHW-6 mg/L	3.80	0.170	0.072	606	576	487	292	285
FHW-10 mg/L	7.25	0.233	0.107	664	656	598	299	287
FHW50-4 mg/L	2.51	0.123	0.055	530	543	503	285	282
FHW50-6 mg/L	4.27	0.163	0.082	611	631	616	286	293
FHW50-10 mg/L	7.92	0.443	0.203	669	683	683	302	293

Table 20. Mean (n=3) survival (standard deviation) for *Escherichia coli* in GSI dose effectiveness tests involving the electrolytic cell component of the Siemens SiCURE™ ballast water management system (BWMS). Measured in Most Probable Number (MPN) per 100 mL.

Sample ID	MPN 0 hours	MPN 24 hours	MPN 48 hours
LW-0 mg/L	1.71E+08 (8.79E+07)	2.44E+07 (3.55E+07)	1.41E+08 (5.85E+07)
FHW-0 mg/L	8.77E+07 (1.34E+08)	1.22E+08 (1.84E+07)	5.58E+07 (4.09E+07)
FHW50-0 mg/L	1.10E+08 (1.01E+08)	5.62E+07 (6.03E+07)	3.99E+07 (4.71E+07)
St.FHW-0 mg/L	-	1.05E+08 (9.13E+07)	8.88E+07(4.06E+07)
SFHW50-0 mg/L	-	9.95E+07 (8.61E+07)	1.12E+08 (5.40E+07)
FHW-4 mg/L	-	<4.0 (0)*	<2.0 (0)*
FHW-6 mg/L	-	<4.0 (0)*	<2.0 (0)*
FHW-10 mg/L	-	2.89E+03 (3.60E+03)*	3.85E+04 (5.23E+04)*
FHW50-4 mg/L	-	14.3§*	<2.0 (0)*
FHW50-6 mg/L	-	<4.0 (0)*	231§*
FHW50-10 mg/L	-	<4.0 (0)*	108§*

§ At least one replicate was below the limit of detection, so half the limit of detection was used to calculate the mean.

* Statistically significant (P value <0.05) reduced MPN when compared to respective control

Table 21. Mean (n=3) survival (standard deviation) for *Enterococcus faecalis* in GSI dose effectiveness tests involving the electrolytic cell component of the Siemens SiCURE™ ballast water management system (BWMS). Measured in Most Probable Number (MPN) per 100 mL.

Sample ID	MPN 0 hours	MPN 24 hours	MPN 48 hours
LW-0 mg/L	1.24E+08 (3.47E+07)	1.08E+07 (6.14E+06)	6.77E+06 (4.55E+06)
FHW-0 mg/L	9.93E+07 (7.34E+07)	4.13E+07 (1.55E+07)	1.43E+07 (1.66E+06)
FHW50-0 mg/L	1.73E+08 (6.87E+07)	3.36E+07 (1.14E+07)	1.34E+07 (1.48E+06)
St.FHW-0 mg/L	-	3.19E+07 (2.87E+07)	1.31E+07 (4.97E+06)
SFHW50-0 mg/L	-	1.86E+08 (2.62E+08)	1.28E+07 (5.36E+06)
FHW-4 mg/L	-	<4.0 (0)*	3.1§
FHW-6 mg/L	-	<4.0 (0)*	<2.0 (0)*
FHW-10 mg/L	-	2.62E+02 (3.71E+02)*	9.65E+01(1.16E+02)
FHW50-4 mg/L	-	<4.0 (0)*	<2.0 (0)*
FHW50-6 mg/L	-	<4.0 (0)*	1.56E+01 (1.66E+01)*
FHW50-10 mg/L	-	<4.0 (0)*	<2.0 (0)*

§ At least one replicate was below the limit of detection, so half the limit of detection was used to calculate the mean.
* Statistically significant (P value <0.05) reduced MPN when compared to respective control in the same time period.

Table 22. Mean (n=3) survival (standard deviation) for heterotrophic bacteria in GSI dose effectiveness tests involving the electrolytic cell component of the Siemens SiCURE™ ballast water management system (BWMS). Measured in Most Probable Number (MPN) per mL.

Sample ID	MPN 0 hours	MPN 24 hours	MPN 48 hours
LW-0 mg/L	2.8E+02 (3.84E+01)	3.5E+04 (3.44E+04)	2.4E+04 (2.40E+03)
FHW-0 mg/L	3.7E+04 (2.55E+03)	2.0E+04(2.76E+03)	> 7.4E+04
FHW50-0 mg/L	2.1E+04 (3.69E+03)	4.3E+04 (6.35E+03)	> 7.4E+04
St.FHW-0 mg/L	-	NR	NR
SFHW50-0 mg/L	-	NR	NR
FHW-4 mg/L	5.8E+01 (2.46E+01)*	3.2E+02 (4.33E+02)*	3.5E+04 (2.39E+04)
FHW-6 mg/L	6.7E+01 (2.68E+01)*	1.1E+02 (7.99E+01)*	> 7.4E+04
FHW-10 mg/L	7.2E+01 (1.70E+01)*	2.2E+02(3.49E+02)*	> 7.4E+04
FHW50-4 mg/L	2.6E+01 (8.19)*	1.4E+01 (4.95)*	NR
FHW50-6 mg/L	2.3E+01 (1.27E+01)*	3.7E+01 (4.88E+01)*	7.3E+03 (1.24E+04)
FHW50-10 mg/L	1.8E+01 (1.11E+01)*	<2.0 (0)*	2.1

NR= Not reportable, bacteria outside countable range.
* Statistically significant (P value <0.05) reduced MPN when compared to respective control in the same time period.

Residual Toxicity Tests

Table 23 describes total residual chlorine concentrations across time measured during residual toxicity tests. Initial (i.e., -120 hour) chlorine concentrations were very close to the desired treatment level of 6 mg/L (Table 23). Five days later (i.e., at 0 hours) the chlorine concentration was 5.02, 0.020, and 0.055 mg/L in LW, FHW, and FHW50, respectively (Table 23). Table 24 describes survival of the three species tested (i.e., the daphnid *C. dubia,* fathead minnows *P. promelas* and amphipods *H. azteca*) with water treated with 6.0 mg/L of chlorine and held for five days. Survival was greater than 96.7 % in all controls tested (Table 24). There was complete mortality by two hours in all three species tested in LW treated with 6.0 mg/L of chlorine (Table 24). There was greater than 96.7 % survival in both FHW and FHW50 treated with 6.0 mg/L of chlorine and held for five days (Table 24). Water chemistry parameters measured during the residual toxicity tests are summarized in Tables 25, 26 and 27, respectively.

Table 23. Total residual chlorine (mg/L) across time measured in GSI residual toxicity tests involving *Pimephales promelas*, *Hyalella azteca*, and *Ceriodaphnia dubia*.

Sample ID	Species	Total Residual Chlorine (mg/L)				
		-120 hr (N=1)	0 hr (N=1)	2 hr (N=1 or N=3)*	24 hr (N=1)	48 hr (N=2)
LW-0 mg/L	*Pimephales promelas*	0.024	0.006	0.004	0.003	< 0.003
FHW-0 mg/L	*Pimephales promelas*	< 0.003	< 0.003	-	< 0.003	< 0.003
SLW-0 mg/L	*Pimephales promelas*	0.024	0.007	0.003	-	< 0.003
SFHW-0 mg/L	*Pimephales promelas*	< 0.003	< 0.003	-	< 0.003	< 0.003
LW-6 mg/L	*Pimephales promelas*	6.10	5.02	0.995	-	-
FHW-6 mg/L	*Pimephales promelas*	5.23	0.020	-	0.011	< 0.003
FHW50-6 mg/L	*Pimephales promelas*	5.19	0.055	-	0.03	0.012
LW-0 mg/L	*Hyalella azteca*	0.024	0.006	0.003	< 0.003	< 0.003
FHW-0 mg/L	*Hyalella azteca*	< 0.003	< 0.003	-	< 0.003	< 0.003
SLW-0 mg/L	*Hyalella azteca*	0.024	0.007	-	< 0.003	< 0.003
SFHW-0 mg/L	*Hyalella azteca*	< 0.003	< 0.003	-	< 0.003	< 0.003
LW-6 mg/L	*Hyalella azteca*	6.10	5.02	2.53	-	-
FHW-6 mg/L	*Hyalella azteca*	5.23	0.020	-	0.013	0.004
FHW50-6 mg/L	*Hyalella azteca*	5.19	0.055	-	0.014	0.006
LW-0 mg/L	*Ceriodaphnia dubia*	0.024	0.006	0.003	< 0.003	< 0.003
FHW-0 mg/L	*Ceriodaphnia dubia*	< 0.003	< 0.003	-	< 0.003	< 0.003
SLW-0 mg/L	*Ceriodaphnia dubia*	0.024	0.007	-	< 0.003	< 0.003
SFHW-0 mg/L	*Ceriodaphnia dubia*	< 0.003	< 0.003	-	< 0.003	< 0.003
LW-6 mg/L	*Ceriodaphnia dubia*	6.10	5.02	3.63	-	-
FHW-6 mg/L	*Ceriodaphnia dubia*	5.23	0.020	-	0.014	0.005
FHW50-6 mg/L	*Ceriodaphnia dubia*	5.19	0.055	-	0.032	0.015

*N=1 for L-0 and N=3 for L-6 mg/L

Table 24. Percent survival of *Pimephales promelas*, *Ceriodaphnia dubia* and *Hyalella azteca* exposed to water held for five days following treatment with the electrolytic cell component of the Siemens SiCURE™ ballast water management system (BWMS).

Sample ID	*Pimephales promelas*		*Ceriodaphnia dubia*		*Hyalella azteca*	
	2 hr (N=3)	48 hr (N=3)	2 hr (N=3)	48 hr (N=3)	2 hr (N=3)	48 hr (N=3)
LW-0 mg/L	100 (0)	100 (0)	100 (0)	96.7 (5.8)	100 (0)	100 (0)
FHW-0 mg/L	100 (0)	100 (0)	100 (0)	96.7 (5.8)	100 (0)	100 (0)
SLW-0 mg/L	100 (0)	100 (0)	100 (0)	96.7 (5.8)	100 (0)	96.7 (5.8)
SFHW-0 mg/L	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)
LW-6 mg/L	0 (0)	-	0 (0)	-	0 (0)	-
FHW-6 mg/L	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)
FHW50-6 mg/L	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	96.7 (5.8)

Table 25. Water Chemistry Measurements for the Residual Toxicity Test with *Hyalella azteca* Exposed to Water treated with the electrolytic cell component of the Siemens SiCURE™ ballast water management system (BWMS) and aged for five days.

Sample ID	Temperature (°C) (N=4)	Dissolved Oxygen (mg/L) (N=4)	pH (N=4)	Conductivity (µS/cm) (N=2)	Hardness (mg/L $CaCO_3$) (N=2)	Alkalinity (mg/L $CaCO_3$) (N=2)
LW-0 mg/L	23.7 (22.9, 24.8)	7.0 (6.7, 7.4)	7.84 (7.72, 7.97)	157.3 (145.0, 169.6)	56.6 (52.4, 60.8)	53.2 (49.6, 56.8)
FHW-0 mg/L	23.5 (22.6, 24.4)	7.0 (6.9, 7.2)	8.10 (8.08, 8.12)	203 (192, 215)	76.6 (69.2, 84.0)	65.2 (62.0, 68.4)
SFHW- 0 mg/L	24.0 (23.2, 25.0)	6.8 (6.4, 7.2)	8.08 (8.04, 8.13)	510 (458, 563)	76.6 (70.4, 82.8)	63.8 (62.0, 65.6)
SLW-0 mg/L	24.1 (23.4, 24.9)	6.7 (6.2, 7.3)	7.79 (7.58, 8.06)	508 (494, 522)	53.6 (51.2, 56.0)	51.6 (48.4, 54.8)
LW-6 mg/L	23.0 (21.5, 24.5)	7.6 (7.2, 7.9)	7.95 (7.86, 8.07)	507 (489, 525)	51.2*	58.4*
FHW-6 mg/L	23.4 (22.2, 24.9)	6.8 (6.5, 7.3)	8.15 (8.13, 8.18)	605 (579, 631)	77.4 (72.4, 82.4)	66.8 (62.4, 71.2)
FHW50-6 mg/L	23.0 (21.6, 24.4)	6.8 (6.5, 7.3)	8.03 (7.94, 8.16)	567 (525, 608)	78.0 (61.2, 94.8)	66.8 (59.6, 74.0)

* N = 1

Table 26. Water Chemistry Measurements for the Residual Toxicity Test with *Pimephales promelas* Exposed to Water treated with the electrolytic cell component of the Siemens SiCURE™ ballast water management system (BWMS) and aged for five days.

Sample ID	Temperature (°C) (N=4)	Dissolved Oxygen (mg/L) (N=4)	pH (N=4)	Conductivity (µS/cm) (N=2)	Hardness (mg/L $CaCO_3$) (N=2)	Alkalinity (mg/L $CaCO_3$) (N=2)
LW-0 mg/L	23.6 (22.7, 24.8)	7.2 (6.9, 7.4)	7.95 (7.92, 7.97)	162 (145, 178)	64.6 (52.4, 76.8)	52.8 (49.6, 56.0)
FHW-0 mg/L	24.4 (24.2, 24.5)	6.8 (6.6, 7.2)	8.10 (8.07, 8.13)	209 (192, 225)	77.0 (69.2, 84.8)	66.8 (62.0, 71.6)
SFHW-0 mg/L	23.3 (22.2, 24.4)	7.0 (6.8, 7.2)	8.07 (8.03, 8.13)	524 (458, 589)	82.4 (70.4, 94.4)	66.0 (62.0, 70.0)
SLW-0 mg/L	24.0 (23.2, 24.8)	6.8 (6.5, 7.3)	7.95 (7.90, 8.06)	496 (494, 497)	55.6 (51.2, 60.0)	50.0 (48.4, 51.6)
LW-6 mg/L	23.0 (21.5, 24.5)	7.6 (7.2, 8.0)	7.93 (7.83, 8.07)	504 (489, 518)	51.2*	58.4*
FHW-6 mg/L	23.2 (21.8, 24.5)	6.8 (6.6, 7.3)	8.15 (8.12. 8.20)	671 (579, 763)	68.6 (64.8, 72.4)	61.2 (60.0, 62.4)
FHW50-6 mg/L	24.5 (23.4, 26.6)	6.6 (6.4, 7.3)	8.06 (8.02, 8.11)	537 (525, 548)	86.0 (61.2,110.8)	59.6*

* N = 1

Table 27. Water Chemistry Measurements for the Residual Toxicity Test with *Ceriodaphnia dubia* Exposed to Water treated with the electrolytic cell component of the Siemens SiCURE™ ballast water management system (BWMS) and aged for five days.

Sample ID	**Temperature (°C) (N=4)**	**Dissolved Oxygen (mg/L) (N=4)**	**pH (N=4)**	**Conductivity (µS/cm) (N=2)**	**Hardness (mg/L $CaCO_3$) (N=2)**	**Alkalinity (mg/L $CaCO_3$) (N=2)**
LW-0 mg/L	23.6 (22.6, 24.8)	7.4 (7.3, 7.5)	8.03 (7.97, 8.09)	158.8 (145.0, 172.6)	55.8 (52.4, 59.2)	51.2 (49.6, 52.8)
FHW-0 mg/L	23.4 (22.5, 24.4)	7.1 (7.0, 7.2)	8.15 (8.10, 8.19)	205 (192, 218)	97.4 (69.2,125.6)	66.4 (62.0, 70.8)
SFHW-0 mg/L	24.1 (23.5. 25.0)	6.8 (6.6, 7.2)	8.16 (8.13, 8.19)	507 (458, 557)	77.0 (70.4, 83.6)	64.2 (62.0, 66.4)
SLW-0 mg/L	24.3 (23.8, 24.8)	6.9 (6.6, 7.3)	8.05 (8.02, 8.08)	494 (494, 494)	55.8 (51.2, 60.4)	50.2 (48.4, 52.0)
LW-6 mg/L	23.1 (21.6, 24.5)	7.6 (7.2, 7.9)	7.96 (7.88, 8.07)	501 (489, 512)	51.2*	58.4*
FHW-6 mg/L	23.5 (22.3, 24.6)	7.0 (6.8, 7.3)	8.15 (8.13, 8.18)	571 (563, 579)	94.6 (72.4,116.8)	78.8 (62.4, 95.2)
FHW50-6 mg/L	23.1 (21.6, 24.4)	7.0 (6.7, 7.3)	8.17 (8.10, 8.32)	682 (525, 839)	72.2 (61.2, 83.2)	63.8 (59.6, 68.0)

* N = 1

GSI QUALITY MANAGEMENT

Standard Operating Procedures

GSI SOPs were used to implement all test activities. This facilitates consistent conformance to technical and quality system requirements and increases data quality. The SOPs include both programmatic and technical processes and procedures such as organism culturing; sample collection, labeling, analysis and custody; and safety. Table 28 outlines the GSI SOPs utilized for these tests. Any deviations made to SOPs during the experiment were recorded and also approved by the GSI Lead On-Site Investigator for Bench-Scale Studies as soon as practicable, as well as communicated to the GSI Senior QAQC officer.

Table 1 outlines the GSI bench-scale SOPs utilized for these tests. In addition, detailed test plans was written for the bench-scale dose-effectiveness and acute residual toxicity testing. These test plans outlined the treatment groups, test organisms, water chemistry and quality measurements, test conditions, and test endpoints for each test that was to be conducted.

Table 28. GSI Bench-Scale Standard Operating Procedures (SOPs) Utilized for SiCURE™ Maritime Ballast Water Treatment System Tests.

SOP Category	Subcategory	SOP Title	SOP Code
General	Administration	Procedure for Record Keeping	GSI/SOP/G/A/RK/1
General	Administration	Procedure for Data Entry, Data Quality Control and Database Management	GSI/SOP/G/RA/DM/1
General	Administration	Procedures for Good Documentation Practices	GSI/SOP/G/A/RK/3
Research Activities	Sample Custody	Procedure for Custody of GSI Samples	GSI/SOP/G/RA/SC/1
Research Activities	Sample Custody	Procedure for Labeling GSI Bench-Scale Samples	GSI/SOP/G/RA/SC/4
Bench Scale	Culturing	Procedure for Culturing the Freshwater Amphipod *Hyalella azteca*	GSI/SOP/BS/RA/C/2
Bench Scale	Culturing	Procedure for Culturing the Cladocerans *Daphnia magna* and *Ceriodaphnia dubia*	GSI/SOP/BS/RA/C/3
Bench Scale	Culturing	Procedure for Culturing *Selenastrum capricornutum*	GSI/SOP/BS/RA/C/4
Bench Scale	Culturing	Procedure for Culturing the Copepod *Eucyclops spp.*	GSI/SOP/BS/RA/C/6
Bench Scale	Dose Effectiveness	Procedure for Assessing Dose Effectiveness of a Ballast Treatment System Using a Copepod	GSI/SOP/BS/RA/EF/1
Bench Scale	Dose Effectiveness	Procedure for Assessing the Dose Effectiveness of a Ballast Treatment System Using the Cladoceran *Daphnia magna*	GSI/SOP/BS/RA/EF/2
Bench Scale	Dose Effectiveness	Bench-Scale Procedure for Assessing Dose Effectiveness of a Ballast Water Treatment System Using the Freshwater Rotifer *Brachionus calyciflorus*	GSI/SOP/BS/RA/EF/3

Bench Scale	Dose Effectiveness	Procedure for Assessing Dose Effectiveness of a Ballast Treatment System Using Cysts of the Freshwater Rotifer *Brachionus calyciflorus*	GSI/SOP/BS/RA/EF/4
Bench Scale	Dose Effectiveness	Procedure for Assessing Dose Effectiveness of a Ballast Treatment System Using *Selenastrum capricornutum*	GSI/SOP/BS/RA/EF/5
Bench Scale	Dose Effectiveness	Procedure for Exposing Test Organisms to an Active Substance	GSI/SOP/BS/RA/DE/7
Bench Scale	Residual Toxicity	Bench-Scale Procedure for Measuring Residual Toxicity using *Ceriodaphnia dubia*	GSI/SOP/BS/RA/WET/1
Bench Scale	Residual Toxicity	Bench-Scale Procedure for Measuring Residual Toxicity using Fathead Minnows (*Pimephales promelas*)	GSI/SOP/BS/RA/WET/2
Bench Scale	Residual Toxicity	Bench-Scale Procedure for Measuring Residual Toxicity Using the Amphipod *Hyalella azteca*	GSI/SOP/BS/RA/RT/4
Bench Scale	Microbial Analysis	Procedure for Quantifying Heterotrophic Plate Counts (HPCs) using IDEXX's SimPlate® for HPC Method	GSI/SOP/BS/RA/MA/1
Bench Scale	Microbial Analysis	Procedure for the Detection and Enumeration of *Enterococcus* using Enterolert™	GSI/SOP/BS/RA/MA/3
Bench Scale	Microbial Analysis	Procedure for the Detection and Enumeration of Total Coliforms and *E.coli* using IDEXX's Colilert®	GSI/SOP/BS/RA/MA/4
Bench Scale	Chemistry	Procedure for Analyzing Total Residual Chlorine Concentrations in Water	GSI/SOP/BS/RA/C/6

Data Management

All original datasheets were stored in three-ring binders, each with a unique identification code specific to the Siemens SiCURE^{TM} electrolytic cell component bench-scale testing. All log books were also given a unique identification code and are specific to electrolytic chlorination bench-scale testing. The raw data has been archived by the GSI Senior QA/QC Officer at the UWS campus for a period of at least five years.

Following the completion of the bench-scale tests described herein, a thorough review of all data sheets and laboratory notebooks was completed to ensure compliance with the documentation procedures outlined in all relevant GSI SOPs. A QA/QC Log Book was used to document the data verification and validation activities.

Quality Assurance/Quality Control (QA/QC)

Tests were initiated with healthy, vigorous animals. Reference toxicant tests were performed with all test organisms, with the exception of *S. capricornutum* and cysts of *B. calyciflorus*, prior to the start of the definitive test on at least a monthly basis. Quality control charts are available upon request. Test conditions, such as water quality, were monitored daily for parameters that might affect the outcome of the test (e.g., temperature, pH, and dissolved oxygen). Weekly calibration of appropriate meters, as well as, daily verification of meter accuracy ensured optimal performance.

The data quality objectives, criteria, and results from acute residual toxicity testing are described in Table 32. Reference toxicant tests were conducted on all test organisms, and all LC_{50} values were within quality control limits (Table 32). A second, quality assurance count was conducted on 33 % to 37 % of the primary counts made on test organisms during acute residual toxicity testing (Table 32), however, the results of the second counts were not recorded and average RPD could not be determined. Average survival in the dechlorinated LW reference control ranged from 96.7 % - 100 % in all acute residual toxicity tests, and survival in the 100 % HW controls ranged from 96.7 % - 100 %.

The data quality objectives, criteria, and results from microbial dose effectiveness testing are described in Table 33. A second, quality assurance count was conducted on a majority of the Quanti-Tray® or SimPlate® primary counts during microbial analysis, the average RPD for total heterotrophic bacteria, *E. coli*, and *Enterococcus spp.* analyses ranged from 0.1% - 1.8% and were well within the GSI data quality criteria (Table 33). In addition, a percentage of the samples collected during heterotrophic bacteria, *E. coli*, and *E. faecalis* analyses were analyzed in duplicate. The average RPD for all duplicate analyses was 51.9 % for *E.coli* and *E. faecalis* analyses and 19.9 % for total heterotrophic bacteria analysis. The RPD from *E. coli* and *E. faecalis* analyses did not meet the GSI data quality criteria and the RPD for heterotrophic bacteria was just below the criteria of less than 20 % RPD (Table 33). Corrective action should be taken to reduce the variability of future duplicate analyses.

The data quality objectives, criteria, and results from chemistry and water quality analyses conducted during bench-scale testing of electrolytic chlorination treatment technology are described in Table 34. All of the results achieved from every performance measurement made were within GSI data quality criteria, with the exception of alkalinity performance measurement completeness (i.e., 79 % of performance measurements met data quality criteria).

Table 31. Biological Data Quality Objectives, Criteria, and Results from GSI Dose Effectiveness Tests of Electrolytic Chlorination Ballast Treatment Technology (Siemens SiCURE™) at the Lake Superior Research Institute (University of Wisconsin-Superior).

Data Quality Indicator	Evaluation Process/ Performance Measurement	Data Quality Objective	Performance Measurement Result	
Bias	**Experiment Bias:** Monthly reference toxicant tests are conducted on test organisms. Performance is measured by **sensitivity of the test organisms relative to historical values**.	LC_{50} value within 2 standard deviations of the historical LC_{50} average	***S. capricornutum:*** No data, reference toxicant test not conducted on this test organism.	
			***B. calyciflorus* (Adults):** LC_{50} value from reference toxicant test performed 18 May 2009 (2069 mg/L KCl) was within 2 SD of the historical average, n=1.	
			***B. calyciflorus* (Cysts):** No data, reference toxicant test not conducted on this test organism.	
			***Eucyclops spp*:** LC_{50} value from reference toxicant test performed 20 May 2009 (756 mg/L KCl) was within 2 SD of the historical average, n=1.	
			***D. magna*:** LC_{50} value from reference toxicant test performed 26 May 2009 (848.5 mg/L KCl) was within 2 SD of the historical average, n=1.	
	Operator Bias: Experimental units (10 %) are counted by two separate analysts – with performance measured by **average relative percent difference (RPD)** of the number of live test organisms counted for all second analyses.	Zooplankton: < 10 % average RPD (±SEM) Phytoplankton: < 40 % average RPD (±SEM)	***S. capricornutum:*** 1**0%** of experimental units counted by a second analyst.	***S. capricornutum:* 14.3 % (± 3.1 %) RPD**, n=10
			***B. calyciflorus* (Adults): 55%** of experimental units counted by a second analyst	***B. calyciflorus* (Adults): 1.3 % (± 1.3 %) RPD**, n=8 with data (other QA counts not recorded)
			***B. calyciflorus* (Cysts): 37 %** of experimental units counted by a second analyst	***B. calyciflorus* (Cysts): 12.2 % (± 6.4 %) RPD**, n=10 with data (other QA counts not recorded)
			***Eucyclops spp*: 57 %** of experimental units counted by a second analyst	***Eucyclops spp*:** No data, QA counts not recorded
			***D. magna*: 39 %** of experimental units counted by a second analyst	***D. magna*:** No data, QA counts not recorded
Comparability	Routine procedures are conducted according to appropriate SOPs to ensure consistency between tests.	Not Applicable – Qualitative.	The following GSI SOPs were used for all Dose-effectiveness Testing conducted during the test trials: **-GSI/SOP/BS/RA/EF/1 -GSI/SOP/BS/RA/EF/2 -GSI/SOP/BS/RA/EF/3 -GSI/SOP/BS/RA/EF/4 - GSI/SOP/BS/RA/EF/5**	

Completeness	Number of valid data obtained from the performance measurement system vs. number of performance measurements collected. Performance is measured by percent completeness (%C).	> 90 % C.	***Dose Effectiveness Testing:*** 25 quantifiable, valid data/31 collected = **81 % C**

Table 32. Biological Data Quality Objectives, Criteria, and Results from Acute Residual Toxicity Testing of Electrolytic Chlorination Ballast Treatment Technology (Siemens SiCURE™) at the Lake Superior Research Institute (University of Wisconsin-Superior).

Data Quality Indicator	Evaluation Process/ Performance Measurement	Data Quality Objective	Performance Measurement Result	
Bias	**Experiment Bias:** Monthly reference toxicant tests are conducted on test organisms. Performance is measured by **sensitivity of the test organisms relative to historical values**.	LC_{50} value within 2 standard deviations of the historical LC_{50} average	***C. dubia:*** LC_{50} value from reference toxicant test performed 06 July 2009 (505 mg/L KCl) was within 2 SD of the historical average, n=1.	
			H. azteca: LC_{50} value from reference toxicant test performed 29 June 2009 (357 mg/L KCl) was within 2 SD of the historical average, n=1.	
			P. promelas: LC_{50} value from reference toxicant test performed 14 July 2009 (7.07 g/L NaCl) was within 2 SD of the historical average, n=1.	
	Operator Bias: Experimental units (10 %) are counted by two separate analysts – with performance measured by **average relative percent difference (RPD)** of the number of live test organisms counted for all second analyses.	Zooplankton: < 10 % average RPD (±SEM) Phytoplankton: < 40 % average RPD (±SEM)	***C. dubia:*** **37 %** of experimental units counted by a second analyst	***C. dubia:*** No data, QA counts not recorded
			H. azteca: **33 %** of experimental units counted by a second analyst	***H. azteca:*** No data, QA counts not recorded
			P. promelas: **37 %** of experimental units counted by a second analyst	***P. promelas:*** No data, QA counts not recorded
Comparability	Routine procedures are conducted according to appropriate SOPs to ensure consistency between tests.	Not Applicable – Qualitative.	The following GSI SOPs were used for all WET Testing conducted during the test trials: **-GSI/SOP/BS/RA/WET/1** **-GSI/SOP/BS/RA/WET/2** **-GSI/SOP/BS/RA/RT/4**	

Completeness	Number of valid data obtained from the performance measurement system vs. number of performance measurements collected. Performance is measured by percent completeness (%C).	> 90 % C.	***Acute Residual Toxicity Testing:*** 3 quantifiable, valid data/3 collected = **100 % C**

Table 33. Microbial Analysis Quality Objectives, Criteria, and Results from Bench-Scale Testing of Electrolytic Chlorination Ballast Treatment Technology (Siemens SiCURE™) at the Lake Superior Research Institute (University of Wisconsin-Superior).

Data Quality Indicator	Evaluation Process/ Performance Measurement	Data Quality Objective	Performance Measurement Result
Precision	Samples (10%) are analyzed in duplicate – with performance measured by **average relative percent difference (RPD)** of all duplicate analyses performed during test trials.	<20 % average (± SEM) RPD.	***E. coli* and *Enterococcus spp*.: 51.9 % (±12.8%) RPD**, n=21
			Total Heterotrophic Bacteria: 19.9 % (±26.1 %) RPD, n=13
Bias	**Samples (10%) are counted by two separate analysts – with performance measured by average relative percent difference (RPD) for all second counts performed during test trials.**	<20 % average (± SEM) RPD.	***E. coli* and *Enterococcus spp.*: 0.1 % (±0.2 %) RPD**, n=153
			Total Heterotrophic Bacteria: 1.8 % (±2.3%) RPD, n=67
Representativeness	Control and treatment samples are handled and analyzed in the same manner.	Not Applicable – Qualitative.	All control and treatment samples, were collected, handled, and analyzed in the same manner (using the appropriate GSI SOPs).
Comparability	Routine procedures are conducted according to appropriate SOPs to ensure consistency between tests.	Not Applicable – Qualitative.	The following GSI SOPs were used for all microbial analyses conducted during the test trials: **-GSI/SOP/BS/RA/MA/1** – Procedure for Quantifying Heterotrophic Plate Counts (HPCs) using IDEXX's SimPlate® for HPC Method **-GSI/SOP/BS/RA/MA/3** – Procedure for the Detection and Enumeration of *Enterococcus* using Enterolert® **-GSI/SOP/BS/RA/MA/4** – Procedure for the Detection and

			Enumeration of Total Coliforms and *E. coli* using IDEXX's Colilert®
Completeness	Number of valid data obtained from the performance measurement system vs. number of performance measurements collected. Performance is measured by percent completeness (%C).	> 90 % C.	***E. coli* and *Enterococcus spp*.:** 161/174 performance measurements met DQO = **93 % C**
			Total Heterotrophic Bacteria: 72/80 performance measurements met DQO = **90 % C**
Sensitivity	The limit of detection (LOD) for the analytical method used is reported.	Dependent upon the analytical technique used.	***E. coli* LOD:** < 1 MPN/100 mL
			***Enterococcus spp*. LOD:** < 1 MPN/100 mL
			Heterotrophic LOD: < 2 MPN/1 mL

Table 34. Chemistry and Water Quality Data Quality Objectives, Criteria, and Results from Bench-Scale Testing of Electrolytic Chlorination Ballast Treatment Technology (Siemens SiCURE™) at the Lake Superior Research Institute (University of Wisconsin-Superior).

Data Quality Indicator	Evaluation Process/ Performance Measurement	Data Quality Objective	Performance Measurement Result	
Precision	Samples (10%) are collected and analyzed in duplicate – with performance measured by **average relative percent difference (RPD)** of all duplicate analyses performed during test trials.	< 20 % average (± SD) RPD.	**Total Residual Chlorine: 4.87 % (± 1.26 %) RPD,** n=54	
			Hardness: 9 % of samples analyzed in duplicate.	**Hardness: 5.84 % (± 1.95 %) RPD**, n=12
			Alkalinity: 12 % of samples analyzed in duplicate.	**Alkalinity: 16.46 % (± 8.64 %) RPD**, n=14
Bias	**Performance is measured by average percent spike-recovery (%SPR) of all analyses performed during test trials.**	75 %-110 % average (± SD) SPR.	**Total Residual Chlorine: 100.41 % (± 1.32 %) SPR**, n=8	
Representativeness	Control and treatment samples are handled and analyzed in the same manner.	Not Applicable – Qualitative.	All control and treatment samples, were collected, handled, and analyzed in the same manner (using the appropriate GSI SOPs).	
Comparability	Routine procedures are conducted according to appropriate SOPs to ensure consistency between tests.	Not Applicable – Qualitative.	The following GSI SOPs were used for all water quality analyses conducted during the test trials: **-GSI/SOP/BS/RA/C/6** – Procedure for Analyzing Total Residual Chlorine (TRC) Concentrations in Water	

Completeness	Number of valid data obtained from the performance measurement system vs. number performance measurements collected. Performance is measured by percent completeness (%C).	> 90 % C.	**Total Residual Chlorine:** 58/62 performance measurements met DQO = **94 % C**
			Hardness: 11/12 performance measurements met DQO = **92 % C**
			Alkalinity: 11/14 performance measurements met DQO = **79 % C**
Sensitivity	The limit of detection (LOD) and quantification (LOQ) for the analytical method used is reported.	Dependent upon the analyte and instrumentation.	**Total Residual Chlorine:** LOD = **3 µg/L**; LOQ = **8 µg/L**

CONCLUSION

GSI bench-scale dose effectiveness test results show that the electrolytic cell component of the Siemens SiCURE™ BWMS produces a chlorine solution that is significantly toxic to *Eucyclops* spp., *Daphnia magna* and adult *Brachionus calyciflorus* at the 4 mg/L, 6 mg/L and 10 mg/L concentrations tested in both FHW and FHW50. In both the *D. magna* and *B. calyciflorus* tests, toxicity was seen in less than 24 hours exposure. With the *B. calyciflorus* resting eggs, hatch rates were significantly impacted in FHW at 4 mg/L and 6 mg/L chlorine concentrations. However, in FHW at 10 mg/L chlorine and in FHW50 at 4 mg/L, 6 mg/L and 10 mg/L chlorine there was no significant effect on hatch rates of *B. calyciflorus* eggs. While all concentrations of chlorine tested on *Selenastrum capricornutum* caused a significant decrease in survival, 91 % of the *S. capricornutum* was still alive after 48 hours in FHW with a concentration of 10 mg/L chlorine. This lower effect at the 10 mg/L chlorine concentration in FHW was seen in tests involving *S. capricornutum*, rotifer resting eggs and *Eucyclops* spp.

The residual toxicity test results show that there is no residual toxicity to *Pimephales promelas, Hyalella azteca* or *Ceriodaphnia dubia* at 6 mg/L chlorine in either FHW or FHW50. However, toxicity to all organisms was seen at that concentration of chlorine in LW. It is likely that the organic matter present in the FHW and FHW50 interacts with the chlorine to break it down more quickly than in LW. This is supported by the total residual chlorine concentrations shown in Table 23.