GREAT SHIPS INITIATIVE
BENCH-SCALE TEST FINDINGS
Technical Report - Public

Sodium Hypochlorite Solution

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ABSTRACT

The Great Ships Initiative (GSI), an innovative collaboration whose objective is to end the problem of ship-mediated invasive species in the Great Lakes-St. Lawrence Seaway System, conducts bench-scale research to aid developers of innovative technologies which could have application as ballast treatment systems. This report describes 2008 findings from bench-scale evaluations of a ballast treatment proposed by researchers from the Michigan Technological University in Houghton, Michigan. The treatment, sodium hypochlorite solution (i.e., common house-hold bleach) in doses of 3-3.5 mg/L, is intended for use in emergency situations to address viral hemorrhagic septicemia (VHS) virus or similar pathogens. The researchers also propose injection of ascorbic acid (i.e. vitamin C) into the treated ballast water to act as a dechlorination agent. GSI tested the proposed formulation to assist range-finding for effective doses, determination of the rates of chemical degradation, and the potential for residual toxicity. Please see www.greatshipsinitiative.org for more information about GSI's bench-scale testing program.

The proposed dose of 3 mg/L sodium hypochlorite solution added to test water, irrespective of water type (i.e., filtered Duluth-Superior Harbor water or laboratory water) proved effective at the bench scale at inactivating most species tested, including bacteria. The test species of green algae, *Selenastrum*, and rotifer resting eggs were somewhat resistant. A lower applied dose of chlorine (0.9 mg/L) was sufficient to achieve this level of effectiveness in laboratory water which had far less dissolved organic material than filtered harbor water.

There was no acute toxicity detected in these test associated with 3 mg/L sodium hypochlorite solution followed by neutralization with 9 mg/L with ascorbic acid. Higher concentrations of ascorbic acid did result in acute toxicity. Our limited chronic toxicity analysis also did not detect an effect, but it should be noted that much further testing would be necessary to conclude with confidence whether or not chronic toxicity would occur as a result of this treatment. Chronic toxicity tests will be especially important to assure that by-products of treatment in waters containing high concentrations of dissolved organic carbon (DOC) do not pose an environmental hazard.

TABLE OF CONTENTS

INTRODUCTION

This technical report presents quantitative and measured findings from GSI bench-scale evaluations of sodium hypochlorite solution, i.e., common house-hold bleach. Proposed by researchers from the Michigan Technological University in Houghton, Michigan, sodium hypochlorite solution in doses of 3-3.5 mg/L is intended for use in emergency situations to address viral hemorrhagic septicemia (VHS) virus or similar pathogens. GSI undertook these bench-scale tests during summer 2008 at the Lake Superior Research Institute's Aquatic Toxicity Laboratory (ATL) of the University of Wisconsin-Superior in Superior, Wisconsin. Tests included range-finding evaluations of dose effectiveness, chemical degradation and residual toxicity. A summary of these findings for non-scientific audiences can be accessed at http://www.nemw.org/GSI/GSI-BS-P-FS-Chlorine.pdf. Please note that GSI's bench-scale tests do not by themselves provide adequate information to assess a prospective ballast treatment's ability to meet a particular discharge standard or to achieve environmental soundness under shipboard application. Instead, these tests provide initial insights into possible strengths and weaknesses of the treatment—information that can be used by developers to better design a more effective system and/or to move to the next stage of treatment evaluation. The tests are also a form of trouble-shooting to encounter possible problems with the proposed treatment in advance of more extensive and larger scale tests.

BACKGROUND

The Great Ships Initiative

The Great Ships Initiative (GSI)[1] is a collaborative effort whose objective is to end the problem of ship-mediated invasive species in the Great Lakes-St. Lawrence Seaway System. To that end, the GSI established sophisticated independent third party ballast treatment evaluation capabilities at three scales—bench, land-based, and shipboard. Each scale is dedicated to addressing specific evaluation objectives:

- GSI Bench-Scale Tests
  - Range finding for effective doses under a range of ambient conditions;
  - Chemical degradation over time under a range of ambient conditions;
  - Detection of any residual toxicity under a range of ambient conditions; and
  - Confirmation of treatment process.

- GSI Land-Based Tests
  - Detection of scale-up, mechanical operation issues;
  - Effectiveness of a dose with respect to the full range of ambient organisms; and
  - Detection of any whole water effluent toxicity.

- GSI Shipboard Tests
  - Confirmation of biological and operational performance as expected in the ship environment; and

1 www.greatshipsinitiative.org

- Confirmation of performance as expected under a broad range of ambient conditions.

Developers of ballast water treatment systems apply for GSI research services online, and awards are offered based on an objective review process, regardless of the state of development of the proposed treatment. GSI status testing will be performed at the scale appropriate to the treatment state of development, with the goal of helping meritorious ballast treatment systems to progress as rapidly as possible to an approval-ready and market-ready condition.

To assure relevancy of test output, GSI test protocols are as consistent with the International Maritime Organization (IMO) Convention and federal requirements as practicable. In particular, bench testing directly supports IMO G9 evaluations, and land-based testing directly supports IMO G8 evaluations.

GSI tests are third party assessments. They are completely independent of any vested interest in outcomes. The GSI tests are supported by general project funds which derive from federal and state agency grants and contributions, Great Lakes port contributions, and in-kind contributions by the local government and universities. None of these funds come to the GSI with any strings (other than public disclosure).

Ms. Allegra Cangelosi of the Northeast-Midwest Institute is the Principal Investigator and Manager of the GSI. Researchers from the University of Wisconsin-Superior's Lake Superior Research Institute, and the University of Minnesota-Duluth's Natural Resources Research Institute, among others, provide critical scientific and technical expertise and implementation services to GSI's biological research activities, and the GSI generally. A GSI Advisory Committee comprising top-level officials of key stakeholder groups helps steer the GSI providing crucial assistance in making GSI award decisions and fund-raising. The GSI Advisory Committee includes elected leadership, environmental organizations, port directors and federal officials from the United States and Canada, and industry representatives. 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.

The largest contributor of GSI operating funds is the United States Department of Transportation, including its Maritime Administration, and the Saint Lawrence Seaway Development Organization. GSI also receives significant funds and in-kind contributions from the National Oceanic and Atmospheric Administration, the Canadian St. Lawrence Seaway Management Corporation, the City of Superior, Wisconsin, and approximately ten U.S. and Canadian ports in the Great Lakes.

GSI Bench Tests

GSI bench-scale tests take place year-round at the Lake Superior Research Institute's Aquatic Toxicity Laboratory (ATL) of the University of Wisconsin-Superior in Superior, Wisconsin. The ATL is amply equipped with staff expertise and resources to conduct the tests, and has a long history of successfully undertaking similar tests.

The overarching goals of GSI bench testing are to explore dose-effectiveness, chemical degradation, residual toxicity, and sensitivity to challenge conditions of a proposed treatment method about which

little is known. To that end, the tests are "range-finding" missions, to determine the optimal treatment dose/intensity that would maximize effectiveness and minimize residual toxicity. Findings help treatment developers better design an effective system and/or to move to the next stage of treatment evaluation. The tests are also a form of trouble-shooting to encounter possible problems with the proposed treatment in advance of more extensive and larger scale tests.

GSI bench-scale dose effectiveness tests help determine the range of concentrations of an active substance that is harmful to a variety of robust freshwater zooplankton, algae and bacteria known to be relatively resilient to stressors. Dose effectiveness test results are expressed as percent survival, percent mortality, and percent hatch. They are also 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 percent 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 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 bench-scale chemical degradation tests determine the effect that various water quality or environmental parameters may have on the rate of chemical degradation of a treatment system 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 expressed as the percent change in active ingredient concentration.

GSI bench-scale residual toxicity tests help estimate the effect that treated water (following neutralization of the active substance, a degradation period, or no treatment at all) may have on non-target organisms in the receiving system. These tests 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, and the tests are performed on sensitive organisms rather than robust species.

ABOUT SODIUM HYPOCHLORITE SOLUTION AND ASCORBIC ACID

Researchers from the Michigan Technological University in Houghton, Michigan proposed a sodium hypochlorite solution (i.e., common household bleach) formulation consisting of approximately 5.25 % sodium hypochlorite (NaOCl) and 94.75 % water, for use in emergency situations to inactivate viral hemorrhagic septicemia (VHS) virus or similar pathogens entrained in ballast water. They propose approximately 3-3.5 mg/L of sodium hypochlorite solution be metered into ballast uptake, or added to full ballast water tanks. A neutralizing agent such as ascorbic acid, or vitamin C, would be added to the tanks after an exposure period as a dechlorination agent prior to or upon discharge. The applicant (MTU researchers) recommended an ascorbic acid concentration of three times the applied chlorine concentration to achieve neutralization. Methods to assure full mixing of the treatment and its neutralization agent if applied in an actual shipboard context are being developed and evaluated separately.

METHODS

GSI bench-scale standard operating protocols (SOPs) are grounded in published standard methods, and modified to reflect ballast treatment circumstances. They are also consistent with international and domestic guidelines where they exist. All SOPs are peer-reviewed prior to acceptance and are subject to periodic review and revision to assure that the most up to date approaches are employed. Table 1 outlines the GSI bench-scale SOPs utilized for the sodium hypochlorite solution tests. In addition, all GSI bench-scale research activities comply strictly with a detailed Quality Assurance Project Plan (QAPP) which is consistent in format, detail and stringency with United States Environmental Protection Agency requirements.

Table 1. GSI Bench-Scale Standard Operating Procedures (SOPs) Utilized for Sodium Hypochlorite Tests.

SOP Category	Subcategory	SOP Title	SOP Code
General	Administration	Procedure for Record Keeping	GSI/SOP/G/A/RK/1
General	Sample Custody	Procedure for Sample Custody	GSI/SOP/G/RA/SC/1
General	Sample Custody	Procedure for Labeling Bench-Scale Samples	GSI/SOP/G/RA/SC/3
Research Activities	Culturing	Procedure for Culturing the Freshwater Rotifer *Branchionus calyciflorus*	GSI/SOP/BS/RA/C/1
Research Activities	Culturing	Procedure for Culturing the Freshwater Amphipod *Hyalella azteca*	GSI/SOP/BS/RA/C/2
Research Activities	Culturing	Procedure for Culturing the Cladocerans *Daphnia magna* and *Ceriodaphnia dubia*	GSI/SOP/BS/RA/C/3
Research Activities	Chemical Degradation	Procedure for Determining Chemical Degradation of a Primary Treatment or Its By-Products	GSI/SOP/BS/RA/CD/1
Research Activities	Dose Effectiveness	Procedure for Assessing Dose-Effectiveness of a Ballast Water Treatment System Using the Copepod *Eucyclops spp*.	GSI/SOP/BS/RA/DE/1
Research Activities	Dose Effectiveness	Procedure for Assessing Dose-Effectiveness of a Ballast Water Treatment System Using the Daphnid *Daphia magna*	GSI/SOP/BS/RA/DE/2
Research Activities	Dose Effectiveness	Procedure for Assessing Dose-Effectiveness of a Ballast Water Treatment System Using the Freshwater Rotifer *Branchionus calyciflorus*	GSI/SOP/BS/RA/DE/3
Research Activities	Dose Effectiveness	Procedure for Assessing Dose-Effectiveness of a Ballast Water Treatment System Using Resting Eggs of the Freshwater Rotifer *Branchionus calyciflorus*	GSI/SOP/BS/RA/DE/4
Research Activities	Dose Effectiveness	Procedure for Assessing Dose-Effectiveness of a Ballast Water Treatment System Using the *Selenastrum capricornutum*	GSI/SOP/BS/RA/DE/5
Research Activities	Dose Effectiveness	Procedure For Quantifying Heterotrophic Plate Counts Using IDEXX's SimPlate for HPC Method	GSI/SOP/BS/RA/MA/1
Research Activities	Dose Effectiveness	Procedure for Assessing Antimicrobial Activity Using Time-Kill Method	GSI/SOP/BS/RA/MA/2
Research Activities	Dose Effectiveness	Procedure for the Detection and Enumeration of *Enterococcus* using Enterolert™	GSI/SOP/BS/RA/MA/3
Research Activities	Dose Effectiveness	Procedure for the Detection and Enumeration of *E. coli* Using IDEXX'sColilert	GSI/SOP/BS/RA/MA/4
Research Activities	Residual Toxicity	Procedure for Measuring Residual Toxicity Using *Ceriodaphnia dubia*	GSI/SOP/BS/RA/RT/1

Research Activities	Residual Toxicity	Procedure for Measuring Residual Toxicity Using Fathead Minnows (*Pimephales promelas*)	GSI/SOP/BS/RA/RT/2
Research Activities	Residual Toxicity	Procedure for Measuring Residual Toxicity Using the Amphipod *Hyalella azteca*	GSI/SOP/BS/RA/RT/4
Research Activities	Residual Toxicity	Procedure for Assessing Chronic Residual Toxicity of a Ballast Water Treatment System Using the Daphnid *Ceriodaphia dubia*	GSI/SOP/BS/RA/RT/6
Research Activities	Chemistry	Procedure for Analyzing Non-Purgeable Total Organic Carbon (NPTOC) and Non-Purgeable Dissolved Organic Carbon (NPDOC) in Water	GSI/SOP/BS/RA/C/3
Research Activities	Chemistry	Procedure for Analyzing the Concentration of Chlorine in Test Water	GSI/SOP/BS/RA/C/6

Bench-Scale Dose Effectiveness Test Methods

Zooplankton and Algae

These GSI dose effectiveness tests measured the range of concentrations of chlorine (measured as total residual chlorine) harmful to juvenile (less than 24 hours old) daphnids, adult copepods, newly hatched rotifers, rotifer resting eggs, and a green alga. The tests evaluated five exposure concentrations of total residual chlorine and a control at a temperature of 25.0 °C ± 1.0 °C. Filtered Duluth-Superior Harbor water (FHW) and laboratory water (LW), which have contrasting physical/chemical properties, were used test water. Harbor water was collected from a depth of approximately 3 m. Alkalinity, as $CaCO_3$, ranged 65 – 69 mg/L. Non-purgeable dissolved organic carbon ranged 10 – 22 mg/L. The water is stained with tannins. Prior to use, the harbor water was passed in sequence through a Whatman GF/B filter followed by a Millipore 0.45 μm membrane filter. The LW consisted of treated Lake Superior water from the City of Superior, Wisconsin that was passed through an activated carbon column. Alkalinity, as $CaCO_3$, ranged 45 – 50 mg/L. Non-purgeable dissolved organic carbon ranged 0.5 – 2.0 mg/L. The water is transparent. Both FHW and LW were not renewed in the tests.

All exposures with the exception of the rotifer adults and rotifer resting eggs took place over 48 hours, involved 50 mL of exposure solution in a 300 mL beaker, and included three replicates. Tests on rotifer adults and rotifer resting eggs involved four replicates and 2.0 mL of exposure solution. Adult rotifers were only exposed for 24 hours, and instead of complete darkness, rotifer resting eggs were exposed to continuous light to stimulate hatching. Daily observations were made on mortality, and measured water quality parameters including total residual chlorine, temperature, dissolved oxygen, pH, conductivity, alkalinity and hardness. Table 2 arrays the types and numbers of organisms analyzed, the exposure concentrations used, and the number of replicates per dose effectiveness test.

GSI zooplankton and algae dose effectiveness test results are generally expressed in terms of a series of absolute quantifications, i.e., LC_{99}, NOEC and LOEC. However in the case of sodium hypochlorite solution tests, NOEC and LOEC values were not calculated because the rate of chlorine degradation was so rapid it became very difficult to obtain a representative measure. This would have resulted in NOEC and LOEC values with high uncertainty associated with them. Instead, findings are expressed as survival/mortality in treated samples compared to controls. In the case of rotifer resting eggs, the occurrence of hatching was used as the benchmark measurement. The average number of young produced when compared to the controls was used to assess chronic-residual toxicity. ANOVA was used to compare differences in the mean values of the treatment and control groups at a significance level of 5 %. When difference among the groups were found to be significant either Dunnett's or Dunn's method was used to determine which specific treatment group differed from the control group.

Table 2. Numbers and Types of Organisms, and Initial Exposure Concentrations of Total Residual Chlorine Used For GSI Dose-Effectiveness Tests on Zooplankton and Algae.

Major Taxonomic Group	Type	Species	Test Water Types	Initial Chlorine Concentration	No. of Organisms per Exposure /Control	No. of Replicates per Exposure /Control	Total Number of Organisms
Zooplankton	Juvenile daphnids	*Daphnia magna*	FHW	<0.002 (control), 0.024, 0.081, 0.27, 0.90 and 3.00 mg/L	10	3	6 x 10 x 3 = 180
			LW	<0.002 (control), 0.031, 0.084, 0.33, 0.86 and 2.88 mg/L	10	3	6 x 10 x 3 = 180
Zooplankton	Adult copepods	*Eucyclops* spp.	FHW	<0.002 (control), 0.024, 0.081, 0.27, 0.90 and 3.00 mg/L	10	3	6 x 10 x 3 = 180
			LW	<0.002 (control), 0.031, 0.084, 0.33, 0.86 and 2.88 mg/L	10	3	6 x 10 x 3 = 180
Zooplankton	Adult rotifers	*Brachionus calyciflorus*	FHW	<0.002 (control), 0.057, 0.12, 0.39, 1.27 and 4.14 mg/L	5	4	6 x 5 x 4 = 120
Zooplankton	Rotifer resting eggs	*Brachionus calyciflorus*	FHW	<0.002 (control), 0.024, 0.081, 0.27, 0.90 and 3.00 mg/L	20	4	6 x 20 x 4 = 480
			LW	0.004 (control), 0.021, 0.064, 0.23, 0.76 and 2.66 mg/L	20	4	6 x 20 x 4 = 480
Algae	Green alga	*Selenastrum spp.*	FHW	<0.002 (control), 0.024, 0.081, 0.27, 0.90 and 3.0 mg/L	200,000 cells/mL	3	6 x 200,000 x 3 = 3,600,000
			LW	<0.002 (control), 0.037, 0.087, 0.30, 1.06 and 3.09 mg/L	200,000 cells/mL	3	6 x 200,000 x 3 = 3,600,000

Bacteria

Dose effectiveness tests were also conducted to determine if the concentrations of sodium hypochlorite (measured as total residual chlorine) proposed are harmful to a range of ambient bacteria contained in FHW. Experiments were conducted in FHW using ambient levels of bacteria and in LW spiked with test organisms (*Escherichia coli* and *Enterococcus faecalis*) to increase

initial densities. Each test evaluated three chlorine exposure concentrations and one control at a temperature of 25.0 °C ± 1.0 °C.. Samples were analyzed following 0 (control samples only), 2, 24 and 48 hours of exposure to the treatment, enumerating total coliforms, *Escherichia coli*, Enterococcus and heterotrophic bacteria. Total residual chlorine was also measured for each sample at all analysis times. The Colilert® and Enterolert ™ tests using Quanti-Tray/2000 and the Quanti-Tray sealer from IDEXX laboratories were used to perform the analyses. The Colilert® test has detection limits for total coliforms and *E. coli* of 1 colony forming unit (cfu) per 100 mL, and the Enterolert ™ test can detect Enterococcus at 1 cfu/100 mL. Both tests use Defined Substrate Technology® (DST) in which the bacteria metabolize the enzymes in the specific media causing the sample to fluoresce. Heterotrophic plate count (HPC) bacteria in both FHW and LW were analyzed using SimPlate for HPC medium which uses IDEXX's Multiple Enzyme Technology™ (MET™). Results are expressed as Most Probable Number (MPN) per 100 mL for the Colilert and Enterolert tests and MPN per 1 mL for the SimPlate tests. MPN is a common method of obtaining quantitative data on concentrations of discrete items from positive/negative (incidence) data and in this case is directly related to colony forming units (cfu).

Future experiments, not reported here, will test the chlorine treatment effectiveness on a viral species, the bacteriophage MS2. These methods and results will be generated and incorporated into this report in Spring 2009.

Bench-Scale Chemical Degradation Test Methods

The GSI chemical degradation tests assessed the effect of various water quality or environmental parameters on the rate of sodium hypochlorite degradation (measured as total residual chlorine). The tests were performed using a series of five concentrations of DHW diluted with LW (resulting in five different levels of non-purgeable dissolved organic carbon), and two different ambient temperatures (15 °C and 25 °C). Three 50 mL replicates per exposure concentration were prepared. Each beaker (300 mL) was covered with a glass plate to minimize evaporation during the test period. During the 96 hours of exposure, the solutions were analyzed for their total residual chlorine concentration using a Thermo Orion Model 97-70 Residual Chlorine Electrode connected to an Orion Model 290A pH/mV/ISE meter. Test results are expressed as the change in total residual chlorine concentration over time. Table 3 arrays the test conditions and exposure concentrations used for this set of chemical degradation tests.

Table 3. Test Conditions and Non-Purgeable Dissolved Organic Carbon Concentrations Used for GSI Chemical Degradation Tests of Sodium Hypochlorite.

Water Type	Light Regime	Non-Purgeable Dissolved Organic Carbon (NPDOC) Concentration	Ambient Temperature	No. of Replicates per Exposure /Control
FHW diluted with LW	Complete darkness	1.8 mg/L	25 °C	3
		3.6 mg/L	25 °C	3
		5.9 mg/L	25 °C	3
		11.2 mg/L	25 °C	3
		21.1 mg/L	15 °C	3
			25 °C	3

Following this set of tests, a second set of chemical degradation experiments were performed. These tests involved examination of the degradation of sodium hypochlorite solution (measured as total residual chlorine) in the presence of fish tissue. Prior to use, the fish tissue was frozen in liquid nitrogen and processed in a blender to produce a much smaller tissue particle size. The tissue was then weighed into a beaker with 200 mL of water (either deionized water or filtered Duluth-Superior Harbor water) spiked to 3.0 mg/L as total residual chlorine. Following 10, 30, 60, and 120 minutes of exposure, the solutions were analyzed for their concentration of chlorine using the residual chlorine electrode. Test results are expressed as the change in total residual chlorine concentration over time. Table 4 arrays the test conditions, exposure concentrations and weight of fish tissue used for this set of chemical degradation tests.

Table 4. Test Conditions, Exposure Concentrations (Measured as Total Residual Chlorine), and Weight of Fish Tissue Used for GSI Chemical Degradation Tests of Sodium Hypochlorite.

Water Type	Initial Chlorine Concentration	Weight of Fish Tissue
Deionized water	3.0 mg/L	0.103 g
		0.500 g
		1.001 g
FHW	3.0 mg/L	0.000 g
		0.103 g
		0.502 g
		1.001 g

A third set of chemical degradation experiments was conducted to estimate the amount of ascorbic acid needed to degrade an applied chlorine concentration of approximately 3 mg/L to below detect. A 20 mg/L chlorine solution was allowed to degrade for approximately one hour and then analyzed and found to have 3.9 mg/L chlorine. The solution was then split into three 300 mL aliquots. Ascorbic acid solution was added to achieve a concentration of 10.0 mg/L in the first beaker, 16 mg/L to the second, and 24 mg/L to the third (n=1). The solutions were analyzed for total residual chlorine at several times (5, 45, 105, 300, 1440 min.) after the addition of ascorbic acid.

Bench-Scale Residual Toxicity Test Methods

Residual toxicity tests were performed to determine if water treated with sodium hypochlorite solution followed by a neutralization step was harmful to a specific suite of sensitive freshwater organisms. The organisms consisted of juvenile (less than 24 hours old) daphnids, juvenile (less than 24 hours old) fathead minnows and amphipods (7-8 days old). Test solutions were prepared by mixing a known amount of sodium hypochlorite solution (i.e., containing 20 mg/L of total residual chlorine) in FHW. Solutions were allowed to degrade for one hour before being neutralized by the addition of enough ascorbic acid to yield an ascorbic acid concentration that was approximately three-times that of the remaining residual chlorine concentration. Following completion of this process, the organisms were added to the solution.

All tests were performed at a temperature of 25.0 °C ± 1.0 °C and the test water was not renewed. Exposures were conducted in a 16 hour/8 hour light/dark cycle, and consisted of 50 mL of solution in a 300 mL beaker. There were three replicates per exposure solution and tests were 48 hours in length. Daily observations of mortality in control and treatment samples were compared with test results expressed as percent survival of organisms following the exposure period. Table 5 arrays the types and numbers of organisms analyzed, the exposure concentrations used (measured as total residual chlorine), and the number of replicates per test.

Table 5. Numbers and Types of Organisms, and Average Exposure Concentrations (Measured as Total Residual Chlorine) Analyzed in GSI Residual Toxicity Tests.

Major Taxonomic Group	Type	Species	Residual Chlorine Concentration Following 1 Hour of Degradation	Amount of Ascorbic Acid Added to Neutralize the Residual Chlorine	No. of Organisms Per Replicate	No. of Replicates
Zooplankton	Juvenile daphnids	*Ceriodaphnia dubia*	3.0 mg/L	9.0 mg/L	10	3
Fish	Juvenile fathead minnows	*Pimephales promelas*	3.0 mg/L	9.0 mg/L	15	3
Zooplankton	Amphipods (7-8 days old)	*Hyalella azteca*	3.0 mg/L	9.0 mg/L	10	3

A residual-chronic toxicity test was performed to examine the treatment/neutralization components individually and in combination for toxic effects. In this test, juvenile (less than 24 hours old)

daphnids were exposed to various concentrations of chlorine and/or ascorbic acid prepared in either LW or FHW. All tests were performed at a temperature of 25.0 °C ± 1.0 °C and the test water was renewed daily. Exposures were conducted in a 16 hour/8 hour light/dark cycle, and consisted of 50 mL of solution in a 300 mL beaker. There were ten replicates containing one organism each per exposure solution and tests were 7 days in length. Test organisms were fed a combination fermented yeast-trout chow-cereal leaves (YTC) and *Selenastrum* algae during the daily renewal. Total residual chlorine concentrations were measured daily on the renewal solutions and selected 24 hour old exposure solutions. Daily observations on survival and the number of young produced were used to determine residual toxicity for the exposure period. Table 6 arrays the water types, treatment/neutralization exposure concentrations, and the number of replicates per test.

Table 6. Water Types, Treatment/Neutralization Exposure Concentrations, and the Number of Replicates in GSI Residual Toxicity Tests Involving Juvenile Daphnids (*Ceriodaphnia dubia*).

Water Type	Residual Chlorine Concentration Following 24 Hours Degradation	Amount of Ascorbic Acid Added to Neutralize the Residual Chlorine	No. of Organisms Per Replicate	Total No. of Replicates
LW	0.0 mg/L	0.0 mg/L	1	10
	0.0 mg/L	7.5 mg/L	1	10
	0.0 mg/L	75 mg/L	1	10
	3.0 mg/L	7.5 mg/L	1	10
	3.0 mg/L	75 mg/L	1	10
FHW	0.0 mg/L	0.0 mg/L	1	10
	0.0 mg/L	7.5 mg/L	1	10
	0.0 mg/L	75 mg/L	1	10
	3.0 mg/L	7.5 mg/L	1	10
	3.0 mg/L	75 mg/L	1	10

FINDINGS

Bench-Scale Dose Effectiveness Test Findings

Zooplankton and Algae

Results of GSI bench-scale dose effectiveness tests involving sodium hypochlorite toxicity on robust species of freshwater zooplankton and an alga are presented in table 7. Findings specific to the species, chlorine doses and water quality conditions tested appear in tables 8-11. Chlorine treatment within the dose range proposed (i.e. up to 3.5 mg/L measured as total residual chlorine) was significantly effective at reducing numbers of live organisms across taxonomic groups tested, but did not inactivate all individuals FHW. There was only 3 percent survival after two hours of the cladoceran *Daphnia magna,* the experimental organism most sensitive to chlorine treatment, in LW after exposure to an initial chlorine concentration of 0.2 mg/L. In contrast, an initial chlorine concentration of 3.0 mg/L (i.e., the highest concentration tested) was required to reduce survival to 3 percent in FHW (table 12). It is likely that the higher organic content naturally present in the FHW reacted with the chlorine diminishing acute toxicity for these organisms. Fewer rotifer cysts hatched following treatment with chlorine in FHW than in LW, but further testing would be needed to distinguish such a finding from random variability.

Table 7. Percent Survival of Species Following 24 and 48 Hours of Exposure to Chlorine (Measured as Total Residual Chlorine) at Doses Less Than or Within the Proposed Dose Range (i.e., up to 3.5 mg/L). Note: ✓ Indicates Species With Less Than 1% Survival.

Major Taxonomic Group	Type	Species	Water Type	Survival (%)	
				24 Hours	48 Hours
Algae	Green alga	*Selenastrum sp.*	FHW	**67 %**	**27 %**
			LW	**✓ (0 %)**	**✓ (0 %)**
Zooplankton	Cladoceran	*Daphnia magna*	FHW	**✓ (0 %)**	**✓ (0 %)**
			LW	**✓ (0 %)**	**✓ (0 %)**
	Copepod	*Eucyclops sp.*	FHW	**57 %**	**33 %**
			LW	**✓ (0 %)**	**✓ (0 %)**
	Rotifer	*Branchionus calyciflorus*	FHW	**✓ (0 %)***	**✓ (0 %)***
			LW	**Not Measured**	**Not Measured**
		*B. calyciflorus cysts***	FHW	**Not Measured**	**✓ (0 %)**
			LW		**10 %**

*Initial chlorine concentration was 4.14 mg/L, i.e., slightly higher than the proposed dose range.
**Resting egg survival is scored as number hatched.

Table 8. Average (n=3) Percent Survival (+/- Standard Error) of Algae (*Selenastrum spp.*) Exposed to Chlorine (Measured as Total Residual Chlorine) in Two Water Types.

Water Type	Applied Chlorine Concentration (mg/L)	Chlorine Concentration at 48 Hours (mg/L)	Percent Survival (%)		
			O Hours Exposure	24 Hours Exposure	48 Hours Exposure
FHW	<0.002	<0.002	100 +/- 0	100 +/- 0	100 +/- 0
	0.024	<0.002	100 +/- 0	100 +/- 0	100 +/- 0
	0.081	<0.002	100 +/- 0	100 +/- 0	100 +/- 0
	0.27	<0.002	100 +/- 0	100 +/- 0	99 +/- 1
	0.90	<0.002	100 +/- 0	100 +/- 0	99 +/- 0
	3.00	<0.002	100 +/- 0	67 +/- 2*	27 +/- 3*
LW	<0.002	<0.002	100 +/- 0	100 +/- 0	100 +/- 0
	0.037	0.004	100 +/- 0	100 +/- 0	100 +/- 0
	0.087	0.008	100 +/- 0	100 +/- 0	97 +/- 0
	0.30	0.024	100 +/- 0	86 +/- 2*	88 +/- 10*
	1.06	0.048	2 +/- 1*	0 +/- 0*	0 +/- 0*
	3.09	0.22	0 +/- 0*	0 +/- 0*	0 +/- 0*

* Using Dunnett's Method there is a statistically significant difference ($p<0.05$) when compared to control.

Table 9. Average (n=4) Percent Hatch (+/- Standard Error) of Rotifer Resting Eggs (*Branchionus calyciflorus)* Exposed to Chlorine (Measured as Total Residual Chlorine) in Two Water Types.

Water Type	Applied Chlorine Concentration (mg/L)	Measured Chlorine Concentration at 48 Hours (mg/L)	Percent Hatch (%) at 48 Hours Exposure
FHW	<0.002	<0.002	24 +/- 1
	0.024	<0.002	14 +/- 2
	0.081	<0.002	14 +/- 3
	0.27	<0.002	10 +/- 4
	0.90	<0.002	13 +/- 4
	3.00	0.003	0 +/- 0*
LW	0.004	0.025	31 +/- 4
	0.021	0.012	18 +/- 7
	0.064	0.009	26 +/- 2
	0.23	0.007	25 +/- 5
	0.76	<0.002	14 +/- 3*
	2.66	0.033	10 +/- 3*

* Using Dunn's or Dunnett's Method there is a statistically significant difference ($p<0.05$) when compared to control.

Table 10. Average (n=4) Percent Survival (+/- Standard Error) of Adult Rotifers (*Branchionus calyciflorus)* Exposed to Chlorine (Measured as Total Residual Chlorine) in Filtered Duluth-Superior Harbor Water (FHW).

Applied Chlorine Concentration (mg/L)	Measured Chlorine Concentration at 24 Hours (mg/L)	Percent Survival (%)	
		2 Hours Exposure	24 Hours Exposure
<0.002	<0.002	100 +/- 0	100 +/- 0
0.057	0.003	100 +/- 0	100 +/- 0
0.12	0.028	100 +/- 0	100 +/- 0
0.39	0.016	100 +/- 0	80 +/- 8*
1.27	0.015	25 +/- 5*	0 +/- 0*
4.14	0.059	0 +/- 0*	0 +/- 0*

* Using Dunnett's Method there is a statistically significant difference ($p<0.05$) when compared to control.

Table 11. Average (n=3) Percent Survival (+/- Standard Error) of Adult Copepods (*Eucyclops sp.*) Exposed to Chlorine (Measured as Total Residual Chlorine) in Two Water Types.

Water Type	Applied Chlorine Concentration (mg/L)	Measured Chlorine Concentration at 48 Hours (mg/L)	Percent Survival (%)		
			2 Hours Exposure	24 Hours Exposure	48 Hours Exposure
FHW	<0.002	<0.002	100 +/- 0	100 +/- 0	100 +/- 0
	0.024	<0.002	100 +/- 0	100 +/- 0	100 +/- 0
	0.081	<0.002	100 +/- 0	100 +/- 0	97 +/- 3
	0.27	<0.002	100 +/- 0	100 +/- 0	100 +/- 0
	0.90	<0.002	100 +/- 0	100 +/- 0	97 +/- 3
	3.00	<0.002	97 +/- 3	57 +/- 7*	33 +/- 8*
LW	<0.002	<0.002	100 +/- 0	100 +/- 0	97 +/- 3
	0.031	0.002	97 +/- 3	97 +/- 3	97 +/- 3
	0.084	0.002	100 +/- 0	97 +/- 3	93 +/- 3
	0.33	0.005	100 +/- 0	100 +/- 0	100 +/- 0
	0.86	Not measured	100 +/- 0	0 +/- 0*	0 +/- 0*
	2.88	Not measured	0 +/- 0*	0 +/- 0*	0 +/- 0*

* Using Dunnett's Method there is a statistically significant difference ($p<0.05$) when compared to control.

Table 12. Average (n=3) Percent Survival (+/- Standard Error) of Cladocerans (*Daphnia magna*) Exposed to Chlorine (Measured as Total Residual Chlorine) in Two Water Types.

Water Type	Applied Chlorine Concentration (mg/L)	Measured Chlorine Concentration at 48 Hours (mg/L)	Percent Survival (%)		
			2 Hours Exposure	24 Hours Exposure	48 Hours Exposure
FHW	<0.002	<0.002	100 +/- 0	100 +/- 0	93 +/- 6
	0.024	<0.002	100 +/- 0	100 +/- 0	97 +/- 3
	0.081	<0.002	100 +/- 0	97 +/- 3	97 +/- 3
	0.27	<0.002	100 +/- 0	100 +/- 0	100 +/- 0
	0.90	Not measured	100 +/- 0	0 +/- 0*	0 +/- 0*
	3.00	Not measured	3 +/- 3*	0 +/- 0*	0 +/- 0*
LW	<0.002	<0.002	100 +/- 0	100 +/- 0	83 +/- 12
	0.031	0.002	100 +/- 0	100 +/- 0	100 +/- 0
	0.084	Not measured	100 +/- 0	0 +/- 0*	0 +/- 0*
	0.33	Not measured	3 +/- 3*	0 +/- 0*	0 +/- 0*
	0.86	Not measured	0 +/- 0*	0 +/- 0*	0 +/- 0*
	2.88	Not measured	0 +/- 0*	0 +/- 0*	0 +/- 0*

* Using Dunnett's Method there is a statistically significant difference ($p<0.05$) when compared to control.

Bacteria

Bacteria results (tables 14 and 15) from the dose effectiveness tests were similar in some respects to those of zooplankton and algae. Again, measured concentrations of chlorine in FHW were significantly lower—even when measurements were taken just minutes after the addition of sodium hypochlorite, i.e., at 0 hours—than the initial concentrations added to the FHW. The measured concentrations of sodium hypochlorite in FHW at 0 hours also were lower than those in LW samples treated with the same initial doses (table 13).

Table 13. Chlorine Concentrations (Applied and Measured as Total Residual Chlorine Over Time) For Spiked Bacteria Dose Effectiveness Tests Involving *E. coli* and Enterococcus Conducted in Filtered Duluth-Superior Harbor Water (FHW) and Laboratory Water (LW).

Water Type	Sample Type and Applied Chlorine Concentration	Measured Chlorine Concentration (mg/L)			
		0 Hours	2 Hours	24 Hours	48 Hours
FHW	Control	<0.002	<0.002	<0.002	<0.002
	0.27 mg/L Chlorine	<0.002	<0.002	<0.002	<0.002
	0.9 mg/L Chlorine (Rep 1)	0.054	0.011	<0.002	<0.002
	0.9 mg/L Chlorine (Rep 2)	0.066	0.013	<0.002	<0.002
	3.0 mg/L Chlorine	0.32	0.22	0.013	0.004
LW	Control	0.003	0.004	<0.002	<0.002
	0.27 mg/L Chlorine (Rep 1)	0.19	0.11	0.043	0.005
	0.27 mg/L Chlorine (Rep 2)	0.21	0.11	0.046	0.012
	0.585 mg/L Chlorine	0.41	0.27	0.14	0.14
	0.9 mg/L Chlorine	0.64	0.35	0.086	0.11

Chlorine treatment reduced the number of total coliforms, *E. coli*, and Enterococcus at applied chlorine concentrations of 3.0 mg/L in FHW water over 48 hours (table 14). Heterotrophic bacteria appeared least sensitive to the treatment. Counts grew significantly in all samples, including controls, over the same exposure time except in spiked samples treated with 3 mg/L applied chlorine (table 14). An initial concentration of 0.585 mg/L chlorine in LW for example, was effective at reducing bacteria to <1 MPN at all observation time periods, with the exception of heterotrophic bacteria (table 15). There was no re-growth observed in the LW samples over the same time period.

Table 14. Most Probable Number (MPN) of Bacteria (Total Coliforms, *E.coli*, Enterococcus and Heterotrophic Bacteria) per 100 mL of Filtered Duluth-Superior Harbor Water (FHW) Spiked with *E. coli* and Enterococcus in Control and Chlorine-Treated Samples Following 2, 24 and 48 Hours of Exposure Time.

	Total Coliforms (MPN)				*E. coli* (MPN)			
Sample	**Initial**	**2 Hrs**	**24 Hrs**	**48 Hrs**	**Initial**	**2 Hrs**	**24 Hrs**	**48 Hrs**
Control	1730	1990	>2420	>2420	454	525	1120	199
0.27 mg/L Chlorine		1.1E+4	1010	>2420		570	792	135
0.9 mg/L Chlorine (# 1)		816	>2420	>2420		73	459	2420
0.9 mg/L Chlorine (# 2)		866	>2420	>2420		78	272	1200
3.0 mg/L Chlorine		<1	<1	<1		<1	<1	<1
	Enterococcus				**Heterotrophic Bacteria**			
Sample	**Initial**	**2 Hrs**	**24 Hrs**	**48 Hrs**	**Initial**	**2 Hrs**	**24 Hrs**	**48 Hrs**
Control	1200	1990	1730	261	N/A	550	>738	266
0.27 mg/L Chlorine		1640	1550	166		480	>738	289
0.9 mg/L Chlorine (# 1)		1050	103	54		153	>738	589
0.9 mg/L Chlorine (# 2)		980	155	29		100	>738	450
3.0 mg/L Chlorine		1	<1	<1		18	31	14

Table 15. Most Probable Number (MPN) of Bacteria (Total Coliforms, *E.coli*, Enterococcus and Heterotrophic Bacteria) per 100 mL of Laboratory Water (LW) Spiked with *E. coli* and Enterococcus in Control and Chlorine-Treated Samples Following 2, 24 and 48 Hours of Exposure Time.

	Total Coliforms (MPN)				*E. coli* (MPN)			
Sample	**Initial**	**2 Hrs**	**24 Hrs**	**48 Hrs**	**Initial**	**2 Hrs**	**24 Hrs**	**48 Hrs**
Control	980	866	>2420	>2420	980	866	>2420	>2420
0.27 mg/L Chlorine (# 1)		435	317	>2420		435	317	>2420
0.27 mg/L Chlorine (# 2)		579	902	>2420		579	902	>2420
0.585 mg/L Chlorine		<1	<1	<1		<1	<1	<1
0.9 mg/L Chlorine		<1	<1	<1		<1	<1	<1
	Enterococcus				**Heterotrophic Bacteria**			
Sample	**Initial**	**2 Hrs**	**24 Hrs**	**48 Hrs**	**Initial**	**2 Hrs**	**24 Hrs**	**48 Hrs**
Control	687	387	921	687	2	470	>738	5.5E+05
0.27 mg/L Chlorine (# 1)		115	1	<1		175	>738	2.2E+05
0.27 mg/L Chlorine (# 2)		125	<1	<1		164	>738	2.4E+05
0.585 mg/L Chlorine		<1	<1	<1		4	<2	<2
0.9 mg/L Chlorine		<1	<1	<1		<2	34	<2

Bench-Scale Chemical Degradation Test Findings

Approximately 1.5 mg/L of chlorine was still present 96 hours after an initial dose of 3.0 mg/L was applied to water with a low organic carbon content (i.e., <2.0 mg/L NPOC). In contrast, concentrations of chlorine added to FHW (>6.0 mg/L NPOC) degraded to below detect within 24 hours (figure 1). Temperature had a slight influence on the rates of degradation (figure 2). The 15 °C test temperature displayed slower degradation of the chlorine than the 25 °C test temperature in the same water type.

Results of the second set of GSI chemical degradation tests on chlorine using either deionized water or FHW containing fish tissue are described in table 16. As expected, organic matter in the form of ground fish tissue reduced the amount of chlorine present in deionized water. The rate of degradation of chlorine caused by ground fish tissue was higher in water that also has a background of elevated organic carbon, i.e., FHW (table 16).

Figure 1. Change in Concentration of Chlorine (Measured as Total Residual Chlorine) in Filtered Duluth-Superior Harbor Water (FHW) Over 96 Hours Exposure to Different Levels of Non-Purgeable Organic Carbon (NPOC).

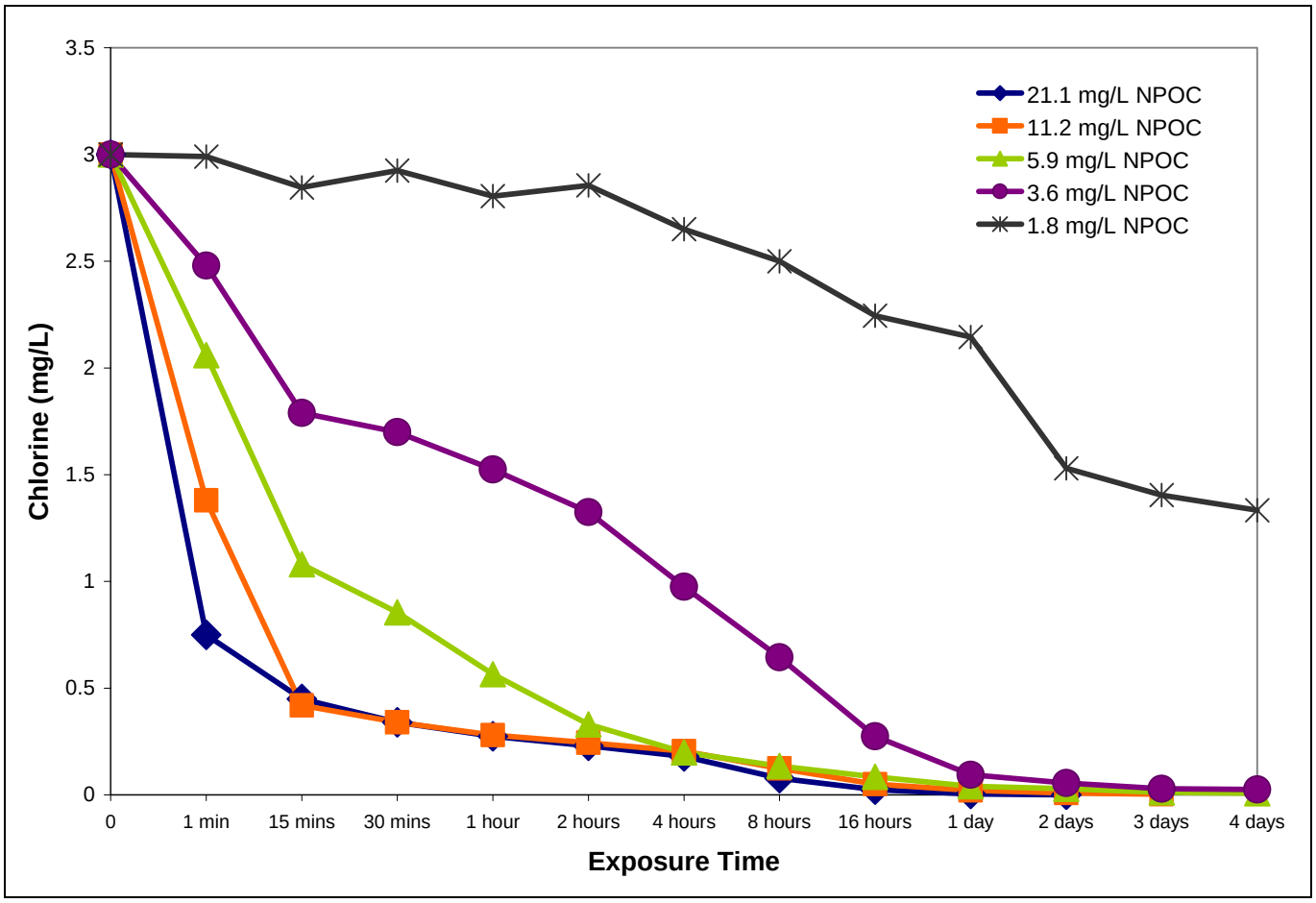

Figure 2. Change in Chlorine Concentration (Measured as Total Residual Chlorine) in Filtered Duluth-Superior Harbor Water (FHW) Over 96 Hours Exposure to Two Temperatures. Note: Non-Purgeable Organic Carbon was 21.1 mg/L.

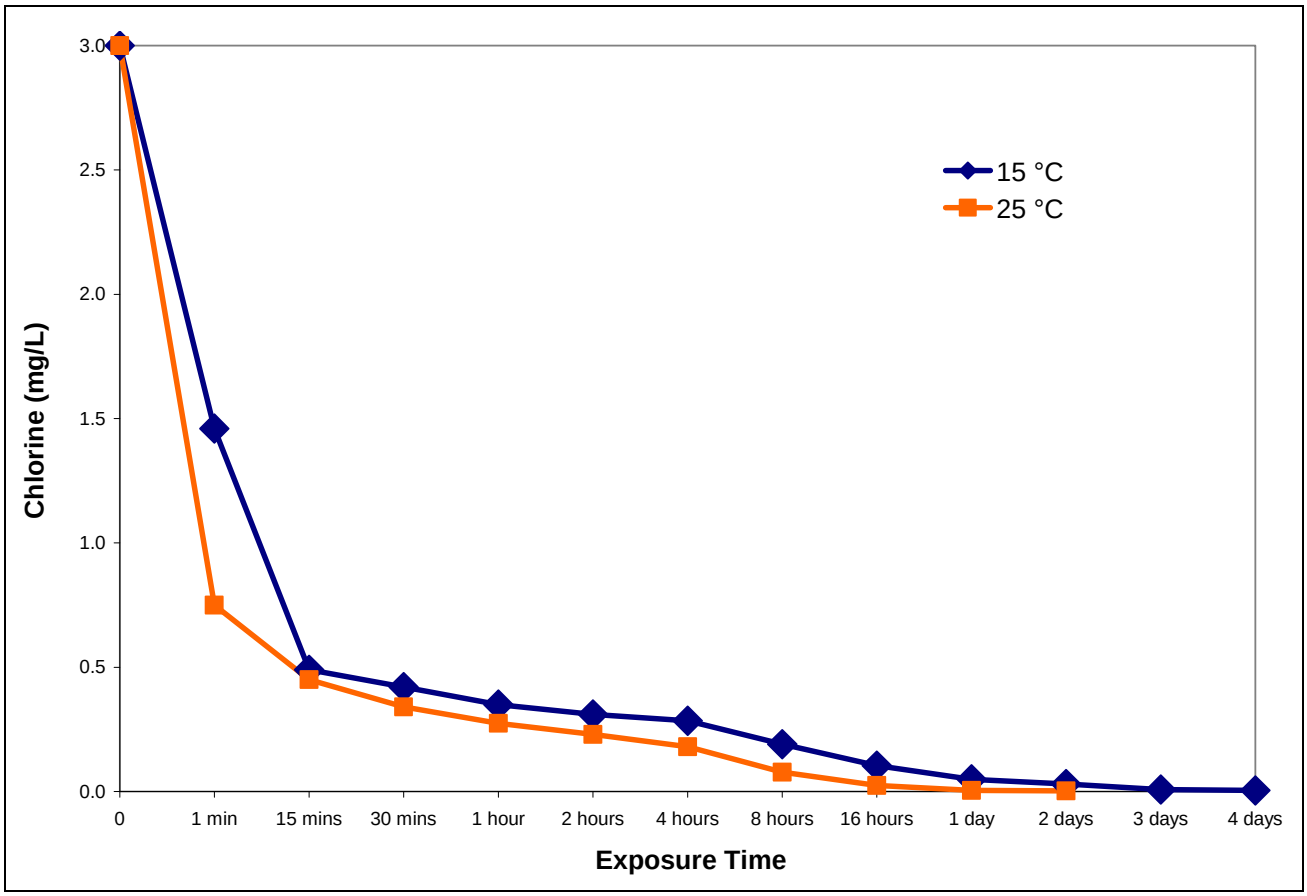

Table 16. Influence of Ground Fish Tissue on Chlorine Concentration (Measured as Total Residual Chlorine) in Two Water Types. Note: Initial Chlorine Concentrations was 3.0 mg/L.

Water Type	Sample #	Wt. of Fish Tissue (g)	Exposure Time			
			10 mins	30 mins	60 mins	120 mins
Deionized Water	1	0.103	2.60	2.19	1.78	0.96
	2	0.500	2.13	1.92	1.93	0.19
	3	1.001	1.75	1.69	1.77	0.019
FHW	1	0.000	0.84	0.76	0.73	0.61
	2	0.103	0.84	0.59	0.47	0.34
	3	0.502	0.70	0.66	0.028	0.014
	4	1.001	0.21	0.047	0.003	<0.002

Results from the third set of chemical degradation experiments indicate that 10 mg/L of ascorbic acid was not adequate to neutralize 3.9 mg/L total residual chlorine to below detection over a 24 hour period due to an apparent rebound effect (table 17). Samples neutralized with 10 mg/L ascorbic acid contained undetectable chlorine concentrations after 5 minutes, but 29.5 µg/L total residual chlorine were detected in the samples after 45 minutes and 122 µg/L after 5 hours. In general, as the levels of ascorbic acid used to neutralize the chorine increased, the duration of the non-detect period grew longer, but all samples had measurable chlorine by the end of the observation period (24 hours) (table 17). It is unclear whether this rebound effect was an artifact of the analytical process or a real change in chlorine concentrations.

Table 17. Total Residual Chlorine Concentrations (µg/L) Over Time After Being Neutralized With Different Amounts of Ascorbic Acid.

Concentration of Ascorbic Acid (mg/L)	Measured Chlorine Concentration (µg/L)				
	5 min.	45 min	1 Hr 45 min	5 Hr	24 Hr
10	<2.3	29.5	94.1	122	68.5
16	<2.3	<2.3	<2.3	<2.3	20.5
24	<2.3	<2.3	<2.3	<2.3	5.8

Bench-Scale Residual Toxicity Test Findings

FHW treated with 3.0 mg/L of chlorine and neutralized with 9.0 mg/L ascorbic acid prior to organism exposure (24- and 48-hours) was not acutely toxic to sensitive freshwater organisms (table 18). Immediately after neutralization, measured chlorine concentrations were below the detection limit of 0.002 mg/L. In all cases, organism survival was equal to or greater than 97 %.

Table 18. Average (n=3) Percent Survival (+/- Standard Error) of Juvenile Daphnids, Juvenile Fathead Minnows and 7-8 Day Old Amphipods Exposed to 3.0 mg/L of Chlorine Neutralized With Ascorbic Acid in Filtered Duluth-Superior Harbor Water (FHW).

Major Taxonomic Group	Type	Species	Average Percent Survival (%)	
			24 Hours	48 Hours
Zooplankton	Juvenile daphnids	*Ceriodaphnia dubia*	97 +/- 3	97 +/- 3
Fish	Juvenile fathead minnows	*Pimephales promelas*	100 +/- 3	100 +/- 3
Zooplankton	Amphipods (7-8 days old)	*Hyalella azteca*	97 +/- 3	97 +/- 3

Ascorbic acid concentrations of 7.5 mg/L alone or in combination with chlorine (2.5 mg/L) were also not acutely toxic. However, a higher level of ascorbic acid (75 mg/L) alone or in combination with 3 mg/L chlorine was acutely toxic in LW, and chronically toxic in FHW (table 19). Chronic toxicity, expressed as reductions in young produced, and acute toxicity expressed as mortality in parents caused by the treatments was statistically significant ($p<0.05$).

Table 19. Actue Toxicity Expressed as Average (n=10) Dead, and Chronic Toxicity Expressed as Average (n=10) Number of Young Produced (+/- Standard Deviation) in Daphnids (*Ceriodaphnia dubia*) Exposed to Various Concentrations of Chlorine and/or Ascorbic Acid in Filtered Duluth-Superior Harbor Water (FHW) or Laboratory Water (LW).

Water Type	Treatment	Average Number of Young Produced	Significant Difference Compared to Controls (p<0.05)*
FHW	Control	20 +/- 7	N/A
	7.5 mg/L Ascorbic Acid	25 +/- 7	No
	75 mg/L Ascorbic Acid	10 +/- 7	Yes (Chronic)
	Chlorine neutralized with 7.5 mg/L Ascorbic Acid	23 +/- 9	No
	Chlorine neutralized with 75 mg/L Ascorbic Acid	9 +/- 6	Yes (Chronic)
LW	Control	25 +/- 8	N/A
	7.5 mg/L Ascorbic Acid	24 +/- 7	No
	75 mg/L Ascorbic Acid	0 +/- 0	Yes (Acute)
	Chlorine neutralized with 7.5 mg/L Ascorbic Acid	23 +/- 10	No
	Chlorine neutralized with 75 mg/L Ascorbic Acid	0 +/- 0	Yes (Acute)

* ANOVA on ranks, multiple comparisons versus control group (Dunn's Method).

Quality Assurance/Quality Control

Quality control (QC) sample analysis for sodium hypochlorite solutions (measured as total residual chlorine) tests consisted of 1) analysis of duplicate samples, and 2) analysis of samples spiked with known amounts of chlorine. Approximately 10 % of samples were analyzed in duplicate. This is also true for spiked samples in dechlorinated LW. An attempt was made to analyze spiked samples using FHW but it was found that chlorine reacted too quickly for accurate determinations of spike recoveries to be made. Table 20 provides the results of the QC sample analysis.

Table 20. Sodium Hypochlorite Solution (Measured as Total Residual Chlorine) Duplicate Agreement and Spike Recovery Results.

	Duplicate Agreement (%)	Spike Recovery (%)
Mean	97.5	105.2
Standard Deviation	4.9	24.0
Maximum Value	100.00	197.3
Minimum Value	75.0	84.8
Number of QC Samples	91	33

Toxicity tests were initiated with healthy, vigorous animals. Reference toxicant tests were performed with all test species prior to the start of the definitive test. Control charts are available upon request. Test conditions were monitored daily for parameters that might affect the outcome of the test (i.e., temperature, and dissolved oxygen). Daily and weekly calibration of test meters ensured optimal performance. The LSRI Quality Assurance and Quality Control Officer performed inspections of logbooks, recorded measurements, and instrumentation used during the tests. Any deviations were discussed with the principal investigator and documented in the study logbook.

CONCLUSION

In conclusion, the proposed dose of 3 mg/L sodium hypochlorite solution added to test water proved effective at the bench scale at inactivating most species tested, including bacteria. The test species of green algae, *Selenastrum*, and rotifer resting eggs were somewhat resistant. Further bench testing, currently under way, will help clarify the concentrations of chlorine that are necessary to inactivate microbial organisms, including viruses, under the various water conditions.

As expected, in the absence of the neutralization step, residual chlorine in water following an applied dose of 3.0 mg/L was a function of the amount of dissolved organic carbon (DOC) contained in the test water. Chlorine did not degrade dramatically in low DOC water, but did degrade dramatically and rapidly in high DOC water, and water with high total organic compounds (e.g. ground fish tissue). As a result, a lower applied dose of chlorine (0.9 mg/L) was sufficient to achieve across the board treatment effectiveness in LW than was required for FHW (3.0 mg/L).

There was also no acute residual toxicity detected in these tests associated with 3 mg/L sodium hypochlorite solution followed by neutralization with 9 mg/L of ascorbic acid. However, residual acute and chronic toxicity were detected in association with a higher level of ascorbic acid (75 mg/L) even in the absence of chlorine. Ascorbic acid commonly known as Vitamin C is a weak acid, and used in large quantities and unbuffered waters lowers pH.

Our limited chronic toxicity analysis also did not detect any effect, but it should be noted that much further testing would be necessary to conclude with confidence whether or not chronic toxicity would occur as a result of this treatment and/or its neutralization process. Chronic toxicity tests will

be especially important to assure that by-products of treatment in waters containing high concentrations of DOC do not pose an environmental problem.