BENCH-SCALE TECHNICAL REPORT

LABORATORY DETERMINATION OF THE EFFECTS OF ULTRAVIOLET IRRADIATION ON SURVIVAL AND REPRODUCTION OF SELECTED PROTIST AND ZOOPLANKTON SPECIES

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ABSTRACT

The Great Ships Initiative (GSI) provides bench-scale (i.e., laboratory scale) status testing services to aid developers of innovative technologies that could have application as ballast water management systems (BWMSs). This report describes findings from GSI bench-scale tests examining the effects of ultraviolet (UV) irradiation on survival and reproduction of select laboratory-cultured freshwater algae and zooplankton. Tests took place in 2014 at the Lake Superior Research Institute (LSRI) of the University of Wisconsin-Superior (UWS) in Superior, Wisconsin, USA, and comprised two iterative stages. First, acute dose effectiveness tests were undertaken to determine UV effect on survival of the green alga *Selenastrum capricornutum* and the cladoceran *Daphnia magna*. Second, chronic toxicity tests were undertaken to determine UV effect on reproduction capacity of these two species. The acute dose effectiveness tests involved monitoring organism survival up to 48 hours following exposure to up to five doses of UV irradiation. The chronic residual toxicity tests involved measuring reproductive counts for up to 21 days following exposure to up to five doses of UV irradiation. In both sets of tests UV dosing was achieved using a Rayonet™ Chamber Reactor (Model RPR-100; Southern New England Ultraviolet Company; Branford, Connecticut, USA), a bench-scale device that utilizes several reactor lamps to provide a known output wavelength of 253.7 nm.

Overall, test results indicate that UV light at 253.7 nm is capable of reducing survival, growth, and reproduction of the two species tested (in laboratory water, LW; transmittance > 95 %). In terms of survival, a complete kill of the green algae *S. capricornutum* occurred within 24 hours of exposure to UV doses greater than 220 mJ/cm^2, while at 48 hours post-exposure survival was less than 20 % at UV doses greater than 67 mJ/cm^2. Survival of the cladoceran *D. magna* was less than 40 % at doses greater than 4.4 mJ/cm^2 at 48 hours post-exposure. In terms of chronic residual toxicity, both *S. capricornutum* and *D. magna* experienced reductions in growth and reproduction at lower UV doses of ≥ 24 mJ/cm^2 and 2.1 mJ/cm^2, respectively.

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1. INTRODUCTION

This Great Ships Initiative (GSI) technical report presents quantitative findings from bench-scale tests evaluating the effects of ultraviolet (UV) irradiation on survival and reproduction of select laboratory-cultured freshwater algae and zooplankton. Tests were conducted at the Lake Superior Research Institute (LSRI) of the University of Wisconsin-Superior (UWS) in Superior, Wisconsin, USA, between February and May of 2014 and involved evaluation of acute dose effectiveness to determine UV effects on survival of the green alga *Selenastrum capricornutum* and the cladoceran *Daphnia magna*. A second set of tests evaluated UV effects on growth and reproduction capacity of these two test species. In both sets of tests UV dosing was achieved using a Rayonet™ Chamber Reactor, a bench-scale device that utilizes several reactor lamps to provide a known output wavelength of 253.7 nm (hereafter referred to as 254 nm). The dose was defined as the product of the depth-averaged fluence (UV dose) rate and the exposure period. Prior to beginning the tests, GSI personnel spent several months developing exposure methods, as well as the chemical actinometry method used to determine UV dose. The tests build on earlier exploratory work undertaken by the Northeast-Midwest Institute (NEMWI), GSI's managing entity (Cangelosi *et al*., 2004).

2. BACKGROUND

GSI is a regional effort managed by the 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 ballast water management systems (BWMSs) at the bench, land-based and shipboard scales. GSI performs informal status tests for systems that are in the research and development stage, and formal certification/verification tests appropriate to market-ready BWMSs. In the process GSI has served as a platform for development and validation of sampling and analysis methods relevant to ballast discharge quality and BWMS performance assessment.

In terms of organization, GSI is a project of NEMWI—a Washington, D.C-based private, non-profit, and non-partisan research organization. The project is led by Ms. Allegra Cangelosi of NEMWI and carried out collaboratively with contracting entities including the UWS, AMI Consulting Engineers, and the University of Minnesota-Duluth. Researchers from these organizations, among others, provide critical scientific and technical expertise and implementation services to GSI.

3. GSI BENCH-SCALE TESTS

In general, GSI bench-scale tests involve 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 processes and configurations 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 relevant GSI testing facility, and costs involved in the installation and removal of the system to and from the GSI testing facility.

GSI bench-scale 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 goals of GSI bench-scale tests are to explore dose effectiveness, chemical fate/degradation, chronic residual toxicity, and/or sensitivity to challenge conditions of a proposed BWMS process and/or configurations thereof.

4. UV EXPOSURE METHODS

4.1. UV Reactor

In these tests UV irradiation was accomplished using a Rayonet™ Merry-Go-Round style reactor (Model RPR-100; Southern New England Ultraviolet Company, Branford, Connecticut, USA; Figure 1), which is a bench-scale device recommended to GSI by Dr. Ernest Blatchley of Purdue University (personal communication, 2014). The sources of UV irradiation for this device are 16 low-pressure UV lamps which provide essentially monochromatic output at a characteristic wavelength of 254 nm. The Rayonet™ Merry-Go-Round style reactor comprises a batch system in which an array of lamps is distributed radially around the center of an enclosed space. A carousel located concentrically within the lamp array is capable of holding up to 18 exposure vessels (Figure 2). The carousel rotates within the enclosed space to allow uniform UV exposure of samples. Given the configuration of this system, GSI personnel undertook exploratory UV dose measurement work over several months using chemical actinometry.

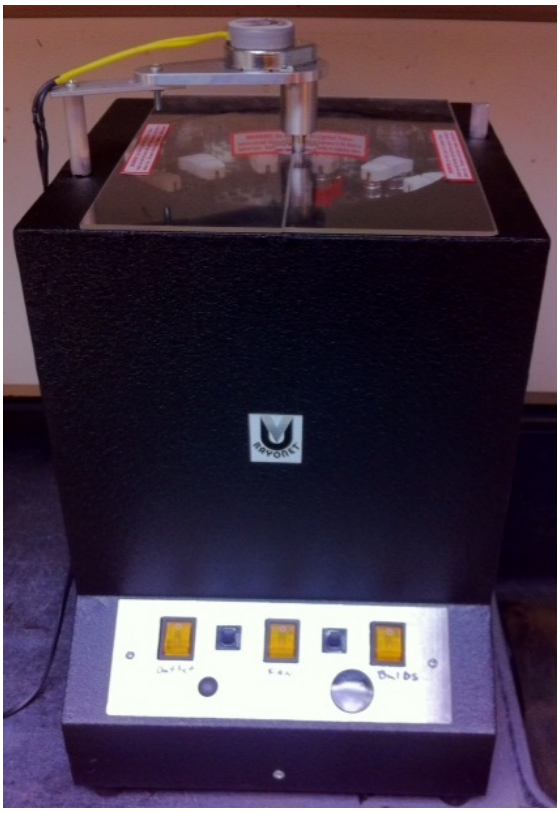

Figure 1. Rayonet™ Merry-Go-Round Reactor (Model RPR-100; Southern New England Ultraviolet Company, Branford, Connecticut, USA).

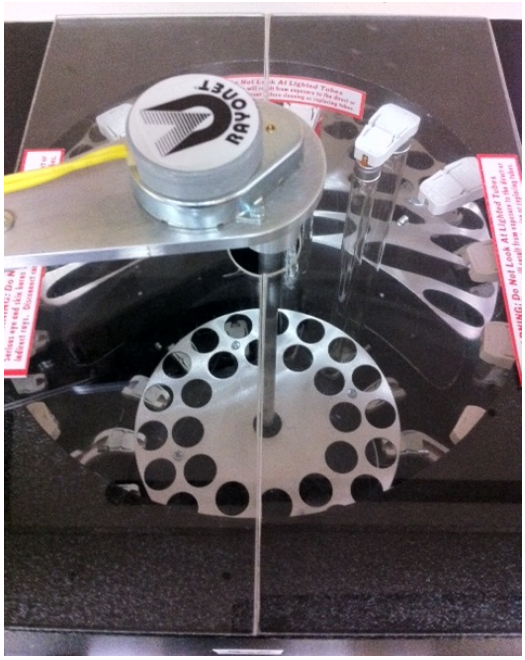

Figure 2. Inside the Rayonet™ Merry-Go-Round Reactor Showing the Single Bulb and Carousel with Places for up to 18 Exposure Vessels.

4.2. Exposure and Chemical Actinometry Methods Development

GSI worked collaboratively with Dr. Blatchley to develop the exposure method and the chemical actinometry method used to determine UV dose. An actinometer refers to any instrument used for measuring the intensity of incident radiation, especially one in which the intensity of radiation is measured by the speed of a photochemical reaction (Merriam-Webster, 2013). Initial work involved exposing the chemical actinometer to UV light at 254 nm supplied by a collimated beam system. The specific UV dose the actinometer was exposed to was then determined by measuring the irradiance using a calibrated radiometer and the length of the exposure. Using the collimated beam system, the UV dose was provided from a single direction. However, in the Rayonet™ Merry-Go-Round style reactor the exposure comes from all directions, thus, the radiometer could not be used to determine UV dose. Instead, work progressed to measuring UV exposures in the Merry-Go-Round style reactor. This work is summarized below.

4.2.1. Determination of UV Dose using Chemical Actinometry

As described by Rahn (1997), a solution of 0.6 M iodide and 0.1 M iodate in 0.01 M borate buffer can be used as a chemical actinometer to measure the incident fluence (i.e., UV dose) from a low-pressure mercury lamp that emits more than 85 % of its energy at 254 nm. The actinometer solution is optically opaque to light below 290 nm and is optically transparent to wavelengths greater than 330 nm. Irradiation results in the linear formation of triiodide, which is quantified by measuring the absorbance of the solution at 420 nm using a UV-Vis Spectrophotometer and a cell with a 1 cm path length. GSI employed two actinometers during each exposure period. The calculated UV doses for the duplicate actinometers were required to yield a relative percent difference (RPD) of less than 20 %. The specific actinometry procedure used is outlined in Appendix 1.

In order to determine the appropriate length of exposure time, an actinometer-reference point was generated using data obtained by exposing an actinometer solution to a dose (based on radiometer measurements and length of exposure) of UV light (at 254 nm) generated by the collimated beam. The concentration of triiodide formed by this exposure was determined by measuring the absorbance of the solution at 420 nm and then using Beer's law ($A=\epsilon bc$) to calculate the molarity. The molar extinction coefficient (ϵ) for triiodide at 420 nm is 3600 M^1cm^{-1}. The number of moles of triiodide/cm^2 produced was calculated by multiplying the molarity by the volume (L) of solution and dividing by the surface area (cm^2) irradiated in the exposure. The UV dose (in mJ/cm^2), determined from the radiometer readings and the length of exposure and the moles of triiodide/cm^2, comprised the reference point that was then used, along with the absorbance measurements of the actinometer solution from the Merry-Go-Round reactor, to determine the UV dose applied during the test organism exposures.

Table 1 lists the approximate UV exposure times GSI proposed for the two species of test organisms involved in both the acute dose effectiveness and chronic residual toxicity tests. Times were based on there being a total of thirteen quartz tubes in the Merry-Go-Round reactor while undertaking each exposure. Two of the thirteen quartz tubes during each exposure period contained the actinometer solution.

Table 1. Approximate Length of UV Exposure by Test Organism Proposed for use in GSI's Acute Dose Effectiveness and Chronic Residual Toxicity Tests.

Test Organism	Proposed UV Dose	Approximate Exposure Time (at 23°C)
Selenastrum capricornutum	25 mJ/cm^2	1 min 8 sec
	50 mJ/cm^2	2 min 13 sec
	75 mJ/cm^2	3 min 19 sec
	100 mJ/cm^2	4 min 25 sec
Daphnia magna	0.5 mJ/cm^2	53 sec
	1.0 mJ/cm^2	1 min 40 sec
	2.0 mJ/cm^2	3 min 13 sec
	4.0 mJ/cm^2	6 min 17 sec
	8.0 mJ/cm^2	12 in 27 sec

4.2.2. Calculation of UV Dose

The fluence (i.e., UV dose) was calculated as the product of UV irradiance and exposure time. For these tests, in agreement with Rahn (1997), a potassium iodide/potassium iodate solution in borate buffer was used as the chemical actinometer to monitor UV radiation (Rahn, 1997). In order to minimize the influence of temperature on the actinometer reaction, the actinometer solution was placed in an incubator set at 23 °C ± 3 °C prior to use in the exposures. The irradiance was measured before conducting each definitive test in order to ensure the reactor was performing as expected. The measured dose was compared with the previously measured value and was required to be within ± 20 % of that value. In addition, the room temperature was measured prior to each test and the temperature of the actinometer solution in the quartz tubes was also measured at the beginning and end of each test. The actinometer solution was required to be 23 °C ± 3 °C in order for the UV dose measurements to be valid. The UV dose was additionally measured during each exposure by placing two actinometer solutions into exposure quartz tubes alongside the tubes holding the test organisms. The actinometers were placed opposite from one another in the carousel.

5. TEST METHODS

5.1. Treatment Application

For both the acute dose effectiveness and chronic residual toxicity tests, the Rayonet™ Merry-Go-Round style reactor was turned on and allowed to warm up for 15 minutes prior to the start of the exposure period. Experimental water qualities were prepared according to "Section 5.3 – Experimental Water Preparation", and 50 mL (for *S. capricornutum* exposures) or 30 mL (for the *D. magna* exposures) of experimental water, along with the appropriate number of test organisms, was added to the cylindrical-quartz exposure tubes (Rayonet, RQV-8). Test organisms were added to the experimental water qualities at least 10 minutes prior to UV irradiation. The reactor was briefly turned off while the exposure tubes were added; the exposure time began when the reactor was turned back on. The tubes containing the test organisms were placed as symmetrically as possible within the carousel.

5.2. Test Facility

Tests took place in LSRI laboratories between February and May of 2014. The LSRI laboratories are equipped with adequate ventilation, electrical connections, and climate control. All exposures were conducted in a laboratory hood, as the Rayonet™ Merry-Go-Round style reactor may create small quantities of ozone.

All personnel present in the laboratory during the time of the exposures were required to wear safety glasses and protective laboratory coats. Additionally, the reactor was turned off during the addition and removal of organisms to minimize personnel exposure.

5.3. Experimental Water Preparation

Experimental water comprised laboratory (LW) which was used as a surrogate for Lake Superior water. This water type has a very low concentration of non-purgeable organic carbon (NPOC, range is 0.3 – 1.1 mg/L) and total suspended solids (TSS), and a very high UV transmittance (> 98 %). Experimental LW was prepared as follows: Municipal water from the City of Superior, Wisconsin, was passed through an activated carbon column in order to remove the majority of the chlorine. The remaining residual chlorine was removed through injection of sodium sulfite, and the resulting total residual chlorine concentration was below the method limit of detection (i.e., < 3 μg/L).

Performance Control Water (PCW) was also prepared for the chronic residual toxicity tests as a quality control (QC) measure. This experimental water type aims to augment optimal conditions for each type of test organism. Tests involving PCW are used to indicate the health of the test organisms and also help demonstrate that the experimental methods themselves do not impact test organism endpoints such as survival and/or reproduction. The PCW for the two species of test organisms used in the chronic residual toxicity tests were:

- *S. capricornutum*: Algae Growth Media (USEPA, 2002).
- *D. magna*: Hard Reconstituted Water (HRW).

Table 2 details the acceptable ranges for water chemistry parameters measured in the experimental water types (prior to UV treatment).

Table 2. Reference Limits for Experimental Water Qualities (Laboratory Water, LW, and Performance Control Water, PCW) Prepared for Use during GSI Bench-Scale UV Irradiation Tests.

Parameter	Units	Water Type	Acceptable Range to Initiate GSI Bench-Scale Testing
Temperature	°C	LW	22 - 28
		PCW	22 – 28
pH	NA	LW	6.5 - 9.0
		PCW	
Specific Conductivity	µS/cm	LW	120 - 150
		PCW	80 - 160
Dissolved Oxygen	mg/L	LW	6 – 12
		PCW	
Percent Transmittance, Unfiltered	%T at 254 nm	LW	97.4 – 99.4
		PCW	USEPA Algae Growth Media: 89.8 – 99.4 Hard Reconstituted Water (HRW): 98 – 99.9

5.4. Experimental Design and Methods

5.4.3. Acute Dose Effectiveness Tests

Acute dose effectiveness tests measured the effects of UV irradiation on the green algae *S. capricornutum* and the cladoceran *D. magna*. Tests involved organisms being exposed to UV irradiation in the Rayonet™ Merry-Go-Round reactor and monitoring of organism survival up to 48 hours following exposure. Table 3 summarizes the overall experimental design for the acute dose effectiveness tests. Briefly, tests involving *S. capricornutum* were conducted according to *GSI/SOP/BS/RA/EF/5 – Procedure for Assessing Dose Effectiveness of a Ballast Treatment System using Selenastrum capricornutum* (GSI, 2013 draft), with the exception that algae were held in constant light, rather than in constant dark, in order to allow photorepair to occur. In addition, counts were made at 48 hours following exposure regardless of results from the previous counts. In this procedure four to seven day old *S. capricornutum* were added to LW to achieve a concentration of 200,000 cells/mL. Five replicates were prepared for each dose with one of the replicates sampled prior to exposure for initial counts, and one replicate used for water chemistry measurements (Table 3). Following exposure, samples were transferred to sterile 125 mL Erlenmeyer flasks and placed into a shaking incubator set at 25 °C, 100 rpm, and in complete light. Live/dead counts were made on the three remaining replicates per exposure at 4, 24 and 48 hours (Table 3).

The *D. magna* acute dose effectiveness tests were conducted according to *GSI/SOP/BS/RA/EF/2 – Bench-Scale Procedure for Assessing Dose Effectiveness of a Ballast Water Management System using a Cladoceran* (GSI, 2013). Less than 48 hour old *D. magna* were exposed to

varying doses of UV irradiation during the tests. Prior to exposure, the organisms were acclimated in 50 % test water/50 % culture water at 25 °C in darkness for 16 – 24 hours. Up to ten replicates per dose were used in the tests, with each replicate containing five organisms (Table 3). One of the replicates was used only for water chemistry measurements. Following exposure, organisms were transferred to cups and placed in an incubator set at 25 °C in complete darkness. Survival counts were made at 4, 24 and 48 hours (Table 3).

Table 3. Experimental Design for GSI Dose Effectiveness Tests of UV Irradiation in Laboratory Water.

Test Organism	Test Number	Treatment Group (Proposed UV Dose)	No. Reps per Treatment Group	No. Organisms per Rep.	Exposure Duration (Hours)	Light:Dark Regime/ Temperature	Analysis Times (Hours)
Selenastrum capricornutum	1	0 mJ/cm^2	3 + 1 replicate for water chemistry + 1 replicate for initial counts	200,000 cells/mL	48	24:0 Hr */ 25 ± 3 ° C	0, 4, 24 and 48 hours
		100 mJ/cm^2					
		200 mJ/cm^2					
		400 mJ/cm^2					
		800 mJ/cm^2					
	2	0 mJ/cm^2	3 + 1 replicate for water chemistry + 1 replicate for initial counts	200,000 cells/mL	48	24:0 Hr */ 25 ± 3 ° C	
		25 mJ/cm^2					
		50 mJ/cm^2					
		75 mJ/cm^2					
		100 mJ/cm^2					
Daphnia magna	1	0 mJ/cm^2	5 + 1 replicate for water chemistry measurements	5	48	0:24 Hr/ 25 ± 3 ° C	0, 4, 24 and 48 hours
		3.125 mJ/cm^2					
		25 mJ/cm^2					
		100 mJ/cm^2					
		400 mJ/cm^2					
	2	0 mJ/cm^2	5 + 1 replicate for water chemistry measurements	5	48	0:24 Hr/ 25 ± 3 ° C	
		0.8 mJ/cm^2					
		1.6 mJ/cm^2					
		6.4 mJ/cm^2					
	3	0 mJ/cm^2	10 + 1 replicate for water chemistry measurements	5	48	0:24 Hr/ 25 ± 3 ° C	
		0.5 mJ/cm^2					
		1.0 mJ/cm^2					
		2.0 mJ/cm^2					
		4.0 mJ/cm^2					
		8.0 mJ/cm^2					

* This light regime is different from what would normally be used during a GSI dose effectiveness test involving *S. capricornutum*. The change was made at the request of the UV expert to allow for algae photorepair mechanisms which are light dependent.

5.4.3. Chronic Residual Toxicity Tests

Chronic residual toxicity tests measured the effects of UV irradiation on reproductive capacity of *S. capricornutum* and *D. magna*. Tests involved organisms being exposed to UV irradiation in the Rayonet™ Merry-Go-Round reactor and monitoring of organism reproduction for up to 21 days. Table 4 summarizes the overall experimental design for the chronic residual toxicity tests. Briefly, the *S. capricornutum* tests were based on *GSI/SOP/BS/RA/WET/3 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Water Treatment System to the Green Alga (Selenastrum capricornutum)*, with some modifications. The range of UV doses used mirrored those used for the second set of acute dose effectiveness tests involving *S. capricornutum* (i.e., 0 – 100 mJ/cm^2; Tables 3 and 4). A control (i.e., 0 mJ/cm^2 UV with spinning for the maximum exposure duration) and a performance control (for QC purposes) were also utilized. Inoculum was prepared on the day of the planned exposure using *S. capricornutum* starter culture; a sufficient volume of inoculum was prepared to provide an initial cell density of 10,000 cells/mL (± 10 %) per test chamber. Five replicates were prepared per treatment group; however, one of the five replicates was used as a chemistry-only replicate for measuring water chemistry parameters (Table 4). Initial cell density was determined prior to exposure by counting three samples.

Following exposure, organisms were transferred to sterile 125 mL Erlenmeyer flasks and placed into a shaking incubator set at 25 °C ± 3, 100 rpm, and in continuous light. Temperature and pH were measured every 24 hours in the chemistry flask from each control and treatment group. Reproduction (i.e., cell growth) was determined via direct cell counts at 96 hours post-exposure. Densities in the control flasks were required to exceed an average of 1 x 10^6 cells/mL and not vary by more than 20 % among replicates in order for the test to be deemed acceptable.

Chronic residual toxicity tests involving *D. magna* were static-renewal tests based on the U.S. Environmental Protection Office (USEPA), Office of Prevention, Pesticides, and Toxic Substances *Guideline OPPTS 850.1300 Daphnid Chronic Toxicity Test* (USEPA OPPTS, 1996). Note: test methods differed from standard life cycle tests in that the toxicant exposure was not conducted throughout the duration of the test, rather, exposure was conducted at the start of the test and organisms were held in optimal culturing conditions to determine reproductive effects due to this initial exposure.

The range of UV doses used for the tests involving *D. magna* were the same as those used for the acute dose effectiveness tests (i.e., 0 – 8 mJ/cm^2; Tables 3 and 4). A control (i.e., 0 mJ/cm^2 UV with spinning for the maximum exposure duration) and a performance control (for QC purposes) were also utilized. There were ten replicates per treatment group with one organism per replicate (Table 4). A water chemistry-only replicate was used to measure temperature, dissolved oxygen, conductivity, and pH of the LW used to set up the test. The test began with less than 48 hour old *D. magna* obtained from cultures at LSRI. Prior to the test, less than 24 hour old organisms were acclimated in 50 % test water (i.e., LW)/50 % culture water (i.e., HRW) at 25 °C with a photoperiod of 16 hours light and 8 hours dark. Each replicate contained approximately 80 mL of LW in 110 mL plastic SOLO® cups. Organisms were batch-exposed to UV radiation. After exposure, organisms were transferred to 110 mL plastic SOLO® cups containing approximately 80 mL of fresh LW and placed into an incubator set at 23 °C ± 3 °C with a photoperiod of 16

hours light and 8 hours dark. Food was added to the test solutions prior to transferring the *D. magna* to new solutions. For example, test solutions received YCT at a rate of 7 mL/L (1800 mg/L TSS) and *S. capricornutum* at a rate of 7 mL/L (stock density of 1.0 x 10^8 cells/mL) on Monday, Wednesday, and Friday of each week. Renewals consisted of placing new solutions of LW or PCW in clean test chambers and transferring the *D. magna* into each new test chamber. Reproductive counts were made during renewals and the number of days to first brood was recorded. The test was terminated after 21 days. For test results to be deemed acceptable at least 80 % survival in the control treatment was required. In addition, each organism in the control treatment must have produced an average of greater than 60 young, with no ephippia produced by the control animals.

Table 4. Experimental Design for GSI Chronic Residual Toxicity Tests of UV Irradiation in Various Experimental Water Qualities.

Test Organism	Experimental Water Quality	Treatment Group (Proposed UV Dose)	No. Reps per Treatment Group	No. Organisms per Rep.	Light:Dark Regime/ Temperature	Analysis Times (Hours)
Selenastrum capricornutum	USEPA Media	Reference Control	4 + 1 replicate for water chemistry	10,000 cells/mL	24:0 Hr / 25 ± 3 ° C	96 hours
		0 mJ/cm^2				
		25 mJ/cm^2				
		50 mJ/cm^2				
		75 mJ/cm^2				
		100 mJ/cm^2				
Daphnia magna	Hard Reconstituted Water (HRW)	Reference Control	10 + 1 replicate for water chemistry measurements	1	16:8 Hr/ 25 ± 3 ° C	21 days
	Laboratory Water (LW)	0 mJ/cm^2	10 + 1 replicate for water chemistry measurements	1	16:8 Hr/ 25 ± 3 ° C	21 days
		0.5 mJ/cm^2				
		1.0 mJ/cm^2				
		2.0 mJ/cm^2				
		4.0 mJ/cm^2				
		8.0 mJ/cm^2				

5.5. Water Chemistry Measurements

To verify that percent transmittance (%T) of the experimental water qualities was within the reference limits outlined in Table 2, discrete grab (i.e., whole water) samples were collected from the LW and PCW stock solutions just prior to each acute dose effectiveness and chronic residual toxicity tests taking place. The exact date and time of sample collection was recorded on a water chemistry sample collection datasheet. One unfiltered portion of each sample collected was used to measure %T.

Sample analysis was conducted according to *GSI/SOP/BS/RA/C/4, v.2 – Procedure for Determining Percent Transmittance (%T) of Water at 254 nm.* Briefly, a UV-Vis

spectrophotometer was used to measure %T of the unfiltered sample aliquot. Deionized water was used as a reference to adjust the spectrophotometer to 100 %T, and then each unfiltered sample aliquot was measured in a pre-rinsed sample cuvette with a 1 cm path length.

Several other water chemistry parameters were also measured during the acute dose effectiveness and chronic residual toxicity tests. These parameters were measured using calibrated meters and included temperature, dissolved oxygen, pH, and specific conductivity.

Temperature was measured prior to UV exposure (i.e., in stock solutions), immediately after UV exposure (i.e., in a chemistry replicate), and daily in selected replicates during all of the acute dose effectiveness and chronic residual toxicity tests involving *S. capricornutum*. For the chronic residual toxicity test involving *D. magna*, temperature was measured prior to UV exposure (i.e., in stock solutions), immediately after exposure, and on test days 7, 14, and 21 in at least two replicates from the PCW, 0 mJ/cm^2, 1.0 mJ/cm^2, and the highest UV treatment sample having surviving organisms. In addition, the temperature of at least one of the replicates was measured immediately after exposure in order to determine any temperature effects of the exposure method. All temperature measurements were taken using a Fisher digital thermometer that was calibrated every seven days using an alcohol thermometer (that is calibrated against a certified National Bureau of Standards mercury thermometer) according to *LSRI/SOP/GLM/17 – Procedure for Measuring Temperature with the Electronic Digital*.

The dissolved oxygen concentration of the test water was measured prior to exposure (in stock solutions), immediately after exposure (in a chemistry replicate), and at the end of the acute dose effectiveness tests for *D. magna* and *S. capricornutum*. For the *D. magna* chronic residual toxicity tests, the dissolved oxygen was measured prior to exposure (in stock solutions), immediately after exposure, and on test days 7, 14, and 21 in at least two replicates from the PCW, 0 mJ/cm^2, 1.0 mJ/cm^2, and the highest UV treatment sample having surviving organisms. All dissolved oxygen concentrations were measured using a YSI Model 58 DO Meter with a YSI Model 5239 DO Probe (Yellow Springs Instruments; Yellow Springs, Ohio) according to *LSRI/SOP/GLM/04 – Calibrating, Maintaining, and Using Dissolved Oxygen Meters*. The dissolved oxygen meter was calibrated at least every seven days.

The pH of the test water was measured prior to exposure (in stock solutions), immediately after exposure (in a chemistry replicate), at the end of the acute dose effectiveness tests for both *D. magna* and *S. capricornutum*, and daily in selected replicates during the *S. capricornutum* chronic residual toxicity tests. For the *D. magna* chronic residual toxicity test, the pH was measured prior to exposure (in stock solutions), immediately after exposure, and on test days 7, 14, and 21 in at least two replicates from the PCW, 0 mJ/cm^2, 1.0 mJ/cm^2, and the highest UV treatment sample having surviving organisms. All pH measurements were made using a pH meter (i.e., Orion 3 Star) with a pH combination electrode and automatic-temperature-compensation (ATC) probe following the method outlined in *GSI/SOP/BS/RA/C/9, v. 3 – Procedure for pH Meter Calibration and pH*. The pH meter was calibrated prior to each use with a three-point calibration, and verified for accuracy using a pH 8.00 check buffer.

The specific conductivity of the test water was measured prior to exposure (in stock solutions), immediately after exposure (in a chemistry replicate), and at the end of the acute dose

effectiveness tests. For the *D. magna* chronic residual toxicity test, the specific conductivity was measured prior to exposure (in stock solutions), immediately after exposure, and on test days 7, 14, and 21 in at least two replicates from the PCW, 0 mJ/cm^2, 1.0 mJ/cm^2, and the highest UV treatment sample having surviving organisms. Conductivity was measured using the Oakton Model CON 110 Conductivity/TDS/Temperature Meter (Oakton Instruments; Vernon Hills, Iillinois), hereafter referred to as the CON 110 Meter. The CON 110 Meter was calibrated on a monthly basis, and was verified for accuracy each day prior to sample analysis using a Daily Check Standard (i.e., 0.0100 M or 0.0010 M potassium chloride). Measurement, verification and calibration was conducted according to *LSRI/SOP/GLM/26 – Procedures for Calibrating and Using the Oakton CON 110 Conductivity/TDS/Temperature Meter*.

5.6. Data Analysis

Data was analyzed using the Comprehensive Environmental Toxicity Information systems program (CETIS™ version 1.7.; Tidepool Scientific Software; McKinleyville, California, USA). For dose effectiveness tests the endpoints of LD_{50} and LD_{99} values, i.e., the doses resulting in death of 50 % and 99 %, respectively, of exposed individuals by a predetermined time, were determined using the Linear Interpolation (ICPIN) Method via a two-point interpolation. In addition to CETIS™, data for the chronic residual toxicity tests involving *D. magna* were assessed using WET Analysis Spreadsheet v. 1.6.1 supplied by the USEPA. For this analysis, the total number of living offspring produced per parent animal which did not die accidentally or inadvertently during the test (OECD) was entered into the WET Analysis Spreadsheet and used to determine the IC_{25}, i.e., the concentration resulting in inhibiting a biological function (e.g. growth, reproduction) of 25 % of exposed individuals by a predetermined time. All statistical significance was at a level of 0.05.

6. FINDINGS

6.1. Acute Dose Effectiveness Tests

6.1.1. *Selenastrum capricornutum*

Results from the two acute dose effectiveness tests involving the green alga *S. capricornutum* are presented in Table 5. For the first test, measured doses of UV were slightly higher than the target doses, particularly for the higher doses of 200 and 400 mJ/cm^2 (Table 5). The three highest measured UV doses (i.e., 220.6, 420.2 and 803.9 mJ/cm^2) resulted in 0 % *S. capricornutum* survival at 24 hours, while the fourth highest measured UV dose (i.e., 101.3 mJ/cm^2) resulted in only 17.3 % survival at 24 hours (Table 5). Owing to these results, the second dose effectiveness test involving *S. capricornutum* was initiated using lower target doses. A new calibration curve was also constructed while the actinometer solution was held at 23 °C ± 3 °C in a water bath. This procedure allowed for a more accurate calculation of the proposed UV dose. Still, similar to the first test, survival of *S. capricornutum* was greatly reduced in the two higher doses (i.e., 66.7 and 88.6 mJ/cm^2, respectively; Table 5). Although the test organisms were held in a 24 hour light cycle, survival decreased throughout the 48 hour test period indicating that the algae photorepair mechanism was not able to overcome the damage caused by UV exposure. The median lethal

UV dose (i.e., LD_{50}), calculated for the second set of tests involving *S. capricornutum*, at 48 hours was 55 mJ/cm^2 (Table 6).

Water chemistry values relative to the two dose effectiveness tests involving the green alga *S. capricornutum* are presented in Table 7. In the first set of tests, temperature remained within a degree of difference across all exposure solutions, while pH increased following the addition of test organisms (Table 7). In contrast, dissolved oxygen decreased following the addition of tests organisms, while conductivity remained relatively steady, ranging from 133.3 to 134.7 μS/cm (Table 7). In the second test, temperature varied by approximately 4 °C over the test period (Table 7). pH increased after the addition of test organisms though values were consistent across the different doses (Table 7). Concentrations of dissolved oxygen and conductivity were also consistent across doses (Table 7).

Table 5. Percent Survival of the Green Alga *Selenastrum capricornutum* at 0, 4, 24 and 48 Hours Following Exposure to Various Doses of UV Irradiation.

Test Organism	Test Number	Treatment Group		Percent (%) Survival (± Standard Deviation)			
		Proposed UV Dose	Measured UV Dose	0 Hours (n = 1)	4 Hours (n = 3)	24 Hours (n = 3)	48 Hours (n = 3)
Selenastrum capricornutum	1	0 mJ/cm^2	3.4 mJ/cm^2	98.6 %	99.8 % (± 1.5)	98.4 % (± 1.5)	N/A *
		100 mJ/cm^2	101.3 mJ/cm^2	100 %	100 % (± 0)	17.3 % (± 0.7)	N/A *
		200 mJ/cm^2	220.6 mJ/cm^2	99.3 %	13.7 % (± 5.2)	0 % (± 0)	N/A *
		400 mJ/cm^2	420.2 mJ/cm^2	100 %	0.4 % (± 0.8)	0 % (± 0)	N/A *
		800 mJ/cm^2	803.9 mJ/cm^2	100 %	0 % (± 0)	0 % (± 0)	N/A *
	2	0 mJ/cm^2	0 mJ/cm^2	100 %	100 % (± 0.0)	98.9 % (± 0.3)	99.5 % (± 0.2)
		25 mJ/cm^2	23.4 mJ/cm^2	100 %	99.6 % (± 0.8)	97.7 % (± 1.4)	91.6 % (± 2.5)
		50 mJ/cm^2	45.6 mJ/cm^2	100 %	99.7 % (± 0.4)	98.0 % (± 0.2)	82.7 % (± 13.6)
		75 mJ/cm^2	66.7 mJ/cm^2	100 %	99.3 % (± 1.2)	74.4 % (± 8.4)	17.0 % (± 8.7)
		100 mJ/cm^2	88.6 mJ/cm^2	100 %	99.7 % (± 0.5)	36.7 % (± 12.2)	5.9 % (± 5.9)

* No counts were conducted owing to a severe weather event that restricted GSI personnel entry into the testing laboratory.

Table 6. Survival of the Green Alga *Selenastrum capricornutum* at 4, 24 and 48 Hours in terms of Lethal Doses (LD_{50} and LD_{99}) of UV.

Test Organism	Time Point	Effect Level	UV Dose	95 Percent (%) Confidence Limit	
				Upper	Lower
Selenastrum capricornutum	4 Hours	LD_{50}	> 90 mJ/cm^2	N/A	N/A
		LD_{99}	> 90 mJ/cm^2	N/A	N/A
	24 Hours	LD_{50}	81 mJ/cm^2	72	N/A
		LD_{99}	> 90 mJ/cm^2	N/A	N/A
	48 Hours	LD_{50}	55 mJ/cm^2	50 mJ/cm^2	59 mJ/cm^2
		LD_{99}	> 90 mJ/cm^2	N/A	N/A

Table 7. Water Chemistry Values (Minimum, Maximum) as Measured in Acute Dose Effectiveness Tests involving the Green Alga *Selenastrum capricornutum*.

Test Organism	Test Number	Treatment Group		Water Chemistry Values (Minimum, Maximum)			
		Proposed UV Dose	Measured UV Dose	Temperature (n = 2)	pH (n = 2)	Dissolved Oxygen (n = 1)*	Conductivity (n = 1)*
Selenastrum capricornutum	1	N/A – Stock Solution		23.9 °C	7.43	8.3 mg/L	133.7 µS/cm
		0 mJ/cm^2	3.4 mJ/cm^2	24.6 °C (24.2, 25.0)	7.75 (7.57, 8.06)	7.5 mg/L	133.7 µS/cm
		100 mJ/cm^2	101.3 mJ/cm^2	24.1 °C (23.6, 24.6)	7.78 (7.68, 7.91)	7.6 mg/L	134.7 µS/cm
		200 mJ/cm^2	220.6 mJ/cm^2	24.6 °C (24.5, 24.7)	7.71 (7.58, 7.90)	7.5 mg/L	134.6 µS/cm
		400 mJ/cm^2	420.2 mJ/cm^2	24.1 °C (24.1, 24.1)	7.76 (7.65, 7.90)	7.5 mg/L	133.3 µS/cm
		800 mJ/cm^2	803.9 mJ/cm^2	24.5 °C (24.1, 24.8)	7.72 (7.61, 7.86)	7.8 mg/L	134.6 µS/cm
	2	N/A – Stock Solution		21.6 °C	7.60	8.2 mg/L	131.5 µS/cm
		0 mJ/cm^2	0 mJ/cm^2	23.6 °C (20.8, 24.9)	7.89 (7.68, 8.08)	8.1 mg/L	130.4 µS/cm
		25 mJ/cm^2	23.4 mJ/cm^2	23.9 °C (21.6, 25.2)	7.82 (7.70, 7.90)	8.1 mg/L	128.4 µS/cm
		50 mJ/cm^2	45.6 mJ/cm^2	23.7 °C (20.9, 25.1)	7.85 (7.72, 7.93)	8.7 mg/L	130.8 µS/cm
		75 mJ/cm^2	66.7 mJ/cm^2	23.9 °C (21.2, 25.1)	7.85 (7.71, 7.96)	8.2 mg/L	130.2 µS/cm
		100 mJ/cm^2	88.6 mJ/cm^2	23.9 °C (21.1, 25.1)	7.84 (7.69, 7.96)	8.4 mg/L	129.5 µS/cm

* Single measurement taken immediately after exposure.

6.1.2. *Daphnia magna*

Results from the three acute dose effectiveness tests involving the cladoceran *D. magna* are presented in Table 8. Target UV doses in the first test ranged from a low of 2.8 mJ/cm^2 to a high of 365.4 mJ/cm^2 and were near to measured doses (Table 8). The exposure solutions containing the three highest measured doses (i.e., 25.1, 94.7 and 365.4 mJ/cm^2) resulted in 0 % *D. magna* survival at 24 hours, while the lowest measured dose (i.e., 2.8 mJ/cm^2) had 40 % survival at 48 hours (Table 8). Owing to these results, further experimentation was carried out to find an effective way to shutter the UV lamp in order to attain the lower doses necessary for survival since exposure time was already at a minimum. This was achieved by wrapping the lamp in sheer fabric and subsequently creating a new calibration curve. Correspondingly, the second set of dose effectiveness tests involving *D. magna* was conducted with the newly shuttered bulb, with target UV doses ranging from a low of 0.8 mJ/cm^2 to a high dose of 6.4 mJ/cm^2 (Table 8). Measured doses were very close to target doses across all exposure solutions and all exposure

solutions had some surviving organisms 48 hours post-exposure, with a 50 % survival rate amongst the two highest doses (i.e., 1.6 and 6.2 mJ/cm^2; Table 8). In the third set of tests involving *D. magna*, also conducted using a shuttered bulb, target UV doses ranged from a low of 0.5 mJ/cm^2 to a high of 8.0 mJ/cm^2 (Table 8). Again, the measured doses were very close to the target doses with all exposure solutions having some surviving organisms 48 hours post-exposure, except for those in the highest measured dose of 9.8 mJ/cm^2 (Table 8). The LD_{50} and LD_{99} values, calculated for the third set of tests involving *D. magna* only are shown in Table 9.

Water chemistry values relative to the three dose effectiveness tests involving *D. magna* are presented in Table 10. The first and second set of tests in the series were conducted solely as range finding tests so there were no water chemistry parameters measured. In the third set of tests, temperature had little variation over the test duration, varying less than 3 °C. Dissolved oxygen levels were lower following the addition of test organisms and UV doses compared to levels in the stock solution (Table 10). Conversely, conductivity was higher in solutions following the addition of test organisms and UV doses, while pH remained fairly consistent over the test duration, with values ranging from 7.71 to 7.96 (Table 10).

Table 8. Percent Survival of the Cladoceran *Daphnia magna* at 0, 4, 24 and 48 Hours Following Exposure to Various Doses of UV Irradiation.

Test Organism	Test Number	Treatment Group		Percent (%) Survival (± Standard Deviation)			
		Proposed UV Dose	Measured UV Dose	0 Hours	4 Hours	24 Hours	48 Hours
Daphnia magna	1 (n=5)	0 mJ/cm^2	0.0 mJ/cm^2	100 % (± 0)	100 % (± 0)	100 % (± 0)	100 % (± 0)
		3.1 mJ/cm^2	2.8 mJ/cm^2	100 % (± 0)	100 % (± 0)	40.0 % (± 54.8)	40.0 % (± 54.8)
		25 mJ/cm^2	25.1 mJ/cm^2	100 % (± 0)	100 % (± 0)	0 % (± 0)	0 % (± 0)
		100 mJ/cm^2	94.7 mJ/cm^2	100 % (± 0)	100 % (± 0)	0 % (± 0)	0 % (± 0)
		400 mJ/cm^2	365.4 mJ/cm^2	100 % (± 0)	0 % (± 0)	0 % (± 0)	0 % (± 0)
	2 (n=5)	0 mJ/cm^2	0.0 mJ/cm^2	100 % (± 0)	100 % (± 0)	100 % (± 0)	100 % (± 0)
		0.8 mJ/cm^2	0.6 mJ/cm^2	100 % (± 0)	100 % (± 0)	100 % (± 0)	100 % (± 0)
		1.6 mJ/cm^2	1.6 mJ/cm^2	100 % (± 0)	100 % (± 0)	100 % (± 0)	80.0 % (± 44.7)
		6.4 mJ/cm^2	6.2 mJ/cm^2	100 % (± 0)	100 % (± 0)	40.0 % (± 54.8)	20.0 % (± 44.7)
	3 (n=10)	0 mJ/cm^2	0.0 mJ/cm^2	100 % (± 0)	100 % (± 0)	100 % (± 0)	100 % (± 0)
		0.5 mJ/cm^2	0.6 mJ/cm^2	100 % (± 0)	100 % (± 0)	80.0 % (± 42.6)	80.0 % (± 42.6)
		1.0 mJ/cm^2	1.2 mJ/cm^2	100 % (± 0)	100 % (± 0)	100 % (± 0)	90.0 % (± 31.6)
		2.0 mJ/cm^2	2.4 mJ/cm^2	100 % (± 0)	100 % (± 0)	100 % (± 0)	100 % (± 0)
		4.0 mJ/cm^2	4.4 mJ/cm^2	100 % (± 0)	100 % (± 0)	60.0 % (± 51.6)	40.0 % (± 51.6)
		8.0 mJ/cm^2	9.8 mJ/cm^2	100 % (± 0)	100 % (± 0)	10.0 % (± 31.6)	0 % (± 0)

Table 9. Third Test Survival of the Cladoceran *Daphnia magna* at 4, 24 and 48 Hours in terms of Median (LD_{50}) and Lethal (LD_{99}) Doses of UV.

Test Organism	Time Point	Effect Level	UV Dose	95 Percent (%) Confidence Limit	
				Upper	Lower
Daphnia magna	4 Hours	LD_{50}	> 9.7 mJ/cm^2	N/A	N/A
		LD_{99}	> 9.7 mJ/cm^2	N/A	N/A
	24 Hours	LD_{50}	5.2 mJ/cm^2	3.7 mJ/cm^2	7.0 mJ/cm^2
		LD_{99}	> 9.7 mJ/cm^2	N/A	N/A
	48 Hours	LD_{50}	4.0 mJ/cm^2	3.3 mJ/cm^2	5.6 mJ/cm^2
		LD_{99}	5.2 mJ/cm^2	9.0 mJ/cm^2	9.6 mJ/cm^2

Table 10. Water Chemistry Values (Minimum, Maximum) as Measured in the Third Set of Acute Dose Effectiveness Tests involving the Cladoceran *Daphnia magna*.

Test Organism	Test Number	Treatment Group		Water Chemistry Values (Minimum, Maximum)			
		Proposed UV Dose	Measured UV Dose	Temperature (n = 3)	pH (n = 2)	Dissolved Oxygen (n = 1)*	Conductivity (n = 1)*
Daphnia magna	3	N/A – Stock Solution		24.4 °C*	7.78*	8.7 mg/L*	137.5 µS/cm
		0 mJ/cm^2	0.0 mJ/cm^2	23.7 °C (22.8, 24.5)	7.83 (7.78,7.96)	8.0 mg/L	157.9 µS/cm
		0.5 mJ/cm^2	0.6 mJ/cm^2	23.7 °C (23.3, 24.0)	7.87 (7.83, 7.93)	7.6 mg/L	146.1 µS/cm
		1.0 mJ/cm^2	1.2 mJ/cm^2	23.7 °C (23.4, 24.0)	7.85 (7.81, 7.89)	7.8 mg/L	152.3 µS/cm
		2.0 mJ/cm^2	2.4 mJ/cm^2	23.5 °C (23.2, 23.6)	7.88 (7.84, 7.92)	7.6 mg/L	152.5 µS/cm
		4.0 mJ/cm^2	4.4 mJ/cm^2	23.5 °C (22.9, 24.4)	7.88 (7.78, 7.95)	8.0 mg/L	171.7 µS/cm
		8.0 mJ/cm^2	9.8 mJ/cm^2	23.5 °C (23.0, 24.1)	7.85 (7.71, 7.93)	7.6 mg/L	142.9 µS/cm

* Single measurement taken immediately after exposure.

6.2. Chronic Residual Toxicity Tests

6.2.1. *Selenastrum capricornutum*

Results from the chronic residual toxicity test involving *S. capricornutum* exposed to up to five doses of UV irradiation are shown in Table 11. Actual doses were very close to the target doses. It is estimated that a dose of only 1.3 mJ/cm^2 is required to inhibit growth of roughly 25 % of the test population, while a dose of 24 mJ/cm^2 is required to inhibit growth of 99 % of the test population. The ID_{25}, ID_{50} and ID_{99} values are shown in Table 12.

Water chemistry values relative to the chronic residual toxicity test involving *S. capricornutum* are presented in Table 13. Temperature stayed fairly consistent, ranging from a low of 21.6 °C to

a high of 25.6 °C (Table 13). pH increased following the addition of test organisms and UV exposure, while dissolved oxygen and conductivity exhibited little variation (Table 13).

Table 11. Average Cell Counts of the Green Alga *Selenastrum capricornutum* at 96 Hours Following Exposure to Various Doses of UV Irradiation.

Test Organism	Treatment Group		Density at 96 Hours Post-Exposure (Average ± Standard Deviation; n = 4)
	Proposed UV Dose	Measured UV Dose	
Selenastrum capricornutum	0 mJ/cm^2 (PCW)	0.0 mJ/cm^2	5.16 x 10^6 cells/mL (± 7.29 x 10^5)
	0 mJ/cm^2	0.1 mJ/cm^2	4.34 x 10^6 cells/mL (± 5.22 x 10^5)
	25 mJ/cm^2	24.4 mJ/cm^2	2.85 x 10^4 cells/mL (± 1.19 x 10^4)
	50 mJ/cm^2	48.2 mJ/cm^2	1.14 x 10^4 cells/mL (± 3.76 x 10^3)
	75 mJ/cm^2	74.1 mJ/cm^2	8.01 x 10^3 cells/mL (± 5.50 x 10^2)
	100 mJ/cm^2	93.9 mJ/cm^2	9.37 x 10^3 cells/mL (± 6.29 x 10^2)

Table 12. Green Alga *Selenastrum capricornutum* Survival at 96 Hours in terms of the UV Doses Required to Inhibit Growth of 25 % (ID_{25}), 50 % (ID_{50}) and 99 % (ID_{99}) of the Population.

Test Organism	Time Point	Effect Level	UV Dose	95 Percent (%) Confidence Limit	
				Upper	Lower
Selenastrum capricornutum	96Hours	ID_{25}	1.3 mJ/cm^2	N/A	N/A
		ID_{50}	4.1 mJ/cm^2	N/A	N/A
		ID_{99}	24 mJ/cm^2	N/A	N/A

Table 13. Water Chemistry Values (Minimum, Maximum) as Measured in the Chronic Residual Toxicity Tests involving the Green Alga *Selenastrum capricornutum*.

Test Organism	Treatment Group		Water Chemistry Values (Minimum, Maximum)			
	Proposed UV Dose	Measured UV Dose	Temperature (n = 5)	pH (n = 5)	Dissolved Oxygen (n = 1)*	Conductivity (n = 1)*
Selenastrum capricornutum	N/A – Stock Solution		23.2 °C	7.04	7.8 mg/L	92.5 µS/cm
	N/A – PCW Control		24.7 °C (22.8, 25.2)	7.48 (7.10, 9.73)	7.7 mg/L	92.8 µS/cm
	0 mJ/cm^2	0.1 mJ/cm^2	24.5 °C (21.6, 25.6)	7.41 (7.00, 9.49)	7.8 mg/L	91.7 µS/cm
	25 mJ/cm^2	24.4 mJ/cm^2	24.5 °C (22.0, 25.4)	7.28 (7.18, 7.50)	7.7 mg/L	90.6 µS/cm
	50 mJ/cm^2	48.2 mJ/cm^2	24.6 °C (22.4, 25.4)	7.24 (7.12, 7.43)	7.8 mg/L	91.0 µS/cm
	75 mJ/cm^2	74.1 mJ/cm^2	24.4 °C (22.0, 25.2)	7.22 (7.08, 7.44)	7.8 mg/L	91.9 µS/cm
	100 mJ/cm^2	93.9 mJ/cm^2	24.5 °C (22.3, 25.2)	7.24 (7.10, 7.41)	7.5 mg/L	91.1 µS/cm

* Single measurement taken immediately after exposure. PCW Control was not inserted into carousel nor exposed to UV radiation.

6.2.2. *Daphnia magna*

Results from the chronic residual toxicity test involving *D. magna* exposed to up to five doses of UV irradiation are shown in Table 14. Actual doses were very close to the target doses (i.e., only 0.1 mJ/cm^2 difference; Table 14). The average number of offspring produced by the adults at 96 hours ranged from 0 in the highest UV dose of 8.1 mJ/cm^2 to 214 in the PCW control (Table 14). It should be noted there was no surviving adults in the highest UV dose (Table 14).

Table 15 contains water chemistry values measured in the chronic residual toxicity test involving *D. magna*. Temperature was similar over the duration of the test period and ranged from a low of 21.5 °C to a high of 24.8 °C (Table 15). pH ranged from 7.64 to 8.58 and dissolved oxygen measurements ranged from 7.1 to 8.5 mg/L (Table 15). Conductivity ranged from a low value of 142.4 μS/cm in the pre-exposure stock solution to a high of 197.3 μS/cm (Table 15). The PCW Control had much higher conductivity though remained within the expected range for this specific water type.

Table 14. Percent (%) Survival and Number of Offspring Produced by the Cladoceran *Daphnia magna* at 21 Days Following Exposure to Various Doses of UV Irradiation.

Test Organism	Treatment Group		Percent (%) Survival at 21 Days Post-Exposure (Mean ± Standard Deviation; n = 10)	Number of Live Offspring Produced by Adults 21 Days Post-Exposure (Mean ± Standard Deviation; n = 10)
	Proposed UV Dose	Measured UV Dose		
Daphnia magna	N/A – PCW Control		90 % (± 31.6)	214.3 ± 38.6
	0 mJ/cm^2	0.0 mJ/cm^2	80 % (± 42.2)	191.7 ± 79.6
	0.5 mJ/cm^2	0.6 mJ/cm^2	70 % (± 48.3)	166.8 ± 92.3
	1.0 mJ/cm^2	0.9 mJ/cm^2	90 % (± 31.6)	207.1 ± 76.4
	2.0 mJ/cm^2	2.1 mJ/cm^2	70 % (± 48.3)	113.4 ± 111.3
	4.0 mJ/cm^2	4.1 mJ/cm^2	30 % (± 48.3)	43.4 ± 75.4
	8.0 mJ/cm^2	8.1 mJ/cm^2	0 % (± 0)	0 ± 0

Table 15. Water Chemistry Values (Minimum, Maximum) as Measured in the Chronic Residual Toxicity Tests involving the Cladoceran *Daphnia magna*.

Test Organism	Treatment Group		Water Chemistry Values (Minimum, Maximum)			
	Proposed UV Dose	Measured UV Dose	Temperature (n = 7)	pH (n = 7)	Dissolved Oxygen (n = 7)	Conductivity (n = 7)
Daphnia magna	N/A – Stock Solution		24.8 °C	7.64	8.5 mg/L	142.4 µS/cm
	N/A – PCW Control		24.2 °C (23.7, 24.8)	8.17 (8.07, 8.28)	7.7 mg/L (7.4, 7.8)	570.1 µS/cm (560.0, 585.0)
	0 mJ/cm^2	0.0 mJ/cm^2	23.7 °C (21.5, 24.7)	7.90 (7.70, 8.19)	7.9 mg/L (7.6, 8.5)	181.7 µS/cm (165.6, 192.3)
	0.5 mJ/cm^2	0.6 mJ/cm^2	21.8* °C	7.79*	8.5*mg/L	163.3* µS/cm
	1.0 mJ/cm^2	0.9 mJ/cm^2	23.5 °C (22.4, 24.1)	7.88 (7.66, 8.31)	7.9 mg/L (7.5, 8.5)	181.5 µS/cm (156.8, 195.2)
	2.0 mJ/cm^2	2.1 mJ/cm^2	22.7* °C	7.71*	8.3* mg/L	156.6* µS/cm
	4.0 mJ/cm^2	4.1 mJ/cm^2	23.6 °C (22.1, 24.6)	7.92 (7.67, 8.58)	7.9 mg/L (7.1, 8.5)	180.5 µS/cm (161.2, 197.3)
	8.0 mJ/cm^2	8.1 mJ/cm^2	21.9* °C	7.77*	8.1* mg/L	169.3* µS/cm

* Single measurement taken immediately after exposure. PCW Control was not inserted into carousel nor exposed to UV radiation.

7. QUALITY MANAGEMENT

7.1. Standard Operating Procedures (SOPs)

GSI standard operating procedures (SOPs) and a Test/Quality Assurance Plan (TQAP), i.e., test plan (GSI, 2013) were used to implement all test activities. This facilitates consistent conformance to technical and quality system requirements and increases data quality. The TQAP included detailed instructions for sample and data collection and analysis, sample handling and preservation, data quality objectives, and QA/QC requirements (GSI, 2013). The SOPs include both programmatic and technical processes and procedures such as organism culturing; sample collection, labeling, analysis and custody; and safety. Table 16 summarizes the GSI SOPs utilized for these tests.

The GSI Senior QA/QC Officer performed routine inspections/spot checks of datasheets, logbooks, recorded measurements, and instrumentation used during the tests. Any deviations made to the TQAP were recorded and also approved by the GSI Lead On-Site Investigator for Bench-Scale Studies, as well as communicated to the GSI Senior QAQC officer. None of these deviations, summarized in Table 17, were deemed consequential to the quality of the findings.

Table 16. GSI Bench-Scale Standard Operating Procedures (SOPs) Utilized during the UV Irradiation Tests.

SOP Category	Subcategory	SOP Title	SOP Code
General	Administration	Procedure for Record Keeping	GSI/SOP/G/A/RK/1
		Procedure for General Data Entry using Microsoft Excel	GSI/SOP/G/RA/DM/2
		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
		Procedure for Labeling GSI Bench-Scale Samples	GSI/SOP/G/RA/SC/4
	Chemistry	Procedure for Determining Percent Transmittance (%T) of Light in Water at 254 nm	GSI/SOP/BS/RA/C/4
		Procedure for pH Meter Calibration and pH Measurement	GSI/SOP/BS/RA/C/9
		Procedure for Measuring Temperature with the Electronic Digital Thermometer	LSRI/SOP/GLM/17
		Procedures for Calibrating and Using the Oakton CON 110 Conductivity/TDS/Temperature Meter	LSRI/SOP/GLM/26
	Calibration	Calibrating Dissolved Oxygen Meters	LSRI/SOP/GLM/4
	Dose Effectiveness	Procedure for Assessing Dose Effectiveness of a Ballast Treatment System using *Selenastrum capricornutum*	GSI/SOP/BS/RA/EF/5
		Bench-Scale Procedure for Assessing Dose Effectiveness of a Ballast Water Management System using a Cladoceran	GSI/SOP/BS/RA/EF/2
	Chronic Residual Toxicity	Procedure for Assessing Chronic Residual Toxicity of a Ballast Treatment System to the Green Alga (*Selenastrum capricornutum*)	GSI/SOP/BS/RA/WET/3
		Guideline OPPTS 850.1300 Daphnid Chronic Toxicity Test	N/A – See: USEPA Office of Prevention, Pesticides, and Toxic Substances (1996)

Table 17. Summary of Deviations to the Test/Quality Assurance Plan (GSI, 2013).

GSI Deviation Code	Description of Deviation	Corrective Action	Potential Impact on Study
BS-UV-01	*Selenastrum capricornutum* were not counted at 48 hours	No corrective action needed; data reported from 0, 4 and 24 hours.	No impact. All algae in the 200, 400 and 800 mJ/cm^2 treatment groups were dead at 24 hours and 80 % of algae in the 100 mJ/cm^2 treatment were dead at 24 hours. The dose effectiveness test was repeated using lower doses.

7.2. Quality Assurance/Quality Control (QA/QC)

7.2.1. Analysis of Ultraviolet Dose using Chemical Actinometry

Table 18 describes the results from analysis of QC samples used to measure the UV dose during bench-scale testing (i.e., chemical antinometry method). By default, all samples were analyzed in duplicate, as two actinometers were used to measure the UV dose applied in each round of testing. All of the measurements that were above 0 mJ/cm^2 met the data quality objective of less than 20 % RPD.

Table 18. Data Quality Objectives, Criteria, and Results Relative to Chemistry Analyses.

Data Quality Indicator	Evaluation Process/ Performance Measurement	Data Quality Objective	Performance Measurement Result
Precision	Two actinometers were used to measure the dose applied in each round of testing with performance measured by average relative percent difference (RPD). Measurements from the control group (i.e., 0 mJ/cm^2) were not used to determine average RPD.	< 20 % average RPD	A total of 52 sets of data from the actinometers were compared to each other. In all cases, results from duplicate analyses had an RPD of less than 20 %.
Representativeness	All samples were collected, handled, and analyzed in the same manner.	N/A – Qualitative	All actinometers were collected, handled, and analyzed according to GSI-BS-QAQC-TQAP-7, v.3.
Comparability	Routine procedures were conducted according to appropriate SOPs to ensure consistency between tests.	N/A – Qualitative	All actinometers were collected, handled, and analyzed according to GSI-BS-QAQC-TQAP-7, v.3.
Completeness	Percentage of valid (i.e., collected, handled, analyzed correctly and meeting data quality objectives) water chemistry samples measured out of the total number of water chemistry samples collected. Performance is measured by percent completeness (%C).	> 90 %C.	45 actinometers analyzed/45 meeting data quality objectives = 100%

7.2.2. Acute Dose Effectiveness Tests

The QA measures for the acute dose effectiveness tests were as follows:

- QA Counts:
  - All tests: At least 10 % of the replicates in each treatment group were counted a second time (QA count) by a qualified GSI staff member. The results of the first and second counts were recorded on a datasheet.
- Reference Toxicant Tests:
  - *D. magna* tests only: In order to determine the health of the test organism population, reference toxicant tests were performed monthly using *D. magna* prior to the start of the acute dose effectiveness tests. Results were compared to historical data using quality control charts.

The data quality objectives and performance result measurements from the acute dose effectiveness tests are presented in Table 19. A reference test involving *D. magna*, conducted one week after the dose effectiveness testing, resulted in an LC_{50} within ± two standard deviations of the historical mean.

Table 19. Data Quality Objectives, Criteria, and Performance Measurement Results from Dose Effectiveness Tests.

Data Quality Indicator	Evaluation Process/ Performance Measurement	Data Quality Objective	Performance Measurement Result
Precision	Samples (10 %) were collected and analyzed in duplicate with performance measured by average relative percent difference (RPD).	< 20% average RPD	*S. capricornutum:* Test #1: 4 of 35 samples (i.e., 11.4 %) analyzed in duplicate; RPD (live cells) = 20.3 % ± 34.2 % Test #2: 6 of 50 samples (i.e., 12 %) analyzed in duplicate; RPD (live cells) = 14.4 % ± 13.2 %
Bias, Experiment	Monthly reference toxicity tests were conducted on control organisms; a reference control group was included in the experimental design	LC_{50} value within 2 standard deviations of the historical LC_{50} average. ≥ 90 % survival of reference control group organisms	*S. capricornutum:* No reference toxicity test. Test #1: 98.4 % ± 1.5 % survival in reference control group at 24 hours (test terminated at 24 hours). Test #2: 99.5 % ± 0.2 % survival in reference control group at 48 hours *D. magna:* Test #1: LC_{50} as measured on 17 April 2014 was within 2 standard deviations of the historical LC_{50} average; 100 % survival of reference control organisms at 48 hours Test #2: LC_{50} as measured on 17 April 2014 was within 2 standard deviations of the historical LC_{50} average; 100 % survival of reference control organisms at 48 hours Test #3: LC_{50} as measured on 17 April 2014 was within 2 standard deviations of the historical LC_{50} average; 100 % survival of reference control organisms at 48 hours

Data Quality Indicator	Evaluation Process/ Performance Measurement	Data Quality Objective	Performance Measurement Result
Bias, Operator	Samples (10 %) were counted by two separate analysts with performance measured by average relative percent difference (RPD) of all second counts.	< 40 % average RPD for *S. capricornutum*, < 10 % average RPD for *D. magna*	*S. capricornutum*: Test #1: 5 of 35 samples (i.e., 14.3 %) counted by a second analyst; RPD (live cells) = 4.2 % ± 6.5 % Test #2: 6 of 50 samples (i.e., 12 %) counted by a second analyst; RPD (live cells) = 3.5 % ± 4.6 % *D. magna:* Test #1: 50 of 95 samples (i.e., 52.6 %) counted by a second analyst; RPD = 0 % Test #2: 40 of 98 samples (i.e., 40.8 %) counted by a second analyst; RPD = 0 % Test #3: 165 of 285 samples (i.e., 57.9 %) counted by a second analyst; RPD = 0 %
Representativeness	All samples were collected, handled, and analyzed in the same manner.	N/A – Qualitative	All samples were collected, handled, and analyzed in the same manner (using the appropriate GSI SOPs).
Comparability	Routine procedures were conducted according to appropriate SOPs to ensure consistency between tests.	N/A – Qualitative	The GSI SOPs listed in Table 16 were used to implement all dose effectiveness tests.
Completeness	Percentage of valid (i.e., collected, handled, analyzed correctly and meeting DQOs) samples measured out of the total number of samples collected. Performance is measured by percent completeness (%C).	> 90 % C	*S. capricornutum:* Test #1: 94.3% Test #2: 98% C *D. magna:* Test #1: 100% C Test #2: 100% C Test #3: 100% C

7.2.3. Chronic Reproduction Tests

For the chronic residual toxicity test involving *S. capricornutum*, the final density in both the dilution and reference water exceeded the USEPA test acceptability criteria for control growth (i.e., minimum final density requirement of 1.0×10^6 cells/mL) with 4.34×10^6 and 5.16×10^6 cells/mL, respectively. In addition, USEPA test acceptability criterion for variation was met, as the final cell counts had coefficient of variation (CV) values of 12.0 % for the dilution water and 14.1 % for the reference water. These values are both within the acceptance range of less than 20 % CV, as specified by the USEPA standard methods.

The data quality objectives and the performance results from the chronic residual toxicity tests are presented in Table 20. The QA measures for the chronic residual toxicity tests were as follows:

- QA Counts:
  - All tests: At least 10 % of the replicates in each treatment group were counted a second time (QA count) by a qualified GSI staff member. The results of the first and second counts were recorded on a datasheet.
- Reference Toxicant Tests
  - *D. magna* test only: In order to determine the health of the test organism population, reference toxicant tests were performed monthly using *D. magna* prior to the start of the chronic residual toxicity tests. Results were compared to historical data using quality control charts.

A reference test was conducted with *D. magna* one week prior to the start of the chronic residual toxicity tests and resulted in an LC_{50} within ± two standard deviations of the historical mean.

Table 20. Data Quality Objectives, Criteria, and Performance Measurement Results from Chronic Residual Toxicity Tests.

Data Quality Indicator	Evaluation Process/ Performance Measurement	Data Quality Objective	Performance Measurement Result
Precision	Samples (10 %) were collected and analyzed in duplicate with performance measured by average relative percent difference (RPD).	< 20 % average RPD	*S. capricornutum*: Test #1: 3 of 27 samples (i.e., 11.1 %) analyzed in duplicate; RPD cells = 13.5 % ± 5.2 %
Bias, Experiment	Monthly reference toxicity tests were conducted on control organisms; a reference control group was included in the experimental design	LC_{50} value within 2 standard deviations of the historical LC_{50} average; ≥ 90 % survival of reference control group organisms	*S. capricornutum*: No reference toxicity test. *D. magna*: Test #1: LC_{50} value as measured on 17 April 2014 was within 2 standard deviations of the historical LC_{50} average; 90 % survival of reference control group organisms
Bias, Operator	Samples (10%) were counted by two separate analysts with performance measured by average relative percent difference (RPD) of all second counts.	<10% average RPD	*S. capricornutum*: Test #1: 3 of 27 samples (i.e., 11.1 %) counted by a second analyst; RPD cells = 13.5 % ± 10.3 % *D.magna*: Test #1: 172 of 494 samples (i.e., 34.8 %) counted by a second analyst; RPD = 0.07 %
Representativeness	All samples were collected, handled, and analyzed in the same manner.	N/A – Qualitative	All samples were collected, handled, and analyzed in the same manner (using the appropriate GSI SOPs).
Comparability	Routine procedures were conducted according to appropriate SOPs to ensure consistency between tests.	N/A – Qualitative	The GSI SOPs listed in Table 16 were used to implement all chronic residual toxicity tests.
Completeness	Percentage of valid (i.e., collected, handled, analyzed correctly and meeting DQOs) samples measured out of the total number of samples collected. Performance is measured by percent completeness (%C).	> 90 %C	*S. capricornutum*: Test #1: 96.3% *D.magna*: Test #1: 100% C

8. CONCLUSION

In general, UV light at 253.7 nm was found to be both acutely and chronically toxic to the green alga *S. capricornutum* and the cladoceran *D. magna.* A complete kill of algae occurred within 24 hours of exposure at UV doses greater than 220 mJ/cm^2 while at 48 hours post-exposure survival was less than 20 % at UV doses greater than 67 mJ/cm^2. *D. magna* survival was less than 40 % at doses greater than 4.4 mJ/cm^2 at 48 hours. Both the green algae and the two species of zooplankton utilized in these tests experienced reductions in growth and reproduction at UV doses greater than 24 mJ/cm^2 and 2.1 mJ/cm^2, respectively.

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APPENDIX 1: Iodide/Iodate Chemical Actinometer

(VERSION: 26 FEBRUARY 2014)

Introduction

This iodide/iodate solution in borate buffer is used as a chemical actinometer to determine the dose of ultraviolet (UV) light provided by a low-pressure mercury lamp emitting over 85 % of its energy at 254 nm. The actinometer solution is opaque to wavelengths below 290 nm and optically transparent to light above 330 nm (Rahn, 1997). The irradiation of the solution with light at 254 nm causes the formation of triiodide ion whose concentration can be determined by measuring absorbance of the solution at a wavelength at which the solution absorbs. The wavelength chosen for this project is 420 nm. The concentration of the triiodide is directly proportional to the dose of UV light to which it is exposed.

Reagents

1. **Borate Buffer**: Dissolve 3.81 g of sodium tetraborate ($Na_2B_4O_7 \cdot 10\ H_2O$) in each 1.0 L of deionized water. Adjust the pH of the buffer to 9.25 by adding 0.5 M sodium hydroxide (NaOH). This solution is stable for two months.
2. **Actinometer Solution**: Weigh 2.14 g of potassium iodate (KIO_3) and 9.96 g of potassium iodide (KI) per 100 mL of **borate buffer** and dissolve in a volumetric flask. Potassium iodate is slow to dissolve. To speed up dissolution, sonicate the solution in an ultrasonic bath. The temperature of the actinometer solution is important; therefore keep the ultrasonic bath temperature in the range of 20-25 °C. After the solids have completely dissolved (15-20 minutes), complete dilution to volume with the **borate buffer** solution. Place the solution in an incubator set at 23°C. This solution is to be prepared daily.

Procedure

1. Turn on the PerkinElmer Lambda 35 spectrophotometer and allow it to warm up for a minimum of 5 minutes before starting the PerkinElmer software.
2. Click on the **PerkinElmer UV WinLab** icon on the desktop.
3. In the Methods, select Triiodide Actinometer (Revised) 300/352/400/420 nm.
4. On the **Sample Info** screen, enter "1" as the **Number of Samples** and the **Sample Description** (Triiodide Blank).
5. Click on **Start** and follow the directions on the computer screen.
6. When the **Remove sample(s) and then press OK to perform a 100%T / 0A correction (Autozero)** message appears, fill the 1.0 cm reference and sample cuvettes with deionized water, wipe with a KimWipe and place in proper cuvette holders. The spectrophotometer should be rezeroed before the absorbance of each actinometer solution is determined.
7. When adding solutions to the cuvettes, rinse cuvette with three or four volumes of the sample before filling the cuvette to make an absorbance reading.
8. The first sample to be read is the unexposed actinometer solution (Triiodide Blank). The absorbance for this solution at 300 nm in the 1.0 cm cuvette should be in the range of 0.58 to 0.61. This absorbance can be used to determine the iodide concentration in the solution. The measured absorbance divided by 1.061 yields the molarity of the iodide solution. If the absorbance of the solution is not in this range the actinometer solution should be remade.
9. After obtaining the reading for the unexposed actinometer solution, click **Output**, then **Print**. The printout should be kept (project 3-ring binder) to prove the iodide concentration was in the correct range.
10. Close out of the software and then repeat steps 2-6. For step four, determine the number of actinometer solutions that will be used for the days exposures. Enter that number for the

Number of Samples. Provide a description for each of the actinometer solutions that will be analyzed.

11. **All absorbance readings for exposed actinometer solutions are made using the 1.0 cm cuvettes.** For the repeat of step six, the 1.0 cm cuvettes are filled with deionized water. After the 100 %T / 0A correction has been performed with the 1.0 cm cuvettes, the instrument is ready to make readings on the exposed actinometer solutions.
12. The actinometer solution is now ready to be transferred into the quartz tubes used in the Merry-Go-Round reactor. The volume of solution used should be the same as the volume being used for the organism exposures. When transferring the solution into the quartz tubes, it is important to minimize the amount of solution on the walls of the tubes above the solution column. The addition of the actinometer solution to the quartz tubes is best done using a pipet.
13. The actinometer tubes are placed into the carousel with even spacing between them. After the exposure has been completed, transfer the actinometer solution into a beaker. Swirl the solution to make sure it is homogeneous.
14. After rinsing the cuvette with 3-4 portions of the exposed actinometer solution, transfer an aliquot of the solution to the cuvette and make absorbance readings.
15. Continue exposing actinometer solutions and making absorbance readings until all samples have been treated and read.
16. When readings have been completed, click **Output**, then **Preview**. After reviewing the data, **Print** the results and place in the project 3-ring binder.