

APPENDIX VI

TOXICITY IDENTIFICATION EVALUATION REPORT

Identification of Causes of Toxicity to Green Algae (*Selenastrum capricornutum*) in Agriculture-Dominated Discharge Samples from the East San Joaquin Water Quality Coalition: January 2012 – December 2012

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TABLE OF CONTENTS

1.0 EXECUTIVE SUMMARY	2
2.0 INTRODUCTION	2
3.0 MATERIALS AND METHODS.....	3
3.1 Test Events.....	3
3.2 Test Samples	4
3.3 Toxicity Test Protocols	5
3.4 Algae Toxicity Tests.....	5
3.5 <i>Ceriodaphnia dubia</i> Toxicity Tests	5
3.6 Fathead Minnow Toxicity Tests	6
3.7 Statistical Analysis	6
3.8 Chemical Analysis	6
3.9 Toxicity Identification Evaluation (TIE) Requirements	7
3.10 Toxicity Identification Evaluations (TIEs).....	7
3.10.1 Algae Phase I TIEs.....	8
4.0 RESULTS AND DISCUSSION	8
REFERENCES	

Identification of Causes of Toxicity to Green Algae (*Selenastrum capricornutum*) in Agriculture-Dominated Discharge Samples from the East San Joaquin Water Quality Coalition: January 2012 – December 2012

1.0 EXECUTIVE SUMMARY

A total of 87 samples of agriculturally-dominated discharge, collected from within the boundaries of the East San Joaquin Water Quality Coalition during twelve test events from January 2012 through December 2012, were screened for toxicity to green algae (*Selenastrum capricornutum*), the invertebrate *Ceriodaphnia dubia*, and/or larval fathead minnows (*Pimephales promelas*). Statistically significant toxicity was detected in 2 of 78 algae tests (3%), while none of the 79 *C. dubia* tests or 74 fathead minnow tests detected significant toxicity. Samples that produced $\geq 50\%$ effect on the test species were subjected to Phase I Toxicity Identification Evaluations (TIEs) to identify chemical class of the toxicant(s) responsible for the toxicity. Phase I chronic TIEs were conducted on the two samples that caused algae chronic toxicity. The Phase I TIE on the Lateral 3 along East Taylor Rd sample collected on 1/10/12 did not identify the cause of the toxicity, however, the TIE results indicated that cationic metals and non-polar organic chemicals were not the cause of the toxicity detected in this sample. The Phase I TIE conducted on the Highline Canal @ Lombardy Rd sample collected on 9/11/12 could not be interpreted since there was no significant toxicity detected in the baseline toxicity test, indicating that the sample lost all toxicity prior to the TIE.

2.0 INTRODUCTION

AQUA-Science (A-S) was retained by MLJ-LLC (Davis, CA) to conduct aquatic toxicity tests and toxicity identification evaluations (TIEs) on samples collected from the East San Joaquin Water Quality Coalition (ESJ) in response to monitoring requirements of the Conditional Waiver of Waste Discharge for Irrigated Lands (Ag Waiver). A total of 87 samples collected during twelve test events (January 2012 through December 2012) were tested using three-species toxicity tests with the cladoceran (*Ceriodaphnia dubia*), green algae (*Selenastrum capricornutum*), and larval fathead minnows (*Pimephales promelas*). A Phase I TIE was conducted on the two samples that caused algae chronic toxicity. Results of these analyses are reported herein.

3.0 MATERIALS AND METHODS

3.1 Test Events

Samples were collected during January 2012 through December 2012, including Management Plan Monitoring (MPM). The number of samples collected and tested for each species in each event is shown in Table 1 below:

Table 1. ESJ Testing Events and Toxic Samples

<i>Test Event</i>	<i>Sample Date (No. Samples)</i>	<i>No. Samples/Species</i>	<i>Toxic Samples/TIEs</i>
ESJ 12-01	1/10/12 (10 samples)	7 <i>Ceriodaphnia</i> 8 fathead minnow 9 algae	Lateral 3 along East Taylor Rd (algae) – Phase I TIE conducted
ESJ 12-02	2/7/12 (13 samples)	8 <i>Ceriodaphnia</i> 7 fathead minnow 12 algae	None
ESJ 12-03	3/6/12 (10 samples)	9 <i>Ceriodaphnia</i> 7 fathead minnow 7 algae	None
ESJ 12-04	4/12/12 (6 samples)	6 <i>Ceriodaphnia</i> 6 fathead minnow 6 algae	None
ESJ 12-05	5/9/12 (6 samples)	6 <i>Ceriodaphnia</i> 5 fathead minnow 5 algae	None
ESJ 12-06	6/12/12 (7 samples)	7 <i>Ceriodaphnia</i> 7 fathead minnow 7 algae	None
ESJ 12-07	7/10/12 (8 samples)	8 <i>Ceriodaphnia</i> 7 fathead minnow 7 algae	None
ESJ 12-08	8/14/12 (6 samples)	6 <i>Ceriodaphnia</i> 6 fathead minnow 6 algae	None
ESJ 12-09	9/11/12 (6 samples)	6 <i>Ceriodaphnia</i> 6 fathead minnow 6 algae	Highline Canal @ Lombardy Rd (algae) – Phase I TIE conducted
ESJ 12-10	10/9/12 (5 samples)	5 <i>Ceriodaphnia</i> 5 fathead minnow 5 algae	None

Table 1. ESJ Testing Events and Toxic Samples (continued)

<i>Test Event</i>	<i>Sample Date (No. Samples)</i>	<i>No. Samples/Species</i>	<i>Toxic Samples/TIEs</i>
ESJ 12-11	11/13/12 (4 samples)	4 <i>Ceriodaphnia</i> 4 fathead minnow 4 algae	None
ESJ 12-12	12/3/12 (6 samples)	6 <i>Ceriodaphnia</i> 6 fathead minnow 6 algae	None
Total	87 samples	79 <i>Ceriodaphnia</i> 74 fathead minnow 78 algae	2 Algae Phase I TIE

3.2 Test Samples

Test samples (4-5 gallons/site) were subsurface grabs obtained by MLJ-LLC sampling personnel under the direction of Dr. Mike Johnson. Sample name and GPS coordinates of the sample sites are shown in Table 2. Field measurements included temperature, dissolved oxygen (DO), pH, conductivity, and flow. Samples were placed in ice chests with sufficient wet ice to maintain sample temperature at ≤ 6 °C. Upon arrival at A-S, temperature, DO, conductivity, hardness, alkalinity, and pH were measured. Samples were stored in the dark at 4 °C until tested, within 24 hours of collection.

Table 2. ESJ Sample Information

<i>Site Name</i>	<i>Site ID</i>	<i>GPS Coordinates</i>
Bear Creek @ Kibby Rd	535XBCAKR	37.31280, -120.41380
Berenda Slough along Ave. 18½	545XBSAAE	37.01820, -120.32650
Deadman Creek @ Hwy 59	535DMCAHF	37.19810, -120.48690
Deadman Creek @ Gurr Rd	535XDCAGR	37.19514, -120.56126
Dry Creek @ Rd 18	545XDCARE	36.98180, -120.21950
Dry Creek @ Wellsford Rd	535XDCAWR	37.66020, -120.87430
Duck Slough @ Gurr Rd	535XDSAGR	37.21420, -120.55960
Highline Canal @ Lombardy Rd	535XHICALR	37.45560, -120.72070
Highline Canal @ Hwy 99	535XHCHNN	37.41530, -120.75570
Livingston Drain @ Robin Ave	535XLDARA	37.31690, -120.74230
Lateral 3 along East Taylor Rd	535LTAETR	37.53673, -120.98410
Levee Drain @ Carpenter Rd	535XLDACR	37.48062, -120.03106
McCoy Lateral @ Hwy 140	535XMLAHO	37.30945, -120.78759

3.3 Toxicity Test Protocols

The 96-hour acute *C. dubia* and 96-hour acute fathead minnow toxicity tests were conducted in accordance with the U.S. Environmental Protection Agency (USEPA) 5th edition protocol (USEPA 2002a). The 96-hour chronic algal toxicity tests were conducted in accordance with the USEPA 4th Edition protocol (USEPA 2002b). Control water for the toxicity tests and TIEs was reverse osmosis and granular carbon-treated well water amended with dry salts to attain USEPA moderately hard specifications (EPAMH).

3.4 Algae Toxicity Tests

Algal toxicity tests were conducted in 4 replicates of 125-mL flasks containing 50-mL of test sample filtrate (0.45 µm). A fifth replicate was used as a surrogate for daily water quality measurements. The flasks, containing algal assay media without EDTA, were inoculated with 1×10^4 cells/mL of a 2-4 day-old culture of *S. capricornutum* (University of Texas Algae Type Collection, Austin, TX) in log phase growth. A sixth replicate was tested without algae inoculate to confirm the absence of indigenous algae. This replicate was also used as a sample blank and for water quality measurements. Flasks were placed on a shaker table (100 rpm) in an environmental chamber at $25 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$ with continuous lighting (400 ± 40 fc) and were randomized twice daily. After the 96-hour test period, the absorbance was measured with a spectrophotometer at 750 nm (Model DR2800, Hach Co., Loveland, CO). The absorbance units were corrected to cell number using a calibration curve as follows:

$$\text{cell number} = (\text{absorbance units @ 750 nm} \times 13.026) - 0.0328 \quad (R^2 = 0.9995)$$

Using this conversion, the test was acceptable if the mean algal density in the control flasks was greater than or equal to 2×10^5 cells/mL and the coefficient of variation in the control replicates was $\leq 20\%$.

3.5 *Ceriodaphnia dubia* Toxicity Tests

C. dubia 96-hour acute toxicity tests were initiated with < 24 hour old neonates collected from in-house cultures. Each sample was tested using four replicates of 5 neonates each in a 20-mL glass scintillation vials containing 18-mL of test solutions. Test duration was 96 hours, and test solutions were renewed daily. *C. dubia* were fed a mixture of green algae (*S. capricornutum*) and YTC (a mixture of yeast, organic alfalfa and trout chow) 4 hours prior to 24-hour test solution renewal. Tests were conducted at $25 \pm 2 \text{ }^\circ\text{C}$ with a 16 hour light:8 hour dark photoperiod. Mortality was noted daily. The test was acceptable if control survival was $\geq 90\%$.

3.6 Fathead Minnow Toxicity Tests

Fathead minnows were obtained from Aquatox, Inc. (Hot Springs, AK), and were maintained in EPA moderately hard (EPAMH) water until tested at 6-10 days old. Each test sample was tested using 4 replicates of 10 fish each in 400 mL glass beakers containing 250 mL of test solutions. Test duration was 96 hours and test solutions were renewed daily. Fish were fed *Artemia* nauplii 4 hours prior to daily sample renewal. Tests were conducted at 25 ± 2 °C with a 16 hour light:8 hour dark photoperiod. Mortality was noted daily. The test was acceptable if control survival was $\geq 90\%$.

3.7 Statistical Analysis

Each test sample was subjected to statistical analysis using CETIS v. 1.8.4.13 (Tidepool Scientific, McKinleyville, CA, USA) according to USEPA procedures (USEPA 2002a,b) to determine if the observed effect was statistically different ($p < 0.05$) from the control. In the TIEs, mortality data from the dilution series tests were used to estimate EC_{50} values, e.g., the calculated concentration of the test sample that results a 50% effect on the test endpoint, respectively. Toxic units (TUs) were calculated from the EC_{50} values ($100/EC_{50}$). *Note that throughout this document, the term “EC” represents EC, IC or LC, as appropriate.*

3.8 Chemical Analysis

Beginning in July 2011, Normal Monitoring for organochlorines, group A pesticides, glyphosate and paraquat dichloride decreased to twice a year (August and a storm event) and arsenic, cadmium, lead and molybdenum monitoring decreased to four times a year (July, August and two storm events). Therefore, from January through March 2012 monthly Normal Monitoring events, selected water samples were collected for chemical analysis of five major groups of pesticides according to the decreased monitoring approval. One high TSS event was monitored in April 2012 and water samples were collected for analysis of pesticides (organochlorines, group A pesticides, organophosphates, triazines, and carbamates), glyphosate and paraquat dichloride, as well as total metals (arsenic, boron, cadmium, copper, lead, molybdenum, nickel, selenium and zinc) and dissolved metals (cadmium, copper, lead, nickel and zinc). Total and dissolved metals were analyzed using inductively coupled argon plasma-mass spectrometry analysis (EPA 200.8). On April 17, 2012 the Coalition was approved to temporarily suspend monitoring at Core and Management Plan Monitoring sites (with the exception of Bear Creek @ Kibby Rd) as well as reduce monitoring at Assessment Monitoring sites for the remainder of 2012 for Group A, paraquat, glyphosate, total kjeldahl nitrogen, total phosphorus (as P), *E. coli* and all metals except copper and zinc. The ESJWQC Quality Assurance Program Plan (QAPP) includes a list of all pesticides and metals that were analyzed, associated analytical methods,

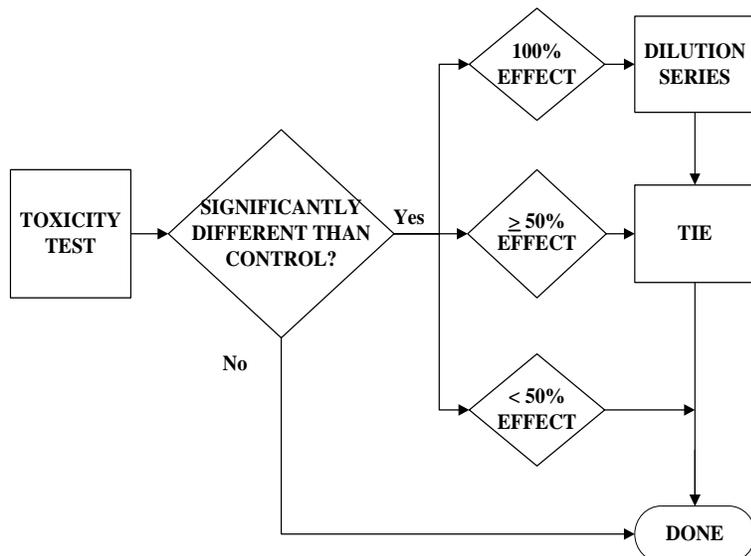
reporting limits and hold times. All samples were collected as outlined in the ESJWQC QAPP and met all quality assurance requirements.

Management Plan Monitoring was conducted at selected sites for toxicity as outlined in the ESJWQC Management Plan (from January through March), then the Coalition was approved to suspend Management Plan Monitoring from April through December 2012. Bear Creek @ Kibby Rd and Assessment Monitoring sites were monitored for management plan constituents throughout the 2012 monitoring year. Depending on the Management Plan schedule, this may or may not have corresponded with specific chemistry analysis.

3.9 Toxicity Identification Evaluation (TIE) Requirements

Figure 1 shows the TIE requirements for samples that show toxicity. Briefly, if the toxicity test detected < 50% effect when compared to the control, no further action was required. If a $\geq 50\%$ effect was detected, a TIE is initiated on the toxic sample. If there was a 100% effect, the TIE incorporated a dilution series toxicity test.

Figure 1. Toxicity Identification Evaluation (TIE) Requirements



3.10 Toxicity Identification Evaluations (TIEs)

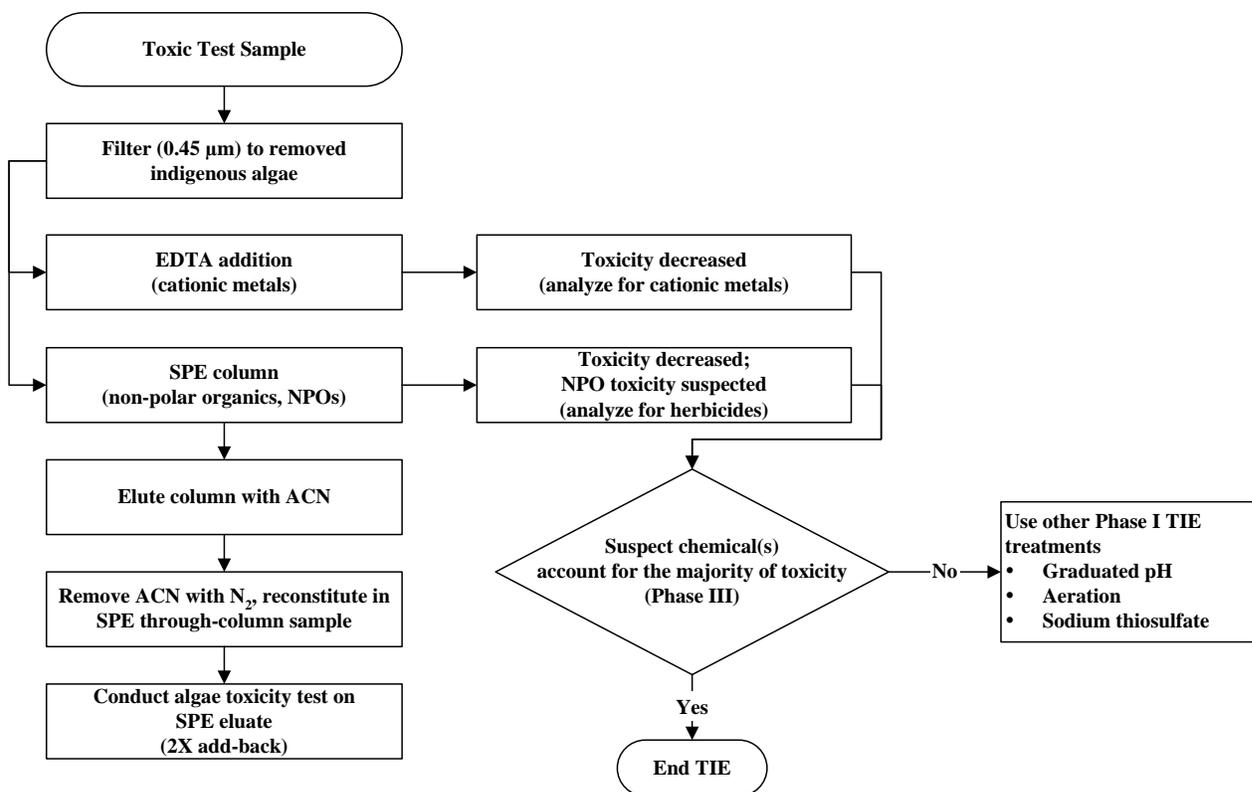
The purpose of the Phase I TIE is to identify the chemical class of the toxicant(s) in the test sample (USEPA 1991). The purpose of the Phase II TIE is to gain the identity of the material(s) responsible for the sample toxicity (USEPA 1993a). The purpose of the Phase III TIE is to

determine if there is a robust relationship between the concentrations of the suspected toxicant(s) identified, and the amount of toxicity measured in the test sample (USEPA 1993b).

3.10.1 Algae Phase I TIEs

Algae Phase I TIEs included a baseline toxicity test, solid phase extraction (SPE) column treatment to identify non-polar organic chemicals (NPOs), and ethylenediamine tetrachloroacetic acid (EDTA) addition to identify cationic metal toxicity. The SPE column was eluted three times with 1-mL of acetonitrile (ACN), which was reduced to dryness using a gentle stream of nitrogen. The ACN was removed from the eluate because algae are affected by even low concentrations of organic solvent (Miller *et al*, 2005). The eluate residue was reconstituted in the SPE through-column sample and added back to the sample at 2X. The algae Phase I TIE flow chart is shown in Figure 2 (Miller *et al*, 2005).

Figure 2. Phase I TIE Flowchart Modified for Algae



4.0 RESULTS AND DISCUSSION

Phase I and Phase III TIE results for algae are summarized below and in Table 3. Raw data for these TIEs is located in Appendix I and Appendix II.

A Phase I chronic algae TIE was conducted with the Lateral 3 along East Taylor Rd sample collected 1/10/12. The initial toxicity test reduced algae growth by 74%. The TIE baseline toxicity test detected 1.6 TUc. However, TIE procedures for cationic metals (EDTA addition) and non-polar organics (SPE column treatment) did not reduce the sample toxicity, indicating that neither of these two classes of toxicants were the cause of the toxicity. No analytical chemistry was available for this sample.

No significant toxicity was detected in the chronic algae Phase I TIE baseline toxicity test with the Highline Canal @ Lombardy Rd sample collected 9/11/12, indicating that the sample lost all detectable toxicity prior to the initiation of the TIE. The analytical results indicated that the dissolved metal concentrations detected in the sample (0.46 ug/L Cu²⁺ and 0.80 ug/L Zn²⁺) were below the toxic threshold for algae (USEPA Ecotox database; www.epa.gov/ecotox).

Approved by/Issue Date: Jeff Miller 1/24/13
Jeff Miller, Ph.D., DABT



Table 3. Summary of Chronic Algae TIEs

<i>Sample Date (TIE Date)</i>	<i>Test Sample (Sample ID)</i>	<i>Inhibition of Algal Growth</i>	<i>Phase I TIE Toxicity Reduced or Eliminated by Indicated Treatment</i>			<i>Suspected Toxicants</i>	<i>Phase III TIE</i>	<i>App. No.</i>
			<i>SPE Column^a</i>	<i>SPE Column Add-Back^b</i>	<i>EDTA Addition^c</i>			
1/10/12 (1/18/12)	Lateral 3 along East Taylor Rd (535LTAETR)		Slight Increase	No Effect	No Effect	Neither cationic metals nor non-polar organics were the cause of sample toxicity	No analytical chemistry conducted on this sample.	I
9/11/12 (9/19/12)	Highline Canal @ Lombardy Rd (535XHCALR)	n/a	n/a	n/a	n/a	No toxicity detected in baseline	Ammonia, 0 mg/L Copper (D), 0.46 ug/L Zinc (D), 0.80 ug/L Copper (T), 0.77 ug/L Zinc (T), 0 ug/L	II

- a Sample treated with C-8 solid-phase (SPE) extraction column
- b SPE column eluted and the eluate added back to the SPE through-column sample
- c Sample amended with ethylenediaminetetraacetic acid (EDTA)
- d Metals are analyzed as total (T) or dissolved (D)

REFERENCES

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APPENDIX I

ALGAE PHASE I TIEs

**ESJ 12-01 TIE
(1/10/12 Sample Date)**

**LATERAL 3 ALONG EAST TAYLOR RD
(535LTAETR)**

**Summary of Chronic Phase I Algae TIE
Lateral 3 along East Taylor Rd 1/10/12 Sample
(535LTAETR)**

<i>Treatment</i>	<i>Conc. (%)</i>	<i>Absorbance @ 750 nm</i>	<i>Cells/mL (x 10⁶)^a</i>	<i>Comments</i>
Baseline Toxicity Test	Control	0.0147	0.1626	EC ₂₅ = 61.9%
	25	0.0213	0.2733	TU _c = 1.6
	50	0.0347	0.4188	
	100	0.0043*	0.0236	
EDTA Tx	Control	0.0227	0.2625	EC ₂₅ = 60.9%
	25	0.0310	0.3710	TU _c = 1.6
	50	0.0300	0.3580	
	100	0.0033*	0.0106	
SPE Column Tx + Add-back	Control	0.0230	0.2668	EC ₂₅ = 35.0%
	25	0.0323	0.3884	TU _c = 2.8
	50	0.0133	0.1409	
	100	0.0037*	0.0150	
	MeOH 2X add-back	0.0245 0.0120	0.2863 0.1235	

Sample Date: 1/10/12

TIE Test Date: 1/18/12

a Cell number x 10⁶ = 13.026 x absorbance @ 750 nm – 0.0328

Conclusion:

TIE procedures for cationic metals (EDTA addition) and non-polar organics (SPE column treatment) did not reduce the sample toxicity, indicating that neither of these two classes of toxicants were the cause of the sample toxicity.

Algae Absorbance Conversion Calculations

ESJ 12-01 TIE (1/10/12): Lateral 3 along East Taylor Rd

Sample	Rep	Absorbance	Mean	% difference (abs)	Cell Number (13.026*abs-0.0328)	Mean	10 ⁶	Mean	% control (cell no)
Control	A	0.017			0.1886		188642		
	B	0.014							
	C	0.013	0.0147	--	0.1365	0.1626	136538	162590	--
25	A	0.023			0.2668		266798		
	B	0.017							
	C	0.024	0.0213	45	0.2798	0.2733	279824	273311	168
50	A	0.033			0.3971		397058		
	B	0.035			0.4231		423110		
	C	0.036	0.0347	136	0.4361	0.4188	436136	418768	258
100	A	0.004			0.0193		19304		
	B	0.005			0.0323		32330		
	C	0.004	0.0043	-70	0.0193	0.0236	19304	23646	15
EDTA Control	A	0.027			0.3189		318902		
	B	0.018			0.2017		201668		
	C	0.023	0.0227	--	0.2668	0.2625	266798	262456	--
25	A	0.035			0.4231		423110		
	B	0.036			0.4361		436136		
	C	0.022	0.0310	111	0.2538	0.3710	253772	371006	141
50	A	0.035			0.4231		423110		
	B	0.024			0.2798		279824		
	C	0.031	0.0300	105	0.3710	0.3580	371006	357980	136
100	A	0.007			0.0584		58382		
	B	0.001			-0.0198		-19774		
	C	0.002	0.0033	-77	-0.0067	0.0106	-6748	10620	4
Column Blank	A	0.023			0.2668		266798		
	B	0.022			0.2538		253772		
	C	0.024	0.0230		0.2798	0.2668	279824	266798	
25	A	0.030			0.3580		357980		
	B	0.032			0.3840		384032		
	C	0.035	0.0323	120	0.4231	0.3884	423110	388374	146
50	A	0.012			0.1235		123512		
	B	0.018			0.2017		201668		
	C	0.010	0.0133	-9	0.0975	0.1409	97460	140880	53
100	A	0.002			-0.0067		-6748		
	B	0.005			0.0323		32330		
	C	0.004	0.0037	-75	0.0193	0.0150	19304	14962	6
MeOH Blank	A	0.027			0.3189		318902		
	B	0.022	0.0245		0.2538	0.2863	253772	286337	
2X Addback	A	0.008			0.0714		71408		
	B	0.016	0.0120		0.1756	0.1235	175616	123512	

CETIS Summary Report

Report Date: 09 Feb-12 17:00 (p 1 of 1)
 Test Code: a1321201Ta | 16-7851-8067

Selenastrum Growth Test (w/o EDTA)

Aqua-Science

Batch ID: 11-2883-5068	Test Type: Cell Growth (w/o EDTA)	Analyst: K. Miller
Start Date: 18 Jan-12 14:26	Protocol: EPA/821/R-02-013 (2002)	Diluent: Laboratory Water w/ EDTA
Ending Date: 22 Jan-12 16:25	Species: Selenastrum capricornutum	Brine: Not Applicable
Duration: 4d 2h	Source: University of Texas-Botany Dept.	Age:
Sample ID: 19-1998-1085	Code: 7270961D	Client: MLJ-LLC
Sample Date: 10 Jan-12 10:50	Material: Ambient Sample	Project: Ag Waiver
Receive Date: 11 Jan-12 09:20	Source: Ag Waiver	
Sample Age: 8d 4h (3.8 °C)	Station: ESJ	

Sample Note: LATERAL 3 ALONG EAST TAYLOR RD - 535LTAETR

Test Note: BASELINE TOXICITY TEST

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
05-6495-8024	Light Absorbance	50	100	70.71	30.3%	2	Dunnett's Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
12-0826-2376	Light Absorbance	IC5	52.18	52.07	52.37	1.916	Linear Interpolation (ICPIN)
		IC10	54.46	54.23	54.85	1.836	
		IC15	56.83	56.47	57.45	1.76	
		IC20	59.31	58.81	60.17	1.686	
		IC25	61.89	61.24	63.01	1.616	
		IC40	70.32	69.14	72.35	1.422	
		IC50	76.55	74.95	79.32	1.306	

Light Absorbance Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	3	0.01467	0.01389	0.01544	0.013	0.017	0.001202	0.002082	14.19%	0.0%
25		3	0.02133	0.01992	0.02275	0.017	0.024	0.002186	0.003786	17.75%	-45.45%
50		3	0.03433	0.0339	0.03476	0.033	0.035	0.0006667	0.001155	3.36%	-134.1%
100		3	0.004333	0.004118	0.004549	0.004	0.005	0.0003333	0.0005774	13.32%	70.45%

Light Absorbance Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3
0	Negative Control	0.017	0.014	0.013
25		0.023	0.017	0.024
50		0.033	0.035	0.035
100		0.004	0.005	0.004

CETIS Summary Report

Report Date: 09 Feb-12 17:04 (p 1 of 1)
 Test Code: a1321201Tb | 00-9486-2113

Selenastrum Growth Test (w/o EDTA)

Aqua-Science

Batch ID:	13-1126-8267	Test Type:	Cell Growth (w/o EDTA)	Analyst:	K. Miller
Start Date:	18 Jan-12 14:26	Protocol:	EPA/821/R-02-013 (2002)	Diluent:	Laboratory Water w/ EDTA
Ending Date:	22 Jan-12 16:35	Species:	Selenastrum capricornutum	Brine:	Not Applicable
Duration:	4d 2h	Source:	University of Texas-Botany Dept.	Age:	
Sample ID:	19-1998-1085	Code:	7270961D	Client:	MLJ-LLC
Sample Date:	10 Jan-12 10:50	Material:	Ambient Sample	Project:	Ag Waiver
Receive Date:	11 Jan-12 09:20	Source:	Ag Waiver		
Sample Age:	8d 4h (3.8 °C)	Station:	ESJ		

Sample Note: LATERAL 3 ALONG EAST TAYLOR RD - 535LTAETR

Test Note: EDTA TREATMENT

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
04-8314-2163	Light Absorbance	50	100	70.71	46.5%	2	Dunnett's Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
16-9814-9258	Light Absorbance	IC5	52.02	16.58	52.54	1.922	Linear Interpolation (ICPIN)
		IC10	54.11	43.88	55.21	1.848	
		IC15	56.29	47.56	58	1.776	
		IC20	58.56	49.97	60.93	1.708	
		IC25	60.91	52.49	64.27	1.642	
		IC40	68.55	60.8	75.59	1.459	
		IC50	74.16	66.71	83.73	1.348	

Light Absorbance Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	3	0.02267	0.02098	0.02435	0.018	0.027	0.002603	0.004509	19.89%	0.0%
25		3	0.03133	0.02863	0.03403	0.023	0.036	0.004177	0.007234	23.09%	-38.24%
50		3	0.03	0.02792	0.03208	0.024	0.035	0.003215	0.005568	18.56%	-32.35%
100		3	0.003333	0.002133	0.004534	0.001	0.007	0.001856	0.003215	96.44%	85.29%

Light Absorbance Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3
0	Negative Control	0.027	0.018	0.023
25		0.035	0.036	0.023
50		0.035	0.024	0.031
100		0.007	0.001	0.002

CETIS Analytical Report

Report Date: 09 Feb-12 17:04 (p 1 of 1)
 Test Code: a1321201Tb | 00-9486-2113

Selenastrum Growth Test (w/o EDTA)

Aqua-Science

Analysis ID: 04-8314-2163 Endpoint: Light Absorbance CETIS Version: CETISv1.8.0
 Analyzed: 09 Feb-12 17:03 Analysis: Parametric-Control vs Treatments Official Results: Yes

Data Transform	Zeta	Alt Hyp	MC Trials	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	0	C > T	Not Run	50	100	70.71	2	46.5%

Dunnett's Multiple Comparison Test

Control	vs	Conc-%	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)
Negative Control		25	-1.988	2.417	4	0.01053	0.9947	Non-Significant Effect
		50	-1.682	2.417	4	0.01053	0.9900	Non-Significant Effect
		100*	4.435	2.417	4	0.01053	0.0028	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.001499667	0.0004998888	3	17.54	0.0007	Significant Effect
Error	0.000228	0.0000285	8			
Total	0.001727667	0.0005283888	11			

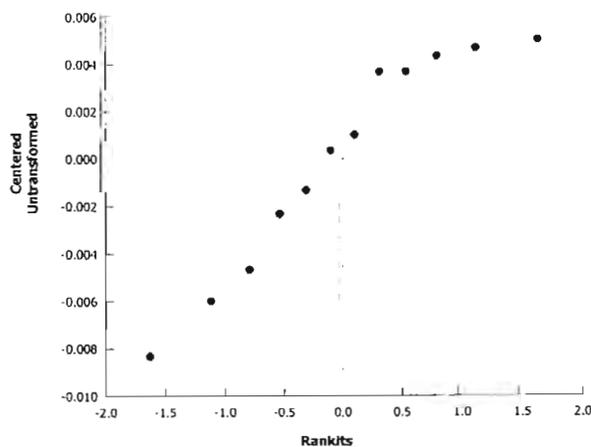
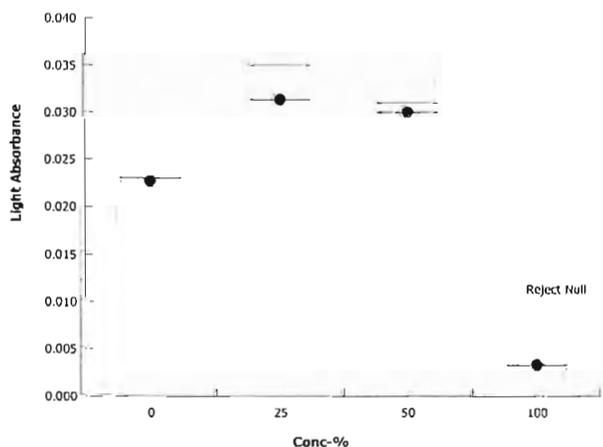
Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.093	11.34	0.7788	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9086	0.8025	0.2046	Normal Distribution

Light Absorbance Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	3	0.02267	0.02095	0.02438	0.018	0.027	0.002603	0.004509	19.89%	0.0%
25		3	0.03133	0.02858	0.03409	0.023	0.036	0.004177	0.007234	23.09%	-38.24%
50		3	0.03	0.02788	0.03212	0.024	0.035	0.003215	0.005568	18.56%	-32.35%
100		3	0.003333	0.002111	0.004556	0.001	0.007	0.001856	0.003215	96.44%	85.29%

Graphics



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ALGAL BIOASSAY - CELL DETERMINATION

Test Number:	ESJ 12-01 TIE	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/013	Technicians:	Walker/Concepcion/Sanford/Berry/Renn
Test Material:	ESJ Ag Waiver Sample Lateral 3 535LTAETR (011012) WITHOUT EDTA Baseline		
Test Species:	<i>Selenastrum capricornutum</i>	Animal Lot No.:	UTEX: 082811
Initiation Date:	January 18, 2012	Termination Date:	January 22, 2012

Absorbance @ 750 nm with Hach DR 2800 Spectrophotometer		Test Terminated @ 1625			
Conc. (%)	Replicate	1	2	3	Mean
Baseline Control	A	0.017	0.017	0.016	0.017
	B	0.014	0.014	0.014	0.014
	wq	0.013	0.013	0.013	0.013
25	A	0.023	0.023	0.024	0.023
	B	0.017	0.017	0.017	0.017
	wq	0.024	0.024	0.023	0.024
50	A	0.033	0.033	0.033	0.033
	B	0.035	0.035	0.035	0.035
	wq	0.035	0.036	0.035	0.035
100	A	0.003	0.005	0.004	0.004
	B	0.006	0.006	0.004	0.005
	wq	0.004	0.004	0.004	0.004

w/o algae

Baseline Control	A	0.002	0.002	0.002	0.002
25%	A	0.002	0.002	0.002	0.002
50%	A	0.002	0.002	0.002	0.002
100%	A	0.002	0.006	0.006	0.006

Technician: BAW

Date: 01/22/12

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Environmental Toxicology Specialists
ALGAL BIOASSAY - CELL DETERMINATION

Test Number:	ESJ 12-01 TIE	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/013	Technicians:	Walker/Concepcion/Sanford/Berry/Renn
Test Material:	ESJ Ag Waiver Sample Lateral 3 535LTAETR (011012) WITHOUT EDTA +1 mg/L EDTA		
Test Species:	<i>Selenastrum capricornutum</i>	Animal Lot No.:	UTEX: 082811
Initiation Date:	January 18, 2012	Termination Date:	January 22, 2012

Absorbance @ 750 nm with Hach DR 2800 Spectrophotometer				Test Terminated @ 1635	
Conc. (%)	Replicate	1	2	3	Mean
EDTA Control	A	0.024	0.028	0.027	0.027
	B	0.018	0.018	0.019	0.018
	C	0.023	0.023	0.023	0.023
	D				
25	A	0.035	0.035	0.035	0.035
	B	0.035	0.035	0.037	0.036
	C	0.022	0.022	0.022	0.022
	D				
50	A	0.034	0.035	0.035	0.035
	B	0.024	0.024	0.24	0.024
	C	0.032	0.031	0.031	0.031
	D				
100	A	0.002	0.002	0.002	0.002
	B	0.002	0.001	0.001	0.001
	C	0.002	0.003	0.002	0.002
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				

w/o algae					
EDTA Control	A	0.002	0.003	0.002	0.002
25%	A	0.001	0.001	0.002	0.001
50%	A	0.004	0.004	0.004	0.004
100%	A	0.008	0.008	0.008	0.008

Technician: 

Date: 01/22/12

AQUA-Science
Environmental Toxicology Specialists
ALGAL BIOASSAY - CELL DETERMINATION

Test Number:	ESJ 12-01 TIE	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/013	Technicians:	Walker/Concepcion/Sanford/Berry/Renn
Test Material:	ESJ Ag Waiver Sample Lateral 3 535LTAETR (011012) WITHOUT EDTA C-8 SPE + Add-back		
Test Species:	<i>Selenastrum capricornutum</i>	Animal Lot No.:	UTEX: 082811
Initiation Date:	January 18, 2012	Termination Date:	January 22, 2012

Absorbance @ 750 nm with Hach DR 2800 Spectrophotometer				Test Terminated @ 1645	
Conc. (%)	Replicate	1	2	3	Mean
Column Blank	A	0.022	0.023	0.023	0.023
	B	0.021	0.023	0.022	0.022
	WR	0.025	0.024	0.024	0.024
25 C-8 TC	A	0.029	0.030	0.030	0.030
	B	0.033	0.032	0.032	0.032
	WR	0.036	0.035	0.035	0.035
50 C-8 TC	A	0.012 0.017	0.012 0.017	0.012 0.017	0.012 0.017
	B	0.013 0.023	0.013 0.023	0.013 0.024	0.013 0.023
	WR	0.016 0.011	0.015 0.010	0.015 0.010	0.015 0.010
100 C-8 TC	A	0.003	0.002	0.002	0.002
	B	0.004	0.005	0.006	0.005
	WR	0.005	0.005	0.003	0.004
Tx. Control	A	0.029	0.027	0.026	0.027
	B	0.022	0.022	0.022	0.022
2X Addback	A	0.008	0.008	0.007	0.008
	B	0.016	0.016	0.016	0.016

w/o algae

Column Blank	A	0.001	0.001	0.001	0.001
25% TC	A	0.002	0.002	0.002	0.002
50% TC	A	0.005	0.005	0.005	0.005
100% TC	A	0.006	0.007	0.006	0.006

Technician:



Date:

01/22/12

ENTRY ERROR ESJ/22/12

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ALGAL BIOASSAY DATA SHEETS

7.0 LAB NOTES

1/18/12

ESJ 12-01 TIE

96 Hr. Static Growth

*Selenastrum capricornutum***1.0 Stock Preparation**

0.8 liters of Ambient samples (1/10/2012) vacuum filtered through 0.22 µm Metrigard filter

1.2 liters of R/O EPAMH vacuum filtered through 0.22 µm Metrigard filter

2.0 Water Quality Measurement

Water quality of dilution and effluent measured after addition of EPA Algal Assay Media (AAM).

Section 3.0, page 1

3.0 Algal Assay Media AdditionAlgal Assay Media prepared as per EPA 821/R-02/013; Section 14 - Tables 1 and 2 (**without EDTA**)

Added 0.39 mL each of #1-5 EPA AAM to 0.39 L:

Lateral 3 along East Taylor Rd

Added 0.39 mL each of #1-5 EPA AAM to 0.39 liters R/O EPAMH:

Control Water

Added 0.39 mL each of #1-5 EPA AAM to 0.39 L Ctrl. Water

+1 mg/L EDTA Control

Added 0.39 mL each of #1-5 EPA AAM to 0.39 L Ambient

+1 mg/L EDTA Control

Added 0.39 mL each of #1-5 EPA AAM to 0.39 L of

C-8 Column Blank

Added 0.39 mL each of #1-5 EPA AAM to 0.39 L of

C-8 SPE TC

Added 0.1 mL each of #1-5 EPA AAM to 0.1 L:

Tx Control

Added 0.1 mL each of #1-5 EPA AAM to 0.1 L:

2X Add-back

Stirred samples on magnetic stir plates for approximately 10 minutes.

4.0 Background Counts

Particle background counts measured with electronic particle counter - Coulter Counter, model ZBI.

Counts recorded on section 5.0, page 2.

5.0 Exposure Series Preparation and Algal Inoculation

All concentrations prepared as described in section 4.0, page 1, and held in 250 mL solution beakers.

All solutions were inoculated with pure culture algal stock in log phase growth to achieve a concentration of 10,000 cells/mL, calculations in section 6.0, page 2. All solutions stirred thoroughly.

Divided the 150 mL in solution beaker into 3-50mL aliquots and distributed into flasks A, B and C (water quality rep). Placed flasks randomly on a shaker table with 100 rpm continuous rotation in environmental chamber.

Continuous light at 25°C ± 1°C.

All flasks randomly rotated twice daily.

Technician:



Date:

01/18/12

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TIE ADD-BACK CALCULATION DATA SHEET

Client:	<u>MLJ - LLC</u>	Test Species:	<u>ALGAE</u>
TIE No.:	<u>ESJ 12-01</u>	TIE Type:	<u>PHASE 1</u>
Sample Date:	<u>01/10/12</u>		

1.0 Through-column volume: 500 mL

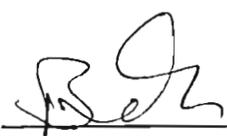
2.0 Eluate concentration factor:

$$\text{Through-column volume } \underline{500} \text{ mL} / \text{eluate volume } \underline{2} \text{ mL} = \underline{250} \text{ X/mL}$$

3.0 Total volume of add-back needed:

$$\underline{2} \text{ reps X } \underline{50} \text{ mL/rep} = \underline{100} \text{ mL}$$

Calculations
Add-Back Level 1: <u>2X</u>
$\underline{100} \text{ mL total volume needed X add-back level } \underline{2} / \text{eluate concentration factor } \underline{250} \text{ X/mL}$ $= \underline{0.8} \text{ mL (} \underline{800} \text{ } \mu\text{L) of eluate}$
Add-Back Level 2: <u>N/A</u>
$\underline{\hspace{2cm}} \text{ mL total volume needed X add-back level } \underline{\hspace{2cm}} / \text{eluate concentration factor } \underline{\hspace{2cm}} \text{ X/mL}$ $= \underline{\hspace{2cm}} \text{ mL (} \underline{\hspace{2cm}} \text{ } \mu\text{L) of eluate}$

Processed by/Date:  01/17/12

AQUA-Science

Environmental Toxicology Specialists

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	ESJ 12-01 TIE	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/013	Technicians:	Walker/Concepcion/Sanford/Berry/Renn
Test Material:	ESJ Ag Waiver Sample Lateral 3 535LTAETR (011012) WITHOUT EDTA		
Test Species:	<i>Selenastrum capricornutum</i>	Animal Lot No.:	UTEX: 082811
Initiation Date:	January 18, 2012	Termination Date:	January 22, 2012

Ambient Concentration (%)	OBSERVATIONS: Day: 0 1/18/2012					96 Hour Observations			
	Temperature (°C)	Dissolved Oxygen*	pH ^{^^}	Alkalinity **/ Hardness~	Conductivity [^]	Temp	D.O.*	pH ^{^^}	Cond. [^]
BASELINE									
Baseline Control	24	6.2	8.11	70/100	371	24	7.7	8.27	464
25	24	6.3	7.99	120/170	501	25	7.6	8.32	519
50	24	6.8	7.94	210/280	639	24	7.7	8.53	652
100	24	7.2	7.88	770/340	916	25	7.7	8.46	698
+1 mg/L EDTA									
EDTA Control	24	6.2	8.13	80/90	374	24	7.7	8.24	410
25	24	6.3	7.98	120/150	503	25	7.7	8.34	516
50	24	6.7	7.93	220/280	638	25	7.6	8.53	648
100	24	7.1	7.91	280/320	919	24	7.7	8.46	699
SPE C-8									
Column Blank	25	6.8	8.16	60/90	389	24	7.7	8.32	421
25	25	6.8	8.07	120/180	510	24	7.6	8.36	521
50	25	6.8	8.03	200/240	651	24	7.6	8.53	657
100	25	6.9	7.96	250/300	907	24	7.7	8.46	700
					Tech Init/Date	SR 01/22/12			

UNIT INSTRUMENTATION LEGEND

*=Dissolved oxygen (mg/L): Meter ID 05

**Alkalinity (mg/L CaCO₃): HACH Test Kit

^^= pH: Meter ID 02

~=Water Hardness (mg/L CaCO₃): HACH Test Kit

^=Conductivity/Salinity (µmohs): Meter ID 03

ADDITIONAL COMMENTS: 1 MEASUREMENT TAKEN IN 10ml SAMPLE VOL. 1/18/12

Lab Control = 2x carbon filtered reverse osmosis water at EPA moderately hard level using EPA salts.

Control water ID = R10 EPAMH # 004 FOR COL BIK

R10 EPAMH # 005 FOR BASELINE AND BASE + EDTA

All surface waters filtered through a 60 µm screen daily

Technician: 

Date: 01/18/12

AQUA-Science
Environmental Toxicology Specialists

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	ESJ 12-01 TIE	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/013	Technicians:	Walker/Concepcion/Sanford/Berry/Renn
Test Material:	ESJ Ag Waiver Sample Lateral 3 535LTAETR (011012) WITHOUT EDTA		
Test Species:	<i>Selenastrum capricornutum</i>	Animal Lot No.:	UTEX: 082811
Initiation Date:	January 18, 2012	Termination Date:	January 22, 2012

Ambient Concentration (%)	Day 1				Day 2				Day 3			
	Temp.	D.O.*	pH^^	Cond.^	Temp.	D.O.*	pH^^	Cond.^	Temp.	D.O.*	pH^^	Cond.^
BASELINE												
Baseline Control	25	7.6	8.22	441	25	7.6	8.15	455	25	8.1	8.19	460
25	25	7.6	8.24	508	25	7.6	8.19	511	25	8.0	8.26	516
50	25	7.5	8.46	891	25	7.6	8.34	653	25	8.0	8.44	653
100	25	7.5	8.46	891	25	7.7	8.34	780	25	7.9	8.38	704
+1 mg/L EDTA												
EDTA Control	25	7.6	8.31	383	25	7.6	8.09	391	25	8.0	8.22	400
25	25	7.6	8.28	511	25	7.6	8.14	511	25	7.9	8.31	512
50	25	7.6	8.27	644	25	7.6	8.30	646	25	8.0	8.36	646
100	25	7.5	8.53	898	25	7.6	8.31	770	25	7.9	8.39	700
SPE C-8												
Column Blank	25	7.5	8.45	398	25	7.6	8.15	408	25	6.9	8.34	418
25	25	7.5	8.34	516	25	7.6	8.20	519	25	6.9	8.38	523
50	25	7.5	8.39	655	25	7.6	8.34	655	26	6.9	8.51	658
100	25	7.5	8.54	896	25	7.6	8.35	810	26	6.9	8.43	707
Tech Init/Date	BES 01/19/12				SR 01/20/12				SR 01/21/12			

UNIT INSTRUMENTATION LEGEND

*=Dissolved oxygen (mg/L): Meter ID 05
 ^^= pH: Meter ID 02
 ^=Conductivity/Salinity (µmohs): Meter ID 03

ADDITIONAL COMMENTS: ENTRY ERROR 01/19/12

Water Quality taken in "C" replicate
 EPA Algal Assay Media (AAM) Without EDTA

APPENDIX II

ALGAE PHASE I TIEs

**ESJ 12-09 TIE
(9/11/12 Sample Date)**

**HIGHLINE CANAL @ LOMBARDY RD
(535XHCALR)**

**Summary of Chronic Phase I Algae TIE
Highline Canal @ Lombardy Rd 9/11/12 Sample
(535XHCALR)**

<i>Treatment</i>	<i>Conc. (%)</i>	<i>Absorbance @ 750 nm</i>	<i>Cells/mL (x 10⁶)^a</i>	<i>Comments</i>
Baseline	Lab Control	0.0425	0.5208	No toxicity detected in baseline
	25	0.0650	0.8139	
	50	0.0615	0.7683	
	100	0.0995	1.2633	
C-8 SPE Column Treatment	Column Blank	0.0290	0.3450	
	25	0.0290	0.3450	
	50	0.0435	0.5338	
	100	0.1085	1.3805	
EDTA Treatment	EDTA Control	0.0685	0.8595	
	25	0.0600	0.7488	
	50	0.0800	1.0093	
	100	0.1250	1.5955	

Sample Date: 9/11/12

Test Date: 9/19/12

a Cell number x 10⁶ = 13.026 x absorbance @ 750 nm – 0.0328

Conclusion:

The initial toxicity test reduced algae growth (cells/mL) by 51%. However, the TIE baseline toxicity test did not detect toxicity, indicating the sample lost all detectable toxicity prior to initiation of the TIE.

Algae Absorbance Conversion Calculations

ESJ 12-09 TIE (9/11/12): HIGHLINE CANAL @ LOMBARDY RD (535XHCALR)

Sample	Rep	Absorbance	% UNIT DIFFERENCE		Cell Number/10 ⁶		Mean	Cell Number	Mean	(cell no) ^b
			Mean	(abs) ^a	(13.026*abs-0.0328)	Mean				
Control	A	0.045			0.5534			553370		
	B	0.040	0.0425	--	0.4882	0.5208	488240	520805	--	
25	A	0.066			0.8269		826916			
	B	0.064	0.0650	53	0.8009	0.8139	800864	813890	156	
50	A	0.059			0.7357		735734			
	B	0.064	0.0615	45	0.8009	0.7683	800864	768299	148	
100	A	0.093			1.1786		1178618			
	B	0.106	0.0995	134	1.3480	1.2633	1347956	1263287	243	
EDTA Control	A	0.071			0.8920		892046			
	B	0.066	0.0685	--	0.8269	0.8595	826916	859481	--	
25	A	0.054			0.6706		670604			
	B	0.066	0.0600	41	0.8269	0.7488	826916	748760	87	
50	A	0.080			1.0093		1009280			
	B	0.080	0.0800	88	1.0093	1.0093	1009280	1009280	117	
100	A	0.125			1.5955		1595450			
	B	0.125	0.1250	194	1.5955	1.5955	1595450	1595450	186	
Column Blank	A	0.032			0.3840		384032			
	B	0.026	0.0290		0.3059	0.3450	305876	344954		
25	A	0.031			0.3710		371006			
	B	0.027	0.0290	-32	0.3189	0.3450	318902	344954	100	
50	A	0.044			0.5403		540344			
	B	0.043	0.0435	2	0.5273	0.5338	527318	533831	155	
100	A	0.104			1.3219		1321904			
	B	0.113	0.1085	155	1.4391	1.3805	1439138	1380521	400	

a Percent difference = [(sample abs/control abs)-1]*100

b Percent control = (sample cell no/control cell no)*100

AQUA-Science
 Environmental Toxicology Specialists
ALGAL BIOASSAY - CELL DETERMINATION

Test Number:	ESJ 12-09 TIE	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/013	Technicians:	Walker/Sanford/Renn/McIntyre/King
Test Material:	ESJ Ag Waiver Sample Highline @ Lombardy - 535XHCALR (091112) WITHOUT EDTA Baseline		
Test Species:	<i>Selenastrum capricornutum</i>	Animal Lot No.:	UTEX: 041212
Initiation Date:	September 19, 2012	Termination Date:	September 23, 2012

Absorbance @ 750 nm with Hach DR 2800 Spectrophotometer					Test Terminated @	1507
Conc. (%)	Replicate	1	2	3	Mean	
Baseline Control	A	0.045	0.045	0.045	0.045	
	B	0.040	0.041	0.040	0.040	
25	A	0.066	0.066	0.066	0.066	
	B	0.064	0.064	0.065	0.064	
50	A	0.059	0.059	0.058	0.059	
	B	0.064	0.064	0.064	0.064	
100	A	0.093	0.093	0.093	0.093	
	B	0.106	0.107	0.106	0.106	

w/o algae

Baseline Control	A	0.004	0.004	0.004	0.004
25%	A	0.012	0.012	0.012	0.012
50%	A	0.015	0.016	0.015	0.015
100%	A	0.007	0.007	0.006	0.007

Technician: BS Date: 9/23/12
 counter error BS 9/23/12

AQUA-Science
 Environmental Toxicology Specialists
ALGAL BIOASSAY - CELL DETERMINATION

Test Number:	ESJ 12-09 TIE	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/013	Technicians:	Walker/Sanford/Renn/McIntyre/King
Test Material:	ESJ Ag Waiver Sample Highline @ Lombardy - 535XHCALR (091112) WITHOUT EDTA +1 mg/L EDTA		
Test Species:	<i>Selenastrum capricornutum</i>	Animal Lot No.:	UTEX: 041212
Initiation Date:	September 19, 2012	Termination Date:	September 23, 2012

Absorbance @ 750 nm with Hach DR 2800 Spectrophotometer		Test Terminated @ <u>1517</u>			
Conc. (%)	Replicate	1	2	3	Mean
EDTA Control	A	0.071	0.071	0.071	0.071
	B	0.065	0.066	0.066	0.066
	C				
	D				
25	A	0.055	0.054	0.054	0.054
	B	0.066	0.067	0.066	0.066
	C				
	D				
50	A	0.080	0.080	0.080	0.080
	B	0.080	0.080	0.081	0.080
	C				
	D				
100	A	0.125	0.125	0.126	0.125
	B	0.127	0.124	0.124	0.125
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				

w/o algae

EDTA Control	A	0.008	0.009	0.008	0.008
25%	A	0.011	0.012	0.012	0.012
50%	A	0.012	0.012	0.012	0.012
100%	A	0.009	0.009	0.009	0.009

Technician: 

Date: 9/23/12

AQUA-Science
 Environmental Toxicology Specialists
ALGAL BIOASSAY - CELL DETERMINATION

Test Number:	ESJ 12-09 TIE	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/013	Technicians:	Walker/Sanford/Renn/McIntyre/King
Test Material:	ESJ Ag Waiver Sample Highline @ Lombardy - 535XHCALR (091112) WITHOUT EDTA C-8 SPE		
Test Species:	<i>Selenastrum capricornutum</i>	Animal Lot No.:	UTEX: 041212
Initiation Date:	September 19, 2012	Termination Date:	September 23, 2012

Absorbance @ 750 nm with Hach DR 2800 Spectrophotometer				Test Terminated @ <u>1525</u>	
Conc. (%)	Replicate	1	2	3	Mean
Column Blank	A	0.031	0.032	0.032	0.032
	B	0.026	0.026	0.026	0.026
25 C-8 TC	A	0.031	0.031	0.030	0.031
	B	0.027	0.026	0.029	0.027
50 C-8 TC	A	0.045	0.045	0.043	0.044
	B	0.043	0.043	0.043	0.043
100 C-8 TC	A	0.105	0.106	0.102	0.104
	B	0.115	0.114	0.110	0.113

w/o algae

Column Blank	A	0.011	0.011	0.012	0.011
25% TC	A	0.008	0.008	0.008	0.008
50% TC	A	0.013	0.013	0.012	0.013
100% TC	A	0.017	0.016	0.016	0.016

Technician: _____



Date: _____

09/23/12

AQUA-Science
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ALGAL BIOASSAY DATA SHEETS

7.0 LAB NOTES

9/19/12

ESJ 12-09 TIE

96 Hr. Static Growth

*Selenastrum capricornutum***1.0 Stock Preparation**

0.8 liters of Ambient samples (9/11/2012) vacuum filtered through 0.22 µm Metrigard filter

1.2 liters of R/O EPAMH vacuum filtered through 0.22 µm Metrigard filter

2.0 Water Quality Measurement

Water quality of dilution and effluent measured after addition of EPA Algal Assay Media (AAM).

Section 3.0, page 1

3.0 Algal Assay Media AdditionAlgal Assay Media prepared as per EPA 821/R-02/013; Section 14 - Tables 1 and 2 (**without EDTA**)

Added 0.39 mL each of #1-5 EPA AAM to 0.39 L:

Highline Canal @ Lombardy Rd

Added 0.39 mL each of #1-5 EPA AAM to 0.39 liters R/O EPAMH:

Control Water

Added 0.39 mL each of #1-5 EPA AAM to 0.39 L Ctrl. Water

+1 mg/L EDTA Control

Added 0.39 mL each of #1-5 EPA AAM to 0.39 L Ambient

+1 mg/L EDTA Control

Added 0.39 mL each of #1-5 EPA AAM to 0.39 L of

C-8 Column Blank

Added 0.39 mL each of #1-5 EPA AAM to 0.39 L of

C-8 SPE TC

Stirred samples on magnetic stir plates for approximately 10 minutes.

4.0 Background Counts

Particle background counts measured with electronic particle counter - Coulter Counter, model ZBI.

Counts recorded on section 5.0, page 2.

5.0 Exposure Series Preparation and Algal Inoculation

All concentrations prepared as described in section 4.0, page 1, and held in 250 mL solution beakers.

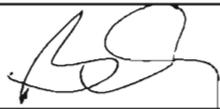
All solutions were inoculated with pure culture algal stock in log phase growth to achieve a concentration of 10,000 cells/mL, calculations in section 6.0, page 2. All solutions stirred thoroughly.

Divided the 150 mL in solution beaker into 3-50mL aliquots and distributed into flasks A, B and C (water quality rep). Placed flasks randomly on a shaker table with 100 rpm continuous rotation in environmental chamber.

Continuous light at 25°C ± 1°C.

All flasks randomly rotated twice daily.

Technician:



Date:

09/19/12

AQUA-Science

Environmental Toxicology Specialists

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	ESJ 12-09 TIE	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/013	Technicians:	Walker/Sanford/Renn/McIntyre/King
Test Material:	ESJ Ag Waiver Sample Highline @ Lombardy - 535XHCALR (091112) WITHOUT EDTA		
Test Species:	<i>Selenastrum capricornutum</i>	Animal Lot No.:	UTEX: 041212
Initiation Date:	September 19, 2012	Termination Date:	September 23, 2012

Ambient Concentration (%)	OBSERVATIONS: Day: 0 9/19/2012					96 Hour Observations			
	Temperature (°C)	Dissolved Oxygen*	pH ^{^^}	Alkalinity **/ Hardness~	Conductivity ^	Temp	D.O.*	pH ^{^^}	Cond. ^
BASELINE									
Baseline Control	25	6.6	7.98	80/100	419	24	8.2	8.04	513
25	25	6.6	8.07	70/70	448344	24	8.2	8.07	386
50	25	6.7	8.14	50/70	346200	24	8.3	8.08	305
100	25	6.4	8.28	30/40	148	24	8.2	8.15	195
+1 mg/L EDTA									
EDTA Control	25	6.6	8.15	—	415	24	8.3	8.01	486
25	25	6.7	8.18	—	346	24	8.2	8.05	367
50	25	6.8	8.22	—	281	24	8.2	8.05	301
100	25	6.8	8.38	—	151	24	8.2	8.13	171
SPE C-8									
Column Blank	25	7.0	8.50	—	434	24	8.2	8.09	511
25	25	7.1	8.57	—	351	24	8.3	8.10	390
50	25	7.1	8.54	—	286	24	8.2	8.09	308
100	25	7.1	8.66	—	161	24	8.2	8.13	180
					Tech Init/Date	SR 09/23/12			

UNIT INSTRUMENTATION LEGEND

*=Dissolved oxygen (mg/L): Meter ID 05

**Alkalinity (mg/L CaCO₃); HACH Test Kit

^^= pH: Meter ID 02

~=Water Hardness (mg/L CaCO₃); HACH Test Kit

^=Conductivity/Salinity (µmohs): Meter ID 07

ADDITIONAL COMMENTS: ① MEASUREMENT TAKEN IN 100ml SAMPLE VOL. EC5 9/19/12

Lab Control = 2x carbon filtered reverse osmosis water at EPA moderately hard level using EPA salts.

Control water ID = 2/0 EPAWH #119

All surface waters filtered through a 60 µm screen daily

Technician: _____



Date: _____

09/19/12

AQUA-Science
Environmental Toxicology Specialists

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	ESJ 12-09 TIE	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/013	Technicians:	Walker/Sanford/Renn/McIntyre/King
Test Material:	ESJ Ag Waiver Sample Highline @ Lombardy - 535XHCALR (091112) WITHOUT EDTA		
Test Species:	<i>Selenastrum capricornutum</i>	Animal Lot No.:	UTEX: 041212
Initiation Date:	September 19, 2012	Termination Date:	September 23, 2012

Ambient Concentration (%)	Day 1				Day 2				Day 3			
	Temp.	D.O.*	pH^^	Cond.^	Temp.	D.O.*	pH^^	Cond.^	Temp.	D.O.*	pH^^	Cond.^
BASELINE												
Baseline Control	24	8.0	8.34	437	24	8.1	8.29	479	25	7.7	8.01	494
25	24	8.0	8.40	399	24	8.0	8.21	367	25	7.7	8.07	374
50	24	7.9	8.44	287	24	8.0	8.20	299	25	7.8	8.10	300
100	24	7.9	8.63	163	24	7.9	8.33	186	25	7.8	8.27	190
+1 mg/L EDTA												
EDTA Control	25	7.9	8.23	434	24	8.1	8.04	463	25	7.8	8.00	468
25	25	7.9	8.26	392	24	8.0	8.09	397	25	7.8	8.08	359
50	25	7.9	8.28	289	24	8.0	8.09	294	26	7.8	8.10	296
100	25	7.9	8.46	158	24	7.9	8.17	163	26	7.7	8.23	167
SPE C-8												
Column Blank	25	8.1	8.13	464	24	8.1	7.83	491	26	7.7	7.98	498
25	25	8.0	8.21	360	24	7.9	7.91	373	26	7.7	7.99	384
50	25	7.9	8.24	293	24	7.9	7.99	298	26	7.8	8.04	301
100	25	7.9	8.38	167	24	7.9	8.06	172	26	7.7	8.16	176
Tech Init/Date	KK 09/20/12				KK 09/21/12				KCM 09/22/12			

UNIT INSTRUMENTATION LEGEND

*=Dissolved oxygen (mg/L): Meter ID 09
 ^^= pH: Meter ID 02
 ^=Conductivity/Salinity (µmohs): Meter ID 03

ADDITIONAL COMMENTS:

Water Quality taken in "C" replicate
 EPA Algal Assay Media (AAM) Without EDTA

AQUA-Science
Environmental Toxicology Consultants

LABORATORY NOTES

Date: 09/17/12

Selenastrum capricornutum

Init: 091712 from 3XB PC

Init: 042412 from 041212 UTEX Slant

Day 0 Date: 09/17/12 Initials: SR

Background:	28, 35, 22	mean	dilution	cells/mL
1X:	7485, 7462, 7489	7479	160:1	1.1966×10^4
2X:	13932, 14192, 14622	14049	160:1	2.2478×10^4
3X:	20403, 20741, 20706	20683	160:1	3.3093×10^4

Day 1 Date: 09/18/12 Initials: SR

Background:	9, 15, 10	mean	dilution	cells/mL	% improvement
1X:	20815, 20997, 21107	20973	160:1	3.3557×10^4	180%
2X:	33571, 33263, 33051	33295	160:1	5.3272×10^4	137%
3X:	51414, 51566, 51383	51454	160:1	8.2326×10^4	149%

Day 2 Date: 9/19/12 Initials: BJS

Background:	10, 9, 15	mean	dilution	cells/mL	% improvement
1X:	62075, 63301, 63205	62860	160:1	10.0576×10^6	200%
2X:	75846, 74409, 74132	74194	160:1	11.8710×10^6	123%
3X:	7876, 79021, 79524	79110	160:1	12.6576×10^6	54%

Used 1X P.C. to start ESD 12-09 TIE algae tests.

Technician: See aboveDate: 09/17/12

