

APPENDIX VI

TOXICITY IDENTIFICATION EVALUATION REPORT

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ENVIRONMENTAL TOXICOLOGY SPECIALISTS

**THREE-SPECIES AQUATIC TOXICITY EVALUATION OF SAN  
JOAQUIN COUNTY AND DELTA WATER QUALITY COALITION  
AMBIENT SAMPLES**

**Event 11-09: 9/20/11 Sample Date**

***C. dubia* Acute Phase I Toxicity Identification Evaluations  
Duck Creek @ Highway 4**

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# THREE-SPECIES AQUATIC TOXICITY EVALUATION OF SAN JOAQUIN COUNTY AND DELTA WATER QUALITY COALITION AMBIENT SAMPLES

## 1.0 EXECUTIVE SUMMARY

A total of eight (8) water samples collected by MLJ LLC personnel in one test event (SJCD 11-09) from waterways within the San Joaquin County and Delta Water Quality Coalition (SJCD) were tested for toxicity using USEPA 3-species toxicity tests. Tests included the acute 96-hour *Ceriodaphnia dubia* survival, acute 96-hour fathead minnow (*Pimephales promelas*) survival, and/or the chronic 4-day algae (*Selenastrum capricornutum*) cell growth toxicity tests. In Test Event SJCD 11-09 (9/20/11 sample date), there was no significant toxicity detected in the fathead minnow or algae toxicity tests with any of the samples tested. However, the Duck Creek @ Hwy 4 (531XDCAHF) sample caused 65% mortality in the *C. dubia* toxicity test. A Phase I *C. dubia* TIE with this sample was conducted, but no toxicity was detected in the TIE baseline toxicity test. However, chemical analysis detected chlorpyrifos at a concentration that is acutely toxic to *C. dubia*. Thus, it is likely that chlorpyrifos was the cause of the sample toxicity.

## 2.0 MATERIALS AND METHODS

### 2.1 Sample Collection and Transport

Subsurface water column grab samples were collected by MLJ LLC personnel on 9/20/11 in 1-gallon amber glass jugs that were transported in ice chests with wet ice to AQUA-Science. A total of 3-5 1-gallon amber jugs were collected to provide adequate sample volume for the three species toxicity tests and any required Toxicity Identification Evaluation (TIE) follow-up. Appropriate chain-of-custody procedures were employed during collection and transport. Table 1 shows the test sample sites in these test events.

**Table 1. Sample Sites for SJCD 11-09 Test Event**

<i>Event ID (Sample Date)</i>	<i>Site Name</i>	<i>Site ID</i>
SJCD 11-09 (9/20/11)	Bear Creek @ North Alpine Rd	531BCANAR
	Duck Creek @ Hwy 4	531XDCAHF
	French Camp Slough @ Airport Way	531SJC504 <sup>a</sup>
	Mokelumne River @ Bruella Rd	531XMRABR
	Mormon Slough @ Jack Tone Rd	544MSAJTR
	Roberts Island Drain @ Holt Rd	544RIDAHT
	Unnamed Drain to Lone Tree Creek @ Jack Tone Rd	531UDLTAJ

a This site also included a field duplicate sample

### 2.2 Water Quality Measurements

Water quality measurements including temperature, dissolved oxygen (D.O.), pH, conductivity, alkalinity, and hardness were made on water from each site at sample log-in (Attachment 1). Meter

calibration/verification and water quality measurements followed the recommended procedures described by the SWRCB QAMP (SWRCB 2002) for SWRCB-compliant data. Temperature was measured in initial and daily test solutions at change-out with a calibrated digital thermometer (Central Co., Friendswood, TX). Temperature was continuously recorded in the test chambers using a Dickson circular chart recorder (Model ICT855, Addison, IL). Dissolved oxygen (YSI Model 550A, Yellow Springs, OH), pH (Beckman 240, Fulton, CO), and conductivity (WTW Model 550, Ft. Myers, FL) were measured in the initial and 24-hour test solutions at change-out. Alkalinity (Hach Model AL-DT) and hardness (Hach HA-DT) were measured with Hach colorimetric tests (Hach Co., Loveland, CO). Ammonia was measured using a Hach DR-2800 Colorimeter (Method 8058).

### **2.3 Sample Storage**

Samples were stored in the dark at 4 °C until used for toxicity tests within 56 hours of sample collection.

### **2.4 Dilution water**

Dilution water was reverse osmosis and granular carbon-treated well water amended with dry salts to attain USEPA moderately hard specifications (EPAMH).

### **2.5 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) 5<sup>th</sup> edition acute protocol (USEPA 2002a). The 96-hour algal toxicity tests were conducted in accordance with the USEPA 4<sup>th</sup> Edition chronic protocol (USEPA 2002b).

### **2.6 *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  $25 \pm 2$  °C with a 16 hour light:8 hour dark photoperiod. Mortality was noted daily. The test was acceptable if control survival was  $\geq 90\%$ .

### **2.7 Fathead Minnow Toxicity Tests**

Fathead minnows were obtained from Aquatox, Inc. (Hot Springs, AK) and Aquatic Biosystems (Fort Collins, CO), and were maintained in EPA moderately hard (EPAMH) water until tested at 7 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 ≥90%.

## 2.8 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 x 10<sup>4</sup> 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 that indigenous algae were not present. This replicate was also used as a sample blank. Flasks were placed on a shaker table (100 rpm) in an environmental chamber at 25 ± 2 °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:

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

Using this conversion, the test was acceptable if the mean algal density in the control flasks was greater than or equal to 2 x 10<sup>5</sup> cells/mL and the coefficient of variation in the control replicates was ≤20%.

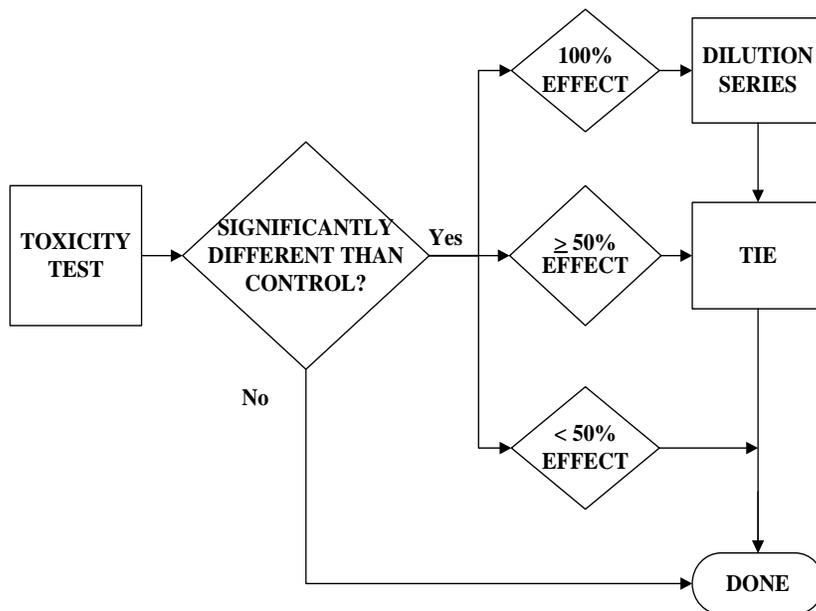
## 2.9 Reference Toxicant Tests

Monthly reference toxicant tests were conducted for each of the three test species on 9/7/11. Sodium chloride was the reference toxicant material for *C. dubia* (control, 0.25, 0.5, 1, 2, and 4 g/L) and fathead minnows (control, 2.5, 5, 7.5, 10 and 15 g/L). Zinc chloride was the reference toxicant material for green algae (control, 1, 2, 4, 8, 16 and 32µg/L).

## 2.10 Toxicity Identification Evaluation (TIE) Requirements

Figure 1 shows the TIE requirements for samples that show toxicity. Briefly, if the sample shows no significant effect or < 50% effect when compared to the control, no further action is required. If there is a ≥50% effect, a TIE is initiated on the toxic sample. If there is a 100% effect, the TIE must incorporate a dilution series toxicity test.

**Figure 1. Toxicity Identification Evaluation (TIE) Requirements**



### 2.11 Chemical Analysis

During monthly normal monitoring events selected water samples were collected for chemical analysis of four major groups of pesticides (organochlorines, organophosphates, triazines, and carbamates) plus glyphosate and paraquat dichloride. In addition, total (arsenic, boron, cadmium, copper, lead, molybdenum, nickel, selenium and zinc) and dissolved (cadmium, copper, lead, nickel and zinc) metals were analyzed using inductively coupled argon plasma-mass spectrometry analysis (EPA 200.8). The SJCDWQC 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 SJCDWQC QAPP and met all quality assurance requirements.

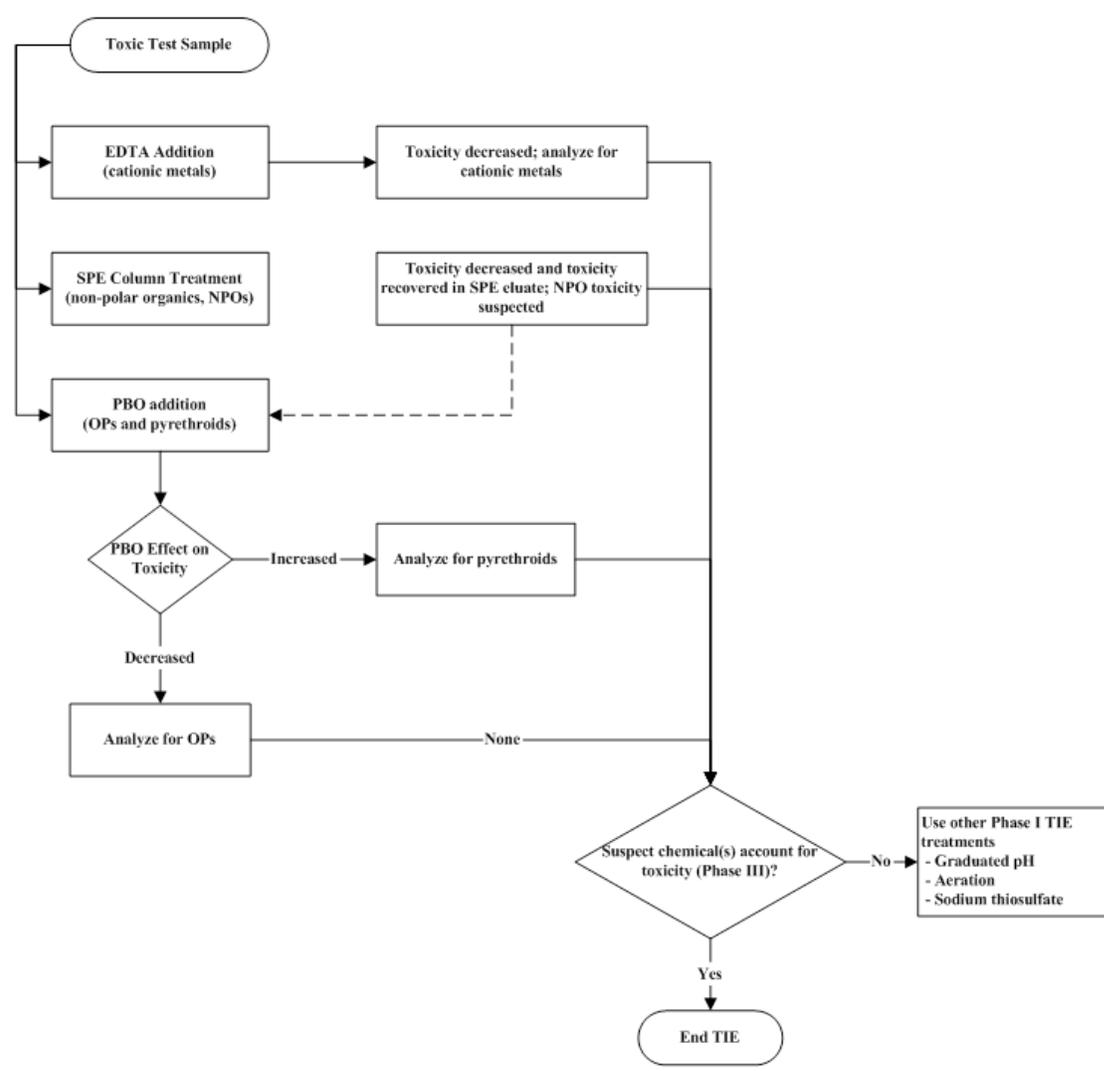
Management Plan Monitoring was conducted at selected sites for toxicity as outlined in the SJCDWQC Management Plan. Depending on the Management Plan schedule, this may or may not have corresponded with specific chemistry analysis.

### 2.12 Acute 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).

The Phase I *C. dubia* TIE 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 eluate residue was reconstituted in the SPE through-column sample and added back to the sample at 2X. In addition, piperonyl butoxide (PBO) and Zeolite column (ammonia) treatments were included. PBO binds *in vivo* with mixed-function oxidase enzymes that metabolize non-polar organic chemicals such as organophosphorous (OP) and pyrethroid insecticides. With OPs, PBO prevents the metabolism to their toxic oxone form, decreasing/preventing toxicity (Ankley et al, 1991). With pyrethroids, PBO prevents metabolism to less toxic forms, increasing/prolonging toxicity (Wheelock et al, 2004). The *C. dubia* Phase I TIE flow chart is shown in Figure 2.

**Figure 2. Phase I TIE Flowchart for *C. dubia***



### 3.0 RESULTS

#### 3.1 Test Event SJCD 11-09

A summary of the toxicity test results from the SJCD 11-09 test event is presented in Table 2. The water quality summary for these samples is found in Attachment 2A-2C. The raw data is found in Appendix I. Raw data for the reference toxicant tests are found in Appendix II.

In Test Event SJCD 11-09 (9/20/11 sample date), In Test Event SJCD 11-09 (9/20/11 sample date), there was no significant toxicity detected in the fathead minnow or algae toxicity tests with any of the samples tested. However, the Duck Creek @ Hwy 4 (531XDCAHF) sample caused 65% mortality in the *C. dubia* toxicity test, and a Phase I TIE was initiated on 9/26/11. There was no significant toxicity detected in the fathead minnow or algae toxicity tests with any of the samples tested. The reference toxicant EC<sub>50</sub> values for *C. dubia*, fathead minnow and algae were 1.9 g/L NaCl, 6.5 g/L NaCl and 19.1 µg/L ZnCl<sub>2</sub>, respectively. These values are within normal limits for this laboratory.

**Table 2. Summary of San Joaquin County and Delta Water Quality Coalition Toxicity Testing Results: SJCD 11-09**

Sample ID	Acute <i>C. dubia</i> (% Survival)	Acute <i>FHM</i> (% Survival)	Chronic Algae <sup>a</sup>			Total NH <sub>3</sub> (mg/L)
			Abs. @ 750 nm	Cells/mL x 10 <sup>6</sup> <sup>a</sup>	% Growth <sup>b</sup>	
Control #1	100	100	0.1068	1.3577	n/a	n/a
Bear Creek @ North Alpine Rd (531BCANAR-GR)	100	100	0.3735	4.8324	356	0.74
Robert's Island Drain @ Holt Rd (544RIDAHT-GR)	100	97.5	0.2643	3.4093	251	0.31
French Camp Slough @ Airport Way (531SJC504-GR)	100	97.5	0.4835	6.2653	461	0.54
French Camp Slough @ Airport Way (531SJC504-GR2)	100	100	0.4858	6.2946	464	0.55
Mokelumne River @ Bruella Rd (531XMRABR-GR)	100	97.5	0.1243	1.5857	117	0.19
Duck Creek @ Hwy 4 (531XDCAHF-GR)	35* <sup>c</sup> (-65%)	n/t	n/t	n/t	n/t	0.59
Unnamed Drain to Lone Tree Creek @ Jack Tone Rd (531UDLTAJ-GR)	100	n/t	n/t	n/t	n/t	0.98
Mormon Slough @ Jack Tone Rd (544MSAJTR-GR)	100	n/t	n/t	n/t	n/t	0.27

Test Date: 9/21/11

a Cell number x 10<sup>6</sup> = 13.026 x absorbance @ 750 nm - 0.0328 (calculated using Excel spreadsheet formula; differences in rounding may occur)

b % Growth = Sample (cells/mL x 10<sup>6</sup>) / Control (cells/mL x 10<sup>6</sup>) X 100

c Phase I TIE initiated on 9/26/11

n/a not applicable

n/t species not tested

### 3.2 Acute *C. dubia* TIE

A summary of the acute *C. dubia* TIE results from Test Event SJCD 11-09 TIE is provided in Attachment 3. Table 3 provides the conclusions from this TIE. Raw data for this TIE is found in Appendix III.

**Table 3. Summary of Acute Phase I *C. dubia* TIE: Duck Creek @ Hwy 5 (531XDCAHF)**

TIE Initiation Date	Baseline Toxicity <sup>a</sup> (TUa)	Toxicity Reduced or Eliminated by TIE Treatment				Phase III TIE	Comments
		SPE Column <sup>b</sup>	SPE Column Add-Back <sup>c</sup>	PBO Addition <sup>d</sup>	EDTA Addition <sup>e</sup>		
		9/26/11	No toxicity detected	n/a	n/a		

- a Toxicity of baseline TIE sample (TUa = 100/EC<sub>25</sub>)
- b Sample treated with C-8 solid-phase (SPE) extraction column
- c SPE column eluted and the eluate added back to the SPE through-column sample
- d Sample amended with piperonyl butoxide (PBO)
- e Sample amended with ethylenediaminetetraacetic acid (EDTA)

The initial toxicity test detected 65% mortality. Although no toxicity was detected in the TIE, the sample contained 0.12 ug/L of chlorpyrifos, an organophosphate insecticide with an acute EC<sub>50</sub> of 0.08 ug/L for *C. dubia* in this laboratory. At the detected concentration, chlorpyrifos could have fully accounted for the toxicity detected in the initial *C. dubia* acute toxicity test. The lack of toxicity detected in the TIE toxicity test was likely related to the loss of sufficient chlorpyrifos from the sample matrix to render the sample non-toxic.

Approved by/Issue date:

 1/30/12  
 Jeff Miller Ph.D., DABT

## REFERENCES

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- Wheelock, CE, JL Miller, MJ Miller, SJ Gee, G Shan, BD Hammock. 2004. Development of toxicity identification evaluation (TIE) procedures for pyrethroid detection using esterase activity. *Environ. Toxicol. Chem.* 23:2699-2708.

# ATTACHMENT 1

## San Joaquin County and Delta Water Quality Coalition

### Sample Receipt Water Quality Measurements

#### 9/20/11 Sample Dates

Sample Name (Sample ID)	Temp (°C)	D.O. (mg/L)	pH	Cond (µmohs)	Alk (mg/L)	Hard (mg/L)
Bear Creek @ North Alpine Rd (531BCANAR-GR)	2.8	8.8	7.39	161	77	69
Robert's Island Drain @ Holt Rd (544RIDAHT-GR)	3.0	9.0	7.42	444	70	96
French Camp Slough @ Airport Way (531SJC504-GR)	2.2	9.0	7.64	94	45	34
French Camp Slough @ Airport Way (531SJC504-GR2)	2.4	9.1	7.74	92	47	35
Mokelumne River @ Bruella Rd (531XMRABR-GR)	2.0	9.6	7.93	40	26	15
Duck Creek @ Hwy 4 (531XDCAHF-GR)	2.5	7.8	7.53	100	55	39
Unnamed Drain to Lone Tree Creek @ Jack Tone Rd (531UDLTAJ-GR)	2.9	8.3	7.78	103	44	35
Mormon Slough @ Jack Tone Rd (544MSAJTR-GR)	2.8	9.8	7.82	75	43	27

## ATTACHMENT 2A

### Water Quality Summary San Joaquin County and Delta Water Quality Coalition

**Event SJCD 11-09 (9/20/11)**

*Ceriodaphnia dubia*

Sample ID	Dissolved Oxygen (mg/L)				pH				Conductivity (µS/cm)				Ammonia (mg/L)	Alkalinity (mg/L)		Hardness (mg/L)		Temperature (°C)			
	initial	final	low	high	initial	final	low	high	initial	final	low	high	initial	initial	final	initial	final	initial	final	low	high
Control	7.9	7.8	7.3	7.9	7.89	8.01	7.89	8.11	301	302	301	368	a	69	69	86	86	25	25	24	25
531BCANAR-GR	7.8	7.7	7.2	7.9	7.68	7.49	7.49	8.08	159	158	158	184	0.74	77	75	69	63	24	26	24	26
544RIDAHT-GR	8.2	8.0	7.0	8.3	7.67	7.63	7.63	7.99	452	451	451	498	0.31	70	70	96	95	25	26	24	26
531SJC504-GR	8.0	7.8	7.2	8.2	7.79	7.61	7.61	7.97	92	93	92	132	0.54	45	46	34	33	25	26	24	26
531SJC504-GR2	7.9	8.0	7.1	8.3	7.73	7.55	7.55	7.95	91	89	89	130	0.55	47	47	35	30	26	26	24	26
531XMRABR-GR	8.5	7.9	7.2	8.5	7.94	7.43	7.43	7.96	41	44	41	79	0.19	26	29	15	12	25	26	24	26
531XDCAHF-GR	6.9	8.3	6.9	8.3	7.73	7.47	7.47	7.93	112	121	112	145	0.59	55	52	39	36	25	24	24	25
531UDLTAJ-GR	7.2	8.3	7.1	8.3	7.70	7.48	7.48	7.83	116	128	110	147	0.98	44	50	35	31	25	24	24	25
544MSAJTR-GR	8.5	8.3	7.3	8.5	7.81	8.20	7.78	8.20	87	108	87	131	0.27	43	44	27	26	25	24	24	25

a Measurement not recorded in control

## ATTACHMENT 2B

### Water Quality Summary San Joaquin County and Delta Water Quality Coalition

#### Event SJCD 11-09 (9/20/11)

##### Fathead Minnow

Sample ID	Dissolved Oxygen (mg/L)				pH				Conductivity (µS/cm)				Ammonia (mg/L)	Alkalinity (mg/L)		Hardness (mg/L)		Temperature (°C)			
	initial	final	low	high	initial	final	low	high	initial	final	low	high	initial	initial	final	initial	final	initial	final	low	high
Control	7.9	7.8	6.9	7.9	7.89	8.01	7.54	8.01	301	302	301	365	a	69	69	86	86	25	25	24	25
531BCANAR-GR	7.8	7.7	5.9	7.9	7.68	7.49	7.48	7.90	159	158	158	197	0.74	77	75	69	63	24	26	24	26
544RIDAHT-GR	8.2	8.0	5.5	8.3	7.67	7.63	7.48	7.85	452	451	451	509	0.31	70	70	96	95	25	26	24	26
531SJC504-GR	8.0	7.8	5.7	8.2	7.79	7.61	7.27	7.91	92	93	92	124	0.54	45	46	34	33	25	26	24	26
531SJC504-GR2	7.9	8.0	5.5	8.3	7.73	7.55	7.22	7.86	91	89	89	134	0.55	47	47	35	30	26	26	24	26
531XMRABR-GR	8.5	7.9	6.1	8.5	7.94	7.43	7.23	7.96	41	44	41	77	0.19	26	29	15	12	25	26	24	26

a Measurement not recorded in control

## ATTACHMENT 2C

### Water Quality Summary San Joaquin County and Delta Water Quality Coalition

#### Event SJCD 11-09 (9/20/11)

#### Algae

Sample ID	Dissolved Oxygen (mg/L)				pH				Conductivity (µS/cm)				Ammonia (mg/L)	Alkalinity (mg/L)	Hardness (mg/L)	Temperature (°C)			
	initial	final	low	high	initial	final	low	high	initial	final	low	high	initial	initial	initial	initial	final	low	high
Control	6.7	7.7	6.7	7.8	7.98	8.10	7.95	8.19	384	442	384	453	a	69	86	24	25	24	25
531BCANAR-GR	5.6	7.9	5.6	7.9	7.95	8.31	7.95	8.68	274	302	274	302	0.74	80	70	24	25	24	25
544RIDAHT-GR	6.0	7.9	6.0	7.9	7.83	8.25	7.83	8.43	545	594	545	603	0.31	70	100	24	25	24	25
531SJC504-GR	5.9	8.1	5.9	8.1	7.85	8.41	7.85	9.05	217	271	217	278	0.54	50	40	24	25	24	25
531SJC504-GR2	6.0	8.1	6.0	8.1	7.85	8.41	7.85	9.23	206	256	206	260	0.55	50	40	24	25	24	25
531XMRABR-GR	6.2	8.0	6.2	8.0	7.86	8.10	7.81	8.28	168	226	168	241	0.19	30	20	24	25	24	25

a Measurement not recorded in control

### ATTACHMENT 3

#### Summary of Acute Phase I *Ceriodaphnia dubia* TIE Duck Creek @ Hwy 4 (531XDCAHF-GR)

<i>Treatment</i>	<i>Purpose</i>	<i>Conc.</i> (%)	<i>96-hr Survival</i> (%)	<i>Comment</i>
<b>Baseline</b>	Determines sample acute toxic units (TUa) <sup>a</sup>	Lab Control	100	No toxicity detected in TIE
		6.25	100	
		12.5	100	
		25	100	
		50	100	
		100	100	
<b>EDTA</b>	Identifies cationic metal toxicity	EDTA Control	95	
		100	100	
<b>PBO</b>	Identifies organophosphate (OP) and pyrethroid toxicity	PBO Control	95	
		6.25	100	
		12.5	100	
		25	100	
		50	95	
		100	100	
<b>C-8 SPE Column</b>	Removes non-polar organic (NPO) toxicants	Column Blank	95	
		100	100	
<b>SPE Add-Back</b>	Determines if NPO toxicants can be recovered from SPE columns	MeOH Control	100	
		100	100	

Sample date: 9/20/11

TIE Test Date: 9/26/11

**Conclusion:**

The initial toxicity test detected 65% mortality. However, no toxicity was detected in the TIE, indicating the sample lost all detectable toxicity prior to initiation of the TIE.

**APPENDIX I**

**SJCD 11-09 Raw Data**

***Ceriodaphnia dubia***

**Fathead Minnow**

**Algae**

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**SJCD 11-09 Raw Data**

***Ceriodaphnia dubia* Acute Toxicity Test**

**CETIS Summary Report**

**Report Date:** 26 Sep-11 10:45 (p 1 of 1)  
**Test Code:** a3011109 | 11-2619-4037

**Ceriodaphnia 96-h Acute Survival Test**

**Aqua-Science**

<b>Batch ID:</b> 13-3079-1841	<b>Test Type:</b> Survival (96h)	<b>Analyst:</b> K. Miller
<b>Start Date:</b> 21 Sep-11 12:30	<b>Protocol:</b> EPA/821/R-02-012 (2002)	<b>Diluent:</b> Laboratory Water
<b>Ending Date:</b> 25 Sep-11 12:25	<b>Species:</b> Ceriodaphnia dubia	<b>Brine:</b> Not Applicable
<b>Duration:</b> 96h	<b>Source:</b> In-House Culture	<b>Age:</b> <24h
<b>Sample ID:</b> 10-4251-1480	<b>Code:</b> 3E237678	<b>Client:</b> MLJ-LLC
<b>Sample Date:</b> 20 Sep-11 11:20	<b>Material:</b> Ambient Sample	<b>Project:</b> Ag Waiver
<b>Receive Date:</b> 21 Sep-11 09:00	<b>Source:</b> Ag Waiver	
<b>Sample Age:</b> 25h (2.5 °C)	<b>Station:</b> SJCD	

**Sample Note:** DUCK CREEK @ HWY 4

**Comparison Summary**

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
17-4127-1254	96h Survival Rate	<100	100	N/A	22.8%	>1	Equal Variance t Two-Sample Test

**Test Acceptability**

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
17-4127-1254	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

**96h Survival Rate Summary**

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	1	1	1	1	1	0	0	0.0%	0.0%
100		4	0.35	0.256	0.444	0	0.6	0.1258	0.2517	71.9%	65.0%

**96h Survival Rate Detail**

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	1	1	1	1
100		0.6	0	0.4	0.4



**AQUA-Science**  
Environmental Toxicology Specialists

**MORTALITY AND BEHAVIOR OBSERVATIONS**

Test Number:	SJCD 11-09	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/012	Technicians:	Walker/Concepcion/Sanford/Berry/Renn
Test Material:	SJCD Ag Waiver Samples (09/20/11)		
Test Species:	<i>Ceriodaphnia dubia</i>	Animal Lot No.:	A/S RO: 092111
Initiation Date:	September 21, 2011	Termination Date:	September 25, 2011

Samples @100%	Vessel No.	Cumulative Mortality				Comments
		Day 1	Day 2	Day 3	Day 4	
Lab Control	A	0	0	0	0	n=5 animals/rep
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
Bear Creek @ North Alpine Rd ①	A	0	0	0	0	Test Initiation Info: Time: 1230 Tech: ML
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
Robert's Island Drain @ Holt Rd ①	A	0	0	0	0	Test Termination Info: Time: 1225 Tech: ML
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
French Camp Slough @ Airport Way ①	A	0	0	0	0	① Heavy Sediment, ML 09/22/11 ② PRECIPITATES PRESENT ML 09/24/11 ③ PRECIPITATES PRESENT ML 09/25/11
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
French Camp Slough @ Airport Way GR2 ①	A	0	0	0	0	
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
Mokelumne River @ Bruejla Rd ①	A	0	0	0	0	
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
Duck Creek @ Hwy 4 ①	A	0	0	2	2	
	B	0	0	1	5	
	C	0	0	3	3	
	D	0	0	1	3	

Technician Initials	ML	AW	AW	ML
Observation Time	1150	1120	1255	1225
Observation Date	09/22/11	09/23/11	09/24/11	09/25/11

**AQUA-Science**  
Environmental Toxicology Specialists

**MORTALITY AND BEHAVIOR OBSERVATIONS**

Test Number:	SJCD 11-09	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/012	Technicians:	Walker/Concepcion/Sanford/Berry/Renn
Test Material:	SJCD Ag Waiver Samples (09/20/11)		
Test Species:	<i>Ceriodaphnia dubia</i>	Animal Lot No.:	A/S RO: 092111
Initiation Date:	September 21, 2011	Termination Date:	September 25, 2011

Samples @100%	Vessel No.	Cumulative Mortality				Comments
		Day 1	Day 2	Day 3	Day 4	
Unnamed Drain to Lone Tree Creek @ Jack Tone Rd	A	0	0	0	3	n=5 animals/rep
	B	0	0	0	3	
	C	0	0	0	3	
	D	0	0	0	3	
Mormon Slough @ Jack Tone Rd	A	0	0	0	3	Test Initiation Info: Time: 12:40 Tech: MC
	B	0	0	0	3	
	C	0	0	0	3	
	D	0	0	0	3	
	A					Test Termination Info: Time: 12:30 Tech: MC
	B					
	C					
	D					
	A					① heavy sediment MC 09/22/11 ② light sediment MC 09/22/11 ③ neonates present MC 09/24/11 ④ neonates present MC 09/25/11
	B					
	C					
	D					
	A					
	B					
	C					
	D					
	A					
	B					
	C					
	D					
	A					
	B					
	C					
	D					

Technician Initials	MC	AW	AW	MC
Observation Time	12:05	12:25	1:05	12:30
Observation Date	09/21/11	09/23/11	09/24/11	09/25/11

**AQUA-Science**  
Environmental Toxicology Specialists

**DOSE PREPARATION SHEET**

09/21/11

**SJCD 11-09**

96 hr. Static Acute bioassay w/ 24 hr. Changeout  
*Ceriodaphnia dubia*

SJCD Ag Waiver Samples (09/20/11)

Control water = Reverse Osmosis water amended with EPA salts to achieve EPAMH specifications

All surface waters filtered through 60 µm screen  
n = 5 animals/replicate - 4 replicates/concentration

Site ID	Site Name	Amount Sample (mL)	Control Water (mL)	Total (mL)*
	Lab Control	0	100	100
531BCANAR	Bear Creek @ North Alpine Rd	100	0	100
544RIDAHT	Robert's Island Drain @ Holt Rd	100	0	100
531SJC504	French Camp Slough @ Airport Way	100	0	100
531SJC504	French Camp Slough @ Airport Way GR2	100	0	100
531XMRABR	Mokelumne River @ Bruella Rd	100	0	100
531XDCAHF	Duck Creek @ Hwy 4	100	0	100
531UDLTAJ	Unnamed Drain to Lone Tree Creek @ Jack Tone Rd	100	0	100
544MSAJTR	Mormon Slough @ Jack Tone Rd	100	0	100

\* 20 mL used to measure pH, 80 mL used for test

	Day 0	Day 1	Day 2	Day 3
Tech	BES	BB	ML	12
Time	10:30	09:55	09:50	09:25
Date	9/21/11	9/22/11	09/23/11	09/24/11









**SJCD 11-09 Raw Data**

**Fathead Minnow Acute Toxicity Test**

**CETIS Summary Report**

**Report Date:** 14 Oct-11 11:46 (p 1 of 1)  
**Test Code:** a3031109a | 17-1356-7922

**Fathead Minnow 96-h Acute Survival Test**

**Aqua-Science**

<b>Batch ID:</b> 03-7907-4719	<b>Test Type:</b> Survival (96h)	<b>Analyst:</b> K. Miller
<b>Start Date:</b> 21 Sep-11 14:20	<b>Protocol:</b> EPA/821/R-02-012 (2002)	<b>Diluent:</b> Laboratory Water
<b>Ending Date:</b> 25 Sep-11 12:40	<b>Species:</b> Pimephales promelas	<b>Brine:</b> Not Applicable
<b>Duration:</b> 94h	<b>Source:</b> Aquatox, AR	<b>Age:</b> 7d
<b>Sample ID:</b> 01-1334-2833	<b>Code:</b> 6C17971	<b>Client:</b> MLJ-LLC
<b>Sample Date:</b> 20 Sep-11 12:50	<b>Material:</b> Ambient Sample	<b>Project:</b> Ag Waiver
<b>Receive Date:</b> 21 Sep-11 09:00	<b>Source:</b> Ag Waiver	
<b>Sample Age:</b> 26h (3 °C)	<b>Station:</b> SJCD	

**Sample Note:** ROBERT'S ISLAND DRAIN @ HOLT RD - 544RIDAHT

**Comparison Summary**

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
18-1984-0526	96h Survival Rate	100	>100	N/A	5.56%	1	Wilcoxon Rank Sum Two-Sample Test

**Test Acceptability**

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
18-1984-0526	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

**96h Survival Rate Summary**

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	1	1	1	1	1	0	0	0.0%	0.0%
100		4	0.975	0.9563	0.9937	0.9	1	0.025	0.05	5.13%	2.5%

**96h Survival Rate Detail**

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	1	1	1	1
100		1	0.9	1	1



**CETIS Summary Report**

Report Date: 14 Oct-11 11:49 (p 1 of 1)  
 Test Code: a3031109b | 15-3691-1820

**Fathead Minnow 96-h Acute Survival Test**

**Aqua-Science**

Batch ID: 03-7907-4719	Test Type: Survival (96h)	Analyst: K. Miller
Start Date: 21 Sep-11 14:20	Protocol: EPA/821/R-02-012 (2002)	Diluent: Laboratory Water
Ending Date: 25 Sep-11 12:40	Species: Pimephales promelas	Brine: Not Applicable
Duration: 94h	Source: Aquatox, AR	Age: 7d
Sample ID: 04-2890-0540	Code: 199080BC	Client: MLJ-LLC
Sample Date: 20 Sep-11 15:30	Material: Ambient Sample	Project: Ag Waiver
Receive Date: 21 Sep-11 09:00	Source: Ag Waiver	
Sample Age: 23h (2.2 °C)	Station: SJCD	

Sample Note: FRENCH CAMP SLOUGH @ AIRPORT WAY

**Comparison Summary**

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
18-1332-0662	96h Survival Rate	100	>100	N/A	5.56%	1	Wilcoxon Rank Sum Two-Sample Test

**Test Acceptability**

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
18-1332-0662	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

**96h Survival Rate Summary**

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	1	1	1	1	1	0	0	0.0%	0.0%
100		4	0.975	0.9563	0.9937	0.9	1	0.025	0.05	5.13%	2.5%

**96h Survival Rate Detail**

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	1	1	1	1
100		1	1	0.9	1

# CETIS Analytical Report

Report Date: 14 Oct-11 11:49 (p 1 of 1)  
 Test Code: a3031109b | 15-3691-1820

## Fathead Minnow 96-h Acute Survival Test

Aqua-Science

Analysis ID: 18-1332-0662      Endpoint: 96h Survival Rate      CETIS Version: CETISv1.8.0  
 Analyzed: 14 Oct-11 11:49      Analysis: Nonparametric-Two Sample      Official Results: Yes

Data Transform	Zeta	Alt Hyp	MC Trials	Test Result	PMSD
Angular (Corrected)	0	C > T	5000 Trials	Sample passes 96h survival rate endpoint	5.56%

### Wilcoxon Rank Sum Two-Sample Test

Control	vs	Conc-%	Test Stat	Critical	DF	Ties	P-Value	Decision(α:5%)
Negative Control		100	16		6	1	0.4984	Non-Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.003319917	0.003319917	1	1	0.3559	Non-Significant Effect
Error	0.0199195	0.003319917	6			
Total	0.02323942	0.006639833	7			

### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1	13.75	0.3559	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.7065	0.6451	0.0027	Non-normal Distribution

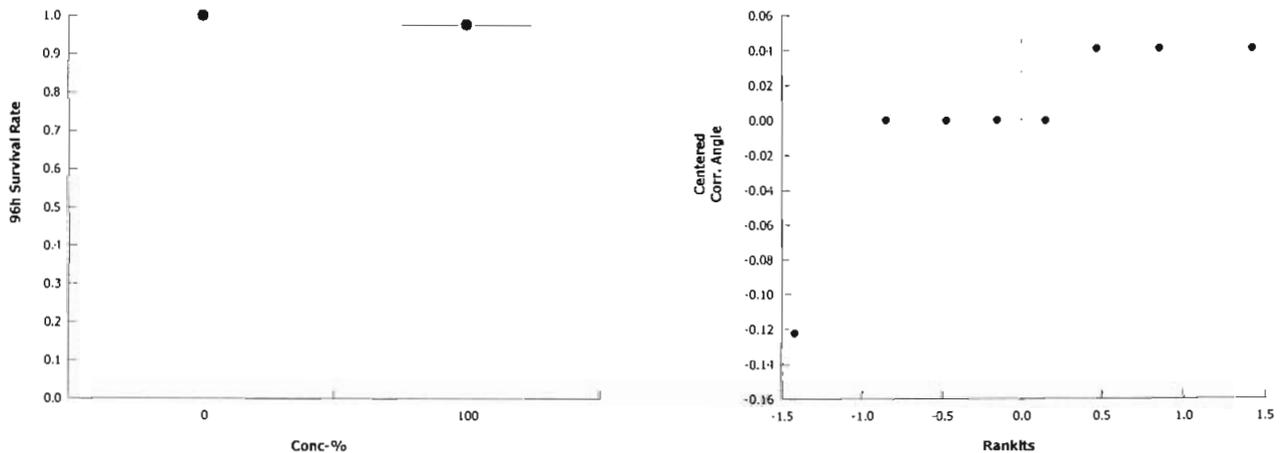
### 96h Survival Rate Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	1	1	1	1	1	0	0	0.0%	0.0%
100		4	0.975	0.956	0.994	0.9	1	0.025	0.05	5.13%	2.5%

### Angular (Corrected) Transformed Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Contr	4	1.412	1.412	1.412	1.412	1.412	0	0	0.0%	0.0%
100		4	1.371	1.34	1.402	1.249	1.412	0.04074	0.08149	5.94%	2.89%

### Graphics



**CETIS Summary Report**

Report Date: 14 Oct-11 11:52 (p 1 of 1)  
 Test Code: a3031109c | 20-9001-4223

**Fathead Minnow 96-h Acute Survival Test**

**Aqua-Science**

<b>Batch ID:</b> 03-7907-4719	<b>Test Type:</b> Survival (96h)	<b>Analyst:</b>
<b>Start Date:</b> 21 Sep-11 14:20	<b>Protocol:</b> EPA/821/R-02-012 (2002)	<b>Diluent:</b> Laboratory Water
<b>Ending Date:</b> 25 Sep-11 12:40	<b>Species:</b> Pimephales promelas	<b>Brine:</b> Not Applicable
<b>Duration:</b> 94h	<b>Source:</b> Aquatox, AR	<b>Age:</b> 7d

<b>Sample ID:</b> 16-6528-3422	<b>Code:</b> 6342355E	<b>Client:</b> MLJ-LLC
<b>Sample Date:</b> 20 Sep-11 08:00	<b>Material:</b> Ambient Sample	<b>Project:</b> Ag Waiver
<b>Receive Date:</b> 21 Sep-11 09:00	<b>Source:</b> Ag Waiver	
<b>Sample Age:</b> 30h (2 °C)	<b>Station:</b> SJCD	

**Sample Note:** MOKELUMNE RIVER @ BRUELLA RD - 531XMRABR

**Comparison Summary**

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
09-1065-6642	96h Survival Rate	100	>100	N/A	5.56%	1	Wilcoxon Rank Sum Two-Sample Test

**Test Acceptability**

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
09-1065-6642	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

**96h Survival Rate Summary**

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	1	1	1	1	1	0	0	0.0%	0.0%
100		4	0.975	0.9563	0.9937	0.9	1	0.025	0.05	5.13%	2.5%

**96h Survival Rate Detail**

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	1	1	1	1
100		1	1	1	0.9

# CETIS Analytical Report

Report Date: 14 Oct-11 11:52 (p 1 of 1)  
 Test Code: a3031109c | 20-9001-4223

## Fathead Minnow 96-h Acute Survival Test

Aqua-Science

Analysis ID: 09-1065-6642      Endpoint: 96h Survival Rate      CETIS Version: CETISv1.8.0  
 Analyzed: 14 Oct-11 11:51      Analysis: Nonparametric-Two Sample      Official Results: Yes

Data Transform	Zeta	Alt Hyp	MC Trials	Test Result	PMSD
Angular (Corrected)	0	C > T	5000 Trials	Sample passes 96h survival rate endpoint	5.56%

### Wilcoxon Rank Sum Two-Sample Test

Control	vs	Conc-%	Test Stat	Critical	DF	Ties	P-Value	Decision(α:5%)
Negative Control		100	16		6	1	0.5022	Non-Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.003319917	0.003319917	1	1	0.3559	Non-Significant Effect
Error	0.0199195	0.003319917	6			
Total	0.02323942	0.006639833	7			

### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1	13.75	0.3559	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.7065	0.6451	0.0027	Non-normal Distribution

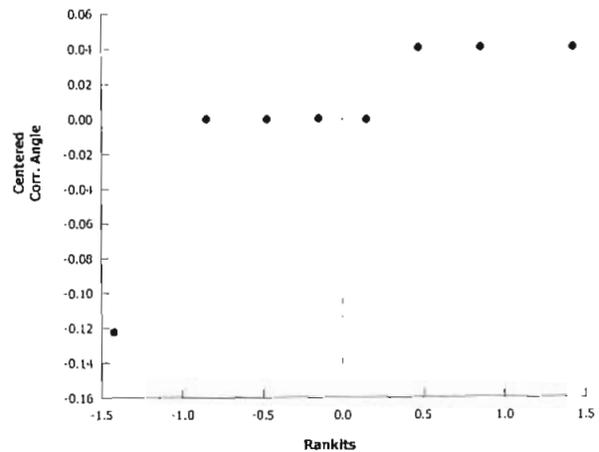
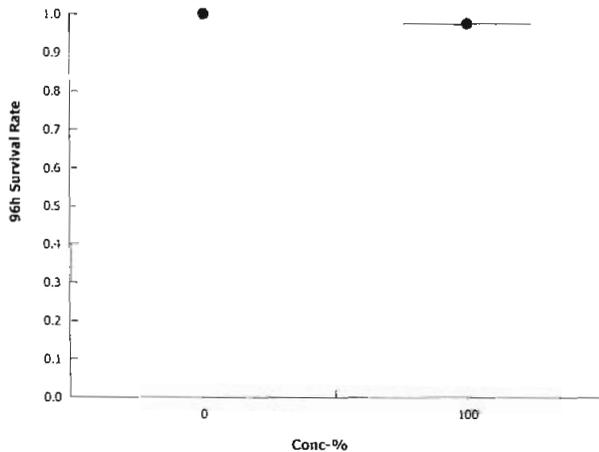
### 96h Survival Rate Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	1	1	1	1	1	0	0	0.0%	0.0%
100		4	0.975	0.956	0.994	0.9	1	0.025	0.05	5.13%	2.5%

### Angular (Corrected) Transformed Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Contr	4	1.412	1.412	1.412	1.412	1.412	0	0	0.0%	0.0%
100		4	1.371	1.34	1.402	1.249	1.412	0.04074	0.08149	5.94%	2.89%

### Graphics



**AQUA-Science**  
Environmental Toxicology Specialists

**MORTALITY AND BEHAVIOR OBSERVATIONS**

Test Number:	SJCD 11-09	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/012	Technicians:	Walker/Concepcion/Sanford/Berry/Renn
Test Material:	SJCD Ag Waiver Samples (09/20/11)		
Test Species:	<i>Pimephales promelas</i>	Animal Lot No.:	AQTX: 091411
Initiation Date:	September 21, 2011	Termination Date:	September 25, 2011

Samples @100%	Vessel No.	Cumulative Mortality				Comments
		Day 1	Day 2	Day 3	Day 4	
Lab Control	A	0	0	0	0	n=10 animals/rep
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
Bear Creek @ North Alpine Rd. ①	A	0	0	0	0	Test Initiation Info: Time: 1420 Tech: RES
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
Robert's Island Drain @ Holt Rd ②	A	0	0	0	0	Test Termination Info: Time: 1240 Tech: SR ① HEAVY SEDIMENT RES 9/22/11 ② MODERATE SEDIMENT 9/22/11
	B	0	0	1	1	
	C	0	0	0	0	
	D	0	0	0	0	
French Camp Slough @ Airport Way ②	A	0	0	0	0	
	B	0	0	0	0	
	C	0	0	0	1	
	D	0	0	0	0	
French Camp Slough @ Airport Way GR2 ②	A	0	0	0	0	
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
Mokelumne River @ Bruella Rd ③	A	0	0	0	0	
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	1	1	

Technician Initials	BES	AW	AW	SR
Observation Time	1539	1605	1555	1240
Observation Date	9/22/11	09/23/11	09/24/11	09/25/11

**AQUA-Science**  
Environmental Toxicology Specialists

**DOSE PREPARATION SHEET**

09/21/11

**SJCD 11-09**

96 hr. Static Acute bioassay w/ 24 hr. Changeout  
*Pimephales promelas*

**SJCD Ag Waiver Samples (09/20/11)**

Control water (R/O EPAMH) = Reverse Osmosis water amended with EPA salts to achieve EPAMH specifications

All surface waters filtered through 60 µm screen

n=10 animals/replicate - 4 replicates/concentration

Site ID	Site Name	Amount Sample (mL)	Dilution Water (mL)	Total (mL)*
-	Lab Control	0	1000	1000
531BCANAR	Bear Creek @ North Alpine Rd.	1000	0	1000
544RIDAHT	Robert's Island Drain @ Holt Rd	1000	0	1000
531SJC504	French Camp Slough @ Airport Way	1000	0	1000
531SJC504	French Camp Slough @ Airport Way GR2	1000	0	1000
531XMRABR	Mokelumne River @ Bruella Rd	1000	0	1000

	Day 0	Day 1	Day 2	Day 3
Tech	RLT	RB	MC	RL
Time	1030	0955	0950	0925
Date	9/21/11	9/22/11	09/23/11	09/24/11









**AQUATOX, INC.**  
416 TWIN POINTS ROAD  
HOT SPRINGS, ARKANSAS 71913  
501-520-0560

**TEST ORGANISM HISTORY**

DATE SHIPPED 9/20/11 CLIENT Agua Science  
Purchase Order #: Cecilia

SPECIES: Pimephales promelas Mysidopsis bahia Cyprinodon variegates

Quantity Shipped: 145

Age: 7 days on 9/21/11

Brood Stock Source: Anderson Farms

Culture Water: Groundwater Artificial Salts Artificial Salts

Hardness (Mg/l CaCO<sub>3</sub>): 160 Salinity (ppt) \_\_\_\_\_

Dissolved Oxygen (Mg/l): 8.1

Feeding: Artemia

Comments: 25.1°C Received by BSI

09/21/11. Temp = 24.5°C

DO = 19.3 mg/L (22°C)

Shipped Via: Federal Express UPS Overnight

Packaged By: \_\_\_\_\_

**SJCD 11-09 Raw Data**  
**Algae Chronic Toxicity Test**

SJCD 11-09 (9/20/11)

Sample	Rep	Absorbance	Mean	% difference (abs)	Cell Number (13.026*abs-0.0328)	Mean	Cell Number x 10 <sup>6</sup>	Mean	% control (cell no)
Control 1	A	0.122			1.5564		1556372		
	B	0.102			1.2959		1295852		
	C	0.114			1.4522		1452164		
	D	0.089	0.1068	--	1.1265	1.3577	1126514	1357726	--
Bear Creek @ North Alpine Rd	A	0.383			4.9562		4956158		
	B	0.322			4.1616		4161572		
	C	0.391			5.0604		5060366		
	D	0.398	0.3735	250	5.1515	4.8324	5151548	4832411	356
Robert's Island Drain @ Holt Rd	A	0.273			3.5233		3523298		
	B	0.218			2.8069		2806868		
	C	0.247			3.1846		3184622		
	D	0.319	0.2643	148	4.1225	3.4093	4122494	3409321	251
French Camp Slough @ Airport May	A	0.483			6.2588		6258758		
	B	0.458			5.9331		5933108		
	C	0.509			6.5974		6597434		
	D	0.484	0.4835	353	6.2718	6.2653	6271784	6265271	461
French Camp Slough @ Airport May GR2	A	0.467			6.0503		6050342		
	B	0.507			6.5714		6571382		
	C	0.508			6.5844		6584408		
	D	0.461	0.4858	355	5.9722	6.2946	5972186	6294580	464
Mokelumne River @ Bruella Rd	A	0.111			1.4131		1413086		
	B	0.139			1.7778		1777814		
	C	0.133			1.6997		1699658		
	D	0.114	0.1243	16	1.4522	1.5857	1452164	1585681	117

*Jen 9/26/11*

# AQUA-Science

Environmental Toxicology Specialists

## ALGAL BIOASSAY - CELL DETERMINATION

Test Number:	SJCD 11-09	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/013	Technicians:	Walker/Concepcion/Sanford/Berry/Renn
Test Material:	SJCD Ag Waiver Samples (09/20/11) WITHOUT EDTA		
Test Species:	<i>Selenastrum capricornutum</i>	Animal Lot No.:	UTEX: 082211
Initiation Date:	September 21, 2011	Termination Date:	September 25, 2011

Absorbance @ 750 nm with Hach DR 2800 Spectrophotometer		Test Terminated @ 1310			
Conc. (%)	Replicate	1	2	3	Mean
Lab Control	A	0.122	0.122	0.122	0.122
	B	0.103	0.102	0.102	0.102
	C	0.114	0.114	0.114	0.114
	D	0.089	0.089	0.089	0.089
Bear Creek @ North Alpine Rd	A	0.384	0.383	0.383	0.383
	B	0.322	0.321	0.323	0.322
	C	0.391	0.392	0.391	0.391
	D	0.399	0.398	0.397	0.398
Robert's Island Drain @ Holt Rd	A	0.274	0.274	0.272	0.273
	B	0.219	0.218	0.218	0.218
	C	0.247	0.247	0.246	0.247
	D	0.319	0.318	0.320	0.319
French Camp Slough @ Airport Way	A	0.483	0.483	0.483	0.483
	B	0.459	0.459	0.457	0.458
	C	0.509	0.509	0.509	0.509
	D	0.484	0.483	0.485	0.484
French Camp Slough @ Airport Way GR2	A	0.468	0.467	0.465	0.467
	B	0.505	0.507	0.508	0.507
	C	0.507	0.508	0.510	0.508
	D	0.460	0.461	0.461	0.461

**w/o algae**

Lab Ctrl	A	0.003	0.003	0.004	0.003
Bear @ North	A	0.004	0.004	0.004	0.004
Robert's @ Holt	A	0.003	0.004	0.004	0.004
French Camp Slough	A	0.005	0.006	0.006	0.006
French Camp Slough GR2	A	0.004	0.005	0.005	0.005

Technician: \_\_\_\_\_

*Sarah R.*

Date: \_\_\_\_\_

09/25/11

**AQUA-Science**  
Environmental Toxicology Specialists

**ALGAL BIOASSAY - CELL DETERMINATION**

Test Number:	SJCD 11-09	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/013	Technicians:	Walker/Concepcion/Sanford/Berry/Renn
Test Material:	SJCD Ag Waiver Samples (09/20/11) WITHOUT EDTA		
Test Species:	<i>Selenastrum capricornutum</i>	Animal Lot No.:	UTEX: 082211
Initiation Date:	September 21, 2011	Termination Date:	September 25, 2011

Absorbance @ 750 nm with Hach DR 2800 Spectrophotometer		Test Terminated @ 1400			
Conc. (%)	Replicate	1	2	3	Mean
Mokelumne River @ Bruella Rd	A	0.110	0.111	0.111	0.111
	B	0.139	0.139	0.139	0.139
	C	0.134	0.133	0.133	0.133
	D	0.115	0.114	0.113	0.114
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				
	A				
	B				
	C				
	D				

**w/o algae**

Mokelumne River	A	0.002	0.002	0.002	0.002
	A				
	A				
	A				
	A				

Technician: Sarah R.

Date: 09/25/11

**AQUA-Science**  
Environmental Toxicology Specialists  
**ALGAL BIOASSAY DATA SHEETS**

**1.0 TEST AND CLIENT INFORMATION**

Test Number:	SJCD 11-09	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/013	Technician(s):	Walker/Concepcion/Sanford/Berry/Renn
Test Material:	SJCD Ag Waiver Samples (09/20/11) WITHOUT EDTA		
Initiation Date:	September 21, 2011	Termination Date:	September 25, 2011

**2.0 TEST CONDITIONS & TEST SPECIES INFORMATION**

Species:	<i>Selenastrum capricornutum</i>	Temperature:	25.0°C ± 1.0°C
Source:	University of Texas (UTEX)	Agitation:	100 RPM continuous
Media:	EPA Algal Assay Media (AAM)	Lighting:	400 ft candles
Inoculation:	10,000 cells/mL		

**3.0 DILUTION WATER INFORMATION**

Dilution Water Source	D.O. (mg/L)	pH (units)	E.C. (µmhos)	Comments
RO EPAMH	6.7	7.98	384	0.22 µm Metrigard vacuum filtered
Bear @ North - 531BCANAR	5.6	7.95	274	0.22 µm Metrigard vacuum filtered
Robert's @ Holt - 544RIDAHT	6.0	7.83	545	0.22 µm Metrigard vacuum filtered
French Camp Slough - 531SJC504	5.9	7.85	217	0.22 µm Metrigard vacuum filtered
French Camp Slough GR2 - 531SJC504	6.0	7.85	206	0.22 µm Metrigard vacuum filtered
Mokelumne River - 531XMRABR	6.2	7.86	168	0.22 µm Metrigard vacuum filtered

**4.0 PREPARATION OF EXPOSURE SOLUTIONS**

Test Solution Conc.	Ctrl.	Sites		Comments
Ambient (mL)	0	320		Time: 1210
Control Water (mL)	320	0		Tech: BS
TOTAL	320 mL	320 mL		

Technician:



Date:

9/21/11

**AQUA-Science**  
Environmental Toxicology Specialists  
**ALGAL BIOASSAY DATA SHEETS**

**5.0 TEST INITIATION**

Without EDTA	Sample	Background Counts		
	Isoton	20	28	21
	R/O EPAMH	38	49	46
	Bear Creek @ North Alpine Rd	30	34	33
	Robert's Island Drain @ Holt Rd	43	56	38
	French Camp Slough @ Airport Way	38	39	37
	French Camp Slough @ Airport Way GR2	38	39	35
	Mokelumne River @ Bruella Rd	35	35	41

**6.0 ALGAE COUNT**

Replicate	Dilution Factor				Cell Counts			Mean
	Dil 1	Dil 2	Count	Factor	1	2	3	
1	0.25 mL SAMPLE	0.5 to 20 mL	0.5 mL	160:1	42869	43087	43132	43029

Calculations: Time of Inoculation: 145

Target: 10,000 cells/mL test solution

Algae Stock Concentration:

$$\frac{43029}{160} = 2.6893 \times 10^5 \text{ cells/mL algal stock}$$

Inoculation:

$$\frac{10,000 \text{ cells/mL solution} \times 250 \text{ mL test solution/concentration}}{2.6893 \times 10^5 \text{ cells/mL algal stock}} = 0.363 \text{ mL stock/conc.} = 363 \text{ } \mu\text{l algal stock/conc.}$$

Final Algal Density Check

Time: 1450	ID	Rep	Count 1	Count 2	Count 3	Dilution	Adj. Avg.
Flask #1	Lab Ctrl	B	307	303	324	40:1	10680
Flask #2	Bear @ North	A	314	309	308	40:1	11120
Flask #3	Robert's @ Holt	D	317	302	305	40:1	10492
Flask #4	French Camp Slough	C	313	307	312	40:1	10908
Flask #5	French Camp Slough GR2	B	306	310	314	40:1	10908

Technician: *Blm B. J.* Date: 9/21/11

**AQUA-Science**  
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**ALGAL BIOASSAY DATA SHEETS**

**6.0 ALGAL COUNT Cont'd**

Final Algal Density Check:	ID	Rep	Count 1	Count 2	Count 3	Dilution	Adj. Avg.
Flask #6	Mokelumne River	D	307	312	304	40:1	10828
Flask #7							
Flask #8							
Flask #9							
Flask #10							
Flask #11							
Flask #12							
Flask #13							
Flask #14							

**7.0 LAB NOTES**

09/21/11

**SJCD 11-09**

96 Hr. Static Growth

*Selenastrum capricornutum*

**1.0 Stock Preparation**

0.32 liters of Ambient samples (9/20/2011) vacuum filtered through 0.22 µm Metrigard filter  
0.8 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 Addition**

Algal Assay Media prepared as per EPA 821/R-02/013; Section 14 - Tables 1 and 2 (**without EDTA**)  
Added 0.32 mL each of #1-5 EPA AAM to 0.32 liters of each ambient site  
Added 0.8 mL each of #1-5 EPA AAM to 0.8 liters R/O EPAMH Lab Control Water.  
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 400 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 250 mL in solution beaker into 5-50mL aliquots and distributed into flasks A-E.  
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: Bl By

Date: 9/21/11



**AQUA-Science**  
Environmental Toxicology Specialists

**WATER QUALITY REPORT FOR AQUATIC BIOASSAYS**

Test Number:	SJCD 11-09	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/013	Technicians:	Walker/Concepcion/Sanford/Berry/Renn
Test Material:	SJCD Ag Waiver Samples (09/20/11) WITHOUT EDTA		
Test Species:	<i>Selenastrum capricornutum</i>	Animal Lot No.:	UTEX: 082211
Initiation Date:	September 21, 2011	Termination Date:	September 25, 2011

Samples @ 100%	Day 1				Day 2				Day 3			
	Temp.	D.O.*	pH^^	Cond.^	Temp.	D.O.*	pH^^	Cond.^	Temp.	D.O.*	pH^^	Cond.^
Lab Ctrl	25	7.8	7.95	403	25	7.4	8.01	418	25	7.7	8.10	442
Bear @ North	25	7.8	8.01	294	25	7.5	8.07	301	25	7.9	8.31	302
Robert's @ Holt	25	7.8	7.96	565	25	7.4	8.06	582	25	7.9	8.25	594
French Camp Slough	25	7.8	7.91	238	25	7.5	8.00	257	25	8.1	8.41	271
French Camp Slough GR2	25	7.8	7.90	227	25	7.4	7.99	242	25	8.1	8.41	256
Mokelumne River	25	7.8	7.81	187	25	7.4	7.88	204	25	8.0	8.10	226
Tech Inits./ Date	BB 9/22/11				JR 09/23/11				JR 09/24/11			

**UNIT INSTRUMENTATION LEGEND**

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

**ADDITIONAL COMMENTS:**

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



**SJCD 11-09 Raw Data**

**Ammonia Analyses**

**AQUA-Science**  
Environmental Toxicology Specialists

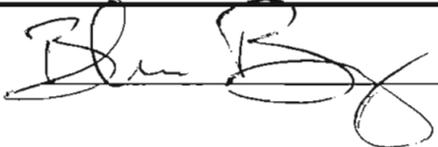
**AMMONIA ANALYSES**

1.0 Client/Event No: **SJCD 11-09**  
 2.0 Method: **HACH DR-2800/Method 8083**  
 3.0 Standard Lot No. **A9100**  
 4.0 Ammonia Standard Curve

Calibrators (mg/L)	Standard (mL)	NH <sub>3</sub> (mg/L)			Comments
		1	2	Mean	
0.1	0.05	0.13	0.13	0.13	
0.5	0.25	0.58	0.58	0.58	
1.0	0.5	1.11	1.11	1.11	
2.0	1.0	2.24	2.24	2.24	

5.0 Test Samples

Sample Name		NH <sub>3</sub> (mg/L)			Comments
		1	2	Mean	
Bear Creek @ North Alpine Rd	- 531BCANAR	0.74	0.74	0.74	
Robert's Island Drain @ Holt Rd	- 544RIDAHT	0.31	0.31	0.31	
French Camp Slough @ Airport Way	- 531SJC504	0.53	0.54	0.54	
French Camp Slough @ Airport Way GR2	- 531SJC504	0.55	0.55	0.55	
Mokelumne River @ Bruella Rd	- 531XMRABR	0.19	0.19	0.19	
Duck Creek @ Hwy 4	- 531XDCAHF	0.58	0.59	0.59	
Unnamed Drain to Lone Tree Creek @ Jack Tone Rd	- 531UDLTAJ	0.97	0.99	0.98	
Mormon Slough @ Jack Tone Rd	- 544MSAJTR	0.27	0.27	0.27	

Technician  Date 9/22/11

**SJCD 11-09 Raw Data**

**Meter Calibration**

**AQUA-Science**  
Environmental Toxicology Specialists

**Meter Calibration Log for Ag Waiver**

Meters calibrated on 09/21/11  
 Sample field date: 09/20/11  
 Test initiation date: 09/21/11  
 Technician's initials: Mc / AW

NOTE: Please enter initial calibration values into calibration logbook.

Initial Calibration

	Temp	EC (μS/cm) Meter- <u>03</u>		DO (mg/L) Meter- <u>09</u>		pH Meter- <u>02</u>			
Calibration specifics	NAP	internal <sup>1</sup>	<u>998</u> μS/cm	<u>99.7</u> μS/cm	internal <sup>2</sup>	water	4 buffer	10 buffer	7 buffer
Calibration readings	NAP	<u>0.466</u>	<u>999</u>	<u>100.1</u>	<u>N/A</u>	<u>7.8</u>	<u>4.00</u>	<u>10.05</u>	<u>7.00</u>

1. Internal cell constant for the below listed sites 0.466
2. Internal cell constant for the below listed sites N/A /water calibrated at 28 °C and 100% saturation ( 7.8 mg/L)

Sample ID:	Species		
	Cerio	FHM	Algae
<b>SJCD 11-09 : Initial Water Quality - Day 0</b>			
Bear Creek @ North Alpine Rd - 531BCANAR	<u>BEJ</u>	<u>BEJ</u>	<u>BB</u>
Robert's Island Drain @ Holt Rd - 544RIDAHT	<u>BEJ</u>	<u>BEJ</u>	<u>BB</u>
French Camp Slough @ Airport Way - 531SJC504	<u>BEJ</u>	<u>BEJ</u>	<u>BB</u>
French Camp Slough @ Airport Way GR2 - 531SJC504	<u>BEJ</u>	<u>BEJ</u>	<u>BB</u>
Mokelumne River @ Bruella Rd - 531XMRABR	<u>BEJ</u>	<u>BEJ</u>	<u>BB</u>
Duck Creek @ Hwy 4 - 531XDCAHF	<u>BEJ</u>		
Unnamed Drain to Lone Tree Creek @ Jack Tone Rd - 531UDLTAJ	<u>BEJ</u>		
Mormon Slough @ Jack Tone Rd - 544MSAJTR	<u>BEJ</u>		

Calibration Verification

	Temp	EC (μS/cm)		DO (mg/L)		pH			
Calibration specifics	NAP	internal <sup>1</sup>	<u>198</u> μS/cm	<u>99.7</u> μS/cm	internal <sup>2</sup>	water	4 buffer	10 buffer	7 buffer check
Calibration readings	NAP	<u>N/A</u>	<u>993</u>	<u>100.3</u>	<u>N/A</u>	<u>7.8</u>	<u>3.92</u>	<u>9.97</u>	<u>6.95</u>

1. Internal cell constant N/A
2. Internal cell constant N/A /water calibrated at 29 °C and 100% saturation ( 7.8 mg/L)















**AQUA-Science**  
Environmental Toxicology Specialists

**Alkalinity and Hardness Calibration Log for Ag Waiver**

Date: 09/21/11  
 Sample field date: 09/20/11  
 Test initiation date: 09/21/11  
 Technician initials: JS

**Initial Calibration**

	Alkalinity	Hardness
Calculated Value	100	100
Actual Value:	104	99

Sample ID:

**SJCD 11-09 : Initial Water Quality - Day 0**

			Alkalinity	Hardness
Bear Creek @ North Alpine Rd	-	531BCANAR	77	69
Robert's Island Drain @ Holt Rd	-	544RIDAHT	70	96
French Camp Slough @ Airport Way	-	531SJC504	45	34
French Camp Slough @ Airport Way GR2	-	531SJC504	47	35
Mokelumne River @ Bruella Rd	-	531XMRABR	26	15
Duck Creek @ Hwy 4	-	531XDCAHF	55	39
Unnamed Drain to Lone Tree Creek @ Jack Tone Rd	-	531UDLTAJ	44	35
Mormon Slough @ Jack Tone Rd	-	544MSAJTR	43	27

**Calibration Verification**

	Alkalinity	Hardness
Calculated Value	100	100
Actual Value:	103	99

# AQUA-Science

Environmental Toxicology Specialists

## Alkalinity and Hardness Calibration Log for Ag Waiver

Date: 09/24/11  
 Sample field date: 09/20/11  
 Test initiation date: 09/21/11  
 Technician initials: SR

**Initial Calibration**

	Alkalinity	Hardness
Calculated Value	100	100
Actual Value:	98	97

Sample ID:

SJCD 11-09 : Initial Water Quality - Day 3			Alkalinity	Hardness
Bear Creek @ North Alpine Rd	-	531BCANAR	75	63
Robert's Island Drain @ Holt Rd	-	544RIDAHT	70	95
French Camp Slough @ Airport Way	-	531SJC504	46	33
French Camp Slough @ Airport Way GR2	-	531SJC504	47	30
Mokelumne River @ Bruella Rd	-	531XMRABR	29	12
Duck Creek @ Hwy 4	-	531XDCAHF	52	36
Unnamed Drain to Lone Tree Creek @ Jack Tone Rd	-	531UDLTAJ	50	31
Mormon Slough @ Jack Tone Rd	-	544MSAJTR	44	26

**Calibration Verification**

	Alkalinity	Hardness
Calculated Value	100	100
Actual Value:	99	98

**SJCD 11-09 Raw Data**  
**Chain-of-Custody Forms**  
**and**  
**Sample Log-in Forms**



# AQUAScience CHAIN-OF-CUSTODY RECORD

Client Name: MLJ-LLC  
 Address: 632 Centrill Dr., Davis, CA 95618  
 Sampled By: F. Wolfe, K. Stovry  
 Phone: (530) 756-5200  
 Fax: (530) 756-5225  
 Project Manager: Michael Johnson  
 Project Name: San Joaquin County & Delta Water Quality Coalition

Acute Ceriodaphnia dubia	X	X	X	X	Chronic Selenastrum capricornutum
Acute Pimephales promelas	X	X	X	X	

Sample Identification	Sample Date	Sample Time	Sample Matrix	Number	Type	Preservative	SAMPLE COMMENTS
531BCANAR-GR	9/20/11	09:10	FW	5	1-G Amber Glass	Ice	
544RIDAHT-GR	9/20/11	12:50	FW	5	1-G Amber Glass	Ice	
531SJC504-GR	9/20/11	15:30	FW	5	1-G Amber Glass	Ice	
531SJC504-GR2	9/20/11	15:30	FW	5	1-G Amber Glass	Ice	
531XMRABR-GR	9/20/11	08:00	FW	5	1-G Amber Glass	Ice	

Comments:

Relinquished By	Signature: <i>[Signature]</i>
Print Name	Frank Wolfe
Organization	MLJ-LLC
Date	9/21/11
Time	9:00
Received By	Signature: <i>[Signature]</i>
Print Name	Cecilia Walker
Organization	AQUA-SCIENCE
Date	09/21/11
Time	09:00

Temperature at Log In: \_\_\_\_\_ (°C)

Matrix codes: SED = sediment, FW = freshwater, WW = wastewater, STRMW = stormwater

Client Name: MLJ-LLC  
 Address: 632 Cantrill Dr., Davis, CA 95618  
 Sampled By: F. Wolf, K. Story  
 Phone: (530) 756-5200  
 Fax: (530) 756-5225  
 Project Manager: Michael Johnson  
 Project Name: San Joaquin County & Delta Water Quality Coalition

Sample Identification	Sample Date	Sample Time	Sample Matrix	Number	Type	Preservative	Acute Ceriodaphnia dubia	Chronic Selenastrum capricornutum	SAMPLE COMMENTS
531XDCAHF-GR	9/20/11	11:20	FW	3	1-G Amber Glass	Ice	X		Ceriodaphnia dubia - Only
531UDLTJ-GR	9/20/11	12:40	FW	3	1-G Amber Glass	Ice	X		Ceriodaphnia dubia - Only
544MSAJTR-GR	9/20/11	10:50	FW	3	1-G Amber Glass	Ice	X		Ceriodaphnia dubia - Only

Comments:

Relinquished By		Received By	
Signature	<i>[Signature]</i>	Signature	
Print Name	Frank Wolfe	Print Name	
Organization	MLJ-LLC	Organization	
Date	9/21/11	Date	
Time	9:00	Time	
Relinquished By		Received By	
Signature	<i>[Signature]</i>	Signature	
Print Name	Cecilia Walker	Print Name	
Organization	AQUA-SCIENCE	Organization	
Date	9/21/11	Date	
Time	09:00	Time	

Temperature at Log In: \_\_\_\_\_ (°C)

Matrix codes: SED = sediment, FW = freshwater, WW = wastewater, STRMW = stormwater



**APPENDIX II**

**SJCD 11-09 Raw Data**

**REFERENCE TOXICANT**

*Ceriodaphnia dubia*

**Fathead Minnow**

**Algae**

**11-09 Raw Data**

**Reference Toxicant**

*Ceriodaphnia dubia*

# CETIS Summary Report

Report Date: 13 Sep-11 14:14 (p 1 of 1)  
 Test Code: r0011109 | 20-0393-2624

## Ceriodaphnia 96-h Acute Survival Test

Aqua-Science

<b>Batch ID:</b> 19-5965-7813	<b>Test Type:</b> Survival (96h)	<b>Analyst:</b> K. Miller
<b>Start Date:</b> 07 Sep-11 12:30	<b>Protocol:</b> EPA/821/R-02-012 (2002)	<b>Diluent:</b> Laboratory Water
<b>Ending Date:</b> 11 Sep-11 16:05	<b>Species:</b> Ceriodaphnia dubia	<b>Brine:</b> Not Applicable
<b>Duration:</b> 4d 4h	<b>Source:</b> In-House Culture	<b>Age:</b> <24h
<b>Sample ID:</b> 16-1541-6575	<b>Code:</b> 60494CFF	<b>Client:</b> Internal Lab
<b>Sample Date:</b> 07 Sep-11	<b>Material:</b> Sodium chloride	<b>Project:</b> Reference Toxicant
<b>Receive Date:</b>	<b>Source:</b> Reference Toxicant	
<b>Sample Age:</b> 13h	<b>Station:</b>	

### Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
09-6028-9404	96h Survival Rate	1	2	1.414	22.9%		Steel Many-One Rank Test

### Point Estimate Summary

Analysis ID	Endpoint	Level	gm/L	95% LCL	95% UCL	TU	Method
14-0397-3575	96h Survival Rate	EC5	1	0.4415	1.191		Linear Interpolation (ICPIN)
		EC10	1.083	0.6577	1.343		
		EC15	1.169	0.8986	1.618		
		EC20	1.259	1.011	1.838		
		EC25	1.352	1.059	2.075		
		EC40	1.656	1.188	2.57		
		EC50	1.881	1.244	2.826		

### Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
09-6028-9404	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria
14-0397-3575	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

### 96h Survival Rate Summary

Conc-gm/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	1	1	1	1	1	0	0	0.0%	0.0%
0.25		4	1	1	1	1	1	0	0	0.0%	0.0%
0.5		4	1	1	1	1	1	0	0	0.0%	0.0%
1		4	0.95	0.9127	0.9873	0.8	1	0.05	0.1	10.53%	5.0%
2		4	0.45	0.3225	0.5775	0	0.8	0.1708	0.3416	75.9%	55.0%
4		4	0	0	0	0	0	0	0		100.0%

### 96h Survival Rate Detail

Conc-gm/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
0.25		1	1	1	1
0.5		1	1	1	1
1		1	1	1	0.8
2		0.6	0.8	0.4	0
4		0	0	0	0

# CETIS Analytical Report

Report Date: 13 Sep-11 14:14 (p 1 of 2)  
 Test Code: r0011109 | 20-0393-2624

## Ceriodaphnia 96-h Acute Survival Test

Aqua-Science

Analysis ID: 09-6028-9404      Endpoint: 96h Survival Rate      CETIS Version: CETISv1.8.0  
 Analyzed: 13 Sep-11 14:09      Analysis: Nonparametric-Control vs Treatments      Official Results: Yes

Data Transform	Zeta	Alt Hyp	MC Trials	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	0	C > T	Not Run	1	2	1.414		22.9%

## Steel Many-One Rank Test

Control	vs	Conc-gm/L	Test Stat	Critical	DF	Ties	P-Value	Decision(α:5%)
Dilution Water		0.25	18	10	6	1	0.8333	Non-Significant Effect
		0.5	18	10	6	1	0.8333	Non-Significant Effect
		1	16	10	6	1	0.6105	Non-Significant Effect
		2*	10	10	6	0	0.0417	Significant Effect
		4*	10	10	6	0	0.0417	Significant Effect

## ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.407496	0.8814992	5	34.08	<0.0001	Significant Effect
Error	0.4656208	0.02586782	18			
Total	4.873117	0.907367	23			

## Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	4.528	4.248	0.0076	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.6492	0.884	<0.0001	Non-normal Distribution

## 96h Survival Rate Summary

Conc-gm/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	1	1	1	1	1	0	0	0.0%	0.0%
0.25		4	1	1	1	1	1	0	0	0.0%	0.0%
0.5		4	1	1	1	1	1	0	0	0.0%	0.0%
1		4	0.95	0.912	0.988	0.8	1	0.05	0.1	10.53%	5.0%
2		4	0.45	0.3201	0.5799	0	0.8	0.1708	0.3416	75.9%	55.0%
4		4	0	0	0	0	0	0	0	100.0%	100.0%

## Angular (Corrected) Transformed Summary

Conc-gm/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	1.345	1.345	1.345	1.345	1.345	0	0	0.0%	0.0%
0.25		4	1.345	1.345	1.345	1.345	1.345	0	0	0.0%	0.0%
0.5		4	1.345	1.345	1.345	1.345	1.345	0	0	0.0%	0.0%
1		4	1.286	1.24	1.331	1.107	1.345	0.05953	0.1191	9.26%	4.43%
2		4	0.7259	0.583	0.8687	0.2255	1.107	0.1878	0.3755	51.74%	46.04%
4		4	0.2255	0.2255	0.2255	0.2255	0.2255	0	0	0.0%	83.24%





**AQUA-Science**  
Environmental Toxicology Specialists

**MORTALITY AND BEHAVIOR OBSERVATIONS**

Test Number:	September Reference Toxicant Study		Director:	J.L. Miller	
Protocol No.:	EPA 821/R-02/012		Technicians:	Walker/Concepcion/Sanford/Berry/Renn	
Test Material:	Reference Toxicant NaCl in Lab Dilution Water				
Test Species:	Ceriodaphnia dubia		Animal Lot No.:	A/S RO: 090711	
Initiation Date:	September 7, 2011		Termination Date:	September 11, 2011	

Conc. (g/L)	Vessel No.	Cumulative Mortality				Comments
		Day 1	Day 2	Day 3	Day 4	
Control	A	0	0	0	0	n=5 animals/rep
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
0.25	A	0	0	0	0	Test Initiation Info: Time: 1230 Tech: W
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
0.5	A	0	0	0	0	Test Termination Info: Time: 1605 Tech: M
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
1	A	0	0	0	0	DINOTRATES PRESENT MC 09/11/11
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
2	A	0	1	2	2	
	B	1	1	1	1	
	C	1	2	3	3	
	D	0	5			
4	A	5				
	B	5				
	C	5				
	D	5				
Technician Initials		W	W	W	M	
Observation Time		1245	1255	1310	1605	
Observation Date		09/08/11	09/09/11	09/10/11	09/11/11	

**AQUA-Science**  
Environmental Toxicology Specialists

**DOSE PREPARATION SHEET**

09/07/11

**September Reference Toxicant**

96 hr. Static Acute bioassay

*Ceriodaphnia dubia*

Reference Toxicant test concentrations: 0, 0.25, 0.5, 1.0, 2.0, and 4.0 g/L NaCl

Ref Tox Std = 40 g/L NaCl

Dilution water=Reverse Osmosis water amended with EPA salts  
to achieve EPAMH specifications (R/O EPAMH)

All surface waters filtered through 60 µm screen

Ref. Tox. Conc. (g/L)	Amount Stock (mL)	Dilution Water (mL)	Total (mL)	
0	0	100	100	
0.25	0.625	QS to 100	100	20 mL used for pH
0.5	1.25	QS to 100	100	measurements
1.0	2.5	QS to 100	100	80 mL used for test
2.0	5.0	QS to 100	100	
4.0	10	QS to 100	100	

n = 5 animals/replicate - 4 replicates/concentration

	Day 0	Day 1	Day 2	Day 3
Tech	BES	ML	GW	GW
Time	1040	0950	1005	0925
Date	9/7/11	09/08/11	09/09/11	09/10/11







**AQUA-Science**  
Environmental Toxicology Specialists

**WATER QUALITY REPORT FOR AQUATIC BIOASSAYS**

Test Number:	September Reference Toxicant	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/012	Technicians:	Walker/Concepcion/Sanford/Berry/Renn
Test Material:	Reference Toxicant NaCl in Lab Dilution Water		
Test Species:	<i>Ceriodaphnia dubia</i>	Animal Lot No.:	A/S RO: 090711
Initiation Date:	September 7, 2011	Termination Date:	September 11, 2011

Conc. (g/L)	OBSERVATION Day: 3 Date: 09/10/11					24 Hour Obsv.			
	Temperature (°C)	Dissolved Oxygen*	pH <sup>^^</sup>	Alkalinity **/ Hardness ~	Conductivity <sup>^</sup>	Temp	D.O.*	pH <sup>^^</sup>	Cond. <sup>^</sup>
Lab Control	25	7.8	8.00	71/76	520	25	7.2	8.19	377
0.25	25	7.8	7.99	80/80	798	25	7.2	8.18	839
0.5	25	7.9	7.96	80/90	1284	25	7.2	8.18	1346
1	25	7.9	7.95	80/90	2200	25	7.1	8.17	2300
2	25	7.9	7.93	-	3810	25	7.2	8.19	3950
4	-	-	-	-	-	-	-	-	-

BES 9/11/11

**UNIT INSTRUMENTATION LEGEND**

\*=Dissolved oxygen (mg/L): Meter ID 05      \*\*Alkalinity (mg/L CaCO<sub>3</sub>); HACH Test Kit

<sup>^^</sup>= pH: Meter ID 02      ~ =Water Hardness (mg/L CaCO<sub>3</sub>); HACH Test Kit

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

**ADDITIONAL COMMENTS:** @ Measurements taken in 10mL sample vol. 09/10/11

Lab Control = 2x carbon filtered reverse osmosis water at EPA moderately hard level using EPA salts.  
Dilution water ID = K10 & PAM #094

All surface waters filtered through a 60 µm screen daily

Technician: Jack      Date: 09/10/11

**11-09 Raw Data**

**Reference Toxicant**

**Fathead Minnow**

# CETIS Summary Report

Report Date: 13 Sep-11 14:14 (p 1 of 1)  
 Test Code: r0031109 | 04-7660-2678

## Fathead Minnow 96-h Acute Survival Test

Aqua-Science

Batch ID: 20-3080-2515	Test Type: Survival (96h)	Analyst: K. Miller
Start Date: 07 Sep-11 15:15	Protocol: EPA/821/R-02-012 (2002)	Diluent: Laboratory Water
Ending Date: 11 Sep-11 13:35	Species: Pimephales promelas	Brine: Not Applicable
Duration: 94h	Source: Aquatox, AR	Age: 7d
Sample ID: 16-1541-6575	Code: 60494CFF	Client: Internal Lab
Sample Date: 07 Sep-11	Material: Sodium chloride	Project: Reference Toxicant
Receive Date:	Source: Reference Toxicant	
Sample Age: 15h	Station:	

### Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
11-0151-9676	96h Survival Rate	5	7.5	6.124	8.37%		Steel Many-One Rank Test

### Point Estimate Summary

Analysis ID	Endpoint	Level	gm/L	95% LCL	95% UCL	TU	Method
02-2176-8741	96h Survival Rate	EC5	5.072	0.9565	5.182		Linear Interpolation (ICPIN)
		EC10	5.22	4.988	5.338		
		EC15	5.371	5.142	5.5		
		EC20	5.526	5.278	5.711		
		EC25	5.685	5.431	5.919		
		EC40	6.184	5.87	6.584		
		EC50	6.538	6.14	7.085		

### Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
02-2176-8741	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria
11-0151-9676	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

### 96h Survival Rate Summary

Conc-gm/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	1	1	1	1	1	0	0	0.0%	0.0%
1.25		4	1	1	1	1	1	0	0	0.0%	0.0%
2.5		4	0.975	0.9563	0.9937	0.9	1	0.025	0.05	5.13%	2.5%
5		4	0.975	0.9563	0.9937	0.9	1	0.025	0.05	5.13%	2.5%
7.5		4	0.25	0.2018	0.2982	0.1	0.4	0.06455	0.1291	51.64%	75.0%
10		4	0	0	0	0	0	0	0		100.0%

### 96h Survival Rate Detail

Conc-gm/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
1.25		1	1	1	1
2.5		1	1	0.9	1
5		1	1	1	0.9
7.5		0.4	0.1	0.2	0.3
10		0	0	0	0



# CETIS Analytical Report

Report Date: 13 Sep-11 14:14 (p 2 of 2)  
Test Code: r0031109 | 04-7660-2678

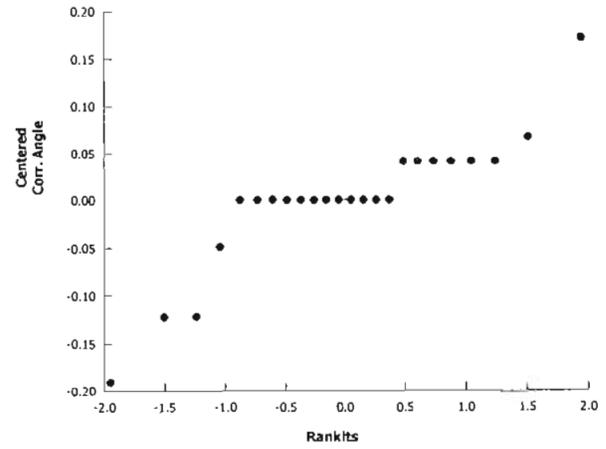
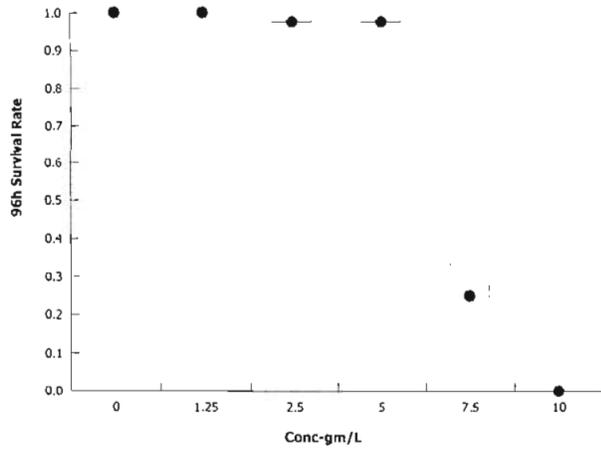
Fathead Minnow 96-h Acute Survival Test

Aqua-Science

Analysis ID: 11-0151-9676      Endpoint: 96h Survival Rate  
Analyzed: 13 Sep-11 14:14      Analysis: Nonparametric-Control vs Treatments

CETIS Version: CETISv1.8.0  
Official Results: Yes

## Graphics



**CETIS Analytical Report**

Report Date: 13 Sep-11 14:14 (p 1 of 1)  
 Test Code: r0031109 | 04-7660-2678

**Fathead Minnow 96-h Acute Survival Test**

**Aqua-Science**

Analysis ID: 02-2176-8741      Endpoint: 96h Survival Rate      CETIS Version: CETISv1.8.0  
 Analyzed: 13 Sep-11 14:14      Analysis: Linear Interpolation (ICPIN)      Official Results: Yes

**Linear Interpolation Options**

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	757303375	200	Yes	Two-Point Interpolation

**Point Estimates**

Level	gm/L	95% LCL	95% UCL
EC5	5.072	0.9565	5.182
EC10	5.22	4.988	5.338
EC15	5.371	5.142	5.5
EC20	5.526	5.278	5.711
EC25	5.685	5.431	5.919
EC40	6.184	5.87	6.584
EC50	6.538	6.14	7.085

**96h Survival Rate Summary**

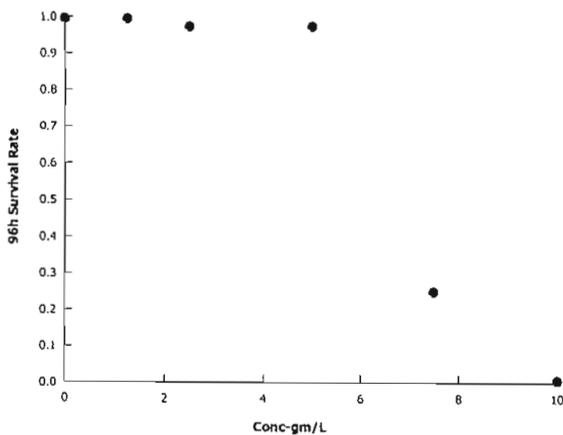
**Calculated Variate(A/B)**

Conc-gm/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	4	1	1	1	0	0	0.0%	0.0%	40	40
1.25		4	1	1	1	0	0	0.0%	0.0%	40	40
2.5		4	0.975	0.9	1	0.025	0.05	5.13%	2.5%	39	40
5		4	0.975	0.9	1	0.025	0.05	5.13%	2.5%	39	40
7.5		4	0.25	0.1	0.4	0.06455	0.1291	51.64%	75.0%	10	40
10		4	0	0	0	0	0	100.0%	100.0%	0	40

**96h Survival Rate Detail**

Conc-gm/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
1.25		1	1	1	1
2.5		1	1	0.9	1
5		1	1	1	0.9
7.5		0.4	0.1	0.2	0.3
10		0	0	0	0

**Graphics**



**AQUA-Science**  
Environmental Toxicology Specialists

**MORTALITY AND BEHAVIOR OBSERVATIONS**

Test Number:	September Reference Toxicant	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/012	Technicians:	Walker/Concepcion/Sanford/Berry/Renn
Test Material:	Reference Toxicant NaCl in Lab Dilution Water		
Test Species:	<i>Pimephales promelas</i>	Animal Lot No.:	AQTX: 083111
Initiation Date:	September 7, 2011	Termination Date:	September 11, 2011

Conc. (g/L)	Vessel No.	Cumulative Mortality				Comments
		Day 1	Day 2	Day 3	Day 4	
Control	A	0	0	0	0	n=10 animals/rep
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
1.25	A	0	0	1	0	Test Initiation Info: Time: 1515 Tech: BCS
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
2.5	A	0	0	0	0	Test Termination Info: Time: 1335 Tech: BCS
	B	0	0	0	0	
	C	0	0	0	1	
	D	0	0	0	0	
5	A	0	0	0	0	
	B	0	0	0	0	
	C	0	0	0	0	
	D	1	1	1	1	
7.5	A	5	6	6	6	
	B	9	9	9	9	
	C	8	8	8	8	
	D	6	7	7	7	
10	A	10	/	/	/	
	B	10	/	/	/	
	C	10	/	/	/	
	D	10	/	/	/	

Technician Initials	BB	BB	BB	BCS
Observation Time	1530	1455	1435	1335
Observation Date	09/08/11	09/09/11	09/10/11	09/11/11

**AQUA-Science**  
Environmental Toxicology Specialists

**DOSE PREPARATION SHEET**

09/07/11

**September Reference Toxicant**  
96-hour Static-Renewal Acute Bioassay  
*Pimephales promelas*

Lab Control = 2X carbon filtered reverse osmosis well water amended to EPA moderately hard specifications.

All surface waters filtered through 60 µm screen  
4 replicates/concentration: 400ml/replicate: 10 animals/replicate

REFERENCE TOXICANT: 10 g/L NaCl Std.

Sample Conc. (g/L)	Amount ref tox (mL)	Dilution Water (mL)	Total (mL)
0	0	1000	1000
1.25	125	QS to 1000	1000
2.5	250	QS to 1000	1000
5	500	QS to 1000	1000
7.5	750	QS to 1000	1000
10	1000	0	1000

Test Day	Day 0	Day 1	Day 2	Day 3
Technician	BES	BB	BB	BB
Time	1055	1035	1025	1027
Date	9/7/11	9/8/11	9/9/11	9/10/11

# AQUA-Science

Environmental Toxicology Specialists

## WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	September Reference Toxicant	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/012	Technicians:	Walker/Concepcion/Sanford/Berry/Renn
Test Material:	Reference Toxicant NaCl in Lab Dilution Water		
Test Species:	<i>Pimephales promelas</i>	Animal Lot No.:	AQTX: 083111
Initiation Date:	September 7, 2011	Termination Date:	September 11, 2011

Conc. (g/L)	OBSERVATIONS: Day 0 Date: 09/07/11					24 Hour Obsv.			
	Temperature (°C)	Dissolved Oxygen*	pH <sup>^^</sup>	Alkalinity**/ Hardness ~	Conductivity ^	Temp	D.O.*	pH <sup>^^</sup>	Cond.^
Lab Control	25	8.0	8.05	71/76	321	24	7.5	7.72	333
1.25	25	8.0	7.89	80/80	2500	24	7.5	7.64	2560
2.5	25	8.0	7.74	-	4720	24	7.4	7.97	4810
5	25	8.0	7.54	80/80	8960	24	7.4	7.54	9170
7.5	25	8.0	7.38	-	12970	24	7.4	7.51	13320
10	25	8.0	7.20	80/90	16900	24	6.9	7.45	17470

MC 09/08/11

### UNIT INSTRUMENTATION LEGEND

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

\*\*Alkalinity (mg/L CaCO<sub>3</sub>): HACH Test Kit

^^= pH: Meter ID 02

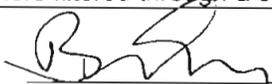
~=Water Hardness (mg/L CaCO<sub>3</sub>): HACH Test Kit

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

**ADDITIONAL COMMENTS:** ① measurement taken in 10ml sample vol. 8:59 AM 9/7/11

Lab Control = 2x carbon filtered reverse osmosis water at EPA moderately hard level using EPA salts.  
Dilution water ID = P/O exam # 094

All surface waters filtered through a 60 µm screen daily

Technician: 

Date: 09/07/11









**AQUATOX, INC.**  
416 TWIN POINTS ROAD  
HOT SPRINGS, ARKANSAS 71913  
501-520-0560

**TEST ORGANISM HISTORY**

DATE SHIPPED 9/6/11 CLIENT Agua Ciencia  
Purchase Order #: Cecilia

SPECIES: Pimephales promelas Mysidopsis bahia Cyprinodon variegates

Quantity Shipped: 745

Age: 7 days in 9/7/11

Brood Stock Source: Anderson Farms

Culture Water: Groundwater Artificial Salts Artificial Salts

Hardness (Mg/l CaCO<sub>3</sub>) 160 Salinity (ppt) \_\_\_\_\_

Dissolved Oxygen (Mg/l): 8.1

Feeding: Artemia

Comments: 25 ± 1°C Login SES 09/07/11

DO ~ 21.9 mg/L (260%)

temp 23.7°C

Shipped Via: Federal Express UPS Overnight

Packaged By: \_\_\_\_\_

**11-09 Raw Data**  
**Reference Toxicant**  
**Algae**

**CETIS Summary Report**

Report Date: 12 Sep-11 11:30 (p 1 of 1)  
 Test Code: r0021109 | 04-5522-4332

**Selenastrum Growth Test (w/o EDTA)**

**Aqua-Science**

Batch ID: 04-3310-5954	Test Type: Cell Growth	Analyst: K. Miller
Start Date: 07 Sep-11 14:05	Protocol: EPA/821/R-02-013 (2002)	Diluent: Laboratory Water
Ending Date: 11 Sep-11 15:00	Species: Selenastrum capricornutum	Brine: Not Applicable
Duration: 4d 1h	Source: University of Texas-Botany Dept.	Age: 1D
Sample ID: 02-1799-0322	Code: CFE44B2	Client: Internal Lab
Sample Date: 06 Sep-11	Material: Zinc chloride	Project:
Receive Date:	Source: Reference Toxicant	
Sample Age: 38h	Station:	

**Comparison Summary**

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
16-1636-8068	Light Absorbance	8	16	11.31	30.0%		Dunnett's Multiple Comparison Test

**Point Estimate Summary**

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
14-6151-0632	Light Absorbance	IC5	2.711	0.7342	11.32		Linear Interpolation (ICPIN)
		IC10	3.59	1.608	12.13		
		IC15	8.539	N/A	10.62		
		IC20	9.514	0.0718	11.54		
		IC25	10.59	6.228	13.03		
		IC40	14.52	10.52	18.9		
		IC50	19.11	12.61	24.72		

**Light Absorbance Summary**

Conc-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.02525	0.02374	0.02676	0.022	0.031	0.002016	0.004031	15.96%	0.0%
1		4	0.03275	0.03052	0.03498	0.024	0.037	0.002983	0.005965	18.21%	-29.7%
2		4	0.0315	0.02947	0.03353	0.026	0.039	0.002723	0.005447	17.29%	-24.75%
4		4	0.02625	0.02415	0.02835	0.02	0.031	0.00281	0.00562	21.41%	-3.96%
8		4	0.02625	0.02484	0.02766	0.023	0.03	0.001887	0.003775	14.38%	-3.96%
16		4	0.0165	0.01561	0.01739	0.014	0.019	0.00119	0.00238	14.43%	34.65%
32		4	0.01025	0.009892	0.01061	0.009	0.011	0.0004787	0.0009574	9.34%	59.41%

**Light Absorbance Detail**

Conc-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.031	0.022	0.023	0.025
1		0.024	0.037	0.036	0.034
2		0.03	0.031	0.039	0.026
4		0.031	0.02	0.023	0.031
8		0.023	0.029	0.03	0.023
16		0.018	0.014	0.015	0.019
32		0.01	0.011	0.011	0.009





**AQUA-Science**  
 Environmental Toxicology Specialists  
**ALGAL BIOASSAY - CELL DETERMINATION**

Test Number:	September Reference Toxicant	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/013	Technicians:	Walker/Concepcion/Sanford/Berry/Renn
Test Material:	Reference Toxicant Zn <sup>2+</sup> in Lab Dilution Water WITHOUT EDTA		
Test Species:	<i>Selenastrum capricornutum</i>	Animal Lot No.:	UTEX: 082211
Initiation Date:	September 7, 2011	Termination Date:	September 11, 2011

Absorbance @ 750 nm with Hach DR 2800 Spectrophotometer		Test Terminated @ 1500			
Conc. (µg/L)	Replicate	1	2		Mean
Control	A	0.030	0.031	0.031	0.031
	B	0.022	0.022	0.021	0.022
	C	0.023	0.023	0.024	0.023
	D	0.025	0.025	0.026	0.025
1	A	0.024	0.024	0.024	0.024
	B	0.037	0.037	0.036	0.037
	C	0.036	0.036	0.036	0.036
	D	0.034	0.034	0.034	0.034
2	A	0.031	0.030	0.030	0.030
	B	0.031	0.030	0.031	0.031
	C	0.039	0.039	0.038	0.039
	D	0.026	0.026	0.026	0.026
4	A	0.031	0.031	0.031	0.031
	B	0.020	0.019	0.020	0.020
	C	0.023	0.023	0.024	0.023
	D	0.030	0.031	0.031	0.031
8	A	0.023	0.023	0.023	0.023
	B	0.029	0.029	0.029	0.029
	C	0.030	0.030	0.030	0.030
	D	0.023	0.023	0.023	0.023
16	A	0.018	0.018	0.018	0.018
	B	0.014	0.014	0.013	0.014
	C	0.015	0.015	0.014	0.015
	D	0.019	0.019	0.019	0.019
32	A	0.010	0.011	0.010	0.010
	B	0.011	0.011	0.011	0.011
	C	0.011	0.010	0.011	0.011
	D	0.009	0.009	0.009	0.009

Technician:

Sarah R

Date:

09/11/11



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Environmental Toxicology Specialists

**WATER QUALITY REPORT FOR AQUATIC BIOASSAYS**

Test Number:	September Reference Toxicant	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/013	Technicians:	Walker/Concepcion/Sanford/Berry/Renn
Test Material:	Reference Toxicant Zn <sup>2+</sup> in Lab Dilution Water	WITHOUT EDTA	
Test Species:	<i>Selenastrum capricornutum</i>	Animal Lot No.:	UTEX: 082211
Initiation Date:	September 7, 2011	Termination Date:	September 11, 2011

Ref. Tox. Conc (µg/L)	Day 1				Day 2				Day 3			
	Temp.	D.O.*	pH <sup>^^</sup>	Cond. <sup>^</sup>	Temp	D.O.*	pH <sup>^^</sup>	Cond. <sup>^</sup>	Temp.	D.O.*	pH <sup>^^</sup>	Cond. <sup>^</sup>
Control ①	25	7.8	7.89	422	24	8.0	8.01	435	26	7.7	8.20	449
1	25	7.9	7.94	430	25	7.9	8.16	483	26	7.7	8.21	542
2	25	7.8	7.98	401	25	7.9	8.15	421	26	7.7	8.22	437
4	25	7.9	7.98	409	26	7.7	8.15	430	26	7.6	8.21	440
8	25	8.0	7.98	408	25	7.8	8.17	412	26	7.7	8.21	421
16	25	8.0	8.00	405	25	7.8	8.17	413	26	7.7	8.21	419
32	25	8.0	7.99	406	25	7.8	8.18	413	26	7.6	8.19	419
Tech Init/Date	CW 09/08/11				SR 09/09/11				SR 09/10/11			

**UNIT INSTRUMENTATION LEGEND**

\*=Dissolved oxygen (mg/L): Meter ID 02  
 ^^= pH: Meter ID 03  
 ^=Conductivity/Salinity (µmohs): Meter ID 01

**ADDITIONAL COMMENTS:** ① Lab control WQ measured using: DO-05, pH-02, EC-03 CW 09/08/11

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

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**ALGAL BIOASSAY DATA SHEETS**

**1.0 TEST AND CLIENT INFORMATION**

Test Number:	September Reference Toxicant	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/013	Technician(s):	Walker/Concepcion/Sanford/Berry/Renn
Test Material:	Reference Toxicant Zn2+ in Lab Dilution Water WITHOUT EDTA		
Initiation Date:	September 7, 2011	Termination Date:	September 11, 2011

**2.0 TEST CONDITIONS & TEST SPECIES INFORMATION**

Species:	<i>Selenastrum capricornutum</i>	Temperature:	25.0°C ± 1.0°C
Source:	University of Texas (UTEX)	Agitation:	100 RPM continuous
Media:	EPA Algal Assay Media (AAM)	Lighting:	400 ft candles
Inoculation:	10,000 cells/mL		

**3.0 DILUTION WATER INFORMATION**

Dilution Water Source	D.O. (mg/L)	pH (units)	E.C. (µmhos)	Other	Comments
RO EPAMH	6.7	8.15	407		0.22 µm Metrigard vacuum filtered
Reference Toxicant	6.4	8.13	400		0.22 µm Metrigard vacuum filtered

**4.0 PREPARATION OF EXPOSURE SOLUTIONS**

Ref. Tox. Conc. (µg/L)	Ctrl.	1	2	4	8	16	32	Time: 1145
ZnCl <sub>2</sub> (µL)	0	19	35	75	139	278	600	Tech: BB
Dilution Water (mL)	250	QS to 250/270 mL						2011-001 ZnCl <sub>2</sub> @
TOTAL	250 mL	270 mL	250 mL	270 mL	250 mL	250 mL	270 mL	14.4 mg/L

Comments:

Technician: Ben Berry Date: 09/07/11



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**ALGAL BIOASSAY DATA SHEETS**

**6.0 ALGAL COUNT Cont'd**

Final Algal Density Check Continued						
	ID	Count 1	Count 2	Count 3	Dilution	Adj. Avg.
Flask #9						
Flask #10						
Flask #11						
Flask #12						
Flask #13						
Flask #14						

**7.0 LAB NOTES**

9/7/11

**September Reference Toxicant**  
96 Hr. Static Growth  
*Selenastrum capricornutum*

**1.0 Stock Preparation**  
0.32 liters of Reference Toxicant vacuum filtered through 0.22 µm Metrigard filter  
0.8 liters of R/O EPAMH vacuum filtered through 0.22 µm Metrigard filter

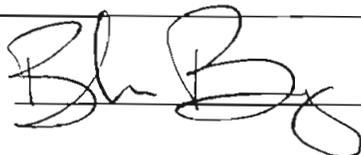
**2.0 Water Quality Measurement**  
Water quality of dilution and effluent measured after addition of EPA Algal Assay Media (AAM).  
Section 3.0, page 1

**3.0 Algal Assay Media Addition**  
Algal Assay Media prepared as per EPA 821/R-02/013; Section 14 - Tables 1 and 2 (**without EDTA**)  
Added 0.32 mL each of #1-5 EPA AAM to 0.32 liters of Reference Toxicant  
Added 0.8 mL each of #1-5 EPA AAM to 0.8 liters R/O EPAMH Lab Control Water.  
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 400 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 250 mL in solution beaker into 5-50mL aliquots and distributed into flasks A-E.  
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: \_\_\_\_\_

09/07/11



**APPENDIX III**

**SJCD 11-09 TIE Raw Data**

*Ceriodaphnia dubia*

**AQUA-Science**  
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**MORTALITY AND BEHAVIOR OBSERVATIONS**

Test Number:	SJCD 11-09 TIE	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/012	Technicians:	Walker/Concepcion/Sanford/Berry
Test Material:	SJCD Ag Waiver Sample <sup>Duck</sup> Dry Creek @ Hwy 4 (09/20/11) 531 X DEAGF 531 X DCAHF <b>Baseline</b>		
Test Species:	<i>Ceriodaphnia dubia</i>	Animal Lot No.:	AS R/O: 092611
Initiation Date:	September 26, 2011	Termination Date:	September 30, 2011

Ambient (%)	Vessel No.	Cumulative Mortality				Comments
		Day 1	Day 2	Day 3	Day 4	
Control	A	0	0	0	0	n=5 animals/rep
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
6.25 (1)	A	0	0	0	0	Test Initiation Info: Time: 1550 Tech: MU
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
12.5 (1)	A	0	0	0	0	Test Termination Info: Time: 1425 Tech: W
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
25 (1)	A	0	0	0	0	① light sediment Mc 0912711 ② heavy sediment Mc 0912711 ③ neonates present Mc 0912911 ④ neonates present W 09/30/11
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
50 (2)	A	0	0	0	0	
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
100 (2)	A	0	0	0	0	
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
Technician Initials		Mc	Mc	Mc	W	
Observation Time		1430	1450	1430	1425	
Observation Date		09/27/11	09/28/11	09/29/11	09/30/11	



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**MORTALITY AND BEHAVIOR OBSERVATIONS**

Test Number:	SJCD 11-09 TIE	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/012	Technicians:	Walker/Concepcion/Sanford/Berry
Test Material:	SJCD Ag Waiver Sample <sup>Duck</sup> Dry Creek @ Hwy 4 (09/20/11) 531XDCAGF 531XDCAHF <b>+PBO</b>		
Test Species:	<i>Ceriodaphnia dubia</i>	Animal Lot No.:	AS R/O: 092611
Initiation Date:	September 26, 2011	Termination Date:	September 30, 2011

Sample	Vessel No.	Cumulative Mortality				Comments
		Day 1	Day 2	Day 3	Day 4	
<b>+PBO @ 100 µg/L</b>						
PBO Control	A	0	0	0	0	Test Initiation Info: Time: 1410 Tech: MC
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	1	1	1	
6.25 µg/L	A	0	0	0	0	(1) light sediment MC 09/27/11 (2) heavy sediment MC 09/27/11 (3) neonates present MC 09/29/11 (4) neonates present WS 09/30/11 Test terminated: WS @ 14:30
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
12.5 µg/L	A	0	0	0	0	
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
25 µg/L	A	0	0	0	0	
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
50 µg/L	A	0	0	0	0	
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	
100 µg/L	A	0	0	0	0	
	B	0	0	0	0	
	C	0	0	0	0	
	D	0	0	0	0	

Technician Initials	MC	MC	MC	WS
Observation Time	1500	1510	1500	1430
Observation Date	09/27/11	09/28/11	09/29/11	09/30/11



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Page \_\_\_\_

**DOSE PREPARATION SHEET**

9/26/11

**SJCD 11-09 TIE**

96 hr. Static Acute bioassay w/ 24 hr. Changeout  
*Ceriodaphnia dubia*

**Baseline**

Test Concentrations: Control, 6.25, 12.5, 25, 50, 100%

Control water = Reverse Osmosis water amended with EPA salts  
to achieve EPAMH specifications (R/O EPAMH)

All surface waters filtered through 60 µm screen

Sample	Amount Sample (mL)	Dilution Water (mL)	Total (mL)*	
Control	0	100	100	*20 mL used
6.25	6.25	QS to 100	100	to measure pH
12.5	12.5	QS to 100	100	
25	25	QS to 100	100	
50	50	QS to 100	100	
100	100	0	100	

	Day 0	Day 1	Day 2	Day 3
Tech	BES	BES	BES	BB
Time	1300	1045	1115	1035
Date	9/26/11	9/27/11	9/28/11	9/29/11

①entry error BES 9/27/11  
n = 5 animals/replicate - 4 replicates/concentration



**AQUA-Science**  
Environmental Toxicology Specialists

**WATER QUALITY REPORT FOR AQUATIC BIOASSAYS**

Test Number:	SJCD 11-09 TIE	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/012	Technicians:	Walker/Concepcion/Sanford/Berry
Test Material:	SJCD Ag Waiver Sample <sup>Duck</sup> Dry Creek @ Hwy 4 (09/20/11) 531XDCAGF		<b>Baseline</b>
Test Species:	<i>Ceriodaphnia dubia</i>	Animal Lot No.:	# AS R/O: 092611
Initiation Date:	September 26, 2011	Termination Date:	September 30, 2011

Ambient (%)	OBSERVATIONS: Day 1 09/27/11					24 Hour Obsv.			
	Temperature (°C)	Dissolved Oxygen*	pH <sup>^^</sup>	Alkalinity **/ Hardness~	Conductivity ^	Temp	D.O.*	pH <sup>^^</sup>	Cond. ^
Lab Control	24	7.8	8.27	57/77	315	25	6.9	7.87	352
6.25	24	7.8	8.21	② 60/60	299	25	6.9	7.86	325
12.5	24	7.9	8.17	-	284	25	6.6	7.83	309
25	24	8.0	8.07	② 50/40	261	25	6.4	7.78	283
50	24	8.1	7.98	-	212	25	6.5	7.73	232
100	24	10.184	7.91	52/34	112	25	6.7	7.71	129

**UNIT INSTRUMENTATION LEGEND**

\*=Dissolved oxygen (mg/L): Meter ID 05                      \*\*Alkalinity (mg/L CaCO<sub>3</sub>); HACH Test Kit

<sup>^^</sup>= pH: Meter ID 02    ~~=Water Hardness (mg/L CaCO<sub>3</sub>); HACH Test Kit

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

**ADDITIONAL COMMENTS:** ① De-aerated before use BCS 9/27/11  
 ② measurement +4.4 in in 1ml sample vol. BCS 9/27/11

Lab Control = 2x carbon filtered reverse osmosis water at EPA moderately hard level using EPA salts.  
 Dilution water ID = R/O EDAM.H # 099

All surface waters filtered through a 60 µm screen daily

Technician:                       Date: 09/27/11

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**WATER QUALITY REPORT FOR AQUATIC BIOASSAYS**

Test Number:	SJCD 11-09 TIE	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/012	Technicians:	Walker/Concepcion/Sanford/Berry
Test Material:	SJCD Ag Waiver Sample Dry Creek @ Hwy 4 (09/20/11) 531XDCAGF		<b>Baseline</b>
Test Species:	<i>Ceriodaphnia dubia</i>	Animal Lot No.:	# AS R/O: 092611
Initiation Date:	September 26, 2011	Termination Date:	September 30, 2011

Ambient (%)	OBSERVATIONS: Day 2 09/28/11					24 Hour Obsv.			
	Temperature (°C)	Dissolved Oxygen*	pH <sup>^^</sup>	Alkalinity **/ Hardness~	Conductivity ^	Temp	D.O.*	pH <sup>^^</sup>	Cond. ^
Lab Control	24	8.1	8.09	57/77	316	25	6.8	7.97	356
6.25	24	8.1	8.04	60/60	297	25	6.7	7.91	323
12.5	24	8.2	8.00	-	284	25	6.5	7.87	308
25	24	8.2	7.92	50/40	259	25	6.4	7.82	283
50	24	8.3	7.85	-	212	25	6.7	7.80	231
100	25	10.1/8.5	7.69	52/34	112	25	6.6	7.79	129

**UNIT INSTRUMENTATION LEGEND**

\*=Dissolved oxygen (mg/L): Meter ID 05                      \*\*Alkalinity (mg/L CaCO<sub>3</sub>); HACH Test Kit  
 ^^= pH: Meter ID 02    ~Water Hardness (mg/L CaCO<sub>3</sub>); HACH Test Kit  
 ^=Conductivity/Salinity (µmhos): Meter ID 03

**ADDITIONAL COMMENTS:** ① Desaturated before use 9/28/11  
 ② measurement taken in 10ml sample vial 9/28/11

Lab Control = 2x carbon filtered reverse osmosis water at EPA moderately hard level using EPA salts.  
 Dilution water ID = R/O ~~05~~ unit # 099

All surface waters filtered through a 60 µm screen daily

Technician:  Date: 9/28/11

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**WATER QUALITY REPORT FOR AQUATIC BIOASSAYS**

Test Number:	SJCD 11-09 TIE	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/012	Technicians:	Walker/Concepcion/Sanford/Berry
Test Material:	SJCD Ag Waiver Sample <u>Duck</u> Dry Creek @ Hwy 4 (09/20/11) 531XDCAF		<b>Baseline</b>
Test Species:	<i>Ceriodaphnia dubia</i>	Animal Lot No.:	# AS R/O: 092611
Initiation Date:	September 26, 2011	Termination Date:	September 30, 2011

Ambient (%)	OBSERVATIONS: Day 3 09/29/11					24 Hour Obsv.			
	Temperature (°C)	Dissolved Oxygen*	pH^^	Alkalinity **/ Hardness~	Conductivity ^	Temp	D.O.*	pH^^	Cond. ^
Lab Control	24	7.7	8.15	57/77	319	25	7.4	8.21	367
6.25	24	7.7	8.13	①60/80	304	25	7.4	8.18	330
12.5	24	7.8	8.09	-	286	25	7.3	8.13	311
25	24	7.8	8.09	②60/70	263	25	7.3	8.10	283
50	25	7.9	8.04	-	214	25	7.3	8.07	233
100	25	①10.4/8.1	7.96	53/36	110	25	7.4	8.02	125

**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 DESATURATED BEFORE USE BB 9/29/11  
② MEASUREMENT TAKEN IN 10ml SAMPLE VOL. BB 9/29/11

Lab Control = 2x carbon filtered reverse osmosis water at EPA moderately hard level using EPA salts.  
Dilution water ID = P/O EPAMH #099

All surface waters filtered through a 60 µm screen daily

Technician: 

Date: 9/29/11

Environmental Toxicology Specialists

DOSE PREPARATION SHEET

9/26/11

SJCD 11-09 TIE

96 hr. Static Acute bioassay w/ 24 hr. Changeout

*Ceriodaphnia dubia*

C-8 SPE Column

Test Concentrations: Column blank, 100% C8TC, MeOH Control, 2X Add-back

C8 Eluate: 250X

Sample	Amount Sample (mL)	Dilution Water (mL)	Total (mL)	Amt MeOH/Eluate (mL)
Column blank	0	100	100*	0
100	100	0	100*	0
MeOH Ctrl.	0	40	40^	0.32
2X add-back	0	40	40^	0.32

\*20 mL used to measure pH.

^ Water quality is not measured.

	Day 0	Day 1	Day 2	Day 3
Tech	BSJ	BSJ	BSJ	BB
Time	1430	1110	1125	1045
Date	9/26/11	9/27/11	9/28/11	9/29/11

n = 5 animals/replicate - 4 replicates/concentration









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TIE ADD-BACK CALCULATION DATA SHEET

Client:	<u>MLJ - LLC</u>	Test Species:	<u>cerio</u>
TIE No.:	<u>JSCD 11-09 TIE</u>	TIE Type:	<u>phase 1</u>
Sample Date:	<u>09/20/11</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:

$$\underline{2} \text{ reps X } \underline{20} \text{ mL/rep} = \underline{40} \text{ mL}$$

Calculations

Add-Back Level 1:

$$\underline{40} \text{ mL total volume needed X add-back level } \underline{2} / \text{eluate concentration factor } \underline{250} \text{ X/mL}$$
$$= \underline{0.32} \text{ mL ( } \underline{320} \text{ } \mu\text{L) of eluate}$$

Add-Back Level 2:

$$\underline{\quad\quad} \text{ mL total volume needed X add-back level } \underline{\quad\quad} / \text{eluate concentration factor } \underline{\quad\quad} \text{ X/mL}$$
$$= \underline{\quad\quad} \text{ mL ( } \underline{\quad\quad} \text{ } \mu\text{L) of eluate}$$

Processed by/Date:

BSH 9/26/11

**AQUA-Science**  
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**DOSE PREPARATION SHEET**

9/26/11

**SJCD 11-09 TIE**

96 hr. Static Acute bioassay w/ 24 hr. Changeout  
*Ceriodaphnia dubia*

**+ PBO @ 100 µg/L**

Test Concentrations: Control and 100% + PBO

PBO Standard: 0.1 mg/mL Piperonyl butoxide (PBO)

Sample	Amount Sample (mL)	Dilution Water (mL)	Total (mL)*	Amt PBO (µL)
Control	0	100	100	100
6.25	6.25	QS to 100	100	100
12.5	12.5	QS to 100	100	100
25	25	QS to 100	100	100
50	50	QS to 100	100	100
100	100	0	100	100

**+ EDTA @ 8 mg/L**

Test Concentrations: Control and 100% + EDTA

EDTA Standard: 10 mg/mL EDTA

Sample	Amount Sample (mL)	Dilution Water (mL)	Total (mL)*	Amt EDTA (µL)
Control	0	100	100	80
100	100	0	100	80

All surface waters filtered through 60 µm screen

\*20 mL used to measure pH.

	Day 0	Day 1	Day 2	Day 3
Tech	BES	BES	BES	BB
Time	1420	1120	1125	1100
Date	9/26/11	9/27/11	9/28/11	9/29/11

n = 5 animals/replicate - 4 replicates/concentration







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Environmental Toxicology Specialists

**WATER QUALITY REPORT FOR AQUATIC BIOASSAYS**

Test Number:	SJCD 11-09 TIE	Study Director:	J.L. Miller
Protocol No.:	EPA 821/R-02/012 <i>DMCK</i>	Technicians:	Walker/Concepcion/Sanford/Berry
Test Material:	SJCD Ag Waiver Sample Dry Creek @ Hwy 4 (09/20/11) 531XDCAF <b>+PBO, +EDTA</b>		
Test Species:	<i>Ceriodaphnia dubia</i>	Animal Lot No.:	# AS R/O: 092611
Initiation Date:	September 26, 2011	Termination Date:	September 30, 2011

Sample @ 100%	OBSERVATIONS: Day 3 09/29/11					24 Hour Obsv.			
	Temperature (°C)	Dissolved Oxygen*	pH^^	Alkalinity **/ Hardness~	Conductivity ^	Temp	D.O.*	pH^^	Cond. ^
<b>+100 µg/L PBO</b>									
PBO Control	24	7.7	8.20	-	320	24	7.5	8.20	366
6.25	24	7.7	8.17	-	298	24	7.4	8.12	325
12.5	24	7.8	8.13	-	286	24	7.3	8.10	309
25	24	7.8	8.11	-	261	24	7.0	8.03	282
50	24	8.0	8.12	-	213	24	6.8	7.99	232
100	25	<i>10.4/8.1</i>	8.02	-	110	24	6.3	7.88	124
<b>+8 mg/L EDTA</b>									
EDTA Control	24	7.8	7.94	-	320	25	7.4	8.16	357
100	25	<i>10.4/8.1</i>	7.75	-	116	24	7.3	8.02	132

**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:** *DESATURATED BEFORE USE PBO 9/29/11*

Lab Control = 2x carbon filtered reverse osmosis water at EPA moderately hard level using EPA salts.  
 Dilution water ID = *P10 EPAMH #009*

All surface waters filtered through a 60 µm screen daily

Technician: *[Signature]*

Date: *9/29/11*

AQUA-Science  
Environmental Toxicology Consultants

## LABORATORY NOTES

Date: 092611

EDTA STANDARD

AT 10 mg/ml

$$\textcircled{1} \text{FW} = 372 - 2\text{H}_2\text{O} = (36) - 2\text{MA} = (46) = 290 \text{g/mol}$$

$$\textcircled{2} \text{Ratio} \quad \frac{372}{290} = 1.3$$

$$\textcircled{3} \left( \frac{10 \text{ mg}}{\text{ml}} \right) (20 \text{ ml}) (1.3) = 260 \text{ mg EDTA in } 20 \text{ ml Ultra Pure}$$

PBO STANDARD

AT 0.1 mg/ml

① PBO 1<sup>o</sup> Standard at 10 mg/ml

a) 200 mg PBO in 20 ml of Methanol  
= 10 mg/ml 1<sup>o</sup> PBO Standard

② PBO 2<sup>o</sup> Standard at 0.1 mg/ml

b) 0.200 ml of 1<sup>o</sup> Standard in 20 ml of Methanol

Technician: \_\_\_\_\_



Date: 9/26/11























