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**Aquatic Toxicity Testing Results for the Grasslands Bypass Project –  
A Three-Species Chronic Screening Bioassay**

February, 2007

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## 1. INTRODUCTION

In February 2007, Block Environmental Services (BES) conducted chronic screening bioassays, using three different species, for the Grasslands Bypass Project. These aquatic bioassay tests were performed to evaluate potential toxicity as part of an on-going monitoring program of agriculture drain water as it is conveyed through the San Luis Drain to Mud Slough and removed from Salt Slough. The toxicity program consists of monthly laboratory bioassay tests using standard protocol testing methods.

The test organisms of interest for the Grasslands Bypass Project are the freshwater green algae (*Selenastrum capricornutum*), the fathead minnow (*Pimephales promelas*), and the daphnid (*Daphnia magna*). Each test was performed under screening conditions (100% sample versus the Delta-Mendota Canal Ambient control). Additional definitive concentrations (75, 50, 25, and 12.5% diluted with Delta-Mendota Canal Ambient control) were tested for the *S. capricornutum* San Luis Drain (Site B) test. As QA/QC measures, Laboratory control water was also evaluated to assess minimum test acceptability criteria, and a standard reference toxicant test was run concurrently for each of the three test species. Due to the unavailability of organisms the *D. magna* bioassays were initiated on February 16, 2007 and required a forth sampling event on February 20, 2007. This report describes the procedures used and the results obtained for the toxicity tests initiated on February 13 and 16, 2007.

BES is an Environmental Laboratory Accreditation Program certified laboratory (#1812).

## 2. MATERIALS AND METHODS



### 2.1 SAMPLE COLLECTION AND HANDLING

**2.1.1 Site Sample Collection** – Water for the laboratory study was collected three times during the seven day testing period. Site samples were collected in a clean one gallon bucket in grab form on days 0, 2, and 4 for the fathead minnow bioassays and on days 0, 2 and 5 for the water flea bioassays. The sample was then transferred to a 2.5 gallon Cubitainer and transported back to the laboratory for testing. In addition, one 250 ml sample was collected for laboratory analysis of selenium by the Bureau of Reclamation. Prior to sample collection, the bucket and sample containers were rinsed three times with station water. Samples for chemical analysis were transferred directly from the bucket to the appropriate sample container. At the laboratory, nitric acid was added to the rinsed 250 ml container for selenium analysis. All samples were transported utilizing chain of custody documentation and stored at 4 °C until use.

**2.1.2 Reference Toxicant** – Sodium Chloride (NaCl) was used as the reference toxicant for both the algae (*S. capricornutum*) and the daphnid (*D. magna*). A stock solution containing 8 g/L NaCl was used for daily preparation of the treatment levels for these organisms. The *S. capricornutum* treatment levels were 8, 4, 2, 1 and 0.5 g/L NaCl in USEPA Moderately Hard Water. The *D. magna* treatment levels were 8, 4, 2, 1, and 0.5 g/L NaCl in Moderately Hard Water (EPA salts added to Arrowhead Distilled Water). Copper sulfate (CuSO<sub>4</sub>) was used as the reference toxicant for the fathead minnow (*P. promelas*). A stock solution containing 100,000 g/L Cu<sup>+2</sup> was used for daily preparation of the treatment levels of 100, 50, 25, 12.5, and 6.25 µg/L Cu<sup>+2</sup> in USEPA Hard Water. The toxicity endpoints from the reference toxicant tests of each test method are subsequently plotted on a running control chart from the last 20 tests. The mean values as well as the upper and lower control limits (± 2 standard deviations) are recalculated with each successive test result. The outliers, which are values falling outside the upper and lower control limits, and trends of increasing or decreasing sensitivity, are readily identified.

## 2.2 TOXICITY TEST PROCEDURES

**2.2.1 Test Procedures** – A detailed procedure for each test is outlined in laboratory standard operating procedures (SOPs) kept at the BES laboratory and outlined on the GBP QA/QC Plan. These SOPs are based upon the following references:

- A Short-Term Chronic Toxicity Test Using *Daphnia magna* (EPA/600/D-87/080)
- Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms (EPA/821/R-02/013)

Test conditions are summarized in Table 2-1.

**2.2.2 Data Analysis** – All toxicity testing results were analyzed using the software program ToxCalc (Version 5.0). This program determines if there is a statistically significant reduction in response at the  $p = 0.05$  level and utilizes the flowchart for statistical analysis outlined in EPA/821/R-02/013. The laboratory testing compared each sample station response with the ambient control water (Delta-Mendota Canal). The parameters of interest for the screening tests are the No Observed Effect Concentration (NOEC), the Lowest Observed Effect Concentration (LOEC), and the resultant Toxic Units ( $TU = 100/NOEC$ ). In addition, the Site B definitive *Selenastrum capricornutum* and *D. magna* tests will address the (25 and 50%) Inhibition Concentrations (IC). The IC values will show the point estimate of the sample concentration that causes a given percent reduction.

**Table 2-1 Summary of Testing Parameters by Organism**

Test Conditions	Organism		
	<i>D. magna</i>	<i>P. promelas</i>	<i>S. capricornutum</i>
<b>Test Type</b>	Static renewal	Static renewal	Static
<b>Temperature</b>	25 ± 1 °C	25 ± 1 °C	25 ± 1 °C
<b>Light Intensity</b>	50 – 100 ft-c	50 - 100 ft-c	400 ± 40 ft-c
<b>Photoperiod</b>	16h light: 8h dark	16h light: 8h dark	Continuous light
<b>Test Chamber</b>	150 ml beaker	600 mL beaker	125 ml flask
<b>Test Solution Volume</b>	50 ml	400 ml	25 ml
<b>Renewal Frequency</b>	Daily	Daily	None
<b>Organism Age</b>	10 days	< 24 hours	4 - 7 days
<b>Organism Source</b>	Aquatic Biosystems (Fort Collins, CO)	Aquatic Biosystems (Fort Collins, CO)	In House Culture
<b>Organisms/Chamber</b>	1	10	10,000 cells/ml
<b>Organisms/Concentration</b>	10	40	1 x 10 <sup>6</sup>
<b>Food Source</b>	YCT & algae	<i>Artemia</i> nauplii	Culture medium
<b>Feeding Frequency</b>	Once/day	Twice/day	None
<b>Cleaning</b>	None	Siphon daily	None
<b>Shaking Rate</b>	None	None	Continuous
<b>Control/Dilution Water</b>	EPA Mod. Hard	EPA Hard	EPA Mod. Hard culture medium
<b>Sample Concentrations</b>	100%	100%	100% (Site B: 75, 50, 25, & 12.5% diluted w/Amb.)
<b>Reference Toxicant</b>	NaCl	CuSO <sub>4</sub> as Cu <sup>+2</sup>	NaCl
<b>Reference Toxicant Concentrations</b>	8, 4, 2, 1, and 0.5 g/L	100, 50, 25, 12.5, and 6.25 µg/L	8, 4, 2, 1, and 0.5 g/L
<b>Test Duration</b>	7 days	7 days	96 hours
<b>Effects Measured</b>	Survival & reproduction	Survival & growth	Growth
<b>Test Acceptability (Laboratory Control)</b>	≥ 80% survival & ≥ 10 young/adult in controls	≥ 80% survival & ≥ 0.25 mg avg. dry weight/survivor in controls	≥ 1 x 10 <sup>6</sup> cells/ml and variance should not exceed 20% in controls

### 3. RESULTS

#### 3.1 ROUTINE REPORTING

##### 3.1.1 Sample Site Identification –

- Site B = SLD at new bridge
- Site C = Mud Slough upstream of SLD discharge
- Site D = Mud Slough downstream of SLD discharge
- Site F = Salt Slough at Highway 165
- Ambient Control = Delta-Mendota Canal

##### 3.1.2 Sample Dates – 02/12/2007, 02/14/2007, 02/16/2007 and 02/20/2007.

##### 3.1.3 Test Species –

- daphnid (*Daphnia magna*) at 10 days old
- fathead minnow (*Pimephales promelas*) at <24 hours old
- freshwater algae (*Selenastrum capricornutum*) between 4 and 7 days old

##### 3.1.4 Test Initiation Dates –

Test Time	<i>D. magna</i>		<i>P. promelas</i>		<i>S. capricornutum</i>	
	Date	Time	Date	Time	Date	Time
Initiation	02/16/07	1115	02/13/07	1200	02/15/07	1300
Termination	02/23/07	1215	02/20/07	1100	02/19/07	1230

**3.1.5 Summary of Water Chemistry of Site Waters** – These values represent the water quality of the samples as received at the BES laboratory.

**Site B**

<b>Water Chemistry</b>	<b>02/12/07</b>	<b>02/14/07</b>	<b>02/16/07</b>	<b>02/20/07</b>
<b>D.O. (mg/L)</b>	11.20	9.80	9.80	11.00
<b>pH</b>	8.10	8.00	7.80	8.00
<b>Conductivity (µS/cm)</b>	3920	3820	710	4130
<b>Salinity (ppt)</b>	2.10	2.00	0.30	2.20
<b>Temperature (°C)</b>	3.00	2.00	4.00	2.50
<b>Total Chlorine (mg/L)</b>	0.06	0.04	ND	0.16
<b>Ammonia (ppm as N)</b>	0.15	0.09	0.09	0.10
<b>Alkalinity (mg/L as CaCO<sub>3</sub>)</b>	176	172	172	190
<b>Hardness (mg/L as CaCO<sub>3</sub>)</b>	>1000	>1000	>1000	>1000
<b>Total Suspended Solids (mg/L)</b>	38	43	74	24

ND – Not Detected (Detection limit 0.03 mg/L)

**Site C**

<b>Water Chemistry</b>	<b>02/12/07</b>	<b>02/14/07</b>	<b>02/16/07</b>	<b>02/20/07</b>
<b>D.O. (mg/L)</b>	9.40	8.90	9.00	9.60
<b>pH</b>	7.80	7.80	7.90	7.90
<b>Conductivity (µS/cm)</b>	1809	1776	2030	2050
<b>Salinity (ppt)</b>	0.90	0.90	1.00	1.00
<b>Temperature (°C)</b>	1.50	2.00	3.00	2.50
<b>Total Chlorine (mg/L)</b>	0.06	0.05	0.03	ND
<b>Ammonia (ppm as N)</b>	0.22	0.26	0.19	0.17
<b>Alkalinity (mg/L as CaCO<sub>3</sub>)</b>	248	250	292	300
<b>Hardness (mg/L as CaCO<sub>3</sub>)</b>	360	380	378	400
<b>Total Suspended Solids (mg/L)</b>	54	52	89	41

ND – Not Detected (Detection limit 0.03 mg/L)

**Site D**

<b>Water Chemistry</b>	<b>02/12/07</b>	<b>02/14/07</b>	<b>02/16/07</b>	<b>02/20/07</b>
<b>D.O. (mg/L)</b>	10.20	8.90	9.10	9.60
<b>pH</b>	7.90	7.80	7.90	8.00
<b>Conductivity (µS/cm)</b>	2210	2370	2520	2740
<b>Salinity (ppt)</b>	1.10	1.20	1.30	1.40
<b>Temperature (°C)</b>	1.00	2.00	2.00	2.50
<b>Total Chlorine (mg/L)</b>	0.04	0.05	0.04	0.05
<b>Ammonia (ppm as N)</b>	0.21	0.19	0.18	0.15
<b>Alkalinity (mg/L as CaCO<sub>3</sub>)</b>	240	238	292	268
<b>Hardness (mg/L as CaCO<sub>3</sub>)</b>	518	524	544	588
<b>Total Suspended Solids (mg/L)</b>	58	39	65	35

**Site F**

<b>Water Chemistry</b>	<b>02/12/07</b>	<b>02/14/07</b>	<b>02/16/07</b>	<b>02/20/07</b>
<b>D.O. (mg/L)</b>	9.50	8.70	8.60	9.30
<b>pH</b>	7.70	7.50	7.70	7.70
<b>Conductivity (µS/cm)</b>	1452	1561	1590	1416
<b>Salinity (ppt)</b>	0.70	0.70	0.80	0.70
<b>Temperature (°C)</b>	3.00	4.00	4.00	4.50
<b>Total Chlorine (mg/L)</b>	0.04	0.04	ND	0.07
<b>Ammonia (ppm as N)</b>	0.22	0.17	0.25	0.22
<b>Alkalinity (mg/L as CaCO<sub>3</sub>)</b>	180	204	190	172
<b>Hardness (mg/L as CaCO<sub>3</sub>)</b>	324	350	350	328
<b>Total Suspended Solids (mg/L)</b>	81	66	161	86

ND – Not Detected (Detection limit 0.03 mg/L)

**Ambient Control**

<b>Water Chemistry</b>	<b>02/12/07</b>	<b>02/14/07</b>	<b>02/16/07</b>	<b>02/20/07</b>
<b>D.O. (mg/L)</b>	10.70	9.60	9.80	9.80
<b>pH</b>	7.70	7.50	7.80	7.80
<b>Conductivity (<math>\mu</math>S/cm)</b>	723	732	710	413
<b>Salinity (ppt)</b>	0.40	0.40	0.30	0.20
<b>Temperature (<math>^{\circ}</math>C)</b>	1.50	2.00	4.00	3.00
<b>Total Chlorine (mg/L)</b>	0.06	0.03	0.04	0.06
<b>Ammonia (ppm as N)</b>	0.15	0.12	0.14	0.05
<b>Alkalinity (mg/L as CaCO<sub>3</sub>)</b>	102	110	102	86
<b>Hardness (mg/L as CaCO<sub>3</sub>)</b>	180	194	160	118
<b>Total Suspended Solids (mg/L)</b>	11	23	20	8

**3.2 *D. magna* End Point Values –**

**3.2.1 100% Site Response versus Ambient Water**

**SURVIVAL**

Sample ID	7 day Survival (%)	NOEC	LOEC	TU
Lab Control	90	-	-	-
Ambient	100	-	-	-
Site B	100	100	>100	1
Site C	80	100	>100	1
Site D	90	100	>100	1
Site F	90	100	>100	1

**REPRODUCTION**

Sample ID	7 Day Neonate Production		NOEC	LOEC	TU
	# per female	Std Dev.			
Lab Control	30.50	16.69	-	-	-
Ambient	28.60	13.45	-	-	-
Site B	31.70	16.05	100	>100	1
Site C	32.90	21.33	100	>100	1
Site D	39.40	11.64	100	>100	1
Site F	31.60	17.69	100	>100	1

3.2.2 Screening Testing Notes

There were not any statistically significant reductions in the *D. magna* survival or reproduction responses for any of the Site waters with respect to the Ambient control water.

The Ambient and Laboratory control waters both met the test acceptability criteria for survival ( $\geq 80\%$ ) and reproduction ( $\geq 10$  neonates/adult).

The concurrent reference toxicant test generated survival (LC50) and reproduction (IC25) endpoints that were within the control chart limitations. This *D. magna* response indicates that the values elicited for the Site Waters are valid.

**3.3 *P. promelas* End Point Values –**

**3.3.1 Site Response versus Ambient Water**

**SURVIVAL**

Sample ID	7 day % Survival		NOEC	LOEC	TU
	Average (%)	Std Dev.			
Lab Control	100	0	-	-	-
Ambient	98	5.00	-	-	-
Site B	98	5.00	100	>100	1
Site C	90	8.16	100	>100	1
Site D	95	5.77	100	>100	1
Site F	88	5.00	100	>100	1

**GROWTH**

Sample ID	7 day Weight		NOEC	LOEC	TU
	Average (mg)	Std Dev.			
Lab Control	0.38	0.04	-	-	-
Ambient	0.37	0.06	-	-	-
Site B	0.45	0.04	100	>100	1
Site C	0.41	0.05	100	>100	1
Site D	0.43	0.03	100	>100	1
Site F	0.33	0.03	100	>100	1

3.3.2 Testing Notes

There were not statistically significant reductions in the *P. promelas* survival or growth response for any of the site waters with respect to the Ambient control water.

The Laboratory and the Ambient control waters passed both the growth ( $\geq 0.25$  mg/surviving adult) and survival ( $\geq 80\%$ ) test acceptability criteria.

The concurrent reference toxicant test generated survival (LC50) and growth (IC25) endpoints that were within the control chart limitations. This *P. promelas* response indicates that the values elicited for the Site Waters are valid.

### 3.4 *S. capricornutum* End Point Values –

#### 3.4.1 100% Site Response versus Ambient Water

##### GROWTH

Sample ID (100%)	96 hour growth		NOEC	LOEC	TU
	Count (10 <sup>5</sup> cells/ml)	Variance (%)			
Lab Control	15.71	7.06	-	-	-
Ambient	13.41	17.87	-	-	-
Site B	7.94	11.49	12.5	25	8
Site C	22.92	3.46	100	>100	1
Site D	17.86	7.17	100	>100	1
Site F	13.84	4.97	100	>100	1

#### 3.4.2 Screening Testing Notes

There were not statistically significant reductions in the growth response for the Site C, D or F waters with respect to the Ambient control water. There was a significant reduction for the Site B water.

Both the Ambient and Laboratory control waters passed the cell density ( $\geq 1 \times 10^6$  cells/ml) and the variance acceptability criteria ( $\leq 20\%$  variance between replicates at test termination).

**3.4.3 Definitive Site B Response versus Ambient Water**

**GROWTH**

Sample ID (% Site B diluted w/Ambient)	96 hour Growth	
	Count (10 <sup>5</sup> cells/ml)	Variance (%)
Laboratory Control	15.71	7.06
Ambient Control	13.41	17.87
12.5	12.76	7.65
25	10.69	9.32
50	10.07	11.83
75	7.75	16.66
100	7.94	11.49

**STATISTICAL ANALYSIS**

End Point	IC 50	IC 25	NOEC	LOEC	Toxic Units
	>100	50.20	12.5	25	8

**3.4.4 Definitive Testing Notes**

There was a statistically significant reduction in the growth response for the Site B water at the 25%, 50%, 75% and 100% concentrations compared to the Ambient control water.

The Ambient and Laboratory control waters passed both the cell density ( $\geq 1 \times 10^6$  cells/ml) and the variance acceptability criteria ( $\leq 20\%$  variance).

The concurrent *S. capricornutum* reference toxicant test generated a growth (IC25) endpoint that was within the control chart limitations.

#### 4. CONCLUSIONS

BES

The objective of these tests was to satisfy the monthly chronic three species bioassay requirements as described in the Grasslands Bypass Project FONSI permit. The results for the chronic bioassay tests using the site water samples collected in February 2007 indicate that:

- *D. magna* survival and reproduction were not adversely affected by any of the Site waters. Results obtained from the concurrent reference toxicant test are typical of in-house sensitivity data to NaCl, indicating that the stock culture that was used was in good condition.
- *P. promelas* survival and growth were not adversely affected by any of the Site waters. Results obtained from the reference toxicant tests are typical of in-house sensitivity data to  $\text{Cu}^{+2}$ , indicating that stock organisms used were in good condition.
- *S. capricornutum* growth was not adversely affected for the Site C, D and F waters. Growth was adversely affected by the Site B water at the 25%, 50%, 75% and 100% concentrations. Results obtained from the concurrent reference toxicant test are typical of in-house sensitivity data to NaCl, indicating that the stock culture that was used was in good condition.