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

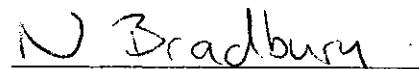
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1. INTRODUCTION

The logo for Block Environmental Services (BES) is a black square with the letters "BES" in white, bold, sans-serif font.

In November 2009, 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. This report describes the procedures used and the results obtained for the toxicity tests initiated on November 3, 2009.

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

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 grab form on days 0, 2, and 4 in a clean one gallon bucket. 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 kept 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 (CuSO₄) was used as the reference toxicant for the fathead minnow (*P. promelas*). A stock solution containing 100,000 g/L Cu⁺² was used for daily preparation of the treatment levels of 100, 50, 25, 12.5, and 6.25 µg/L Cu⁺² 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 DPHS-ELAP approved standard operating procedures (SOPs), which are on file at the BES laboratory and outlined in 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>
Test Type	Static renewal	Static renewal	Static
Temperature	25 ± 1 °C	25 ± 1 °C	25 ± 1 °C
Light Intensity	50 – 100 ft-c	50 - 100 ft-c	400 ± 40 ft-c
Photoperiod	16h light: 8h dark	16h light: 8h dark	Continuous light
Test Chamber	150 ml beaker	600 mL beaker	125 ml flask
Test Solution Volume	50 ml	400 ml	25 ml
Renewal Frequency	Daily	Daily	None
Organism Age	10 days	< 24 hours	4 - 7 days
Organism Source	Aquatic Biosystems (Fort Collins, CO)	Aquatic Biosystems (Fort Collins, CO)	In House Culture
Organisms/Chamber	1	10	10,000 cells/ml
Organisms/Concentration	10	40	1 x 10 ⁶
Food Source	YCT & algae	<i>Artemia nauplii</i>	Culture medium
Feeding Frequency	Once/day	Twice/day	None
Cleaning	None	Siphon daily	None
Shaking Rate	None	None	Continuous
Control/Dilution Water	EPA Mod. Hard	EPA Hard	EPA Mod. Hard culture medium
Sample Concentrations	100%	100%	100% (Site B: 75, 50, 25, & 12.5% diluted w/Amb.)
Reference Toxicant	NaCl	CuSO ₄ as Cu ⁺²	NaCl
Reference Toxicant Concentrations	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
Test Duration	7 days	7 days	96 hours
Effects Measured	Survival & reproduction	Survival & growth	Growth
Test Acceptability (Laboratory Control)	≥ 80% survival & ≥ 10 young/adult in controls	≥ 80% survival & ≥ 0.25 mg avg. dry weight/survivor in controls	≥ 1 x 10 ⁶ 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 – 11/02/2009, 11/04/2009 and 11/06/09

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	11/03/09	1145	11/03/09	1245	11/05/09	1350
Termination	11/10/09	1145	11/10/09	1425	11/09/09	1350



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

Water Chemistry	11/02/09	11/04/06	11/06/09
D.O. (mg/L)	14.60	13.00	13.50
pH	8.40	8.30	8.40
Conductivity (µS/cm)	4150	3890	3890
Salinity (ppt)	2.20	2.10	2.10
Temperature (°C)	2.10	2.00	NM
Total Chlorine (mg/L)	0.16	0.14	0.14
Ammonia (ppm as N)	0.03	ND	ND
Alkalinity (mg/L as CaCO₃)	188	198	196
Hardness (mg/L as CaCO₃)	>1000	>1000	>1000
Total Suspended Solids (mg/L)	42	35	34

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

Site C

Water Chemistry	11/02/09	11/04/06	11/06/09
D.O. (mg/L)	10.50	9.20	10.20
pH	7.70	7.80	7.90
Conductivity (µS/cm)	16.05	1687	1788
Salinity (ppt)	0.80	0.90	0.90
Temperature (°C)	2.30	2.30	NM
Total Chlorine (mg/L)	ND	ND	ND
Ammonia (ppm as N)	0.23	0.24	0.19
Alkalinity (mg/L as CaCO₃)	272	284	280
Hardness (mg/L as CaCO₃)	360	344	354
Total Suspended Solids (mg/L)	18	38	44

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

Site D

Water Chemistry	11/02/09	11/04/06	11/06/09
D.O. (mg/L)	10.30	9.80	10.60
pH	7.60	7.90	7.90
Conductivity (µS/cm)	2280	2580	2440
Salinity (ppt)	1.20	1.30	1.30
Temperature (°C)	3.10	2.10	NM
Total Chlorine (mg/L)	ND	ND	0.05
Ammonia (ppm as N)	0.16	0.12	0.10
Alkalinity (mg/L as CaCO₃)	240	252	270
Hardness (mg/L as CaCO₃)	520	592	532
Total Suspended Solids (mg/L)	42	53	63

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

Site F

Water Chemistry	11/02/09	11/04/06	11/06/09
D.O. (mg/L)	10.70	9.00	10.40
pH	7.60	7.70	7.90
Conductivity (µS/cm)	1340	1334	1425
Salinity (ppt)	0.70	0.70	0.70
Temperature (°C)	4.60	3.50	NM
Total Chlorine (mg/L)	ND	ND	0.07
Ammonia (ppm as N)	0.09	0.09	0.09
Alkalinity (mg/L as CaCO₃)	180	180	182
Hardness (mg/L as CaCO₃)	312	276	286
Total Suspended Solids (mg/L)	45	43	59

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



Ambient Control

Water Chemistry	11/02/09	11/04/06	11/06/09
D.O. (mg/L)	12.40	10.50	11.90
pH	8.10	7.80	8.00
Conductivity (μS/cm)	377	403	465
Salinity (ppt)	0.20	0.20	0.20
Temperature ($^{\circ}$C)	4.10	2.50	NM
Total Chlorine (mg/L)	ND	ND	0.06
Ammonia (ppm as N)	ND	ND	ND
Alkalinity (mg/L as CaCO₃)	68	68	64
Hardness (mg/L as CaCO₃)	86	88	84
Total Suspended Solids (mg/L)	4	9	13

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

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	70	-	-	-
Ambient	70	-	-	-
Site B	90	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	18.60	12.67	-	-	-
Ambient	16.40	12.45	-	-	-
Site B	38.50	16.96	100	>100	1
Site C	21.30	13.58	100	>100	1
Site D	29.10	25.02	100	>100	1
Site F	21.80	11.39	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 reproduction (≥ 10 neonates/adult) test acceptability criteria but not the survival ($\geq 80\%$) criteria. Calculated effect concentrations (NOEC, LOEC, LC/IC values) determined from the survival and reproduction response pattern are reliable and should be reported.

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.

3.3.1 Site Response versus Ambient Water

SURVIVAL

Sample ID	7 day % Survival		NOEC	LOEC	TU
	Average (%)	Std Dev.			
Lab Control	100	0	-	-	-
Ambient	95	5.77	-	-	-
Site B	100	0	100	>100	1
Site C	93	9.57	100	>100	1
Site D	90	5.77	100	>100	1
Site F	83	17.08	100	>100	1

GROWTH

Sample ID	7 day Weight		NOEC	LOEC	TU
	Average (mg)	Std Dev.			
Lab Control	0.43	0.02	-	-	-
Ambient	0.36	0.01	-	-	-
Site B	0.38	0.02	100	>100	1
Site C	0.40	0.05	100	>100	1
Site D	0.37	0.03	100	>100	1
Site F	0.38	0.02	100	>100	1

3.3.2 Testing Notes

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

The Laboratory and the Ambient control waters passed both the growth (≥ 0.25 mg/surviving adult) and survival ($\geq 80\%$) test acceptability criteria. Calculated effect concentrations (NOEC, LOEC, LC/IC values) determined from the survival and reproduction response pattern are reliable and should be reported.

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 ⁵ cells/ml)	Variance (%)			
Lab Control	28.11	7.77	-	-	-
Ambient	26.23	5.66	-	-	-
Site B	20.59*	10.18			
Site C	39.03	4.12	100	>100	1
Site D	35.84	8.55	100	>100	1
Site F	33.52	4.92	100	>100	1

* Statistically significant reduction compared to Ambient control ($p = 0.05$)

3.4.2 Screening Testing Notes

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

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). Calculated effect concentrations (NOEC, LOEC, LC/IC values) determined from the survival and reproduction response pattern are reliable and should be reported.

3.4.3 Definitive Site B Response versus Ambient Water



GROWTH

Sample ID (% Site B diluted w/Ambient)	96 hour Growth	
	Count (10 ⁵ cells/ml)	Variance (%)
Laboratory Control	28.11	7.77
Ambient Control	26.22	5.66
12.5	29.48	7.22
25	26.96	7.83
50	27.78	6.58
75	25.06	3.68
100	20.59*	10.18

* Statistically significant reduction compared to Ambient control ($p = 0.05$)

STATISTICAL ANALYSIS

End Point	IC 50	IC 25	NOEC	LOEC	Toxic Units
	>100	98.34	75	100	1.33

3.4.4 Definitive Testing Notes

There was a statistically significant reduction in the growth response for the Site B water at the 100% concentration 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). Calculated effect concentrations (NOEC, LOEC, LC/IC values) determined from the survival and reproduction response pattern are reliable and should be reported.

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

4. CONCLUSIONS

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 November 2008 indicate that:

- *D. magna* survival and reproduction were not adversely affected by any of the Site waters. The Laboratory and Ambient control water did not pass the survival test acceptability criteria. 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 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. Site B showed a statistically significant reduction in growth at the 100% concentration. 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.