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September 19, 2024

Chris:

I have enclosed our report “Evaluation of the Toxicity of Grasslands Bypass Project Ambient Water Sample: Event 113” for the sample that was collected August 21, 2024. The results of this testing are summarized below.

Toxicity summary for Grasslands Bypass Project ambient water and sediment samples.			
Sample Station	Toxicity relative to the Lab Control treatment?		
	<i>Selenastrum capricornutum</i>	<i>Daphnia magna</i>	Fathead Minnow
	Growth	Survival	Survival
Site D	No	No	No

**Chronic Toxicity of Grasslands Bypass Project Ambient Water to *Selenastrum capricornutum***

There was **no** significant reduction in algal growth in the Grasslands Bypass Project ambient water sample.

**Acute Toxicity of Grasslands Bypass Project Ambient Water to *Daphnia magna***

There was **no** significant reduction in survival in the Grasslands Bypass Project ambient water sample.

**Acute Toxicity of Grasslands Bypass Project Ambient Water to Fathead Minnows**

There was **no** significant reduction in survival in the Grasslands Bypass Project ambient water sample.

### 3. RESULTS

#### 3.1 Effects of the Grasslands Bypass Project Ambient Water on *Selenastrum capricornutum*

The results for this testing are summarized in Table 2. There was ***no*** significant reduction in algal growth in the Grasslands Bypass Project ambient water sample. The test data and summary of statistical analyses for this testing are presented in Appendix B.

Table 2. Effects of Grasslands Bypass Project ambient water on <i>Selenastrum capricornutum</i>		
Test Initiation Date (Time)	Treatment/Sample ID	Mean Algal Cell Density (cells/mL x 10 <sup>6</sup> )
8/21/24 (1431)	Lab Water Control	2.26
	GBP-113-D-TE	3.24

#### 3.2 Effects of the Grasslands Bypass Project Ambient Water on *Daphnia magna*

The results for this testing are summarized in Table 3. There was ***no*** significant reduction in survival in the Grasslands Bypass Project ambient water sample. The test data and summary of statistical analyses for this testing are presented in Appendix C.

Table 3. Effects of Grasslands Bypass Project ambient water on <i>Daphnia magna</i> .		
Test Initiation Date (Time)	Treatment/Sample ID	Mean % Survival
8/21/24 (1443)	Lab Water Control	100
	GBP-113-D-TE	100

#### 3.3 Effects of the Grasslands Bypass Project Ambient Water on Fathead Minnows

The results for this testing are summarized in Table 4. There were ***no*** significant reductions in survival in the Grasslands Bypass Project ambient water sample. The test data and summary of statistical analyses for this testing are presented in Appendix D.

Table 4. Effects of Grasslands Bypass Project ambient water on fathead minnows.		
Test Initiation Date (Time)	Treatment/Sample ID	Mean % Survival
8/21/24 (1445)	Lab Water Control	100
	GBP-113-D-TE	97.5



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October 29, 2024

Chris:

I have enclosed our report “Evaluation of the Toxicity of Grasslands Bypass Project Ambient Water and Sediment Samples: Event 114” for the samples that were collected September 23, 2024. The results of this testing are summarized below.

Toxicity summary for Grasslands Bypass Project ambient water and sediment samples.				
Sample Station	Toxicity relative to the Lab Control treatment?			
	<i>Selenastrum capricornutum</i>	<i>Daphnia magna</i>	Fathead Minnow	<i>Hyaella azteca</i>
	Growth	Survival	Survival	Survival
Site D	No	No	No	Yes
Site B3	No	No	No	
Site F	No	No	No	
Site R	No	No	No	

**Chronic Toxicity of Grasslands Bypass Project Ambient Water to *Selenastrum capricornutum***

There was **no** significant reduction in algal growth in the Grasslands Bypass Project ambient water samples.

**Acute Toxicity of Grasslands Bypass Project Ambient Water to *Daphnia magna***

There were **no** significant reductions in survival in any of the Grasslands Bypass Project ambient water samples.

**Acute Toxicity of Grasslands Bypass Project Ambient Water to Fathead Minnows**

There were **no** significant reductions in survival in the Grasslands Bypass Project ambient water samples.

**Acute Toxicity of Grasslands Bypass Ambient Sediment to *Hyaella azteca***

There was a significant reduction in survival in the Site D sediment tested with *H. azteca*.

### 3. RESULTS

#### 3.1 Effects of the Grasslands Bypass Project Ambient Water on *Selenastrum capricornutum*

The results for this testing are summarized in Table 2. There were ***no*** significant reductions in algal growth in the Grasslands Bypass Project ambient water samples. The test data and summary of statistical analyses for this testing are presented in Appendix B.

Table 2. Effects of Grasslands Bypass Project ambient waters on <i>Selenastrum capricornutum</i> .		
Test Initiation Date (Time)	Treatment/Sample ID	Mean Algal Cell Density (cells/mL x 10 <sup>6</sup> )
9/24/24 (1505)	Lab Water Control	1.72
	GBP-114-D-TE	5.79
	GBP-114-B3-TE	4.21
	GBP-114-F-TE	5.50
	GBP-114-R-TE	4.46

#### 3.2 Effects of the Grasslands Bypass Project Ambient Water on *Daphnia magna*

The results for this testing are summarized in Table 3. There were ***no*** significant reductions in survival in any of the Grasslands Bypass Project ambient water samples. The test data and summary of statistical analyses for this testing are presented in Appendix C.

Table 3. Effects of Grasslands Bypass Project ambient water on <i>Daphnia magna</i> .		
Test Initiation Date (Time)	Treatment/Sample ID	Mean % Survival
9/24/24 (1503)	Lab Water Control	95.0
	GBP-114-D-TE	100
	GBP-114-B3-TE	100
	GBP-114-F-TE	95.0
	GBP-114-R-TE	100



### 3.3 Effects of the Grasslands Bypass Project Ambient Water on Fathead Minnows

The results for this testing are summarized in Table 4. There were ***no*** significant reductions in survival in the Grasslands Bypass Project ambient water samples. The test data and summary of statistical analyses for this testing are presented in Appendix D.

Test Initiation Date (Time)	Treatment/Sample ID	Mean % Survival
9/24/24 (1631)	Lab Water Control	100
	GBP-114-D-TE	100
	GBP-114-B3-TE	100
	GBP-114-F-TE	100
	GBP-114-R-TE	100

### 3.4 Effects of the Grasslands Bypass Project Sediment on *Hyaella azteca*

The results of this test are summarized in Table 5. There was a 41.6% effect in survival in the Grasslands Bypass Project sediment sample; the TST analysis resulted in a Fail. The test data and summary of statistical analyses for this test is present in Appendix E.

Test Initiation Date (Time)	Treatment/Sample ID	Mean % Survival	TST Analysis	% Effect
9/26/24 (1421)	Lab Control	96.2		
	GBP-114-D-SE	56.2	Fail	41.6%





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November 7, 2024

Chris:

I have enclosed our report “Evaluation of the Toxicity of Grasslands Bypass Project Ambient Water Sample: Event 115” for the sample that was collected October 16, 2024. The results of this testing are summarized below.

Toxicity summary for Grasslands Bypass Project ambient water sample.			
Sample Station	Toxicity relative to the Lab Control treatment?		
	<i>Selenastrum capricornutum</i>	<i>Daphnia magna</i>	Fathead Minnow
	Growth	Survival	Survival
Site D	No	No	No

**Chronic Toxicity of Grasslands Bypass Project Ambient Water to *Selenastrum capricornutum***

There was **no** significant reduction in algal growth in the Grasslands Bypass Project ambient water sample.

**Acute Toxicity of Grasslands Bypass Project Ambient Water to *Daphnia magna***

There was **no** significant reduction in survival in the Grasslands Bypass Project ambient water sample.

**Acute Toxicity of Grasslands Bypass Project Ambient Water to Fathead Minnows**

There was **no** significant reduction in survival in the Grasslands Bypass Project ambient water sample.

### 3. RESULTS

#### 3.1 Effects of the Grasslands Bypass Project Ambient Water on *Selenastrum capricornutum*

The results for this testing are summarized in Table 2. There was **no** significant reduction in algal growth in the Grasslands Bypass Project ambient water sample. The test data and summary of statistical analyses for this testing are presented in Appendix B.

Table 2. Effects of Grasslands Bypass Project ambient water on <i>Selenastrum capricornutum</i>		
Test Initiation Date (Time)	Treatment/Sample ID	Mean Algal Cell Density (cells/mL x 10 <sup>6</sup> )
10/16/24 (1520)	Lab Water Control	2.82
	GBP-115-D-TE	6.54

#### 3.2 Effects of the Grasslands Bypass Project Ambient Water on *Daphnia magna*

The results for this testing are summarized in Table 3. There was **no** significant reduction in survival in the Grasslands Bypass Project ambient water sample. The test data and summary of statistical analyses for this testing are presented in Appendix C.

Table 3. Effects of Grasslands Bypass Project ambient water on <i>Daphnia magna</i> .		
Test Initiation Date (Time)	Treatment/Sample ID	Mean % Survival
10/16/24 (1527)	Lab Water Control	100
	GBP-115-D-TE	100

#### 3.3 Effects of the Grasslands Bypass Project Ambient Water on Fathead Minnows

The results for this testing are summarized in Table 4. There were **no** significant reductions in survival in the Grasslands Bypass Project ambient water sample. The test data and summary of statistical analyses for this testing are presented in Appendix D.

Table 4. Effects of Grasslands Bypass Project ambient water on fathead minnows.		
Test Initiation Date (Time)	Treatment/Sample ID	Mean % Survival
10/16/24 (1456)	Lab Water Control	100
	GBP-115-D-TE	100

