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January 5, 2024

Chris:

I have enclosed our report “Evaluation of the Toxicity of Grasslands Bypass Project Ambient Water Sample: Event 105” for the sample that was collected December 12, 2023. 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 test 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 test 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> )
12/12/23 (1710)	Lab Water Control	1.74
	GBP-105-D-TE	6.58

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

The results for this test 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 test 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
12/12/23 (1518)	Lab Water Control	100
	GBP-105-D-TE	100

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

The results for this test 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 test 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
12/12/23 (1635)	Lab Water Control	97.5
	GBP-105-D-TE	95.0





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March 8, 2024

Chris:

I have enclosed our report “Evaluation of the Toxicity of Grasslands Bypass Project Ambient Water Sample: Event 106” for the sample that was collected January 26, 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 test 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 test 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> )
1/26/24 (1405)	Lab Water Control	1.64
	GBP-106-D-TE	6.04

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

The results for this test 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 test 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
1/26/24 (1502)	Lab Water Control	100
	GBP-106-D-TE	100

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

The results for this test 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 test 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
1/26/24 (1515)	Lab Water Control	100
	GBP-106-D-TE	100

