TRI-STATE GENERATION AND TRANSMISSION ASSOCIATION, INC.
HEADQUARTERS: P.O. BOX 33695 DENVER, COLORADO 80233-0695 303-452-6111

June 29, 2018

Submitted via email eric.mink@state.co.us

Mr. Eric Mink
Water Quality Control Division
Colorado Department of Public Health and Environment
4300 Cherry Creek Drive South
Denver, Colorado 80246-1530

RE: CDPS Permit #CO-0000213
New Horizon Mine (Outfall 013)
120-Day Status Report on Whole Effluent Toxicity Testing

Dear Mr. Mink:

As reported on March 7, 2018, Whole Effluent Toxicity (WET) was identified during the first quarter 2018 sampling for Outfall 013 at the Elk Ridge Mining and Reclamation, LLC (Elk Ridge) New Horizon Mine (CDPS Permit #CO-0000213). Tri-State Generation and Transmission Association, Inc. (Tri-State) is the parent of Elk Ridge. Instead of proceeding with the accelerated testing, the facility moved directly to the Toxicity Identification Evaluation (TIE) commencing on March 8, 2018, in accordance with Part I.D.1.c.ii. of the permit.

SeaCrest Group is conducting the TIE on behalf of the facility. To date, SeaCrest Group has wrapped up Phase I and II of the TIE in accordance with EPA's methodology (reports enclosed). Initial observations indicated that total dissolved solids (TDS) is the "primary toxicant, secondary toxicant, or a mask to other toxicants." Phase II testing found reduction of toxicity through pH adjustment and filtration. Further investigation of specific ions precipitated is ongoing.

The current permit for this facility is implemented with a delayed effective date for chronic WET in the first and fourth quarters, beginning October 1, 2020. In these quarters, the effluent limit is "report only" for chronic WET testing until September 30, 2020; however, the automatic compliance response(s) are required to be implemented in these "report only" quarters.

The last required submittal is the 180-day TIE or TRE report (due by August 29th). We are committed to meeting all permit requirements, including reporting deadlines. We will also be contacting you to discuss the findings and further investigations.

If you have any questions on this status report, please contact Chantell Johnson at 303-254-3185 (cjohnson@tristategt.org).

Sincerely,

Barbara A. Walz
Senior Vice President
Policy and Compliance
Chief Compliance Officer

BAW:CJ:der

Enclosures

cc: Brock Bowles, DRMS (via email)
Chantell Johnson (via email)
File G474-11.3(10)a-5

SeaCrestGroup
AN ENVIRONMENTAL SERVICES COMPANY

May 2, 2018

Chantell Johnson
Tri-State Generation and Transmission Association, Inc.
1100 W 116th Ave.
Westminster, CO 80234

Mr. Thomas Fry
Elk Ridge Mining and Reclamation
27646 W 5th Ave.
Nucla, CO 81424

Dear Ms. Johnson and Mr. Fry:

SeaCrest Group has undertaken the TIE (Toxicity Identification Evaluation) at the request of the Elk Ridge Mining and Reclamation. This testing is in response to a WET result that required an automatic compliance response in Q1 of 2018 suggesting toxicity to the *Ceriodaphnia dubia* test species. This series of manipulations and tests is intended to characterize the most potential group of toxicants responsible for the observed toxicity. This report represents Tier i and ii of Phase I of the TIE protocol. The TIE was performed in accordance with EPA protocols for the conduct of such investigations.

Initial observations from Phase I of the TIE are as follows:

1) Toxicity was removed for lethal and sub-lethal resulting in an IC_{25} of >100% for both endpoints after the pH 10 filtration treatment, despite the elevated conductivity in this test (average = 3,330 µhmos/cm). This suggests that the toxicant was precipitated out of solution at pH 10 and filtered off..
2) A visible precipitate was formed when the effluent pH was raised above 8.5.
3) The conductivity exceeded 1,000 µhmos/cm at the LOEC of all baseline/ initial tests. This may suggest that TDS is a primary toxicant, secondary toxicant, or a mask to other toxicants.

If you have any questions or concerns, please do not hesitate to contact me at (303) 661-9324.

Best regards,

Kyra Brisson
Laboratory Director
Enclosure(s): Report
Invoice

RESULTS OF PHASE I OF THE CHRONIC TIE (TOXICITY IDENTIFICATION EVALUATION) CONDUCTED FOR ELK RIDGE MINING AND RECLAMATION ON THE NEW HORIZON MINE OUTFALL 013 SITE

Prepared for:

Chantell Johnson
Tri-State Generation and Transmission Association, Inc.
1100 W 116th Ave.
Westminster, CO 80234

Mr. Thomas Fry
Elk Ridge Mining and Reclamation
P.O. Box 628
Nucla, CO 801424

Prepared by:

SeaCrest Group
500 S Arthur Ave. Suite 450
Louisville, Colorado 80027-3065
(303) 661-9324

May 2, 2018

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Chronic Toxicity Test Summary

Test: 7-day static renewal using *Ceriodaphnia dubia*

Client: Elk Ridge Mining and Reclamation

Test Procedure/Method Followed: EPA/821/R-02-013. Method 1002.0 (2002)

Sample Description: 418208.B

Sample	Time of Collection	Date of Collection	Time of Receipt	Date of Receipt
Effluent	0930; 0940; 0950; 0955	03-07-2018	1255	03-08-2018

	CONTROL (Initial/Tier i/ Tier ii)	100%
Alkalinity (mg/L as $CaCO_3$)	59/60/64	511
Hardness (mg/L as $CaCO_3$)	83/87/100	1998
Total residual chlorine (mg/L)	<0.01	<0.01
Total ammonia (mg/L as NH_3)	<0.03	2.29

Dilution Water:
- Moderately hard laboratory reconstituted water

Test Organism Source:
- *Ceriodaphnia dubia*
  SeaCrest Group

Reference Toxicant:
- Sodium Chloride

Tier i

	Initial	Baseline
Test Initiation Time	1515	1100
Test Initiation Date	03-08-2018	03-15-2018
Test Completion Time	1530	1147
Test Completion Date	03-15-2018	03-22-2018

	Filtered (pH i)	Aerated (pH i)
Test Initiation Time	1130	1130
Test Initiation Date	03-15-2018	03-15-2018
Test Completion Time	1220	1200
Test Completion Date	03-22-2018	03-22-2018

	pH Adjusted (pH~6.5)	pH Adjusted (pH~8.5)
Test Initiation Time	1300	1400
Test Initiation Date	03-15-2018	03-15-2018
Test Completion Time	1310	1340
Test Completion Date	03-22-2018	03-22-2018

	$Na_2S_2O_3$ a (pH i)	$Na_2S_2O_3$ b (pH i)
Test Initiation Time	1200	1230
Test Initiation Date	03-15-2018	03-15-2018
Test Completion Time	1215	1225
Test Completion Date	03-22-2018	03-22-2018

	EDTA a (pH i)	EDTA b (pH i)
Test Initiation Time	1320	1340
Test Initiation Date	03-15-2018	03-15-2018
Test Completion Time	1250	1300
Test Completion Date	03-22-2018	03-22-2018

	C18 (pH i)
Test Initiation Time	1210
Test Initiation Date	03-15-2018
Test Completion Time	1250
Test Completion Date	03-22-2018

Tier ii

	Baseline
Test Initiation Time	1230
Test Initiation Date	03-27-2018
Test Completion Time	1205
Test Completion Date	04-03-2018

	pH 3	pH 10
Test Initiation Time	1325	1330
Test Initiation Date	03-27-2018	03-27-2018
Test Completion Time	1240	1300
Test Completion Date	04-03-2018	04-03-2018

	pH 3 Filtered	pH 10 Filtered
Test Initiation Time	1515	1500
Test Initiation Date	03-27-2018	03-27-2018
Test Completion Time	1500	1426
Test Completion Date	04-03-2018	04-03-2018

	pH 3 Aerated	pH 10 Aerated
Test Initiation Time	1240	1315
Test Initiation Date	03-27-2018	03-27-2018
Test Completion Time	1215	1235
Test Completion Date	04-03-2018	04-03-2018

	pH 3 C18	pH 10 C18
Test Initiation Time	1510	1525
Test Initiation Date	03-27-2018	03-27-2018
Test Completion Time	1442	1450
Test Completion Date	04-03-2018	04-03-2018

Abstract of Initial Test Results

Test Concentrations:	Control (0%), 25%, 50%, 75%, 100%
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**59.5%**
Temperature Range (°C)	24.1 – 25.0
Dissolved Oxygen Range (mg/L)	6.4 – 7.6
pH Range	6.7 – 8.2

Abstract of Baseline Test Results- Tier i

Test Concentrations:	Control (0%), 50%, 60%, 70%, 100%
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**39.4%**
Temperature Range (°C)	24.1 – 25.2
Dissolved Oxygen Range (mg/L)	6.7 – 8.0
pH Range	6.7 – 8.2

Abstract of Filtered (pH i) Test Results

Test Concentrations:	Control (0%), 50%, 60%, 70%, 100%, Method Control
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**74.4%**
Temperature Range (°C)	24.1 – 25.3
Dissolved Oxygen Range (mg/L)	6.6 – 7.9
pH Range	7.3 – 8.1

Abstract of Aerated (pH i) Test Results

Test Concentrations:	Control (0%), 50%, 60%, 70%, 100%, Method Control
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**76.3%**
Temperature Range (°C)	24.1 – 25.4
Dissolved Oxygen Range (mg/L)	6.7 – 8.5
pH Range	7.8 – 8.3

Abstract of pH Adjusted (~6.5) Test Results

Test Concentrations:	Control (0%), 50%, 60%, 70%, 100%, Method Control
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**71.2%**
Temperature Range (°C)	24.1 – 25.2
Dissolved Oxygen Range (mg/L)	6.6 – 8.2
pH Range	6.5 – 8.1

Abstract of pH Adjusted (~8.5) Test Results

Test Concentrations:	Control (0%), 50%, 60%, 70%, 100%, Method Control
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**53.7%**
Temperature Range (°C)	24.1 – 25.4
Dissolved Oxygen Range (mg/L)	5.9 – 8.1
pH Range	7.7 – 8.5

Abstract of $Na_2S_2O_3$ a (pH i) Test Results

Test Concentrations:	Control (0%), 50%, 60%, 70%, 100%, Method Control
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**72.0%**
Temperature Range (°C)	24.1 – 25.4
Dissolved Oxygen Range (mg/L)	6.3 – 8.2
pH Range	6.9 – 8.2

Abstract of $Na_2S_2O_3$ b (pH i) Test Results

Test Concentrations:	Control (0%), 50%, 60%, 70%, 100%, Method Control
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**41.1%**
Temperature Range (°C)	24.1 – 25.2
Dissolved Oxygen Range (mg/L)	6.1 – 7.7
pH Range	6.8 – 8.2

Abstract of EDTA a (pH i) Test Results

Test Concentrations:	Method Control (0%), 50%, 60%, 70%, 100%, Control
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**41.5%**
Temperature Range (°C)	24.1 – 25.2
Dissolved Oxygen Range (mg/L)	6.2 – 8.2
pH Range	7.1 – 8.4

Abstract of EDTA b (pH i) Test Results

Test Concentrations:	Control (0%), 50%, 60%, 70%, 100%, Method Control
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**62.1%**
Temperature Range (°C)	24.1 – 25.4
Dissolved Oxygen Range (mg/L)	6.2 – 8.8
pH Range	6.9 – 8.7

Abstract of C18 (pH i) Test Results

Test Concentrations:	Control (0%), 50%, 60%, 70%, 100%, Method Control
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**73.4%**
Temperature Range (°C)	24.1 – 25.3
Dissolved Oxygen Range (mg/L)	5.0 – 7.4
pH Range	7.4 – 8.2

Abstract of Baseline Test Results- Tier ii

Test Concentrations:	Control (0%), 50%, 60%, 70%, 100%
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**41.4%**
Temperature Range (°C)	24.1 – 25.5
Dissolved Oxygen Range (mg/L)	6.7 – 7.5
pH Range	6.8 – 8.1

Abstract of pH 3 Test Results

Test Concentrations:	Control (0%), 50%, 60%, 70%, 100%, Method Control
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**53.4%**
Temperature Range (°C)	24.1 – 25.2
Dissolved Oxygen Range (mg/L)	6.5 – 7.7
pH Range	6.5 – 8.1

Abstract of pH 10 Test Results

Test Concentrations:	Control (0%), 50%, 60%, 70%, 100%, Method Control
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**62.9%**
Temperature Range (°C)	24.1 – 25.3
Dissolved Oxygen Range (mg/L)	6.4 – 7.6
pH Range	6.7 – 8.1

Abstract of pH 3 Filtered Test Results

Test Concentrations:	Control (0%), 50%, 60%, 70%, 100%, Method Control
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**35.6%**
Temperature Range (°C)	24.1 – 25.6
Dissolved Oxygen Range (mg/L)	6.7 – 8.0
pH Range	6.6 – 8.4

Abstract of pH 10 Filtered Test Results

Test Concentrations:	Control (0%), 50%, 60%, 70%, 100%, Method Control
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**>100%**
Temperature Range (°C)	24.1 – 24.7
Dissolved Oxygen Range (mg/L)	6.7 – 8.1
pH Range	6.7 – 8.3

Abstract of pH 3 Aerated Test Results

Test Concentrations:	Control (0%), 50%, 60%, 70%, 100%, Method Control
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**57.8%**
Temperature Range (°C)	24.1 – 25.3
Dissolved Oxygen Range (mg/L)	7.1 – 8.3
pH Range	6.5 – 8.2

Abstract of pH 10 Aerated Test Results

Test Concentrations:	Control (0%), 50%, 60%, 70%, 100%, Method Control
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**73.6%**
Temperature Range (°C)	24.1 – 25.5
Dissolved Oxygen Range (mg/L)	7.0 – 8.1
pH Range	6.7 – 8.5

Abstract of pH 3 C18 Test Results

Test Concentrations:	Control (0%), 50%, 60%, 70%, 100%, Method Control
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**84.9%**
Temperature Range (°C)	24.1 – 25.3
Dissolved Oxygen Range (mg/L)	5.4 – 8.0
pH Range	6.7 – 8.3

Abstract of pH 10 C18 Test Results

Test Concentrations:	Control (0%), 50%, 60%, 70%, 100%, Method Control
Number of Organisms/Concentration:	5 for *Ceriodaphnia dubia*
Replicates at each Concentration:	5 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Reproduction IC_{25}	**>100%**
Temperature Range (°C)	24.1 – 25.6
Dissolved Oxygen Range (mg/L)	5.8 – 7.8
pH Range	6.6 – 8.4

INTRODUCTION

Toxicity was demonstrated to the *Ceriodaphnia dubia* test species after the Elk Ridge Mining and Reclamation New Horizon Mine 013 effluent failed sub-lethal statistical endpoints for quarterly Whole Effluent Toxicity (WET) test during the first quarter of 2018. In accordance with generally accepted Environmental Protection Agency (EPA) and Colorado Department of Health and Environment (CDPHE) procedures, this triggers the need to initiate a TIE (Toxicity Identification Evaluation) to characterize the possible cause of the observed toxicity. Accordingly, an initial, baseline, and series of TIE Phase I manipulation tests were performed. After each manipulation shortened chronic toxicity tests are run to determine the effects of the manipulation on the toxicity of the effluent. Based on the manipulations that reduce or increase toxicity, inferences about the chemical and physical characteristics of the toxicant(s) are made. This report details the results of these investigations.

MATERIALS AND METHODS

Sample Collection

A sample of 20 effluent gallons was collected from the discharge system. The sample was delivered chilled to the SeaCrest lab where it was held at 0-6°C. Chain of custody forms showing sample collection and lab arrival times are included in Appendix 1.

Dilution Water

Laboratory reconstituted water was used as both the dilution water source and the control for the tests. Reconstituted water was produced by adding sodium bicarbonate, calcium sulfate, magnesium sulfate, potassium chloride, and sodium selenate to deionized water.

Test Organisms

The biomonitoring tests were conducted with *Ceriodaphnia dubia*. *Ceriodaphnia dubia* is cultured in the SeaCrest laboratory. Stock cultures are maintained in 5-gallon aquaria. Brood females are cultured in individual plastic beakers on brood boards for a period of up to 14-days. Neonates less than 24-hours old released from third or subsequent broods of eight or more within an 8-hour period are removed from the brood chambers and used in tests. Brood and stock organisms are fed daily with a mixture of Yeast, Cereal Leaves and Trout Chow (YCT). This is supplemented with an equal volume of green algae *(Selenastrum capricornutum)*.

Test Procedures

Upon receipt at the lab, samples are analyzed for alkalinity, hardness, conductivity, dissolved oxygen, ammonia, chlorine and pH. Alkalinity and hardness are determined titrimetrically according to methods described in Hach Chemical Company[1]. Ammonia is measured by a Thermo Orion ion-selective electrode according to the procedures in APHA/AWWA/WEF[2]. Conductivity, dissolved oxygen and pH probes were used to take these measurements.

The initial test was started on 03-08-2018. The baseline and Tier 1 manipulation tests were started on 03-15-2018. The baseline and Tier 2 manipulation tests were started on 03-28-2018. The tests were performed according to the procedures outlined in USEPA[3] and the Colorado Department of Public Health and Environment[4].

The TIE test guidelines (EPA/600/6-91/005F[7]) necessitate an "initial" test to determine if the present discharge sample exhibits the toxicity seen in previous samples. The guidelines describe the adjustments that the effluent should undergo and stipulate that a "baseline" test is run concurrently with other tests to monitor any change in the toxicity of the samples during the testing period.

Individual organisms were placed in 30 ml plastic containers containing approximately 15 ml of exposure medium. Five replicates at each concentration were used for the initial, baseline, and manipulation tests. The animals were fed daily with the YCT mixture and an equal volume of the green algae *(Selenastrum capricornutum)*. Routine measurements were made each day of temperature, dissolved oxygen, conductivity and pH identified in the guidelines.

Tier i tests

Filtration Test

The filtration test is used to determine if toxicity is associated with filterable material in the effluent. The effluent is filtered through a DI-rinsed 1.5 micron glass fiber filter using a vacuum pump.

Aeration Test

The aeration test is used to determine if toxicity is due to volatile or oxidizable compounds. The effluent is aerated for 60 minutes prior to the initiation of the test.

pH Adjusted Tests

Graduated pH tests are used to determine compounds with pH dependent toxicity. For example, metal toxicity can be affected by pH differences through changes in solubility and speciation. For these tests, the pH is adjusted to bracket the original pH of the effluent.

$Na_2S_2O_3$ Tests

Na2S2O3 tests are used to determine whether toxicity can be attributed to oxidative compounds. Oxidative compounds (such as chlorine) and metal compounds (such as copper and manganese) can be made less toxic or non-toxic by additions of $Na_2S_2O_3$. Reductions in toxicity may also be due to the formation of metal complexes. Two aliquots of effluent dilutions with different amounts of $Na_2S_2O_3$ are prepared.

EDTA Tests

These tests are designed to detect toxicity caused by certain cationic metals. EDTA is a strong chelating agent and, because of its complexing strength, it will often displace other soluble forms of many metals. Two aliquots of effluent are prepared with separate amounts of EDTA.

C18 SPE (Solid Phase Extraction) Filtration test

The C18 SPE filtration test is used to determine the extent of the effluent's toxicity that is due to compounds that are removed or sorbed onto the SPE column at pH i. The effluent is first passed through a 1.5 micron glass fiber filter. Non-polar compounds, some metals, and some surfactants are removed from the sample after it is passed through a SPE column,.

Tier ii test

pH 3 and pH 10 Tests

For these tests, the effluent is adjust to a pH of 3 and a pH of 10 and then these aliquots are adjusted back to pH i. Often precipitation occurs after a drastic pH change.

pH Adjustment and Filtration Tests

Since a pH change can cause toxicants to precipitate or cause solubilized toxicants to sorb on particles, filtration at altered pH values can be used as a tool in characterizing the effluent. Therefore, by filtering pH adjusted effluent, compounds that were in solution without a pH adjustment may no longer be in solution or any toxicants associated with particles may be removed by the filtration process.

pH Adjustment and Aeration Tests

Aeration at pH 3 or pH 10 may make toxicants oxidizable, spargeable, or sublatalbe, that are not so at pH i. For these tests, two aliquots are adjusted to pH 3 or pH 10, next aerated for one hour, then adjusted back to pH i.

pH Adjustment and C18 SPE Filtration Tests

Shifting the ionization equilibrium at high and low pHs may cause the C18 SPE column to extract different compounds than at pH i. Organic acids or bases may be made less polar by shifting their equilibrium to the un-ionized state. Since the pH 3 C18 SPE Filtration test showed reduced toxicity and overburden of the C18 SPE column was suspected, an additional test (pH 3 Multiple C18 SPE Filtration) was run, in which the aliquot was passed through multiple C18 SPE columns.

Data Analysis

Data from the test(s) were analyzed on a personal computer using the TOXCALC package developed by Tidepool Scientific Software. Statistical tests used in the analyses are shown in Table 1. Test acceptability was determined using control survival and performance criteria, concentration-response relationships and percent minimum significant differences (USEPA [5,6]).

INITIAL TEST RESULTS

Initial Test Results

Test results for the Initial test are summarized in Table 1 and provided on the data sheets located in Appendix 2. Survival was 40% in the 100% effluent and ranged from 40% to 100% in the remaining effluent concentrations. Control survival was 80%. The IC_{25} for survival was 60.9%.

Table 1. Summary of Initial test results.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control (0%)	4	22.6	1	30
25%	5	26.4	21	30
50%	5	26.2	20	29
75%	2	8.6	0	22
100%	2	3.8	0	6

Average numbers of neonates in the 100% effluent was 3.8 and ranged from 8.6 – 26.4 in the remaining effluent concentrations. Average number of neonates in the control was 22.6. The IC_{25} for reproduction was estimated at 59.5%.

Tier i
BASELINE TEST RESULTS

Baseline Test Results

Test results for the Baseline test are summarized in Table 2 and provided on the data sheets located in Appendix 2. Survival was 20% in the 100% effluent and ranged from 60% to 80% in the remaining effluent concentrations. Control survival was 100%. The IC_{25} for survival was estimated at 62.5%.

Table 2. Summary of Baseline test results.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control	5	29.0	27	31
50%	4	19.8	4	32
60%	4	19.8	3	28
70%	3	10.8	0	19
100%	1	0.6	0	3

Average numbers of neonates in the 100% effluent was 0.6 and ranged from 10.8 – 19.8 in the remaining effluent concentrations. Average number of neonates in the control was 29.0. The IC_{25} for reproduction was estimated at 39.4%.

FILTERED (pH i) TEST RESULTS

Filtered (pH i) Test Results

Test results for the Filtered (pH i) test are summarized in Table 3 and provided on the data sheets located in Appendix 2. Survival was 20% in the 100% effluent and ranged from 60% to 100% in the remaining effluent concentrations. Control survival was 100%. Method control survival was 100%. The IC_{25} for survival was 56.3%.

Table 3. Summary of Filtered (pH i) test results.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control	5	27.4	24	34
50%	5	26.0	21	31
60%	3	23.2	17	28
70%	3	22.0	19	24
100%	1	12.0	0	21
Method Control	5	29.2	22	39

Average numbers of neonates was 12.0 in the 100% effluent and ranged from 22.0 – 26.0 in the remaining effluent concentrations. Average number of neonates in the control was 27.4. Average number of neonates in the method control was 29.2. The IC_{25} for reproduction was 74.4%.

AERATED (pH i) TEST RESULTS

Aerated (pH i) Test Results

Test results for the Aerated (pH i) test are summarized in Table 4 and provided on the data sheets located in Appendix 2. Survival was 80% in the 100% effluent and 100% in the remaining effluent concentrations. Control survival was 100%. Method control survival was 100% The IC_{25} for survival was >100%.

Table 4. Summary of Aerated (pH i) test results.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control	5	26.0	19	31
50%	5	21.8	19	24
60%	5	20.0	8	25
70%	5	22.8	18	27
100%	4	12.4	3	19
Method Control	5	25.8	17	31

Average numbers of neonates was 12.1 in the 100% effluent and ranged from 20.0 – 22.8 in the remaining effluent concentrations. Average number of neonates in the control was 26.0. Average number of neonates in the method control was 25.8. The IC_{25} for reproduction was 76.3%.

pH Adjusted (~6.5) TEST RESULTS

pH Adjusted (~6.5) Test Results

Test results for the pH Adjusted (~6.5) test are summarized in Table 5 and provided on the data sheets located in Appendix 2. Survival was 60% in the 100% effluent and 100% in the remaining effluent concentrations. Control survival was 100%. Method control survival was 100%. The IC_{25} for survival was 88.8%.

Table 5. Summary of pH Adjusted (~6.9) test results.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control	5	25.8	18	35
50%	5	23.4	20	29
60%	5	19.4	15	23
70%	5	19.8	16	22
100%	3	13.2	0	26
Method Control	5	27.4	19	30

Average numbers of neonates was 13.2 in the 100% effluent and ranged from 19.4 – 23.4 in the remaining effluent concentrations. Average number of neonates in the control was 25.8. the average number of neonates in the method control was 27.4. The IC_{25} for reproduction was 71.2%.

pH Adjusted (~8.5) TEST RESULTS

pH Adjusted (~8.5) Test Results

Test results for the pH Adjusted (~8.5) test are summarized in Table 6 and provided on the data sheets located in Appendix 2. Survival was 80% in the 100% effluent and ranged from 80% to 100% in the remaining effluent concentrations. Control survival was 100%. Method control survival was 80%. The IC_{25} for survival was >100%.

Table 6. Summary of pH Adjusted (~8.5) test results.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control	5	27.4	24	30
50%	5	21.8	15	26
60%	4	18.4	5	26
70%	5	18.2	16	20
100%	4	13.2	4	20
Method Control	4	27.2	21	30

Average numbers of neonates was 13.2 in the 100% effluent and ranged from 18.2 – 21.8 in the remaining effluent concentrations. Average number of neonates in the control was 27.4. Average number of neonates in the method control was 27.2. The IC_{25} for reproduction was 53.7%.

$Na_2S_2O_3$ A TEST RESULTS

$Na_2S_2O_3$ A Test Results

Test results for the $Na_2S_2O_3$ A test are summarized in Table 7 and provided on the data sheets located in Appendix 2. Survival was 100% in the 100% effluent and ranged from 80% to 100% in the remaining effluent concentrations. Control survival was 100%. Method control was 100%. The IC_{25} for survival was >100%.

Table 7. Summary of $Na_2S_2O_3$ A test results.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control	5	27.0	23	31
50%	5	24.0	20	28
60%	5	24.2	18	30
70%	4	20.6	13	30
100%	5	15.4	11	17
Method Control	5	27.4	23	31

Average numbers of neonates was 15.4 in the 100% effluent and ranged from 20.6 – 24.2 in the remaining effluent concentrations. Average number of neonates in the control was 27.0. Average number of neonates in the method control was 27.4 The IC_{25} for reproduction was 72.0%.

$Na_2S_2O_3$ B TEST RESULTS

$Na_2S_2O_3$ B Test Results

Test results for the $Na_2S_2O_3$ B test are summarized in Table 8 and provided on the data sheets located in Appendix 2. Survival was 60% in the 100% effluent and ranged from 60% to 100% in the remaining effluent concentrations. Control survival was 100%. Method control was 100%. The IC_{25} for survival was 41.1%.

Table 8. Summary of $Na_2S_2O_3$ B test results.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control	5	27.6	24	30
50%	5	18.8	15	25
60%	5	19.6	18	23
70%	3	16.2	0	22
100%	3	9.8	0	18
Method Control	5	27.2	25	29

Average numbers of neonates was 9.8 in the 100% effluent and ranged from 16.2 – 19.6 in the remaining effluent concentrations. Average number of neonates in the control was 27.6. The average number of neonates in the method control was 27.2 The IC_{25} for reproduction was estimated to be 41.1%.

EDTA A TEST RESULTS

EDTA A Test Results

Test results for the EDTA A test are summarized in Table 9 and provided on the data sheets located in Appendix 2. Survival was 60% in the 100% effluent and ranged from 80% to 100% in the remaining effluent concentrations. Control survival was 100%. Method control was 100%. The IC_{25} for survival was 77.5%.

Table 9. Summary of EDTA A test results.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control	5	32.2	29	39
50%	4	23.2	19	27
60%	5	22.4	20	24
70%	4	20.2	15	26
100%	4	15.0	6	19
Method Control	5	27.4	24	31

Average numbers of neonates was 15.0 in the 100% effluent and ranged from 20.2 – 23.2 in the remaining effluent concentrations. Average number of neonates in the control was 32.2. The average number of neonates in the method control was 27.4. The IC_{25} for reproduction was estimated to be 41.5%.

EDTA B TEST RESULTS

EDTA B Test Results

Test results for the EDTA B test are summarized in Table 10 and provided on the data sheets located in Appendix 2. Survival was 100% in the 100% effluent and ranged from 60% to 100% in the remaining effluent concentrations. Control survival was 100%. Method control survival was 100%. The IC_{25} for survival was 59.4%.

Table 10. Summary of EDTA B test results.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control	5	30.4	23	39
50%	5	24.0	19	32
60%	3	25.8	20	29
70%	3	15.0	6	22
100%	5	14.4	9	19
Method Control	5	25.8	17	35

Average numbers of neonates was 14.4 in the 100% effluent and ranged from 15.0 – 25.8 in the remaining effluent concentrations. Average number of neonates in the control was 30.4. The average number of neonates in the method control was 25.8. The IC_{25} for reproduction was 62.1%.

C18 TEST RESULTS

C18 Test Results

Test results for the C18 test are summarized in Table 11 and provided on the data sheets located in Appendix 2. Survival was 20% in the 100% effluent and ranged from 80% to 100% in the remaining effluent concentrations. Control survival was 100%. Method control survival was 100%. The IC_{25} for survival was 72.5%.

Table 11. Summary of C18 test results.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control	5	26.8	24	29
50%	5	26.0	20	35
60%	4	24.8	20	30
70%	4	21.4	10	29
100%	1	9.8	1	17
Method Control	5	28.6	23	35

Average numbers of neonates was 9.8 in the 100% effluent and ranged from 21.4 – 26.0 in the remaining effluent concentrations. Average number of neonates in the control was 26.8. The average number of neonates in the method control 28.6. The IC_{25} for reproduction was estimated at 73.4%.

TIER ii

BASELINE TEST RESULTS

Baseline Test Results

Test results for the Baseline test are summarized in Table 12 and provided on the data sheets located in Appendix 2. Survival was 20% in the 100% effluent and ranged from 40% to 100% in the remaining effluent concentrations. Control survival was 100%. The IC_{25} for survival was estimated at 54.2%.

Table 12. Summary of Baseline test results.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control	5	33.8	28	36
50%	5	23.6	15	27
60%	2	8.8	0	23
70%	2	4.0	0	13
100%	1	6.0	0	11

Average numbers of neonates in the 100% effluent was 6.0 and ranged from 4.0 – 23.6 in the remaining effluent concentrations. Average number of neonates in the control was 33.8. The IC_{25} for reproduction was estimated at 41.4%.

pH 3 TEST RESULTS

pH 3 Test Results

Test results for the pH 3 test are summarized in Table 13 and provided on the data sheets located in Appendix 2. Survival was 0% in the 100% effluent and ranged from 40% to 100% in the remaining effluent concentrations. Control survival was 100%. Method control survival was 80%. The IC_{25} for survival was 56.3%.

Table 13. Summary of pH 3 test results.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control	5	29.6	24	34
50%	5	26.2	23	29
60%	3	14.6	0	24
70%	2	8.0	0	21
100%	0	1.6	0	8
Method Control	4	25.6	22	30

Average numbers of neonates was 1.6 in the 100% effluent and ranged from 8.0 – 26.2 in the remaining effluent concentrations. Average number of neonates in the control was 29.6. The average number of neonates in the control was 25.6. The IC_{25} for reproduction was 53.4%.

pH 10 TEST RESULTS

pH 10 Test Results

Test results for the pH 10 test are summarized in Table 14 and provided on the data sheets located in Appendix 2. Survival was 100% in the 100% effluent and 100% in the remaining effluent concentrations. Control survival was 100%. Method control survival was 80%. The IC_{25} for survival was >100%.

Table 14. Summary of pH 10 test results. Five animals were exposed at each concentration.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control	5	29.2	23	35
50%	5	22.8	21	25
60%	5	24.6	21	30
70%	5	17.4	12	23
100%	5	13.0	8	17
Method Control	4	24.4	7	37

Average numbers of neonates was 13.0 in the 100% effluent and ranged from 17.4 – 24.6 in the remaining effluent concentrations. Average number of neonates in the control was 29.2. The average number of neonates in the method control was 24.4. The IC_{25} for reproduction was estimated at 62.9%.

pH 3 FILTERED TEST RESULTS

pH 3 Filtered Test Results

Test results for the pH 3 Filtered test are summarized in Table 15 and provided on the data sheets located in Appendix 2. Survival was 80% in the 100% effluent and ranged from 60% to 80% in the remaining effluent concentrations. Control survival was 100%. Method control survival was 80%. The IC_{25} for survival was 68.3%.

Table 15. Summary of pH 3 Filtered test results. Five animals were exposed at each concentration.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control	5	33.0	29	37
50%	5	21.4	18	24
60%	5	18.4	14	21
70%	3	12.0	6	16
100%	4	8.0	4	14
Method Control	4	23.8	1	34

Average numbers of neonates was 8.0 in the 100% effluent and ranged from 12.0 – 21.4 in the remaining effluent concentrations. Average number of neonates in the control was 33.0. The average number of neonates in the method control was 23.8. The IC_{25} for reproduction was estimated to be 35.6%.

pH 10 FILTERED TEST RESULTS

pH 10 Filtered Test Results

Test results for the pH 10 Filtered test are summarized in Table 16 and provided on the data sheets located in Appendix 2. Survival was 100% in the 100% effluent and 100% in the remaining effluent concentrations. Control survival was 80%. Method control survival was 100%. The IC_{25} for survival was >100%.

Table 16. Summary of pH 10 Filtered test results. Five animals were exposed at each concentration.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control	4	25.8	16	32
50%	5	30.0	27	33
60%	5	27.4	25	30
70%	5	26.6	22	30
100%	5	26.8	24	29
Method Control	5	29.0	26	30

Average numbers of neonates was 26.8 in the 100% effluent and ranged from 26.6 – 30.0 in the remaining effluent concentrations. Average number of neonates in the control was 25.8. The average number of neonates in the method control was 29.0. The IC_{25} for reproduction was >100%.

pH 3 AERATED TEST RESULTS

pH 3 Aerated Test Results

Test results for the pH 3 Aerated test are summarized in Table 17 and provided on the data sheets located in Appendix 2. Survival was 100% in the 100% effluent and 100% in the remaining effluent concentrations. Control survival was 100%. Method control survival was 100%. The IC_{25} for survival was >100%.

Table 17. Summary of pH 3 Aerated test results. Five animals were exposed at each concentration.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control	5	24.0	17	29
50%	5	23.0	18	32
60%	5	16.6	12	19
70%	5	15.4	6	22
100%	5	5.4	2	8
Method Control	5	24.4	22	27

Average numbers of neonates was 5.4 in the 100% effluent and ranged from 15.4 – 23.0 in the remaining effluent concentrations. Average number of neonates in the control was 24.0. The average number of neonates in the method control was 5.4. The IC_{25} for reproduction was 57.8%.

pH 10 AERATED TEST RESULTS

pH 10 Aerated Test Results

Test results for the pH 10 Aerated test are summarized in Table 18 and provided on the data sheets located in Appendix 2. Survival was 100% in the 100% effluent and was 100% in the remaining effluent concentrations. Control survival was 100%. Method control survival was 80%. The IC_{25} for survival was estimated at >100%.

Table 18. Summary of pH 10 Aerated test results. Five animals were exposed at each concentration.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control	5	21.0	0	30
50%	5	18.0	9	23
60%	5	20.6	18	24
70%	5	16.2	12	19
100%	5	12.4	6	24
Method Control	4	18.4	8	25

Average numbers of neonates was 12.1 in the 100% effluent and ranged from 16.2 – 20.6 in the remaining effluent concentrations. Average number of neonates in the control was 21.0. The average number of neonates in the method control was 18.4. The IC_{25} for reproduction was estimated at 73.6%.

pH 3 C18 TEST RESULTS

pH 3 C18 Test Results

Test results for the pH 3 C18 test are summarized in Table 19 and provided on the data sheets located in Appendix 2. Survival was 100% in the 100% effluent and 100% in the remaining effluent concentrations. Control survival was 100%. Method control survival was 100%. The IC_{25} for survival was >100%.

Table 19. Summary of pH 3 C18 test results. Five animals were exposed at each concentration.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control	5	24.2	19	33
50%	5	20.8	4	35
60%	5	22.6	18	26
70%	5	22.8	19	31
100%	5	14.2	11	22
Method Control	5	27.0	20	34

Average numbers of neonates was 14.2 in the 100% effluent and ranged from 20.8 – 22.8 in the remaining effluent concentrations. Average number of neonates in the control was 24.2. The average number of neonates in the method control was 27.0. The IC_{25} for reproduction was 84.9%.

pH 10 C18 TEST RESULTS

pH 10 C18 Test Results

Test results for the pH 10 C18 test are summarized in Table 20 and provided on the data sheets located in Appendix 2. Survival was 100% in the 100% effluent and 100% in the remaining effluent concentrations. Control survival was 100%. Method control survival was 100%. The IC_{25} for survival was >100%.

Table 20. Summary of pH 10 C18 test results. Five animals were exposed at each concentration.

Concentration	No. Surviving	Mean Births	Min.	Max.
Control	5	28.8	20	35
50%	5	26.8	23	30
60%	5	30.0	18	36
70%	5	26.2	19	30
100%	5	24.6	22	30
Method Control	5	28.6	24	34

Average numbers of neonates was 24.6 in the 100% effluent and ranged from 26.2 – 30.0 in the remaining effluent concentrations. Average number of neonates in the control was 28.8. The average number of neonates in the method control was 28.6. The IC_{25} for reproduction was >100%.

DISCUSSION

SeaCrest Group has undertaken the TIE at the request of Elk Ridge Mining and Reclamation and Tri-State Generation and Transmission Association, Inc. for the New Horizon Mine discharge 013. This testing is in response to historical data suggesting toxicity to the *Ceriodaphnia dubia* test species during the first and fourth quarters of the year. The TIE is being performed in accordance with EPA protocols for the conduct of such investigations[7].

An initial toxicity test with the following dilution series, 0%, 12.5%, 50%, 75%, and 100%, was initiated on March 8, 2018. This test confirmed the persistence of toxicity to the *C. dubia* test species resulting in a sub-lethal IC_{25} of 59.5%. The IC_{25} of this initial toxicity test was used to develop the following dilution series, 0%, 50%, 60%, 70%, and 100%, for Tier i and Tier ii of Phase I with the intention of bracketing toxicity. All manipulations for Phase I were run in accordance with EPA guidelines [8].

The majority of Tier i manipulations demonstrated reductions in toxicity compared with the Tier i baseline test, which had an IC_{25} of 39.4%, producing IC_{25}'s ranging from 41.1% - 76.3%. Similarly, the majority of Tier ii manipulations experiences a decrease in toxicity with IC_{25}'s ranging from 53.4% - >100%, compared to the baseline IC_{25} of 41.4%. The test that experienced an increase in toxicity in Tier 2 was filtration treatment at pH 3 resulting in an IC_{25} of 35.6%. The most successful manipulation of either tier was achieved in Tier ii by raising the pH of the effluent to pH 10, passing the effluent through a glass fiber filter with a pore size of 1.2 microns and then adjusting the pH back to the original pH. This manipulation produced an IC_{25} of >100%.

In addition to the removal of toxicity in the pH 10 filtration treatment, there are several other important observations regarding this manipulation. For one, sub-lethal toxicity was removed during this treatment, despite the elevated conductivity in this test (average = 3,330 µhmos/cm). This suggests that the toxicant was precipitated out of solution and filtered off at pH 10 and that elevated conductivity may contribute to toxicity but is not necessarily the primary cause. Additionally, a visible precipitate was formed when the effluent pH was raised above 8.5. This precipitate was light orange in color and easily suspended throughout the effluent. A light pink precipitate was observed in the unaltered effluent water.

Another important factor in the characterization of this water is the elevated TDS levels. For the purposes of WET testing, TDS is often measured as conductivity µhmos/cm. The TDS is considered elevated when it exceeded 1,000 µhmos/cm at the LOEC of any test[9]. In both tiers of Phase I this was the case. This may suggest that TDS is a primary toxicant, secondary toxicant, or a mask to other toxicants. TDS has long been recognized as a difficult means of identifying or projecting potential toxicity in the field of aquatic toxicology[10, 11]. This is due to the complex interactions between the major ions that contribute to TDS, including, but not limited to, chloride, sodium, calcium, magnesium, potassium, bicarbonate, and sulfate.

Phase II was initiated the week of April 23, 2018. The intention of Phase II of the TIE is to identify a most probable toxicant or group of toxicants[12]. This testing will be scaled up to 10 replicates to reduce the potential need for re-runs or confirmation of data. This testing will be based on the observations and data collected in Phase I. The first set of tests will address the removal of toxicity observed in the pH 10 filtration test and will involve three tests. The purpose of these three tests will be to confirm the reduction of toxicity in the pH 10 filtration test, attempt to recapture toxicity from the filtered precipitate, and to determine the major ion and metals concentration of the precipitate. The outcome of this testing will dictate if further Phase II testing will be necessary and may determine the direction of Phase III in which the toxicant or group of toxicants is confirmed and the TIE is concluded[13].

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Appendix 1 – Chain of Custody Form

SeaCrestGroup
AN ENVIRONMENTAL SERVICES COMPANY

CHAIN OF CUSTODY

500 S. Arthur Avenue, Unit 450 - Louisville, CO 80027
(303) 661.9324 - FAX (303) 661.9325

Client/Project Name: Elk Ridge Mining & Reclamation

P. O./Project Number: New Horizon Mine

Contact: T Fry

Address: 27646 West 5th, Nucla, Co. 81424

Phone # 970 424 3050 E-Mail: tfry@tristategt.org

Fax # 970 864 2168 Sampler: T FRY

Report By: ☐ Mail ☒ PDF ☐ FAX

Analysis (Check all applicable)

Sample Location or ID	Date	Time	Grab/ Comp	Lab ID (LAB Use Only)	WET: Acute (Indicate Below)	WET: Chronic (Indicate Below)	WET: Accelerated (Indicate Below)	WET: PTI/TIE/TRE (Indicate Below)	Metals (List Below)	Solids (TS/TDS/TSS) (Circle)	Anions (List Below)	Chromium III/VI (Circle)	Oil and Grease	Coliform (Total/Fecal/E-Coli) (Circle)	BOD/COD (Circle)	Other Analysis (List Below)				Number of Containers	Total Volume
NPDES 013	3.7.18	0930	G	418208.B												✓				S	G

Turnaround Requirements (Analytical Testing Only)

_____ Standard (10 days) _____ 6-9 Day

_____ 3-5 Day _____ 1-2 Day

Requested Report Date:

Test Species: ☐ Fathead Minnow ☐ Cerio daphnia ☐ Daphnia magna ☐ Daphnia pulex ☐ Other (List Below)

Special Instructions/Comments: T.I.E. ANALYSIS

Relinquished By (1)		Received By (1)		Relinquished By (2)		Received By (2)	
Signature	Date/Time	Signature	Date/Time	Signature	Date/Time	Signature	Date/Time
	3.7.18 1200	UPS		UPS			030818 1255

SeaCrestGroup
AN ENVIRONMENTAL SERVICES COMPANY

CHAIN OF CUSTODY

500 S. Arthur Avenue, Unit 450 - Louisville, CO 80027
(303) 661.9324 - FAX (303) 661.9325

Client/Project Name: Elk Ridge Mining & Reclamation

P. O./Project Number: New Horizon Mine

Contact: T Fry

Address: 27646 West 5th, Nucla, Co. 81424

Phone # 970 424 3050 | E-Mail: tfry@tristategt.org

Fax # 970 864 2168 | Sampler: TFRY

Report By: ☐ Mail ☒ PDF ☐ FAX

Analysis (Check all applicable)

Sample Location or ID	Date	Time	Grab/ Comp	Lab ID (LAB Use Only)	WET: Acute (Indicate Below)	WET: Chronic (Indicate Below)	WET: Accelerated (Indicate Below)	WET: PTI/TIE/TRE (Indicate Below)	Metals (List Below)	Solids (TS/TDS/TSS) (Circle)	Anions (List Below)	Chromium III/VI (Circle)	Oil and Grease	Coliform (Total/Fecal/E-Coli) (Circle)	BOD/COD (Circle)	Other Analysis (List Below)				Number of Containers	Total Volume
NPDES 013	3.7.18	0940	G	418208.B												✓				5	6

Turnaround Requirements (Analytical Testing Only)

_____ Standard (10 days) _____ 6-9 Day

_____ 3-5 Day _____ 1-2 Day

Requested Report Date:

Test Species: ☐ Fathead Minnow ☐ Cerio daphnia ☐ Daphnia magna ☐ Daphnia pulex ☐ Other (List Below)

Special Instructions/Comments: T.I.E. ANALYSIS

Relinquished By (1)		Received By (1)		Relinquished By (2)		Received By (2)	
Signature	Date/Time	Signature	Date/Time	Signature	Date/Time	Signature	Date/Time
	3.7.18 1200	UPS		UPS			030818 1255

SeaCrestGroup
AN ENVIRONMENTAL SERVICES COMPANY

CHAIN OF CUSTODY

500 S. Arthur Avenue, Unit 450 - Louisville, CO 80027
(303) 661.9324 - FAX (303) 661.9325

Client/Project Name: Elk Ridge Mining & Reclamation

P. O./Project Number: New Horizon Mine

Contact: T Fry

Address: 27646 West 5th, Nucla, Co. 81424

Phone # 970 424 3050 E-Mail: tfry@tristategt.org

Fax # 970 864 2168 Sampler: T Fry

Report By: ☐ Mail ☒ PDF ☐ FAX

Analysis (Check all applicable)

Sample Location or ID	Date	Time	Grab/ Comp	Lab ID (LAB Use Only)	WET: Acute (Indicate Below)	WET: Chronic (Indicate Below)	WET: Accelerated (Indicate Below)	WET: PTI/TIE/TRE (Indicate Below)	Metals (List Below)	Solids (TS/TDS/TSS) (Circle)	Anions (List Below)	Chromium III/VI (Circle)	Oil and Grease	Coliform (Total/Fecal/E-Coli) (Circle)	BOD/COD (Circle)	Other Analysis (List Below)				Number of Containers	Total Volume
NPDES 013	3.7.18	0950	G	418208-B												✓				5	G

Turnaround Requirements (Analytical Testing Only)

_____ Standard (10 days) _____ 6-9 Day

_____ 3-5 Day _____ 1-2 Day

Requested Report Date:

Test Species: ☐ Fathead Minnow ☐ Cerio daphnia ☐ Daphnia magna ☐ Daphnia pulex ☐ Other (List Below)

Special Instructions/Comments: T. I. E. ANALYSIS

Relinquished By (1)		Received By (1)		Relinquished By (2)		Received By (2)	
Signature	Date/Time	Signature	Date/Time	Signature	Date/Time	Signature	Date/Time
[signature] ✓	3.7.18 1200	UPS		UPS		[signature]	030818 1255

SeaCrestGroup
AN ENVIRONMENTAL SERVICES COMPANY

CHAIN OF CUSTODY

500 S. Arthur Avenue, Unit 450 - Louisville, CO 80027
(303) 661.9324 - FAX (303) 661.9325

Client/Project Name: Elk Ridge Mining & Reclamation

P. O./Project Number: New Horizon Mine

Contact: T Fry

Address: 27646 West 5th, Nucla, Co. 81424

Phone # 970 424 3050 E-Mail: tfry@tristategt.org

Fax # 970 864 2168 Sampler: TFRY

Report By: ☐ Mail ☒ PDF ☐ FAX

Analysis (Check all applicable)

Sample Location or ID	Date	Time	Grab/ Comp	Lab ID (LAB use Only)	WET: Acute (Indicate Below)	WET: Chronic (Indicate Below)	WET: Accelerated (Indicate Below)	WET: PTI/TIE/TRE (Indicate Below)	Metals (List Below)	Solids (TS/TDS/TSS) (Circle)	Anions (List Below)	Chromium III/VI (Circle)	Oil and Grease	Coliform (Total/Fecal/E-Coli) (Circle)	BOD/COD (Circle)	Other Analysis (List Below)				Number of Containers	Total Volume
NPDES 013	3.7.18	0955	G	418208.B												✓				S	G

Turnaround Requirements (Analytical Testing Only)

_____ Standard (10 days) _____ 6-9 Day

_____ 3-5 Day _____ 1-2 Day

Requested Report Date:

Test Species: ☐ Fathead Minnow ☐ Cerio daphnia ☐ Daphnia magna ☐ Daphnia pulex ☐ Other (List Below)

Special Instructions/Comments: T.I.E. ANALYSIS

Relinquished By (1)		Received By (1)		Relinquished By (2)		Received By (2)	
Signature	Date/Time	Signature	Date/Time	Signature	Date/Time	Signature	Date/Time
	3.7.18 1200	UPS		UPS			030818 1255

SeaCrest Group
Louisville, CO

Sample Receipt Form

Form #: 42
Effective: December 2008

Project #: 418 208.B
Date: 030818
Sample #:
Initials: ND

Samples Were:
1. (Shipped) Hand Delivered Messengered (circle one)
Notes: UPS

3. Chilled to Ship — Ambient (Chilled) (circle one)
Notes:
Wet Ice (Blue Ice) (circle one)

4. Cooler Received Broken or Leaking — Y (N) NA
Notes:

5. Sample Received Broken or Leaking — Y (N) NA
Notes:

6. Received Within Holding Times — (Y) N
Notes:

7. Aeration necessary — Y (N) NA
Notes:

8. Sample Received at Temperature between 0-6° C . — (Y) N NA
Notes:

9. Description of Sample (Color, Odor, and/or Presence of Particulate Matter):
eff: clear, some PM

rec'g initial chemistries taken on several bottles to capture variation

	Temp	DO (mg/L)	DO (%Sat)	pH	Cond	Aeration Time	Aeration DO (mg/L)	Aeration DO (%Sat)	Aeration pH
208.B	5.8	11.9	101.4	11.7	3080				
208.B	5.4	11.4	101.9	11.6	3030				
208.B	5.7	11.5	99.7	11.6	2910				

Custody Seals:
1. Present on Outer Package — Y (N)
2. Unbroken on Outer Package — Y N (NA)
3. Present on Sample — Y (N)
4. Unbroken on Sample — Y N (NA)

Custody Documentation:
1. Present Upon Receipt of Sample — (Y) N

Appendix 2 – Data Sheets for the *Ceriodaphnia dubia* Test

WET TEST REPORT FORM – CHRONIC
INITIAL TEST

Permittee: Elk Ridge Mining and Reclamation **Outfall:** 013
Permit No.: CO0000213

Test Type: Routine ☐ TIE ☒
Test Species: *Ceriodaphnia dubia* **IWC:** 100%

Test Start Time	Test Start Date	Test End Time	Test End Date
1515	03-08-2018	1530	03-15-2018

Test Results	Lethality	Reproduction
IC_{25}	60.9%	59.5%

Dilution(s) - % Effluent

Measurements	Control (0%)	25%	50%	75%	100%
% Survival for day 1	100	100	100	100	100
% Survival for day 2	100	100	100	60	100
% Survival for day 3	100	100	100	40	80
% Survival for day 4	80	100	100	40	80
% Survival for day 5	80	100	100	40	60
% Survival for day 6	80	100	100	40	60
% Survival for day 7	80	100	100	40	40
3 Mean Brood Total	22.6	26.4	26.2	8.6	3.8

Recon Water: 83
Recon Water: 59
pH (initial/final) – Control: 7.9/8.0 100%: 6.8/8.1

Were all Test Conditions in Conformance with Division Guidelines? YES ☒ NO ☐
If **NO**, list deviations from test specifications:
Laboratory: SeaCrest Group
Comments:
Analyst's Name: Madison Reese and Tessa Hunt-Woodland

Signature [signature] *Date* 5/2/2018

initial

The SeaCrest Group
Louisville, CO

Ceriodaphnia Chronic TIE Initial/Baseline Benchsheet

Form #: 113a
Effective: January 2006

Permittee: Elk Ridge Lab #: 418208.B Site: NPDES 013
IWC %: 100 Template #: 4 Dilution Water: MH 1604 Sample Date: 030718
Age & Source: cerio 030818 Test Start: 030818 1515 Test End: 031518 1530
Test Conditions: ACUTE TEMPLATE

	0	1	2	3	4	5	6	7	8	Total
(C) 0	0	0	0	1	D					1
	0	0	0	5	9	10	13	9		28
	0	0	0	5	0	11	14	10		30
	0	0	0	4	0	8	12	8		24
	0	0	0	8+1	0	9	12	10		30
DO	7.3	10.9	10.4 | 10.9	7.2	7.0	6.9	7.3	6.9	7.0	
Temp	24.1	24.1	24.1 | 24.2	24.2	24.3	24.1	24.8	24.7	24.6	22.6
pH	7.9	8.0	7.9 | 8.2	7.8	7.9	8.1	7.8	7.9	8.5	
Cond	311	320	310 | 285	311	288	285	283	280		290
(1) 25	0	0	0	6	0	11	13	8		30
	0	0	0	5	0	13	11	10		29
	0	0	0	6+2	0	4	9	10		21
	0	0	0	4	7	0	12	7		23
	0	0	0	6	0	11	12	9		29
DO	7.2	10.9	10.4 | 10.9	7.3	7.1	6.9	7.4	6.9	7.2	
Temp	24.3	24.1	24.1 | 24.4	24.2	24.3	24.1	24.8	24.7	24.6	26.4
pH	7.0	8.0	7.9 | 7.0	8.2	8.3	8.0	8.3	8.3		
Cond	1283	1329	1315 | 1330	1296	1169	1085	1067	1073		
(2) 50	0	0	0	4+1	0	11	13	7		29
	0	0	0	4	0	6	10	9		20
	0	0	0	3+1	0	11	13	8		28
	0	0	0	2+1	0	10	12	8		26
	0	0	0	5	0	10	14	8		29
DO	7.1	7.0	10.5 | 10.8	7.4	7.2	6.9	7.4	7.0	7.2	
Temp	24.5	24.1	24.1 | 24.0	24.2	24.3	24.2	24.8	24.7	24.6	26.2
pH	10.9	7.9	7.8 | 10.8	8.4	8.4	6.8	8.3	8.5	8.0	
Cond	1710	1915	1811 | 1902	1856	1716	1712	1739	1730		
(3) 75	0	0	0	D						0
	0	0	0	3	0	8	10	11		21
	0	0	0	3	0	7	11	10		22
	0	0	0 D							0
	0	0	0 D							0
DO	7.0	7.0	10.10 | 10.7	7.4	7.2	6.8	7.5	7.1	7.3	
Temp	24.1	24.1	24.1 | 24.8	24.2	24.3	24.3	24.8	24.7	24.6	8.6
pH	10.8	7.9	7.8 | 10.8	8.0	8.2	6.8	8.3	8.3	8.2	
Cond	2500	2810	2750 | 2710	2620	2350	2340	2310	2330		
(4) 100	0	0	0	2	0	0	0	2		6
	0	0	0	3	0	3	0	0		6
	0	0	0	D						0
	0	0	0	3	0	2D				5
	0	0	0	0	0	0	1	1D		2
DO	10.9	7.1	10.7 | 10.10	7.5	7.3	6.8	7.6	7.2	7.3	
Temp	24.9	24.1	24.1 | 25.0	24.2	24.3	24.4	24.8	24.7	24.6	3.4
pH	10.8	7.9	7.8 | 10.7	7.9	8.1	6.7	7.9	8.0	8.1	
Cond	3100	3310	3350 | 3310	3320	2900	2900	2900	2800		
Algae	ABS/ABD	ABS/ABD	ABS/ABD	ABS/ABD	ABS/ABD	ABS/ABD	ABS/ABD	ABS/ABD		
YCT	1501	1501	1501	1501	1501	1501	1501	1501		
H_2O	(day 0)	(day 1)	(day 2)	(day 3)	(day 4)	(day 5)	(day 6)	(day 7)		
Initials	TWL	TWL	TWL	THW	THW	THW	THW	THW		

	Eff #1	Eff #2	Eff #3	Recon #1	Recon #2	Recon #3
Hardness				83		
Alkalinity				59		
Chlorine				<0.01		
Ammonia				<0.03		

1	2	3	4	5
A3	A11	A7	A10	B2

x:y:z = board #:row:column

1. Exposure Chamber
Total Capacity: 30 ml
Test Solution Surface Area: ____ cm^2
Test Solution Volume: 10 ml
Water Depth (constant): ____ cm
(cyclic): to ____ cm

2. Feeding Schedule
Not fed: ____
Fed Irregularly: ____
Fed Daily: X
Food Used: YCT, algae

3. Aeration
#1 None: ____
#2 None: ____
#3 None: ____
Before Use: ____
(____ minutes @ ~100 bubbles/min)
Before Use: ____
(____ minutes @ ~100 bubbles/min)
Before Use: ____
(____ minutes @ ~100 bubbles/min)

4. Screened Animal Enclosers
Not Used: X
Used: ____

5. Condition/appearance of surviving organisms at end of test (i.e., alive but immobile; loss of orientation; erratic movement; etc,):
D

6. Comments:
ABS/ABD 1501 (day 6) TWL
(day 7) THW

Ceriodaphnia Survival and Reproduction Test-7 Day Survival

Start Date:	3/8/2018	Test ID:	418208I	Sample ID:	EFFFIN-Effluent Final
End Date:	3/15/2018	Lab ID:	SCG-Seacrest Group	Sample Type:	EFF2-Industrial
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5
ON-Control	1.0000	1.0000	1.0000	1.0000	1.0000
SN--Control	0.0000	1.0000	1.0000	1.0000	1.0000
25	1.0000	1.0000	1.0000	1.0000	1.0000
50	1.0000	1.0000	1.0000	1.0000	1.0000
75	0.0000	1.0000	1.0000	0.0000	0.0000
100	1.0000	1.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
ON-Control	1.0000	1.2500	1.0472	1.0472	1.0472	0.000	5		
SN--Control	0.8000	1.0000	0.9425	0.5236	1.0472	24.845	5	0.9333	1.0000
25	1.0000	1.2500	1.0472	1.0472	1.0472	0.000	5	0.9333	1.0000
50	1.0000	1.2500	1.0472	1.0472	1.0472	0.000	5	0.9333	1.0000
75	0.4000	0.5000	0.7330	0.5236	1.0472	39.123	5	0.4000	0.4286
100	0.4000	0.5000	0.7330	0.5236	1.0472	39.123	5	0.4000	0.4286

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.9016	0.918	-2E-16	-0.1393
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.35)	1	2.306		

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	52.188	4.719	51.180	70.821	4.1950
IC10	54.375	6.035	52.361	84.492	3.0195
IC15	56.563				
IC20	58.750				
IC25	60.938				
IC40	67.500				
IC50	71.875				

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	3/8/2018	Test ID:	418208I	Sample ID:	EFFFIN-Effluent Final
End Date:	3/15/2018	Lab ID:	SCG-Seacrest Group	Sample Type:	EFF2-Industrial
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5
ON-Control	1.000	28.000	30.000	24.000	30.000
SN--Control	28.000	30.000	24.000	30.000	
25	30.000	29.000	21.000	23.000	29.000
50	29.000	20.000	28.000	25.000	29.000
75	0.000	21.000	22.000	0.000	0.000
100	6.000	6.000	0.000	5.000	2.000

			Transform: Untransformed					Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
ON-Control	22.600	0.8071	22.600	1.000	30.000	54.516	5	25.067	1.0000
SN--Control	28.000	1.0000	28.000	24.000	30.000	10.102	4		
25	26.400	0.9429	26.400	21.000	30.000	15.526	5	25.067	1.0000
50	26.200	0.9357	26.200	20.000	29.000	14.634	5	25.067	1.0000
75	8.600	0.3071	8.600	0.000	22.000	136.992	5	8.600	0.3431
100	3.800	0.1357	3.800	0.000	6.000	70.613	5	3.800	0.1516

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.93904	0.918	-0.7848	1.91485
Bartlett's Test indicates equal variances (p = 0.01)	12.7227	13.2767		
The control means are not significantly different (p = 0.42)	0.84772	2.36462		

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	51.903	15.095	0.000	56.912	-1.1739
IC10	53.806	9.241	4.915	63.825	-2.0994
IC15	55.709	4.677	39.875	70.737	0.7872
IC20	57.611	4.716	47.904	77.649	1.8910
IC25	59.514	5.136	49.735	83.541	1.8836
IC40	65.223	5.979	56.230	90.198	1.4197
IC50	69.028	6.570	58.843	94.889	1.1172

TIER 1

WET TEST REPORT FORM – CHRONIC BASELINE TEST

Permittee: Elk Ridge Mining and Reclamation **Outfall:** 013

Permit No.: CO0000213

Test Type: Routine ☐ TIE ☒

Test Species: *Ceriodaphnia dubia* **IWC:** 100%

Test Start Time	Test Start Date	Test End Time	Test End Date
1100	03-15-2018	1147	03-22-2018

Test Results	Lethality	Reproduction
IC_{25}	62.5%	39.4%*

* Estimated

Dilution(s) - % Effluent

Measurements	Control (0%)	50%	60%	70%	100%
% Survival for day 1	100	100	100	100	100
% Survival for day 2	100	100	100	60	40
% Survival for day 3	100	100	100	60	20
% Survival for day 4	100	80	100	60	20
% Survival for day 5	100	80	100	60	20
% Survival for day 6	100	80	80	60	20
% Survival for day 7	100	80	80	60	20
3 Mean Brood Total	29.0	19.8	19.8	10.8	0.6

Recon Water: 87
Recon Water: 60
pH (initial/final) – Control: 7.9/8.0 100%: 6.8/8.1

Were all Test Conditions in Conformance with Division Guidelines? YES ☒ NO ☐
If **NO**, list deviations from test specifications:
Laboratory: SeaCrest Group
Comments:
Analyst's Name: Tessa Hunt-Woodland and Dan Hillenburg

Signature [signature] *Date* 05/02/2018

BASELINE

The SeaCrest Group
Louisville, CO

Ceriodaphnia Chronic TIE Initial/Baseline Benchsheet

Form #: 113a
Effective: January 2006

Permittee: Elk Ridge Lab #: 418208 Site: 013
IWC %: 100 Template #: 5 Dilution Water: MH 18-005 Sample Date: 030718
Age & Source: cerio 2005 031518 Test Start: 031518 1100 Test End: 032218 1147
Test Conditions:

	0	1	2	3	4	5	6	7	8	Total
(C) 0	0	0	0	5	9 +1	~~4~~	15	16		30
	0	0	0	7	5	4 +2	0	13		27
	0	0	0	4	9	0	17	17		30
	0	0	0	5	7	0	19	18		31
	0	0	0	3	8 +1	~~10~~ 15	15	18		27
DO	7.0	7.1	7.1 | 7.3	7.3	7.1 | 6.9	7.1	7.2	6.7		29.0
Temp	24.2	24.1	24.2 | 24.1	25.2	24.3 | 24.1	24.2	24.1	24.2		
pH	7.9	8.0	7.8 | 7.9	8.6	8.2 | 8.1	8.1	8.1	8.0		
Cond	297	317	| 307	352	| 313	308	312	308		
(1) 50	0	0	0	3	7	+20	5	8		17
	0	0	0	2	+2 OD					4
	0	0	0	7	9	0	~~14~~	9		32
	0	0	0	6	5	+10	13	~~11~~		25
	0	0	0	3	7	0	11	12		21
DO	6.9	7.1	7.1 | 7.0	7.4	7.1 | 7.4	7.1	7.4	6.9		19.4
Temp	24.2	24.1	24.2 | ~~24.1~~	25.2	24.3 | 24.6	24.2	24.1	24.2		
pH	7.7	8.0	7.8 | 6.8	7.9	8.2 | 7.0	8.0	8.1	8.0		
Cond	1791	—	1873	—	1826	—		—		
(2) 100	0	0	0	3	7	0	11	0		21
	0	0	0	0	0	+30	D	—	—	3
	0	0	0	4	8	+20	~~14~~	6		28
	0	0	0	6	7	+20	8	8		23
	0	0	0	5	8	0	11	9		24
DO	6.8	7.1	7.1 | 6.8	7.4	7.1 | 7.5	7.1	7.8	7.1		19.8
Temp	24.2	24.1	24.2 | 24.2	25.2	24.3 | 24.6	24.2	24.2	24.2		
pH	7.4	8.0	7.8 | 6.8	7.8	8.2 | 6.9	7.9	8.0	8.0		
Cond	1927	—	2240	—	1978	—		—		
(3) 70	0	0	0	3	8	8	0	11		19
	0	0	0 D	—	—	—	—	—	—	0
	0	0	0	4	8	0	7	5		19
	0	0	0 D	—	—	—	—	—	—	0
	0	0	0	1	+2	0	6	7		16
DO	6.7	7.2	7.0 | 6.8	7.5	7.2 | 7.7	7.2	8.0	7.3		10.8
Temp	24.1	24.2	24.1 | 24.2	25.2	24.3 | 24.6	24.2	24.2	24.1		
pH	7.1	7.9	7.8 | 6.8	7.8	8.2 | 6.9	7.9	8.0	8.1		
Cond	2370	—	2490	—	2530	—		—		
(4) 100	0	0	0	D	—	—	—	—	—	0
	0	0	0 D	—	—	—	—	—	—	0
	0	0	0 D	—	—	—	—	—	—	0
	0	0	0 D	—	—	—	—	—	—	0
	0	0	0	2	+1	~~7.2~~ 7.9	0	0		3
DO	6.7	7.2	7.0 | 6.8	7.5	7.2 | 7.7	~~24.2~~ 7.9	8.0	7.5		0.6
Temp	24.1	24.2	24.1 | 24.2	25.2	24.3 | 24.6	24.2	24.2	24.1		
pH	6.8	7.9	8.0 | 6.7	7.8	8.2 | 6.8	7.9	8.0	8.1		
Cond	3140	3120	3240	3080	3180	3080	2920	2770		
Algae	ABS/ARE	ABS/ARE		ABS/ARE	ABS/ARE		ABS			
YCT	1801	1801		1801	1801		ARE			
H_2O					1801		1801			
Initials	THW	DH		DH	THW		DH	DH		

Day 0-7 days 5

	Eff #1	Eff #2	Eff #3	Recon #1	Recon #2	Recon #3
Hardness				87		
Alkalinity				60		
Chlorine				<0.01		
Ammonia				<0.05		

1	2	3	4	5
A2	A4	A5	A7	A8

x:y:z = board #:row:column

1. Exposure Chamber
Total Capacity: 30 ml
Test Solution Surface Area: ____ cm^2
Test Solution Volume: 10 ml
Water Depth (constant): ____ cm
(cyclic): to ____ cm

2. Feeding Schedule
Not fed: ____
Fed Irregularly: ____
Fed Daily: X
Food Used: YCT, algae

3. Aeration
#1 None: ____
#2 None: ____
#3 None: ____
Before Use: ____
(____ minutes @ ~100 bubbles/min)
Before Use: ____
(____ minutes @ ~100 bubbles/min)
Before Use: ____
(____ minutes @ ~100 bubbles/min)

4. Screened Animal Enclosers
Not Used: X
Used: ____

5. Condition/appearance of surviving organisms at end of test (i.e., alive but immobile; loss of orientation; erratic movement; etc,):

6. Comments:

Day 5 signing:
ABS/ARE
1801 YCT
THW

Ceriodaphnia Survival and Reproduction Test-7 Day Survival

Start Date:	3/15/2018	Test ID:	208base	Sample ID:	EFFFIN-Effluent Final
End Date:	3/21/2018	Lab ID:		Sample Type:	EFF2-Industrial
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000
50	1.0000	0.0000	1.0000	1.0000	1.0000
60	1.0000	0.0000	1.0000	1.0000	1.0000
70	1.0000	0.0000	1.0000	0.0000	1.0000
100	0.0000	0.0000	0.0000	0.0000	1.0000

			Transform: Arcsin Square Root					Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
D-Control	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	5	1.0000	1.0000
50	0.8000	0.8000	0.9425	0.5236	1.0472	24.845	5	0.8000	0.8000
60	0.8000	0.8000	0.9425	0.5236	1.0472	24.845	5	0.8000	0.8000
70	0.6000	0.6000	0.8378	0.5236	1.0472	34.233	5	0.6000	0.6000
100	0.2000	0.2000	0.6283	0.5236	1.0472	37.268	5	0.2000	0.2000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.91779	0.918	-0.5613	0.3531
Equality of variance cannot be confirmed				

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05*	12.500	19.915	3.125	85.625	0.7627
IC10*	25.000	18.382	6.250	81.344	0.2763
IC15*	37.500	14.942	9.375	82.500	-0.0594
IC20	60.000	13.811	7.500	75.000	-1.0395
IC25	62.500	12.234	15.625	79.375	-0.9319
IC40	70.000				
IC50	77.500				

* indicates IC estimate less than the lowest concentration

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	3/15/2018	Test ID:	208base	Sample ID:	EFFFIN-Effluent Final
End Date:	3/21/2018	Lab ID:		Sample Type:	EFF2-Industrial
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5
D-Control	30.000	27.000	30.000	31.000	27.000
50	17.000	4.000	32.000	25.000	21.000
60	21.000	3.000	28.000	23.000	24.000
70	19.000	0.000	19.000	0.000	16.000
100	0.000	0.000	0.000	0.000	3.000

			Transform: Untransformed					Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
D-Control	29.000	1.0000	29.000	27.000	31.000	6.451	5	29.000	1.0000
50	19.800	0.6828	19.800	4.000	32.000	52.656	5	19.800	0.6828
60	19.800	0.6828	19.800	3.000	28.000	49.148	5	19.800	0.6828
70	10.800	0.3724	10.800	0.000	19.000	91.989	5	10.800	0.3724
100	0.600	0.0207	0.600	0.000	3.000	223.607	5	0.600	0.0207

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.90857	0.918	-0.9088	0.80465
Bartlett's Test indicates unequal variances (p = 1.29E-03)	17.8968	13.2767		

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05*	7.880	8.487	3.555	52.296	3.0648
IC10*	15.761	11.061	7.109	70.320	1.5695
IC15*	23.641	12.284	10.664	71.356	0.8889
IC20*	31.522	12.618	14.219	75.665	0.4706
IC25*	39.402	11.529	17.774	73.174	0.0986
IC40	62.667	7.309	28.626	70.548	-1.2495
IC50	65.889	5.225	48.251	78.256	-0.7137

* indicates IC estimate less than the lowest concentration

WET TEST REPORT FORM – CHRONIC
FILTERED TEST

Permittee: Elk Ridge Mining and Reclamation **Outfall:** 013
Permit No.: CO0000213

Test Type: Routine ☐ TIE ☒
Test Species: *Ceriodaphnia dubia* **IWC:** 100%

Test Start Time	Test Start Date	Test End Time	Test End Date
1130	03-15-2018	1220	03-22-2018

Test Results	Lethality	Reproduction
IC_{25}	56.3%	74.4%

Dilution(s) - % Effluent

Measurements	Control (0%)	50%	60%	70%	100%	Method Control
% Survival for day 1	100	100	100	100	100	100
% Survival for day 2	100	100	100	100	80	100
% Survival for day 3	100	100	100	100	80	100
% Survival for day 4	100	100	100	100	80	100
% Survival for day 5	100	100	100	100	40	100
% Survival for day 6	100	100	80	80	20	100
% Survival for day 7	100	100	60	60	20	100
Mean 3 Brood Total	27.4	26.0	23.2	22.0	12.0	29.2

Recon Water: 87
Recon Water: 60
pH (initial/final) – Control: 7.9/7.8 100%: 7.4/7.8

Were all Test Conditions in Conformance with Division Guidelines? YES ☒ NO ☐
If **NO**, list deviations from test specifications:
Laboratory: SeaCrest Group
Comments:
Analyst's Name: Tessa Hunt-Woodland, Madison Reese, and Dan Hillenburg

Signature [signature] *Date* 05/02/2018

Effective: January 2006

Permittee: ELK RIDGE Lab #: 418 208 Site: 013

IWC %: 100 Template #: 5 Dilution Water: MRI 15-005

Age & Source: cerio 2005 CB1518 Test Start: CB1518 1150 Test End: 032218 1220

Test Conditions: filtered through glass fiber filter

	0	1	2	3	4	5	6	7	8	Total
(C) 0	0	0	0	4	10	12	0	10		26
	0	0	0	7	7	0	11	4		25
	0	0	0	6	14	11+3	0	11		34
	0	0	0	6	8	13+1	0	15		28
	0	0	0	6	7	+1	10	13		24
DO	7.0	11.8	11.9 7.0	6.8	7.0 6.9	7.3	7.7	11.9		27.4
Temp	24.2	24.1	25.2 24.3	24.9	25.1 24.3	25.3	24.3	24.1		24.2
pH	7.9	7.8	7.8 8.0	7.9	8.0 8.1	8.0	8.0	7.8		
Cond	783	314	314	304	301	314	320	325		
(1) 50	0	0	0	3	9	12+1	0	12		26
	0	0	0	4	9	7	0	13		21
	0	0	0	4	8	0	11	10		23
	0	0	0	7	11	+1	11	9		31
	0	0	0	5	11	+1	12	10		29
DO	6.8	11.7	11.9 7.1	6.8	7.0 7.6	7.4	7.8	11.1		26.0
Temp	24.2	24.1	25.2 24.1	24.9	25.1 24.6	25.3	24.3	24.1		
pH	7.7	7.9	7.8 7.5	7.9	8.0 7.6	7.9	7.9	7.8		
Cond	1799	—	1825	—	1749	—	—	—		
(2) 60	0	0	0	1	10	4+2	0	D —		17
	0	0	0	6	11	+2	9	0 —		28
	0	0	0	5	9	0	8	12		22
	0	0	0	5	10	0	8	10		23
	0	0	0	6	10	+1	N9	8		24
DO	6.7	11.7	7.0 7.6	6.8	7.1 7.7	7.5	7.8	11.8		23.2
Temp	24.3	24.1	25.2 24.8	24.9	25.1 24.7	25.3	24.2	24.1		
pH	7.6	7.9	7.8 7.4	7.9	8.0 7.5	7.9	7.9	7.8		
Cond	1942	—	1958	—	1981	—	—	—		
(3) 70	0	0	0	0	8	8+3	0	5		24
	0	0	0	4	8	+1	9 0 —	—		24
	0	0	0	4	7	8+2	X 0	4		19
	0	0	0	3	9	6+2	0	10		20
	0	0	0	4	11	0	8	D —		23
DO	6.6	11.8	7.1 7.3	6.9	7.1 7.9	7.5	7.8	11.8		22.0
Temp	24.3	24.1	25.2 24.9	24.9	25.1 24.8	25.3	24.2	24.1		
pH	7.5	8.0	7.9 7.3	7.9	7.9 7.5	7.8	7.9	7.8		
Cond	2360	—	2380	—	2450	—	—	—		
(4) 100	0	0	0 D —	—	—	—	—	—		0
	0	0	0	4	7	D —	—	—		11
	0	0	0	3	11	0	7	5		21
	0	0	0	4	10	D —	—	—		14
	0	0	0	1	8	0	SD —	—		14
DO	6.6	11.8	7.2 7.4	6.9	7.1 7.9	7.5	7.9	11.8		*
Temp	24.4	24.1	25.2 25.3	24.9	25.1 24.8	25.3	24.1	24.1		12.0
pH	*6.6E 8.0	7.9	7.9 7.3	7.9	7.9 7.4	7.8	7.8	7.8		
Cond	3060	3210	3020	3000	3120	3000	3000	3080		
dilution water blank	0	0	0	0	9	13	17	0		39
	0	0	0	5	11	9+3	0	14		24
	0	0	0	5	12	11	5	14		28
	0	0	0	3	0	0	7	17		22
	0	0	0	6	12	11+4	0	14		29
DO	7.2	11.9	7.0 7.8	7.0	7.0 7.3	7.5	7.3	11.6		29.2
Temp	24.2	24.1	25.2 24.5	24.9	25.1 24.6	25.3	24.2	24.1		
pH	*7.2E 7.8	8.0	7.9 7.9	7.9	7.9 7.9	8.0	8.1	7.9		
Cond	2972	3271	3011	2998	3000	2999	3111	3130		
Algae	ABS/ABD	ABS/ABD	MIXED	MIXED	ABS/ABD	ABS	ABS	ABS/ABD		
YCT	1801	1801	X	1801	1801	ABD	ABD			
H_2O						1801	1801	1801		
Initials	TMW	TMW		TMW	TMW	TMW	TMW	DH		

	Eff #1	Eff #2	Eff #3	Recon #1	Recon #2	Recon #3
Hardness				87		
Alkalinity				100		
Chlorine				<0.11		
Ammonia				<0.03		

1. Exposure Chamber
   - Total Capacity: 30 ml
   - Test Solution Surface Area: ____ cm^2
   - Test Solution Volume: 10 ml
   - Water Depth (constant): ____ cm
   - (cyclic): to ____ cm
2. Feeding Schedule
   - Not fed: ____
   - Fed Irregularly: ____
   - Fed Daily: X
   - Food Used: YCT, algae
3. Aeration
   - #1 None: ____
   - #2 None: ____
   - #3 None: ____
   - Before Use: ____ (____ minutes @ ~100 bubbles/min)
   - Before Use: ____ (____ minutes @ ~100 bubbles/min)
   - Before Use: ____ (____ minutes @ ~100 bubbles/min)
4. Screened Animal Enclosers
   - Not Used: X
   - Used: ____
5. Condition/appearance of surviving organisms at end of test (i.e., alive but immobile; loss of orientation; erratic movement; etc,):

remaining adults in 70. and 100. were pale and moving erratically

right: round + healthy

6. Comments:

1	2	3	4	5
R10	C2	C3	C4	C5

x:y:z = board #:row:column

Ceriodaphnia Survival and Reproduction Test-7 Day Survival

Start Date:	3/15/2018	Test ID:	208plFilt	Sample ID:	EFFFIN-Effluent Final
End Date:	3/22/2018	Lab ID:	SCG-Seacrest Group	Sample Type:	EFF2-Industrial
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000
50	1.0000	1.0000	1.0000	1.0000	1.0000
60	0.0000	0.0000	1.0000	1.0000	1.0000
70	1.0000	0.0000	1.0000	1.0000	0.0000
100	0.0000	0.0000	1.0000	0.0000	0.0000
DWB	1.0000	1.0000	1.0000	1.0000	1.0000

			Transform: Arcsin Square Root					Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
D-Control	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	5	1.0000	1.0000
50	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	5	1.0000	1.0000
60	0.6000	0.6000	0.8378	0.5236	1.0472	34.233	5	0.6000	0.6000
70	0.6000	0.6000	0.8378	0.5236	1.0472	34.233	5	0.6000	0.6000
100	0.2000	0.2000	0.6283	0.5236	1.0472	37.268	5	0.2000	0.2000
DWB	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	5		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.87439	0.927	0	0.45312
Equality of variance cannot be confirmed				

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	51.250	3.200	50.446	66.250	2.8841
IC10	52.500	4.284	50.893	78.750	2.4213
IC15	53.750	4.565	51.339	81.339	2.1554
IC20	55.000	6.115	51.786	83.929	1.2984
IC25	56.250	6.648	52.232	86.518	1.2925
IC40	70.000				
IC50	77.500				

Reviewed by: MK

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	3/15/2018	Test ID:	208pIFilt	Sample ID:	EFFFIN-Effluent Final
End Date:	3/22/2018	Lab ID:	SCG-Seacrest Group	Sample Type:	EFF2-Industrial
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5
D-Control	26.000	25.000	34.000	28.000	24.000
50	26.000	21.000	23.000	31.000	29.000
60	17.000	28.000	22.000	23.000	26.000
70	24.000	24.000	19.000	20.000	23.000
100	0.000	11.000	21.000	14.000	14.000
DWB	39.000	28.000	28.000	22.000	29.000

			Transform: Untransformed					Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
D-Control	27.400	1.0000	27.400	24.000	34.000	14.507	5	27.400	1.0000
50	26.000	0.9489	26.000	21.000	31.000	15.858	5	26.000	0.9489
60	23.200	0.8467	23.200	17.000	28.000	18.134	5	23.200	0.8467
70	22.000	0.8029	22.000	19.000	24.000	10.660	5	22.000	0.8029
100	12.000	0.4380	12.000	0.000	21.000	63.738	5	12.000	0.4380
DWB	29.200	1.0657	29.200	22.000	39.000	21.028	5		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.97654	0.927	-0.1203	0.98706
Bartlett's Test indicates equal variances (p = 0.35)	5.57915	15.0863		

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05*	48.929	16.346	0.000	69.309	-0.2977
IC10	54.786	11.578	10.505	77.183	-0.9855
IC15	59.679	9.245	27.007	80.320	-0.9517
IC20	70.240	7.941	40.676	80.517	-1.1362
IC25	74.350	6.441	48.317	84.710	-1.5004
IC40	86.680				
IC50	94.900				

* indicates IC estimate less than the lowest concentration

Reviewed by: MP

WET TEST REPORT FORM – CHRONIC
AERATED TEST

Permittee: Elk Ridge Mining and Reclamation **Outfall:** 013
Permit No.: CO0000213

Test Type: Routine ☐ TIE ☒
Test Species: *Ceriodaphnia dubia* **IWC:** 100%

Test Start Time	Test Start Date	Test End Time	Test End Date
1130	03-15-2018	1200	03-22-2018

Test Results	Lethality	Reproduction
IC_{25}	>100%	76.3%

Dilution(s) - % Effluent

Measurements	Control (0%)	50%	60%	70%	100%	Method Control
% Survival for day 1	100	100	100	100	100	100
% Survival for day 2	100	100	100	100	100	100
% Survival for day 3	100	100	100	100	100	100
% Survival for day 4	100	100	100	100	100	100
% Survival for day 5	100	100	100	100	80	100
% Survival for day 6	100	100	100	100	80	100
% Survival for day 7	100	100	100	100	80	100
Mean 3 Brood Total	**26.0**	**21.8**	**20.0**	**22.8**	**12.4**	**25.8**

Recon Water: 87
Recon Water: 60
pH (initial/final) – Control: 8.1/7.9 100%: 8.3/8.0

Were all Test Conditions in Conformance with Division Guidelines? YES ☒ NO ☐
If **NO**, list deviations from test specifications:
Laboratory: SeaCrest Group
Comments:
Analyst's Name: Madison Reese, Tessa Hunt-Woodland, Dan Hillenburg, and Nicole Denkinger

Signature *Date* 05/02/2018

The SeaCrest Group
Louisville, CO

Ceriodaphnia Chronic TIE Aerated Benchsheet

Form #: 113e
Effective: January 2006

Permittee: Elk Ridge Lab #: LAB 208 Site: 013
IWC %: 100 Template #: 5 Dilution Water: MH 18005 Sample Date: 0307
Age & Source: cerio 205 021518 Test Start: 031518 1130 Test End: 032218 - 1200
Test Conditions: aerated 1 hour

	0	1	2	3	4	5	6	7	8	Total
(C) 0	0	0	0	5	10	10	0	10		25
	0	0	0	4	6	0	9	11		19
	0	0	0	5	5	+1	17	14		31
	0	0	0	6	9	0	13	16		29
	0	0	0	6	7	0	14	13		27
DO	10.9	10.7	10.9 / 7.0	7.0	7.2 / 6.9	7.2	7.7	7.2		26.0
Temp	24.1	24.1	24.9 / 24.2	25.1	24.3 / 24.1	24.2	24.4	24.4		
pH	8.1	8.0	7.8 / 8.0	8.0	8.1 / 8.1	8.1	8.1	7.9		
Cond	292	300	314	331	305	309	320	320		
(1) 50	0	0	0	4	5	10	+11	9		22
	0	0	0	4	7	11	12	9		24
	0	0	0	3	6	0	10	14		19
	0	0	0	6	7	+10	10	10		23
	0	0	0	5	5	+10	11	11		21
DO	7.3	10.7	7.0 / 7.5	7.2	7.3 / 7.9	7.3	7.9	7.3		21.8
Temp	24.3	24.1	24.9 / 24.5	25.1	24.3 / 24.7	24.2	24.4	24.4		
pH	8.4	8.0	7.8 / 8.0	8.0	8.1 / 8.0	8.0	8.0	8.0		
Cond	1752	—	1908	—	1770	—	—			
(2) 60	0	0	0	5	5	9	13	10		22
	0	0	0	0	3	0	0	5		8
	0	0	0	5	9	0	11	9		25
	0	0	0	5	7	0	13	12		25
	0	0	0	3	7	0	10	8		20
DO	7.5	10.8	7.2 / 7.8	7.2	7.3 / 7.5	7.4	8.0	7.4		20.0
Temp	24.5	24.1	24.9 / 24.7	25.1	24.3 / 24.7	24.2	24.4	24.4		
pH	8.4	8.0	7.9 / 8.0	7.9	8.1 / 8.6	7.9	8.0	8.0		
Cond	1832	—	1878	—	1950	—	—			
(3) 40	0	0	0	3	7	8	11	11		18
	0	0	0	2	+3 10	+1	11	11		27
	0	0	0	4	8	0	13	12		25
	0	0	0	5	6	6	+12	9		19
	0	0	0	5	9	0	11	13		25
DO	7.1	10.9	7.3 / 7.10	7.3	7.3 / 7.5	7.4	8.2	7.6		22.8
Temp	24.1	24.1	24.9 / 24.9	25.1	24.3 / 24.7	24.2	24.9	24.3		
pH	8.3	7.9	7.9 / 8.0	7.8	8.1 / 7.9	7.9		8.0		
Cond	2180	—	1878 / 2000	—	1970	—	—			
(4) 100	0	0	0	1	+1	0	4	8		14
	0	0	0	1	+3	HEI	7	7		19
	0	0	0	0	+3	0	+2	5		10
	0	0	0	0	+3	D	—	—		3
	0	0	0	3	+3	2 → 8		6		16
DO	8.1	7.0	7.4 / 7.7	7.3	7.4 / 7.6	7.4	8.5	7.8		* 12.4
Temp	25.0	24.1	24.9 / 25.4	25.1	24.3 / 24.8	24.2	24.9	24.3		
pH	8.3	7.9	8.0 / 7.9	7.8	8.1 / 7.9	7.9	8.0	8.0		
Cond	3000	3280	2980	2890	3050	2970	3160	2880		
dilution water blank	0	0	0	4	0	9	4	14		17
	0	0	0	1	7	0	11	11		30
	0	0	0	5	0	0	12	14		~~31~~ 28
	0	0	0	4	10	0	14	12		
	0	0	0	5	6	11	4	13		23
DO	7.7	7.1	7.2 / 7.8	7.2	7.3 / 7.6	7.4	8.1	7.6		25.4
Temp	24.2	24.1	24.9 / 24.6	25.1	24.3 / 24.9	24.2	24.4	24.3		
pH	8.0	7.9	7.9 / 8.1	7.9	8.2 / 8.1	8.2	8.1	8.1		
Cond	314	341	309	305	301	302	335	313		
Algae	ABS/ARD	ABS/ARD	ABS/ARD	ABS/ARD	ABS/ARD	ABS	ABS/ARD	ABS/ARD		
YCT	1801	1801	1801	1801	1801	1801	1801	1801		
H_2O					1801	1801				
Initials	MK	MK	MK	TRW	TRW	TRW	ND	DH		

	Eff #1	Eff #2	Eff #3	Recon #1	Recon #2	Recon #3
Hardness				87		
Alkalinity				60		
Chlorine				<0.01		
Ammonia				<0.03		

1. Exposure Chamber
Total Capacity: 30 ml
Test Solution Surface Area: ____ cm^2
Test Solution Volume: 10 ml
Water Depth (constant): ____ cm
(cyclic): to ____ cm

2. Feeding Schedule
Not fed:
Fed Irregularly:
Fed Daily: X
Food Used: YCT, algae

3. Aeration
#1 None:
#2 None:
#3 None:
Before Use: ____ (____ minutes @ ~100 bubbles/min)
Before Use: ____ (____ minutes @ ~100 bubbles/min)
Before Use: ____ (____ minutes @ ~100 bubbles/min)

4. Screened Animal Enclosers
Not Used: X
Used:

5. Condition/appearance of surviving organisms at end of test (i.e., alive but immobile; loss of orientation; erratic movement; etc,):

6. Comments:

1	2	3	4	5
A2	A4	A5	A7	A8

x:y:z = board #:row:column

Ceriodaphnia Survival and Reproduction Test-7 Day Survival

Start Date:	3/15/2018	Test ID:	208plae	Sample ID:	EFFFIN-Effluent Final
End Date:	3/22/2018	Lab ID:	SCG-Seacrest Group	Sample Type:	EFF2-Industrial
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000
50	1.0000	1.0000	1.0000	1.0000	1.0000
60	1.0000	1.0000	1.0000	1.0000	1.0000
70	1.0000	1.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	1.0000	0.0000	1.0000
DWB	1.0000	1.0000	1.0000	1.0000	1.0000

			Transform: Arcsin Square Root					Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
D-Control	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	5	1.0000	1.0000
50	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	5	1.0000	1.0000
60	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	5	1.0000	1.0000
70	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	5	1.0000	1.0000
100	0.8000	0.8000	0.9425	0.5236	1.0472	24.845	5	0.8000	0.8000
DWB	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	5		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.41613	0.927	-3.8705	19.8512
Equality of variance cannot be confirmed				

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)	Skew
IC05	77.500			
IC10	85.000			
IC15	92.500			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	3/15/2018	Test ID:	208plae	Sample ID:	EFFFIN-Effluent Final
End Date:	3/22/2018	Lab ID:	SCG-Seacrest Group	Sample Type:	EFF2-Industrial
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5
D-Control	25.000	19.000	31.000	28.000	27.000
50	22.000	24.000	19.000	23.000	21.000
60	22.000	8.000	25.000	25.000	20.000
70	18.000	27.000	25.000	19.000	25.000
100	14.000	19.000	10.000	3.000	16.000
DWB	17.000	30.000	31.000	28.000	23.000

			Transform: Untransformed					Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
D-Control	26.000	1.0000	26.000	19.000	31.000	17.201	5	26.000	1.0000
50	21.800	0.8385	21.800	19.000	24.000	8.824	5	21.800	0.8385
60	20.000	0.7692	20.000	8.000	25.000	35.178	5	21.400	0.8231
70	22.800	0.8769	22.800	18.000	27.000	17.653	5	21.400	0.8231
100	12.400	0.4769	12.400	3.000	19.000	49.909	5	12.400	0.4769
DWB	25.800	0.9923	25.800	17.000	31.000	22.501	5		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.91475	0.927	-0.9653	0.36605
Bartlett's Test indicates equal variances (p = 0.32)	5.82839	15.0863		

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05*	15.476	15.071	6.942	98.703	2.2029
IC10*	30.952	16.452	13.885	98.276	0.9938
IC15*	46.429	15.984	20.827	97.294	0.2100
IC20	72.000				
IC25	76.333				
IC40	89.333				
IC50	98.000				

* indicates IC estimate less than the lowest concentration

WET TEST REPORT FORM – CHRONIC
pH ADJUSTED (~6.5) TEST

Permittee: Elk Ridge Mining and Reclamation **Outfall:** 013
Permit No.: CO0000213

Test Type: Routine ☐ TIE ☒
Test Species: *Ceriodaphnia dubia* **IWC:** 100%

Test Start Time	Test Start Date	Test End Time	Test End Date
1300	03-15-2018	1310	03-22-2018

Test Results	Lethality	Reproduction
IC_{25}	88.8%	71.2%

Dilution(s) - % Effluent

Measurements	Control (0%)	50%	60%	70%	100%	Method Control
% Survival for day 1	100	100	100	100	100	100
% Survival for day 2	100	100	100	100	100	100
% Survival for day 3	100	100	100	100	100	100
% Survival for day 4	100	100	100	100	100	100
% Survival for day 5	100	100	100	100	80	100
% Survival for day 6	100	100	100	100	60	100
% Survival for day 7	100	100	100	100	60	100
Mean 3 Brood Total	25.8	23.4	19.4	19.8	13.2	27.4

Recon Water: 87
Recon Water: 60
pH (initial/final) – Control: 8.0/8.0 100%: 6.6/8.1

Were all Test Conditions in Conformance with Division Guidelines? YES ☒ NO ☐
If **NO**, list deviations from test specifications:
Laboratory: SeaCrest Group
Comments:
Analyst's Name: Madison Reese, Tessa Hunt-Woodland, Dan Hillenburg, and Nicole Denkinger

Signature [signature] *Date* 05/02/2018

Crest Group
CO

Ceriodaphnia Chronic TIE pH Adjusted (~6.5) Benchsheet

Form #: 113j
Effective: January 2006

Client: Elk Ridge Lab #: 418 208 Site: 013
100 Template #: 5 Dilution Water: MH 18-005 Sample Date: 0307
Source: cerio 7105 031518 Test Start: 031518 1300 Test End: 032218 - 1310
Test Conditions: pH adjusted to ~6.5 ( )

	0	1	2	3	4	5	6	7	8	Total
(C) 0	0	0	0	4	[illegible] 8 14	+1 0	16	19		35
	0	0	0	3	6	0	13	15		22
	0	0	0	3	6	+1 0	17	0		27
	0	0	0	3	6	0	9	16		18
	0	0	0	7	8	0	12	15		27
DO	6.9	6.9	6.6 / 7.0	7.1	7.4 / 6.9	7.5	7.5	7.3		25.8
Temp	24.1	24.9	24.1 / 24.2	24.8	25.0 / 24.2	24.2	24.3	24.3		
pH	8.0	7.9	7.7 / 8.0	8.0	8.0 / 8.0	8.0	7.9	8.0		
Cond	302	319	307	331	301	307	316	324		
(1) 50	0	0	0	5	9	0	15	16		29
	0	0	0	4	8	+1 0	10	18		24
	0	0	0	5	5	0	11	12		21
	0	0	0	6	7	0	10	15		23
	0	0	0	2	+2	7	9	11		20
DO	7.0	6.9	6.7 / 7.1	7.1	7.4 / 6.9	7.5	7.8	7.4		23.4
Temp	24.3	24.9	24.1 / 24.6	24.8	25.0 / 24.2	24.2	24.3	24.3		
pH	6.8	7.9	7.7 / 7.0	8.0	8.0 / 6.8	8.0	8.0	8.0		
Cond	1810	—	1813	—	1756	—	—	—		
(2) 60	0	0	0	4	3 +2	+2 0	6	7		15
	0	0	0	3	8 +1	+1 0	8	14		20
	0	0	0	5	0	6	7	11		18
	0	0	0	4	5	0	12	18		21
	0	0	0	2	+1 0	9	11	13		23
DO	7.0	7.0	6.8 / 7.2	7.2	7.5 / 6.9	7.5	7.9	7.4		19.4
Temp	24.5	24.9	24.1 / 24.1	24.8	25.0 / 24.2	24.2	24.3	24.3		
pH	6.7	7.9	7.8 / 7.0	7.9	7.9 / 6.8	8.0	8.0	8.0		
Cond	1658	—	2230	—	1925	—	—	—		
(3) 70	0	0	0	4	7	0	9	13		20
	0	0	0	3	5	0	8	12		16
	0	0	0	5	6 +1	+1 0	8	8		20
	0	0	0	5	6	0	10	12		21
	0	0	0	5	6 +1	+1 0	10	18		22
DO	7.1	7.0	6.8 / 7.2	7.3	[illegible] / 7.0	7.5	8.0	7.4		19.8
Temp	24.7	24.9	24.1 / 24.8	24.8	25.0 / 24.2	24.2	24.3	24.2		
pH	6.7	8.0	7.8 / 6.9	7.8	7.8 / 6.7	8.0	8.0	8.1		
Cond	1210	—	2420	—	2300	—	—	—		
(4) 100	0	0	0	3	0	5	5	8		13
	0	0	0	3	5 +1	+1 0	8	8		17
	0	0	0	1	0	7	9	9		26
	0	0	0	4 +1	+2 0	4 D	—	—		10
	0	0	0	D	D	D	D	—		0
DO	7.2	7.0	6.9 / 7.4	7.3	7.5 / 7.0	7.5	8.2	7.5		* 13.2
Temp	25.1	24.9	24.1 / 25.2	24.8	25.0 / 24.1	24.2	24.3	24.2		
pH	6.6	8.0	7.9 / 6.7	7.8	7.8 / 6.5	8.0	8.0	8.1		
Cond	3010	3250	2980	3020	3040	3180	3100	3060		
dilution water blank	0	0	0	6	9 +1	+1 0	14	12		30
	0	0	0	5	4 +1	+1 0	9	10		19
	0	0	0	6	9	0	14	10		29
	0	0	0	6	8 +1	+1 0	14	12		29
	0	0	0	7	8	0	15	12		30
DO	7.0	7.1	6.7 / 7.4	7.4	7.6 / 7.0	7.5	8.1	7.1		27.4
Temp	24.4	24.9	24.1 / 24.3	24.8	25.0 / 24.2	24.2	24.3	24.1		
pH	6.7	7.7	7.8 / 6.6	7.7	7.8 8.0 / 6.5	7.8	7.8	7.8		
Cond	314	321	311	317	314	318	326	350		
Initials	MR	MR	X	[illegible]	ABS	X / ABS ABC	ABS ABC	ABS/AO	ABS/AEO	EE DK
CO_2										
Algae	[illegible]	[illegible]		1801	1801	1801	1801	1801	1801	
YCT	1801	1801		MR	TW	TW	TW	ND	DH	
H_2O										

	Eff #1	Eff #2	Eff #3	Recon #1	Recon #2	Recon #3
Hardness				87		
Alkalinity				60		
Chlorine				<0.01		
Ammonia				<0.03		

1. Exposure Chamber
Total Capacity: 30 ml
Test Solution Surface Area: ____ cm^2
Test Solution Volume: 10 ml
Water Depth (constant): ____ cm
(cyclic): ____ to ____ cm

2. Feeding Schedule
Not fed:
Fed Irregularly:
Fed Daily: X
Food Used: YCT, algae

3. Aeration
#1 None:
#2 None:
#3 None:
Before Use:
(____ minutes @ ~100 bubbles/min)
Before Use:
(____ minutes @ ~100 bubbles/min)
Before Use:
(____ minutes @ ~100 bubbles/min)

4. Screened Animal Enclosers
Not Used: X
Used:

5. Condition/appearance of surviving organisms at end of test (i.e., alive but immobile; loss of orientation; erratic movement; etc,):

6. Comments:

1	2	3	4	5
H8	A9	B3	B4	B8

x.y.z = board #.row.column

HCl/NaOH:

Ceriodaphnia Survival and Reproduction Test-7 Day Survival

Start Date:	3/15/2018	Test ID:	208plpH6.5	Sample ID:	EFFFIN-Effluent Final
End Date:	3/22/2018	Lab ID:	SCG-Seacrest Group	Sample Type:	EFF2-Industrial
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000
50	1.0000	1.0000	1.0000	1.0000	1.0000
60	1.0000	1.0000	1.0000	1.0000	1.0000
70	1.0000	1.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	1.0000	0.0000	0.0000
DWB	1.0000	1.0000	1.0000	1.0000	1.0000

			Transform: Arcsin Square Root					Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
D-Control	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	5	1.0000	1.0000
50	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	5	1.0000	1.0000
60	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	5	1.0000	1.0000
70	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	5	1.0000	1.0000
100	0.6000	0.6000	0.8378	0.5236	1.0472	34.233	5	0.6000	0.6000
DWB	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	5		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.55935	0.927	-1.0534	4.98677
Equality of variance cannot be confirmed				

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)	Skew
IC05	73.750			
IC10	77.500			
IC15	81.250			
IC20	85.000			
IC25	88.750			
IC40	>100			
IC50	>100			

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	3/15/2018	Test ID:	208plpH6.5	Sample ID:	EFFFIN-Effluent Final
End Date:	3/22/2018	Lab ID:	SCG-Seacrest Group	Sample Type:	EFF2-Industrial
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5
D-Control	35.000	22.000	27.000	18.000	27.000
50	29.000	24.000	21.000	23.000	20.000
60	15.000	20.000	18.000	21.000	23.000
70	20.000	16.000	20.000	21.000	22.000
100	13.000	17.000	26.000	10.000	0.000
DWB	30.000	19.000	29.000	29.000	30.000

			Transform: Untransformed					Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
D-Control	25.800	1.0000	25.800	18.000	35.000	24.727	5	25.800	1.0000
50	23.400	0.9070	23.400	20.000	29.000	14.988	5	23.400	0.9070
60	19.400	0.7519	19.400	15.000	23.000	15.720	5	19.600	0.7597
70	19.800	0.7674	19.800	16.000	22.000	11.517	5	19.600	0.7597
100	13.200	0.5116	13.200	0.000	26.000	72.149	5	13.200	0.5116
DWB	27.400	1.0620	27.400	19.000	30.000	17.235	5		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.95209	0.927	-0.1291	1.83752
Bartlett's Test indicates equal variances (p = 0.08)	9.90568	15.0863		

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05*	26.875	17.423	1.353	70.572	0.1570
IC10	50.474	13.474	4.344	68.373	-0.4690
IC15	53.868				
IC20	57.263				
IC25	71.172				
IC40	89.313				
IC50	>100				

* indicates IC estimate less than the lowest concentration