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

September 26, 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)
180-Day 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 conducted the TIE on behalf of the facility. To date, SeaCrest Group recently completed Phase III of the TIE in accordance with EPA's methodology (reports enclosed). As reported earlier, the 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. Phase III results are enclosed.

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.

As approved in the email correspondence on August 15th, we are submitting this final 180-day notice by 9/30/2018. Based on these results and previous results from the facility's discharges, we are moving forward on the Toxicity Reduction Evaluation (TRE), to be completed within 180 days from the completion of the TIE. We would like to discuss both the TIE results and TRE plan with you in the next couple of weeks to confirm our plans moving forward.

If you have any questions on this TIE report, please contact Chantell Johnson at 303-254-3185 (cjohnson@tristategt.org) or Chris Gilbreath at 303-254-3291 (cgilbreath@tristategt.org).

Sincerely,

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

BAW:CJ:der

Enclosure

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

SeaCrestGroup
AN ENVIRONMENTAL SERVICES COMPANY

September 28, 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 Elk Ridge Mining and Reclamation. The TIE is in response to the WET (Whole Effluent Toxicity) results of a test initiated on February 14th, 2018 showing toxicity to the *Ceriodaphnia dubia* test species. Water was collected on March 8th, 2018 and has been used since to conduct the TIE testing. The purpose of this letter is to summarize the findings of the TIE thus far, to highlight the characteristics that are still unknown, and to present a plan for future work.

Phase I indicated the following:

1. Toxicity was removed for lethal and sub-lethal parameters 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 and filtered off at pH 10.
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.

Phase II indicated the following:

1. Analytical testing of the filtrate and precipitate showed the following ions were reduced in the effluent during the pH 10 filtration manipulation: calcium, iron, magnesium, manganese, strontium, and bicarbonate.
2. Mock precipitate testing, spiking dilution water with the known concentration of the above ions, did not provide further data due to the insufficient dissolution of the ions into the diluent.
3. Baseline testing with the original water collected in March 2018 has presented the following over time:
    - A disappearance of lethal toxicity
    - An increase in sub-lethal toxicity.
4. The persistence of toxicity over multiple seasons (quarters) has been demonstrated outside of the TIE with the most recent Q3 test failing sub-lethal endpoints.

The following points remain unclear at this point:

1. Whether the toxicity is from the chemicals present in the pH 10 precipitate alone or a combination of the properties of the precipitate and the filtrate.
2. Which chemical properties and/or constituents of the effluent are changing over time from the original March 2018 sample.
3. If the pH 10 filtration treatment removes toxicity over multiple toxic events and seasons.

SeaCrest Group proposes the following steps to move ahead with Phase III moving towards a TRE:

1. For future sampling events that exhibit toxicity:
    a. Conducting bench-scale treatment of raising the pH of the effluent to 10, filtering, lowering the filtrate to pHi and conducting WET testing with the *C. dubia* species.
    b. Conducting analytical testing on effluent, precipitate, and filtrate to determine the ionic concentration
2. For future sampling event that do not exhibit toxicity:
    a. Conducting analytical testing on effluent to determine the ionic concentration.
3. Using the above data to determine potential ionic thresholds for future treatment options.

The project will continue to be refined and developed with SeaCrest Group, Tri-State Generation and Transmission Association, Inc. and potentially additional consultants and engineers with the aims of removing toxicity from the New Horizon Mine 013 outfall.

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

Best regards,

Kyra Brisson
Laboratory Director

SeaCrestGroup
AN ENVIRONMENTAL SERVICES COMPANY

September 24, 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 Mr. Fry and Ms. Johnson:

SeaCrest Group has undertaken the TIE (Toxicity Identification Evaluation) at the request of the Elk Ridge Mining and Reclamation. The TIE is in response to a WET result in Q1 of 2018 showing toxicity to the *Ceriodaphnia dubia* test species.

Thus far, phase I and II have indicated that toxicity is removed from the NH Mine effluent when brought to pH 10, filtered, and brought back to pHi.

The purpose of these tests, now reported, was to evaluate the potential toxicity of the cations of highest concentration in the precipitate from the pH 10 filtrate. The potential toxicity of the cations, calcium, magnesium, iron, manganese, and strontium were evaluated by spiking the ions into dilution water and measuring the resulting toxicity. Because toxicity may be caused by a combination of many ions that exert their influence together, all cations were added to one solution that was then evaluated using the *C. dubia* test species. This report represents a baseline test as part of the TIE protocol. The TIE was performed in accordance with EPA protocols for the conduct of such investigations.

Initial observations from the mock precipitate testing are as follows:

1) The baseline test, using water that was sampled in March of 2018, exhibited no lethal toxicity. However, the effluent showed greater sub-lethal toxicity than previous test using the same water. This indicates that the properties of the water have changed over time. The changes to the chemical properties or constituents of the water are currently unclear.

2) Two mock precipitate tests were conducted to evaluate the solubility of calcium when introduced in mock 1 as calcium carbonate and mock 2 as calcium sulfate.
3) Apart from the calcium concentration in the mock test using calcium sulfate, none of the cations of concern reached target concentrations.
4) No toxicity was observed in mock precipitate test using calcium carbonate or calcium sulfate.

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): Invoice
Report

SeaCrestGroup
AN ENVIRONMENTAL SERVICES COMPANY

1341 Cannon Street
Louisville, CO 80027-1455
(303) 661-9324 Phone
(303) 661-9325 Fax

Invoice

Invoice Number:
418208.B_Mock
Invoice Date:
September 24, 2018

BILL TO:
Western Fuels 27646 W. 5th Ave. Nucla, CO 81424

Customer Contact	Customer PO#	Terms	Customer ID
Mr. Thomas Fry	0212454	Payable Upon Receipt	Western Fuels

QTY	Description	Unit Price	Extended Price
	WET TIE Phase II using *Ceriodaphnia dubia*		
	Mock Precipitate Testing		
1	- Baseline Test	$780.00	$780.00
2	- Five Ions of Concern (Ca, Fe, Mn, Ni, Mg)	$1,100.00	$2,200.00
1	- XRD Semi Quantitative Analysis	$295.00	$295.00
1	- 24 analyte ICP-MS Analysis	$120.00	$120.00
2	- Confirmation of 5 cations of concern	$120.00	$240.00
5	- Consulting, preparation and management of study method and equipment, specified general in-house chemistries and final report	$120.00	$600.00

Total: $4,235.00

All invoices are due and payable upon receipt.
Outstanding balances over 30-days are subject to a finance charge of 1.5% per month.

THANK YOU FOR YOUR BUSINESS!

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

Prepared for:

Ms. 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

September 24, 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 Followed: • *Ceriodaphnia dubia*: EPA/821/R-02-013. Method 1002.0 (2002)

Sample Number: 418208.B

Ceriodaphnia dubia	*Baseline*	*Mock 1*	*Mock 2*
Test Initiation Time	1610	1623	1245
Test Initiation Date	09-07-2018	09-07-2018	09-10-2018
Test Completion Time	1617	1626	1330
Test Completion Date	09-13-2018	09-13-2018	09-16-2018

Dilution Water: • Moderately hard laboratory reconstituted water

Test Organism Source: • *Ceriodaphnia dubia*
SeaCrest Group

Reference Toxicant: • Sodium Chloride

Abstract of Results

Test Concentrations: Control (0%), 20%, 40%, 60%, 80%, 100%

Number of Organisms/Concentration: 10 for *Ceriodaphnia dubia*

Replicates at each Concentration: 10 for *Ceriodaphnia dubia*

Ceriodaphnia dubia	*Baseline*	*Mock 1*	*Mock 2*
Test vessel size	30ml	30ml	30ml
Exposure volume	15ml	15ml	15ml
Pass/Fail Status	**N/A**	**N/A**	**N/A**
Temperature Range (°C)	24.1 – 25.9	24.1 – 25.4	24.2 – 25.8
Dissolved Oxygen Range (mg/L)	6.2 – 7.2	5.4 – 7.1	6.1 – 7.1
pH Range	7.6 – 8.1	7.1 - 8.6	6.8 - 8.2

INTRODUCTION

Biomonitoring provides an effective means by which the toxicity of discharges from municipal, industrial and mining operations can be tested. Among the advantages of biomonitoring is the ability to test complex effluents containing a broad range of contaminants. Biomonitoring, when used in conjunction with chemical analyses, can generate data capable of identifying a much wider range of contaminants.

The Colorado Water Quality Control Division requires certain NPDES permittees to perform acute and/or chronic biomonitoring tests. The chronic test measures significant differences in lethality and in reproduction (*Ceriodaphnia dubia*) or growth (Fathead minnow – *Pimephales promelas*) between control and exposed organisms.

The present report discusses the results of chronic TIE mock precipitate and baseline tests. The mock precipitate tests were run using spiked diluent water aimed at matching precipitate cation concentrations. The baseline test was conducted on effluent from Elk Ridge Mining and Reclamation New Horizon Mine 013 discharge. These tests were conducted in September 2018.

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 Baseline test was started on 09-07-2018, the Mock 1 test was started on 09-07-2018, and the Mock 2 test was started on 09-10-2018. The tests were performed according to the

procedures outlined in USEPA[3] and the Colorado Department of Public Health and Environment[4].

Individual *Ceriodaphnia dubia* were placed in 30ml plastic containers containing approximately 15ml of exposure medium. Ten replicates at each concentration were used. The animals were fed daily with the YCT mixture and an equal volume of the green algae *(Selenastrum capricornutum)*. The exposure medium was changed daily in each container and the number of young released overnight were counted and recorded. Young were removed from the containers daily and discarded. Routine measurements were made each day of temperature, dissolved oxygen and pH before and after the water changes.

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]).

Table 1. Statistics methods used in testing for significant differences in test parameters.

Species	Normality	Homogeneity
Ceriodaphnia dubia	Kolmogorov Test	Bartlett's Test
Statistical Difference		

Ceriodaphnia dubia	Survival	Reproduction	IC_{25}
Baseline	Fisher's Exact Test	Steel's Many-One Rank Test	ICp
Mock 1	Fisher's Exact Test	Dunnett's Test	ICp
Mock 2	Fisher's Exact Test	Dunnett's Test	ICp

RESULTS

Baseline Test Results

Test results for the *Ceriodaphnia dubia* Baseline test are summarized in Table 2 and provided on the data sheets located in Appendix 1. Survival was 90% in the 100% effluent concentration and ranged from 70% - 100% in the remaining effluent concentrations. Control survival was 100%. The No Observable Effect Concentration (NOEC) for lethality was 100%. The IC_{25} for lethality was >100%.

Table 2. Summary of Baseline test results. Ten animals were exposed at each concentration. An asterisk (*) denotes a statistically significant difference from the control.

Concentration	No. Surviving	Mean Births	Min.	Max.	Significant Difference	
					Lethality	Reprod.
Control (0%)	10	27.3	14	36		
20%	10	17.7	8	24		
40%	8	10.1	0	23		*
60%	7	6.0	0	17		*
80%	9	2.5	0	6		*
100%	9	0.5	0	2		*

Average numbers of neonates was 0.5 in the 100% effluent concentration and ranged from 2.5 – 17.7 in the remaining effluent concentrations. Average number of neonates in the control was 27.3 for statistical analyses and for test acceptability criteria. The NOEC for reproduction was 20%. The IC_{25} for reproduction was estimated to be 14.2%.

Mock 1 Test Results

Test results for the *Ceriodaphnia dubia* Mock 1 test are summarized in Table 3 and provided on the data sheets located in Appendix 2. Survival was 90% in the 100% mock concentration and ranged from 90% - 100% in the remaining mock concentrations. Control survival was 100%. The No Observable Effect Concentration (NOEC) for lethality was 100%. The IC_{25} for lethality was >100%.

Table 3. Summary of Mock 1 test results. Ten animals were exposed at each concentration. An asterisk (*) denotes a statistically significant difference from the control.

Concentration	No. Surviving	Mean Births	Min.	Max.	Significant Difference	
					Lethality	Reprod.
Control (0%)	10	26.3	16	35		
20%	10	27.3	19	34		
40%	10	27.2	16	36		
60%	10	31.4	22	42		
80%	9	27.5	13	43		
100%	9	24.4	17	37		

Average numbers of neonates was 24.4 in the 100% mock concentration and ranged from 27.2 – 31.4 in the remaining concentrations. Average number of neonates in the control was 26.3

for statistical analyses and for test acceptability criteria. The NOEC for reproduction was 100%. The IC_{25} for reproduction was estimated to be >100%.

Mock 2 Test Results

Test results for the *Ceriodaphnia dubia* Mock 2 test are summarized in Table 4 and provided on the data sheets located in Appendix 3. Survival was 100% in the 100% mock concentration and ranged from 90% - 100% in the remaining mock concentrations. Control survival was 90%. The No Observable Effect Concentration (NOEC) for lethality was 100%. The IC_{25} for lethality was >100%.

Table 4. Summary of Mock 2 test results. Ten animals were exposed at each concentration. An asterisk (*) denotes a statistically significant difference from the control.

Concentration	No. Surviving	Mean Births	Min.	Max.	Significant Difference	
					Lethality	Reprod.
Control (0%)	9	25.8	15	33		
20%	9	26.5	13	40		
40%	9	26.0	20	35		
60%	10	25.5	19	37		
80%	10	26.5	21	30		
100%	10	22.0	11	30		

Average numbers of neonates was 22.0 in the 100% mock concentration and ranged from 25.5 – 26.5 in the remaining mock concentrations. Average number of neonates in the control was 25.8 for statistical analyses and 27.0 for test acceptability criteria. The NOEC for reproduction was 100%. The IC_{25} for reproduction was estimated to be >100%.

Test Acceptability

Acceptable control survival was achieved in the tests. Similarly, *Ceriodaphnia dubia* reproduction in control organisms met required levels. The PMSD for each test was within the required limits for an acceptable test (Table 5).

Table 5. PMSD for chronic test parameters.

	Reproduction	**Result**		
Baseline PMSD (% Minimum significant difference)	*Ceriodaphnia dubia*	Lower bound	Upper bound	Test parameters deemed to be acceptable if numbers fall within limits for the test.
		13	47	
	N/A	20.6		
	Reproduction	**Result**		
Mock 1 PMSD (% Minimum significant difference)	*Ceriodaphnia dubia*	Lower bound	Upper bound	Test parameters deemed to be acceptable if numbers fall within limits for the test.
		13	47	
	N/A	27.1		
	Reproduction	**Result**		
Mock 2 PMSD (% Minimum significant difference)	*Ceriodaphnia dubia*	Lower bound	Upper bound	Test parameters deemed to be acceptable if numbers fall within limits for the test.
		13	47	
	N/A	22.6		

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. Phase I and II have indicated that toxicity is removed from the NH Mine effluent when brought to pH 10, filtered, and brought back to pHi, in effect, removing some of the dissolved constituents. The TIE is being performed in accordance with EPA protocols for the conduct of such investigations[7].

A baseline toxicity test with the following dilution series, 0%, 20%, 40%, 60%, 80%, and 100%, was initiated on September 7, 2018, as well as a Mock 1 toxicity test with the above dilution series initiated on September 7, 2018, and a Mock 2 toxicity test with the above dilution series initiated on September 10, 2018.

The baseline test was performed to evaluate the persistence of toxicity to the *C. dubia* test species from the sample collected on March 7, 2018. The purpose of Mock 1 and Mock 2 tests was to evaluate the potential toxicity of the cations of highest concentration in the precipitate from the pH 10 filtrate. The cations, calcium, magnesium, iron, manganese, and strontium were evaluated by spiking the ions into dilution water and measuring the resulting toxicity. Because toxicity may be caused by a combination of many ions that exert their influence together, all cations were added to one solution that was then evaluated using the *C. dubia* test species. This report represents a baseline test as part of the TIE protocol. The TIE was performed in accordance with EPA protocols for the conduct of such investigations.

Initial observations from the mock precipitate testing are as follows:

1) The baseline test exhibited statistically significant sub-lethal toxicity, resulting in an estimated sub-lethal IC_{25} of 14.2%. The baseline test exhibited no statistically significant lethal toxicity. This indicates that the properties of the water have changed over time. The changes to the chemical properties or constituents are unclear at this time.
2) Two tests were conducted to evaluate the solubility of two calcium compounds, calcium carbonate and calcium sulfate.
3) Apart from the calcium concentration in mock 1 test using calcium sulfate, none of the cations of concern reached target concentrations. This could be due in part to the elevated pH of the mock solutions.
4) No toxicity was observed in either mock precipitate test. The Mock 1 using, using calcium carbonate, did not exhibit statistically significant sub-lethal toxicity, resulting in an IC_{25} of >100%. The Mock 2 test, using calcium sulfate, did not exhibit statistically significant sub-lethal toxicity, resulting in an IC_{25} of >100%.

REFERENCES

1. **Hach Chemical Company.** 2002. *Hach Water Analysis Handbook.* Hach Chemical Company, Loveland, Colorado. 1260pp.
2. **APHA/AWWA/WEF.** 1998. *Standard Methods for the Examination of Water and Wastewater.* 20th Edition. American Public Health Association, Washington, D.C.
3. **USEPA.** 2002. *Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms.* EPA-821-R-02-013. 335 pp.
4. **CDPHE (Colorado Department of Public Health and Environment).** 1998. *Laboratory Guidelines for Conducting Whole Effluent Toxicity Tests.* Water Quality Control Division.
5. **USEPA.** 2000. *Method of Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing* (40 CFR Part 136). EPA/821/B-00/013.
6. **USEPA**. 2000. *Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications under the National Pollutant Discharge Elimination System Program.* EPA/833/R-00/003.
7. **USEPA. 1991.** *Methods for Aquatic Toxicity Identification Evaluations.* EPA/600/6-91/003
8. **USEPA. 1992.** *Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents Phase I.* EPA600/6-91-005F
9. **USEPA. 1999.** *Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants*. EPA/833B-99/002
10. **USEPA. 1993.** *Methods for aquatic toxicity Identification Evaluations: Phase II Toxicity Identification Procedures for Sample Exhibiting Acute and Chronic Toxicity.* EPA/600/R-92-080
11. **USEPA. 1993.** *Methods for Aquatic Toxicity Identification Evaluations: Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity*. EPA/600/R-92-081

Appendix 1 – Data Sheets for the *Ceriodaphnia dubia* Baseline Test

WET TEST REPORT FORM – CHRONIC BASELINE

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

Permit No.: CO-0000213

Test Type: Routine ☐ TIE ☒

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

Test Start Time	Test Start Date	Test End Time	Test End Date
1610	09-07-2018	1617	09-13-2018

Test Results	Lethality	Reproduction
NOEC	100%	20%
IC_{25}	>100%	14.2%

Dilution(s) - % Effluent

Measurements	Control (0%)	20%	40%	60%	80%	100%
% 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	90	100
% Survival for day 4	100	100	90	90	90	90
% Survival for day 5	100	100	90	70	90	90
% Survival for day 6	100	100	80	70	90	90
Mean 3 Brood Total	27.3	17.7	10.1	6.0	2.5	0.5

Hardness (mg/L) – Recon Water: 95
Alkalinity (mg/L) – Recon Water: 57
pH (initial/final) – Control: 8.0/8.0 100%: 7.6/8.1

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: Tessa Hunt-Woodland and Corey Sutton

Signature [signature] *Date* 09/24/18

SeaCrest Group
Louisville, CO

TLC

Ceriodaphnia Chronic Benchsheet

Form #: 101a
Effective: January 2009

Permittee: Baseline (Elk Ridge) Lab #: 418 208.B Site: NPDES 013
IWC %: Template #: 5 Dilution Water: MH 18-018 Sample Date:
Age & Source: cerio 2018 090718 Test Start: 090718 1610 Test End: 091318 1617
Test Conditions:

	0	1	2	3	4	5	6	7	8	Total
(C) 0	0	0	0	2 →	7	13	12			34
	0	0	0	4	0	14	15			33
	0	0	0	6	9 +2	0	15			32
	0	0	0	3	9	6	0			18
	0	0	0	0	8	15	13			36
	0	0	0	2 →	11	7	15			35
	0	0	0	6	11	13 +1	0			31
	0	0	0	0	8 +1	0	13			22
	0	0	0	0	7	6 +1	0			14
	0	0	0	2 +1	0	8	7			18
DO	7.0	6.9 6.9	6.5 7.1	6.4 7.0	6.5 7.1	6.4	6.9 6.3			STAT
Temp	24.5	24.1 24.2	24.4 25.1	24.3 24.6	24.6 25.1	24.9	25.0 24.2			27.3
pH	8.0	7.9 7.9	8.1 8.1	8.1 8.1	8.1 8.1	7.7	7.8 8.0			ACCP
Cond	305	300	315	310	309	304				—
(1) 20	0	0	0	0	6	5	6			17
	0	0	0	6	0	10	14			24
	0	0	0	5	5	0	11			21
	0	0	0	4	8	11	0			23
	0	0	0	0	10	6	0			16
	0	0	0	3	11	0	0			14
	0	0	0	2	6	0	11			19
	0	0	0	3	7	0	13			23
	0	0	0	0	4	4	0			8
	0	0	0	0	0	0	12			12
DO	7.1	6.9 6.9	6.5 7.0	6.4 6.9	6.6 7.0	6.4	6.7 6.3			
Temp	24.5	24.1 24.2	24.4 25.2	24.3 24.6	24.6 25.2	24.9	25.0 24.2			17.7
pH	7.8	7.9 7.9	8.1 7.9	8.0 7.9	8.0 7.9	7.7	7.8 8.0			
Cond	1160	1023	992	1051	1011	945				

	0	1	2	3	4	5	6	7	8	Total
(2) 40	0	0	0	0	6	9	8			23
	0	0	0	1	4 +1	0	0			6
	0	0	0	0	8	8 +1	8			17
	0	0	0	3	6	0	0			9
	0	0	0	0	OD					0
	0	0	0	0	5	3 +3	0			11
	0	0	0	0	5	4 +2	0			11
	0	0	0	6	4	0 +	2			6
	0	0	0	6	4	6	5 D			15
	0	0	0	6	3	0	6			3
DO	7.3	7.0 6.9	6.6 6.9	6.5 6.8	6.7 6.8	6.4	6.5 6.3			
Temp	24.4	24.1 24.2	24.4 25.2	24.3 24.7	24.6 25.5	24.9	25.2 24.2			10.1
pH	7.7	7.9 7.8	8.0 7.8	7.9 7.8	7.9 7.8	7.7	7.7 8.0			
Cond	1579	1520	1538	1610	1554	1544				
(3) 60	0	0	0	3 →	3	3	8			17
	0	0	0	0	OD					0
	0	0	0	6	2	0 D				2
	0	0	0	1	0	4	0			5
	0	0	0	0	3	2	4			9
	0	0	0	0	1	3	3			7
	0	0	0	0	0	0	8			8
	0	0	0	0	0	5	7			12
	0	0	0	0	0	0	0			0
	0	0	0	0	0	0 D				0
DO	7.4	7.0 6.9	6.4 6.8	6.6 6.7	6.9 6.7	6.5	6.4 6.4			
Temp	24.4	24.1 24.3	24.4 25.3	24.3 24.8	24.6 25.4	24.9	25.3 24.2			6.0
pH	7.7	7.8 7.8	8.0 7.7	7.8 7.7	7.8 7.8	7.8	7.7 8.1			
Cond	1978	1961	1959	1995	1958	1941				

	0	1	2	3	4	5	6	7	8	Total
(4) 80	0	0	0	0	0	0	3			3
	0	0	0	0	0	3	0			3
	0	0	0	0	4	0	0			4
	0	0	0	0 D						0
	0	0	0	0	0	3	3			6
	0	0	0	0	2	0	0			2
	0	0	0	1	0	0	0			1
	0	0	0	0	0	0	4			4
	0	0	0	0	0	0	0			0
	0	0	0	0	0	0	2			2
DO	7.6	7.1 | 6.9	6.7	6.7	6.7 | 6.6	7.0 | 6.5	6.5 | 6.3	6.4		
Temp	24.3	24.1 | 24.3	24.4	25.3	24.3 | 24.8	24.9 | 25.7	24.9 | 25.5	24.2		2.5
pH	7.6	7.8 | 7.7	7.9	7.7	7.7 | 7.7	7.8 | 7.7	7.8 | 7.6	8.1		
Cond	2620	2630	2580		2650	2630	2560			
(5) 100	0	0	0	0	0	0	0			0
	0	0	0	0	0 D					0
	0	0	0	0	0	0	2			2
	0	0	0	0	0	0	0			0
	0	0	0	0	0	0	0			0
	0	0	0	0	0	1	0			1
	0	0	0	0	0	0	0			0
	0	0	0	0	0	0	0			0
	0	0	0	0	0	0	0			0
	0	0	0	0	0	2	0			2
DO	7.6	7.1 | 6.9	6.7	6.6	6.7 | 6.5	7.2 | 6.4	6.5 | 6.2	6.4		
Temp	24.2	24.1 | 24.3	24.4	25.4	24.3 | 24.9	24.9 | 25.9	24.9 | 25.6	24.2		0.5
pH	7.6	7.8 | 7.7	7.9	7.6	7.7 | 7.6	7.7 | 7.6	7.8 | 7.6	8.1		
Cond	3130	3110	3040		3140	3110	3050			
Algae	ABS/ARO	ABS/ARO	ABS/ARO		ABS/ARO	1832/ARO	1832/ARO			
YCT	1806	1806	1806		1806	1806	1806			
H_2O	—	—	—		—	—	—			
Initials	TMW	TMW	CS		CS	CS	CS	CS		

	Eff #1	Eff #2	Eff #3	Rec'g #1	Rec'g #2	Rec'g #3	Recon #1	Recon #2	Recon #3
Hardness									
Alkalinity									
Chlorine									
Ammonia									

1. Exposure Chamber
   - Total Capacity: 30 ml
   - Test Solution Volume: 15 ml
   - Test Solution Surface Area: _____ cm^2
   - Water Depth (constant): _____ cm
   - (cyclic): _____ to _____ cm
2. Feeding Schedule
   - Not fed: _____
   - Fed Daily: X
   - Fed Irregularly: _____
   - Food Used: YCT, algae
3. Aeration
   - #1 None: _____ Before Use: _____ (_____ minutes @ ~100 bubbles/min)
   - #2 None: _____ Before Use: _____ (_____ minutes @ ~100 bubbles/min)
   - #3 None: _____ Before Use: _____ (_____ minutes @ ~100 bubbles/min)
4. Screened Animal Enclosers
   - Not Used: X
   - Used: _____ cm diameter
5. Condition/appearance of surviving organisms at end of test (i.e., alive but immobile; loss of orientation; erratic movement; etc,): _____
6. Comments: Active & Mobile

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

1	2	3	4	5	6	7	8	9	10
A1	A2	A4	A5	A6	A8	A10	B1	B3	B5

Ceriodaphnia Survival and Reproduction Test-7 Day Survival

Start Date:	9/7/2018	Test ID:	418208MBas	Sample ID:	EFFFIN-Effluent Final
End Date:	9/13/2018	Lab ID:		Sample Type:	EFF1-POTW
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
20	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
40	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	0.0000	1.0000
60	1.0000	0.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000
80	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
100	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Conc-%	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical
D-Control	1.0000	1.0000	0	10	10	10		
20	1.0000	1.0000	0	10	10	10	1.0000	0.0500
40	0.8000	0.8000	2	8	10	10	0.2368	0.0500
60	0.7000	0.7000	3	7	10	10	0.1053	0.0500
80	0.9000	0.9000	1	9	10	10	0.5000	0.0500
100	0.9000	0.9000	1	9	10	10	0.5000	0.0500

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	100	>100		1
Treatments vs D-Control				

Ceriodaphnia Survival and Reproduction Test-7 Day Survival

Start Date:	9/7/2018	Test ID:	418208MBas	Sample ID:	EFFFIN-Effluent Final
End Date:	9/13/2018	Lab ID:	.	Sample Type:	EFF1-POTW
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
20	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
40	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	0.0000	1.0000
60	1.0000	0.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000
80	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
100	1.0000	0.0000	1.0000	1.0000	1.0000	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	10	1.0000	1.0000
20	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	10	1.0000	1.0000
40	0.8000	0.8000	0.9425	0.5236	1.0472	23.424	10	0.8250	0.8250
60	0.7000	0.7000	0.8901	0.5236	1.0472	28.414	10	0.8250	0.8250
80	0.9000	0.9000	0.9948	0.5236	1.0472	16.644	10	0.8250	0.8250
100	0.9000	0.9000	0.9948	0.5236	1.0472	16.644	10	0.8250	0.8250

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.05)	3.00752	0.895	-1.996	3.15271
Equality of variance cannot be confirmed				

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL	Skew
IC05	25.714			
IC10	31.429			
IC15	37.143			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	9/7/2018	Test ID:	418208MBas	Sample ID:	EFFFIN-Effluent Final
End Date:	9/13/2018	Lab ID:		Sample Type:	EFF1-POTW
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	34.000	33.000	32.000	18.000	36.000	35.000	31.000	22.000	14.000	18.000
20	17.000	24.000	21.000	23.000	16.000	14.000	19.000	23.000	8.000	12.000
40	23.000	6.000	17.000	9.000	0.000	11.000	11.000	6.000	15.000	3.000
60	17.000	0.000	2.000	5.000	9.000	7.000	8.000	12.000	0.000	0.000
80	3.000	3.000	4.000	0.000	6.000	2.000	1.000	4.000	0.000	2.000
100	0.000	0.000	2.000	0.000	0.000	1.000	0.000	0.000	0.000	2.000

			Transform: Untransformed					Rank	1-Tailed
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
D-Control	27.300	1.0000	27.300	14.000	36.000	30.552	10		
20	17.700	0.6484	17.700	8.000	24.000	29.901	10	75.50	75.00
*40	10.100	0.3700	10.100	0.000	23.000	68.190	10	61.00	75.00
*60	6.000	0.2198	6.000	0.000	17.000	95.581	10	56.00	75.00
*80	2.500	0.0916	2.500	0.000	6.000	76.012	10	55.00	75.00
*100	0.500	0.0183	0.500	0.000	2.000	169.967	10	55.00	75.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.05)	0.79007	0.895	-0.1154	0.1896
Bartlett's Test indicates unequal variances (p = 1.76E-07)	39.6441	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test Treatments vs D-Control	20	40	28.2843	5

Reviewed by:

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	9/7/2018	Test ID:	418208MBas	Sample ID:	EFFFIN-Effluent Final
End Date:	9/13/2018	Lab ID:		Sample Type:	EFF1-POTW
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	34.000	33.000	32.000	18.000	36.000	35.000	31.000	22.000	14.000	18.000
20	17.000	24.000	21.000	23.000	16.000	14.000	19.000	23.000	8.000	12.000
40	23.000	6.000	17.000	9.000	0.000	11.000	11.000	6.000	15.000	3.000
60	17.000	0.000	2.000	5.000	9.000	7.000	8.000	12.000	0.000	0.000
80	3.000	3.000	4.000	0.000	6.000	2.000	1.000	4.000	0.000	2.000
100	0.000	0.000	2.000	0.000	0.000	1.000	0.000	0.000	0.000	2.000

			Transform: Untransformed						1-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	27.300	1.0000	27.300	14.000	36.000	30.552	10			
*20	17.700	0.6484	17.700	8.000	24.000	29.901	10	3.895	2.287	5.636
*40	10.100	0.3700	10.100	0.000	23.000	68.190	10	6.979	2.287	5.636
*60	6.000	0.2198	6.000	0.000	17.000	95.581	10	8.642	2.287	5.636
*80	2.500	0.0916	2.500	0.000	6.000	76.012	10	10.062	2.287	5.636
*100	0.500	0.0183	0.500	0.000	2.000	169.967	10	10.874	2.287	5.636

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.05)	0.79007	0.895	-0.1154	0.1896
Bartlett's Test indicates unequal variances (p = 1.76E-07)	39.6441	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	<20	20			5.63581	0.20644	1036.58	30.3722	1.5E-15	5, 54

Reviewed by:

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	9/7/2018	Test ID:	418208MBas	Sample ID:	EFFFIN-Effluent Final
End Date:	9/13/2018	Lab ID:		Sample Type:	EFF1-POTW
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	34.000	33.000	32.000	18.000	36.000	35.000	31.000	22.000	14.000	18.000
20	17.000	24.000	21.000	23.000	16.000	14.000	19.000	23.000	8.000	12.000
40	23.000	6.000	17.000	9.000	0.000	11.000	11.000	6.000	15.000	3.000
60	17.000	0.000	2.000	5.000	9.000	7.000	8.000	12.000	0.000	0.000
80	3.000	3.000	4.000	0.000	6.000	2.000	1.000	4.000	0.000	2.000
100	0.000	0.000	2.000	0.000	0.000	1.000	0.000	0.000	0.000	2.000

			Transform: Untransformed					Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
D-Control	27.300	1.0000	27.300	14.000	36.000	30.552	10	27.300	1.0000
20	17.700	0.6484	17.700	8.000	24.000	29.901	10	17.700	0.6484
40	10.100	0.3700	10.100	0.000	23.000	68.190	10	10.100	0.3700
60	6.000	0.2198	6.000	0.000	17.000	95.581	10	6.000	0.2198
80	2.500	0.0916	2.500	0.000	6.000	76.012	10	2.500	0.0916
100	0.500	0.0183	0.500	0.000	2.000	169.967	10	0.500	0.0183

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.05)	0.79007	0.895	-0.1154	0.1896
Bartlett's Test indicates unequal variances (p = 1.76E-07)	39.6441	15.0863		

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL		Skew
IC05*	2.844	1.034	2.094	5.309	3.1776
IC10*	5.688	2.067	4.189	10.618	3.1776
IC15*	8.531	2.730	6.283	15.895	2.4379
IC20*	11.375	3.170	8.378	20.548	1.6963
IC25*	14.219	3.482	10.472	23.255	1.2376
IC40	23.474	4.584	16.756	33.771	0.6697
IC50	30.658	5.025	23.115	42.335	0.3283

* indicates IC estimate less than the lowest concentration

Reviewed by:

Appendix 2 – Data Sheets for the *Ceriodaphnia dubia Mock 1* Test

WET TEST REPORT FORM – CHRONIC MOCK 1 CaCO3

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

Permit No.: CO-0000213

Test Type: Routine ☐ TIE ☒

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

Test Start Time	Test Start Date	Test End Time	Test End Date
1623	09-07-2018	1627	09-13-2018

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

Dilution(s) - % Effluent

Measurements	Control (0%)	20%	40%	60%	80%	100%
% 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	90	90
% Survival for day 6	100	100	100	100	90	90
Mean 3 Brood Total	26.3	27.3	27.2	31.4	27.5	24.4

Hardness (mg/L) – Recon Water: 95
Alkalinity (mg/L) – Recon Water: 57
pH (initial/final) – Control: 8.0/8.1 100%: 7.1/8.6

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 Corey Sutton

Signature *Date* 09/24/18

SeaCrest Group
Louisville, CO

Ceriodaphnia Chronic Benchsheet

Form #: 101a
Effective: January 2009

Permittee: TIE MUCK 1 Lab #: 418 208.M1 Site: NPDES 013
IWC %: _____ Template #: 5 Dilution Water: MH 15-018 Sample Date: _____
Age & Source: cerio 2018 090718 Test Start: 090718 1623 Test End: 091348 1640
Test Conditions:

	0	1	2	3	4	5	6	7	8	Total
(C) 0	0	0	0	6	0	16	12			34
	0	0	0	7	10	0	14			31
	0	0	0	0	9	10	16			25
	0	0	0	6	9	12	0			27
	0	0	0	6 +1	0	13	15			35
	0	0	0	0	8	12	10			30
	0	0	0	7	7	7	08			21
	0	0	0	0	8 +1	0	13			22
	0	0	0	6	7	9	0			22
	0	0	0	4 AB	0	6.1 0	9			16
DO	7.0	6.6 6.9	6.4 6.9	6.5 7.0	6.4 6.8	8.0 7.0	6.3			STAT
Temp	24.2	24.1 24.2	24.2 25.0	24.8 24.6	25.1 24.2	25.3 24.8	24.8			26.3
pH	8.0	8.0 8.0	7.9 8.1	8.0 8.0	8.1 8.1	6.1 7.9	8.1			ACCP
Cond	308	299	300	302	308	302				—
(1) 20	0	0	0	3	6	13	9			31
	0	0	0	7	0	8	15			30
	0	0	0	9	9	0	16			34
	0	0	0	6	10	12 +1	0			29
	0	0	0	4	0	14	12			30
	0	0	0	0	9	12	0			21
	0	0	0	0	8	11	0			19
	0	0	0	7	9	0	16			32
	0	0	0	2	10	11 +1	0			24
	0	0	0	5	7	0	11			23
DO	6.8	6.6 6.8	6.4 6.8	6.6 6.8	6.4 6.7	6.1 6.9	6.3			
Temp	24.4	24.1 24.2	24.2 25.0	24.8 24.8	25.1 24.2	25.3 24.8	24.8			27.3
pH	7.5	8.0 7.8	7.9 7.8	8.0 7.9	8.2 8.1	8.1 7.8	8.2			
Cond	379	383	364	374	365	350				
	0	1	2	3	4	5	6	7	8	Total
(2) 40	0	0	0	8 +1	0	12	15			36
	0	0	0	5	0	17	12			34
	0	0	0	0	8	12	14			34
	0	0	0	6	8	13	0			27
	0	0	0	0	7	15	13			35
	0	0	0	8	10	8	10			28
	0	0	0	5	11	0	0			16
	0	0	0	4	9	0	6			19
	0	0	0	0	7	12	0			19
	0	0	0	5	8	0	11			24
DO	6.4	6.6 6.7	6.4 6.8	6.7 6.6	6.5 6.7	6.2 6.9	6.4			
Temp	24.4	24.1 24.2	24.2 25.0	24.8 25.0	25.1 24.3	25.3 24.8	24.8			27.2
pH	7.2	8.0 7.7	7.9 7.7	8.1 7.8	8.3 8.0	8.2 7.8	8.3			
Cond	467	464	447	442	425	400				
(3) 60	0	0	0	7	0	11	16			34
	0	0	0	6	0	17	19			42
	0	0	0	1	8	16	13			38
	0	0	0	5	9	10	0			24
	0	0	0	6	12 EE	13	17			36
	0	0	0	2	12+1	0	11			26
	0	0	0	5	11	14	4			30
	0	0	0	5	8	0	12			25
	0	0	0	0	8	14	15			37
	0	0	0	5	6	0	11			22
DO	6.1	6.5 6.7	6.3 6.7	6.8 6.5	6.5 6.6	6.3 6.0	6.4			
Temp	24.6	24.1 24.2	24.2 25.1	24.8 25.2	25.1 24.4	25.3 24.7	24.8			31.4
pH	7.2	8.0 7.7	7.8 7.5	8.2 7.8	8.3 7.9	8.3 7.7	8.4			
Cond	557	548	521	508	495	459				

	0	1	2	3	4	5	6	7	8	Total
(4)	0	0	0	9	0	11	15			35
80	0	0	0	7	0	9	17			43
	0	0	0	6	0	11	12			29
	0	0	0	4	8	9	0			21
	0	0	0	5	11 +1	0	14			31
	0	0	0	3 →	10	12	11			36
	0	0	0	5	8	0 D				13
	0	0	0	6	10	0	11			27
	0	0	0	0	8	10	0			18
	0	0	0	4	8	0	10			22
DO	5.7 6.5	6.5	6.3 6.7	6.9 6.4	6.6	6.5	6.4 6.7	6.5		
Temp	24.7	24.1 24.1	24.2 25.1	24.8 25.3	25.1	24.5	25.3 24.7	24.8		17.5
pH	7.1	8.0 7.7	7.8 7.5	8.3 7.7	8.4	7.8	8.4 7.7	8.5		
Cond	6.42	6.67	596	585	6.12	516				
(5)	0	0	0	7	0	13	17			37
100	0	0	0	4	0	8	14			26
	0	0	0	6	9	CS Eec 7 0	12			27
	0	0	0	3	10	7	0			20
	0	0	0	5 +1	0	11	12			29
	0	0	0	1	9	7 D				17
	0	0	0	6	10	12	0			28
	0	0	0	4	8	0	7			19
	0	0	0	0	9	8	0			17
	0	0	0	4	9	6.4 0	11			24
DO	5.4 6.5	6.4	6.3 6.6	7.1 6.2	6.6	6.5	8.5 6.7	6.5		
Temp	24.7	24.1 24.1	24.2 25.1	24.8 25.4	25.1	24.6	25.3 24.7	24.8		24.4
pH	7.1	8.0 7.7	7.8 7.4	8.3 7.7	8.5	7.8	8.5 7.8	8.6		
Cond	728	711	6.94	649	620	573				
Algae	ABS/ARC	ABS/ARC	ABS/ARC	ABS/ARC	ABS/ARC	1832/ARC				
YCT	1806	1806	1806	1806	1806	1806				
H_2O	—	—	—	—	—	—				
Initials	THW	THW	CS	CS	THW	CS	CS			

	Eff #1	Eff #2	Eff #3	Rec'g #1	Rec'g #2	Rec'g #3	Recon #1	Recon #2	Recon #3
Hardness									
Alkalinity									
Chlorine									
Ammonia									

1. Exposure Chamber
   - Total Capacity: 30 ml
   - Test Solution Volume: 15 ml
   - Test Solution Surface Area: _____ cm^2
   - Water Depth (constant): _____ cm
   - (cyclic): _____ to _____ cm
2. Feeding Schedule
   - Not fed: _____
   - Fed Daily: X
   - Fed Irregularly: _____
   - Food Used: YCT, algae
3. Aeration
   - #1 None: _____ Before Use: _____ (_____ minutes @ ~100 bubbles/min)
   - #2 None: _____ Before Use: _____ (_____ minutes @ ~100 bubbles/min)
   - #3 None: _____ Before Use: _____ (_____ minutes @ ~100 bubbles/min)
4. Screened Animal Enclosers
   - Not Used: X
   - Used: _____ cm diameter
5. Condition/appearance of surviving organisms at end of test (i.e., alive but immobile; loss of orientation; erratic movement; etc,): _____

6. Comments: Active & mobile

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

1	2	3	4	5	6	7	8	9	10
A1	A3 Eec	A4	A5	A6	A8	A10	B1	B3	B5

Ceriodaphnia Survival and Reproduction Test-7 Day Survival

Start Date:	9/7/2018	Test ID:	418208M1	Sample ID:	EFFFIN-Effluent Final
End Date:	9/13/2018	Lab ID:		Sample Type:	EFF1-POTW
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
20	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
40	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
60	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
80	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000

Conc-%	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical
D-Control	1.0000	1.0000	0	10	10	10		
20	1.0000	1.0000	0	10	10	10	1.0000	0.0500
40	1.0000	1.0000	0	10	10	10	1.0000	0.0500
60	1.0000	1.0000	0	10	10	10	1.0000	0.0500
80	0.9000	0.9000	1	9	10	10	0.5000	0.0500
100	0.9000	0.9000	1	9	10	10	0.5000	0.0500

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test Treatments vs D-Control	100	>100		1

Reviewed by:

Ceriodaphnia Survival and Reproduction Test-7 Day Survival

Start Date:	9/7/2018	Test ID:	418208M1	Sample ID:	EFFFIN-Effluent Final
End Date:	9/13/2018	Lab ID:		Sample Type:	EFF1-POTW
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
20	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
40	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
60	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
80	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	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	10	1.0000	1.0000
20	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	10	1.0000	1.0000
40	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	10	1.0000	1.0000
60	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	10	1.0000	1.0000
80	0.9000	0.9000	0.9948	0.5236	1.0472	16.644	10	0.9000	0.9000
100	0.9000	0.9000	0.9948	0.5236	1.0472	16.644	10	0.9000	0.9000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.05)	3.66133	0.895	-4.7381	23.3311
Equality of variance cannot be confirmed				

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL	Skew
IC05	70.000			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	9/7/2018	Test ID:	418208M1	Sample ID:	EFFFIN-Effluent Final
End Date:	9/13/2018	Lab ID:		Sample Type:	EFF1-POTW
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	34.000	31.000	25.000	27.000	35.000	30.000	21.000	22.000	22.000	16.000
20	31.000	30.000	34.000	29.000	30.000	21.000	19.000	32.000	24.000	23.000
40	36.000	34.000	34.000	27.000	35.000	28.000	16.000	19.000	19.000	24.000
60	34.000	42.000	38.000	24.000	36.000	26.000	30.000	25.000	37.000	22.000
80	35.000	43.000	29.000	21.000	31.000	36.000	13.000	27.000	18.000	22.000
100	37.000	26.000	27.000	20.000	29.000	17.000	28.000	19.000	17.000	24.000

			Transform: Untransformed					1-Tailed		
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	26.300	1.0000	26.300	16.000	35.000	23.511	10			
20	27.300	1.0380	27.300	19.000	34.000	18.761	10	-0.320	2.287	7.136
40	27.200	1.0342	27.200	16.000	36.000	27.447	10	-0.288	2.287	7.136
60	31.400	1.1939	31.400	22.000	42.000	22.023	10	-1.634	2.287	7.136
80	27.500	1.0456	27.500	13.000	43.000	33.339	10	-0.385	2.287	7.136
100	24.400	0.9278	24.400	17.000	37.000	25.935	10	0.609	2.287	7.136

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.05)	0.83894	0.895	0.02104	-0.7507
Bartlett's Test indicates equal variances (p = 0.64)	3.38361	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	100	>100		1	7.1355	0.27131	52.51	48.687	0.38265	5, 54

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	9/7/2018	Test ID:	418208M1	Sample ID:	EFFFIN-Effluent Final
End Date:	9/13/2018	Lab ID:		Sample Type:	EFF1-POTW
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	34.000	31.000	25.000	27.000	35.000	30.000	21.000	22.000	22.000	16.000
20	31.000	30.000	34.000	29.000	30.000	21.000	19.000	32.000	24.000	23.000
40	36.000	34.000	34.000	27.000	35.000	28.000	16.000	19.000	19.000	24.000
60	34.000	42.000	38.000	24.000	36.000	26.000	30.000	25.000	37.000	22.000
80	35.000	43.000	29.000	21.000	31.000	36.000	13.000	27.000	18.000	22.000
100	37.000	26.000	27.000	20.000	29.000	17.000	28.000	19.000	17.000	24.000

			Transform: Untransformed					Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
D-Control	26.300	1.0000	26.300	16.000	35.000	23.511	10	28.050	1.0000
20	27.300	1.0380	27.300	19.000	34.000	18.761	10	28.050	1.0000
40	27.200	1.0342	27.200	16.000	36.000	27.447	10	28.050	1.0000
60	31.400	1.1939	31.400	22.000	42.000	22.023	10	28.050	1.0000
80	27.500	1.0456	27.500	13.000	43.000	33.339	10	27.500	0.9804
100	24.400	0.9278	24.400	17.000	37.000	25.935	10	24.400	0.8699

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.05)	0.83894	0.895	0.02104	-0.7507
Bartlett's Test indicates equal variances (p = 0.64)	3.38361	15.0863		

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL	Skew
IC05	85.500			
IC10	94.548			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			

Appendix 3 – Data Sheets for the *Ceriodaphnia dubia* Mock 2 Test

WET TEST REPORT FORM – CHRONIC MOCK 2 CaSO4

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

Permit No.: CO-0000213

Test Type: Routine ☐ TIE ☒

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

Test Start Time	Test Start Date	Test End Time	Test End Date
1245	09-10-2018	1330	09-16-2018

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

Dilution(s) - % Effluent

Measurements	Control (0%)	20%	40%	60%	80%	100%
% 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	90	100	90	100	100	100
% Survival for day 6	90	90	90	100	100	100
Mean 3 Brood Total	25.7	26.5	26.0	25.5	26.5	22.0

Hardness (mg/L) – Recon Water: 95
Alkalinity (mg/L) – Recon Water: 57
pH (initial/final) – Control: 8.0/7.9 100%: 6.8/7.9

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 Corey Sutton

Signature *Date* 09/24/18

SeaCrest Group
Louisville, CO

Ceriodaphnia Chronic Benchsheet

Form #: 101a
Effective: January 2009

Permittee: TIE; MOCK B (2 EE) Lab #: 418 208.M3 Site: NEFPDES 013
IWC %: Template #: 5 Dilution Water: MH18-018 Sample Date:
Age & Source: cerio 1018 091018 Test Start: 091018 1245 Test End: 091618 1330
Test Conditions:

	0	1	2	3	4	5	6	7	8	Total
(C) 0	0	0	0	7	0	8 D				15
	0	0	0	3	8	9	0			20
	0	0	0	8	5	0	14			27
	0	0	0	6	6	10+1	0			23
	0	0	0	6	9	8+1	0			24
	0	0	0	0	6	12	15			33
	0	0	0	10	10	11+1	0			32
	0	0	0	5	0	9	14			28
	0	0	0	3	9	10+3	0			25
	0	0	0	6	9	0	16			31
DO	7.0	6.4 6.9	6.1 6.9	6.3 6.8	7.0 6.9	6.5 6.9	6.1			STAT 25
Temp	25.0	25.6 24.2	25.7 25.1	25.4 24.7	25.8 24.6	25.1 24.3	24.4		13	27.0
pH	8.0	8.2 7.8	8.0 8.0	7.9 7.9	7.9 7.8	8.1 8.1	7.9		11.0	ACCP
Cond	309	308	304	307	311	304				256
(1) 20	0	0	0	5 +2	6	14	19			40
	0	0	0	7	6+2	0	11			31
	0	0	0	0	0	10	16			26
	0	0	0	2	11	0	OD			13
	0	0	0	6	7+1	0	14			28
	0	0	0	0	4	9	9			22
	0	0	0	7	9	13	0			29
	0	0	0	7	8	12	13			28
	0	0	0	0	9	12	0			21
	0	0	0	3	8+1	0	15			27
DO	6.9	6.4 6.8	6.1 6.9	6.3 6.8	7.0 6.9	6.5 6.8	6.1			26.5
Temp	25.0	25.6 24.2	25.7 25.0	25.4 24.7	25.8 24.6	25.1 24.4	24.4			
pH	7.4	8.2 7.7	8.0 7.8	7.9 7.8	7.9 7.7	8.0 8.0	7.9			
Cond	614	580	597	611	615	582				
	0	1	2	3	4	5	6	7	8	Total
(2) 40	0	0	0	9	0	11	15			35
	0	0	0	9	11	0	1			21
	0	0	0	6	6	0	12			24
	0	0	0	7	9	0	12			28
	0	0	0	5	11	5 D				21
	0	0	0	0	5	13	9			30
	0	0	0	4	7	9	10			20
	0	0	0	7	1 +8		16			32
	0	0	0	8	12	9	0			29
	0	0	0	4	6	0	10			20
DO	6.7	6.4 6.7	6.2 6.9	6.3 6.8	7.0 6.9	6.5 6.8	6.1			26.0
Temp	25.0	25.6 24.3	25.7 24.7	25.4 24.8	25.8 24.6	25.1 24.6	24.4			
pH	7.2	8.1 7.5	7.9 7.7	7.9 7.6	7.9 7.6	8.0 7.8	7.9			
Cond	877	867	821	878	853	847				
(3) 60	0	0	0	11 12	0	10	14			37
	0	0	0	6	10	6	0			22
	0	0	0	5	6+2	0	7			20
	0	0	0	6	8	8+1	0			23
	0	0	0	5	10	7	0			19
	0	0	0	1 +6	7	13	11			32
	0	0	0	7	9	9	0			25
	0	0	0	5	8+2	0	12			27
	0	0	0	7	7	7	0			21
	0	0	0	4	9	0	16			29
DO	6.5	6.5 6.6	6.2 6.9	6.4 6.9	7.1 6.9	6.4 6.7	6.1			26.5
Temp	25.0	25.6 24.3	25.7 24.8	25.4 24.9	25.8 24.6	25.1 24.7	24.4			
pH	7.0	8.1 7.3	7.9 7.5	7.8 7.5	7.9 7.5	7.9 7.7	7.9			
Cond	1101	1087	1073	1092	1108	1075				

	0	1	2	3	4	5	6	7	8	Total
(4)	0	0	0	0	10	0	4			24
80	0	0	0	5	10	15	6			30
	0	0	0	5	10	0	14			29
	0	0	0	6	8	0	10			24
	0	0	0	5	10	10 +1	6			26
	0	0	0	1 →	8	12	9			30
	0	0	0	7	10	9	0			26
	0	0	0	5	8	6	8			21
	0	0	0	7	10	0	10			27
	0	0	0	5	9 +1	0	13			28
DO	6.4	6.5 6.5	6.3 6.9	6.4 6.9	7.1 6.9	6.4 6.6	6.1			
Temp	25.0	25.6 24.4	25.7 24.7	25.4 24.9	25.8 24.7	25.1 24.9	24.4			765
pH	6.9	8.0 7.2	7.8 7.4	7.8 7.5	7.9 7.4	7.8 7.7	7.9			
Cond	1338	1311	1280	1310	1320	1290				
(5)	0	0	0	6	0	6	13			25
100	0	0	0	5	7	0	0			12
	0	0	0	5	9	8	17			30
	0	0	0	6	8	0	10			24
	0	0	0	7	10	9	0			26
	0	0	0	2 →	6	10	8			26
	0	0	0	6	12	9	0			27
	0	0	0	6	0	0	5			11
	0	0	0	3	11	5	0			19
	0	0	0	6	0	9	5			20
DO	6.2	6.5 6.5	6.3 6.9	6.4 6.9	7.1 6.9	6.4 6.6	6.1			
Temp	25.1	25.1 24.5	25.7 24.6	25.4 25.0	25.8 24.7	25.1 25.0	24.4			220
pH	6.8	8.0 7.0	7.8 7.5	7.8 7.4	7.9 7.4	7.8 7.6	7.9			
Cond	1533	1523	1498	1512	1542	1508				
Algae	ABS/ARO	ARO / ABS	ABS/ARO	ABS/ARO	ABS/ARO	ABS/ARO				
YCT	18006	18006	18006	18006	18006	18006				
H_2O	—	—	—	—	—	—				
Initials	CS	MW	CS	CS	MW	CS	CS			

	Eff #1	Eff #2	Eff #3	Rec'g #1	Rec'g #2	Rec'g #3	Recon #1	Recon #2	Recon #3
Hardness									
Alkalinity									
Chlorine									
Ammonia									

1. Exposure Chamber
   - Total Capacity: 30 ml
   - Test Solution Surface Area: _____ cm^2
   - Test Solution Volume: 15 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: _____ Before Use: _____ (_____ minutes @ ~100 bubbles/min)
   - #2 None: _____ Before Use: _____ (_____ minutes @ ~100 bubbles/min)
   - #3 None: _____ Before Use: _____ (_____ minutes @ ~100 bubbles/min)
4. Screened Animal Enclosers
   - Not Used: X
   - Used: _____ cm diameter
5. Condition/appearance of surviving organisms at end of test (i.e., alive but immobile; loss of orientation; erratic movement; etc,): _____

6. Comments: Active & Mobile

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

1	2	3	4	5	6	7	8	9	10
A1	A2	A6	A7	A9	A10	B1	B3	B4	B6

Ceriodaphnia Survival and Reproduction Test-7 Day Survival

Start Date:	9/10/2018	Test ID:	418208M2	Sample ID:	EFFFIN-Effluent Final
End Date:	9/16/2018	Lab ID:	SCG-Seacrest Group	Sample Type:	EFF1-POTW
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
ON-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
SN--Control	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
20	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
40	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000
60	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
80	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Conc-%	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical
ON-Control	1.0000	1.1111	0	10	10	10	0.5619	
SN--Control	0.9000	1.0000	1	9	10	10	*	
20	0.9000	1.0000	1	9	10	10	0.7632	0.0500
40	0.9000	1.0000	1	9	10	10	0.7632	0.0500
60	1.0000	1.1111	0	10	10	10	0.5000	0.0500
80	1.0000	1.1111	0	10	10	10	0.5000	0.0500
100	1.0000	1.1111	0	10	10	10	0.5000	0.0500

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	100	>100		1
Treatments vs SN--Control				

Reviewed by:_____

Ceriodaphnia Survival and Reproduction Test-7 Day Survival

Start Date:	9/10/2018	Test ID:	418208M2	Sample ID:	EFFFIN-Effluent Final
End Date:	9/16/2018	Lab ID:	SCG-Seacrest Group	Sample Type:	EFF1-POTW
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
ON-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
SN--Control	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
20	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
40	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000
60	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
80	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	1.0000	1.0000	1.0000	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
ON-Control	1.0000	1.1111	1.0472	1.0472	1.0472	0.000	10		
SN--Control	0.9000	1.0000	0.9948	0.5236	1.0472	16.644	10	0.9500	1.0000
20	0.9000	1.0000	0.9948	0.5236	1.0472	16.644	10	0.9500	1.0000
40	0.9000	1.0000	0.9948	0.5236	1.0472	16.644	10	0.9500	1.0000
60	1.0000	1.1111	1.0472	1.0472	1.0472	0.000	10	0.9500	1.0000
80	1.0000	1.1111	1.0472	1.0472	1.0472	0.000	10	0.9500	1.0000
100	1.0000	1.1111	1.0472	1.0472	1.0472	0.000	10	0.9500	1.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.05)	3.53057	0.895	-3.8686	14.5011
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.33)	1	2.10092		

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL	Skew
IC05	>100			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	9/10/2018	Test ID:	418208M2	Sample ID:	EFFFIN-Effluent Final
End Date:	9/16/2018	Lab ID:	SCG-Seacrest Group	Sample Type:	EFF1-POTW
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
ON-Control	15.000	20.000	27.000	23.000	24.000	33.000	32.000	28.000	25.000	31.000
SN--Control	20.000	27.000	23.000	24.000	33.000	32.000	28.000	25.000	31.000	
20	40.000	31.000	26.000	13.000	28.000	22.000	29.000	28.000	21.000	27.000
40	35.000	21.000	24.000	28.000	21.000	30.000	20.000	32.000	29.000	20.000
60	37.000	22.000	20.000	23.000	19.000	32.000	25.000	27.000	21.000	29.000
80	24.000	30.000	29.000	24.000	26.000	30.000	26.000	21.000	27.000	28.000
100	25.000	12.000	30.000	24.000	26.000	26.000	27.000	11.000	19.000	20.000

			Transform: Untransformed						1-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
ON-Control	25.800	0.9556	25.800	15.000	33.000	21.834	10	*		
SN--Control	27.000	1.0000	27.000	20.000	33.000	16.355	9			
20	26.500	0.9815	26.500	13.000	40.000	26.579	10	-0.275	2.287	5.820
40	26.000	0.9630	26.000	20.000	35.000	21.144	10	-0.079	2.287	5.820
60	25.500	0.9444	25.500	19.000	37.000	22.660	10	0.118	2.287	5.820
80	26.500	0.9815	26.500	21.000	30.000	11.002	10	-0.275	2.287	5.820
100	22.000	0.8148	22.000	11.000	30.000	29.066	10	1.493	2.287	5.820

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.05)	0.58998	0.895	-0.0996	0.13229
Bartlett's Test indicates equal variances (p = 0.27)	6.36627	15.0863		
The control means are not significantly different (p = 0.61)	0.51243	2.10982		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs ON-Control	100	>100		1	5.82007	0.22558	29.0167	32.3907	0.49058	5, 54

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	9/10/2018	Test ID:	418208M2	Sample ID:	EFFFIN-Effluent Final
End Date:	9/16/2018	Lab ID:	SCG-Seacrest Group	Sample Type:	EFF1-POTW
Sample Date:		Protocol:	EPAFW02-EPA/821/R-02-01	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
ON-Control	15.000	20.000	27.000	23.000	24.000	33.000	32.000	28.000	25.000	31.000
SN--Control	20.000	27.000	23.000	24.000	33.000	32.000	28.000	25.000	31.000	
20	40.000	31.000	26.000	13.000	28.000	22.000	29.000	28.000	21.000	27.000
40	35.000	21.000	24.000	28.000	21.000	30.000	20.000	32.000	29.000	20.000
60	37.000	22.000	20.000	23.000	19.000	32.000	25.000	27.000	21.000	29.000
80	24.000	30.000	29.000	24.000	26.000	30.000	26.000	21.000	27.000	28.000
100	25.000	12.000	30.000	24.000	26.000	26.000	27.000	11.000	19.000	20.000

			Transform: Untransformed					Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
ON-Control	25.800	0.9556	25.800	15.000	33.000	21.834	10	26.150	1.0000
SN--Control	27.000	1.0000	27.000	20.000	33.000	16.355	9		
20	26.500	0.9815	26.500	13.000	40.000	26.579	10	26.150	1.0000
40	26.000	0.9630	26.000	20.000	35.000	21.144	10	26.000	0.9943
60	25.500	0.9444	25.500	19.000	37.000	22.660	10	26.000	0.9943
80	26.500	0.9815	26.500	21.000	30.000	11.002	10	26.000	0.9943
100	22.000	0.8148	22.000	11.000	30.000	29.066	10	22.000	0.8413

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.05)	0.58998	0.895	-0.0996	0.13229
Bartlett's Test indicates equal variances (p = 0.27)	6.36627	15.0863		
The control means are not significantly different (p = 0.61)	0.51243	2.10982		

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL	Skew
IC05	85.788			
IC10	92.325			
IC15	98.863			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			

Appendix 4 – QA/QC and Reference Toxicant Test Chart

Quality Assurance Check List – Chronic Whole Effluent Toxicity Test

Client: Elk Ridge Mining and Reclamation **SeaCrest Sample No.:** 418208.B

Species Tested: *Ceriodaphnia dubia*

	Start Date of Test (*Ceriodaphnia dubia*)
Baseline	09-07-2018
Mock 1	09-07-2018
Mock 2	09-10-2018

Test protocol conforms to CDPHE guidelines?	**Y**
Average test temp. ±1°C?	**Y**
DO level ≥4.0mg/L; no super-saturation?	**Y**
Survival in control ≥80%?	**Y**
Ceriodaphnia dubia neonates <24-hours old?	**Y**
Appropriate reference toxicity test conducted?	**Y**
Lab. Ref. Tox. test results within the confidence limits for the lab?	**Y**

Signature _____ *Date* 09/24/18
Position: Laboratory Director

SeaCrestGroup
AN ENVIRONMENTAL SERVICES COMPANY

METHOD QC

Method	Analyte	Date	LCS (rec)	%REC	%RPD	QC LIMITS
2320 B	Alkalinity - Total	8/2/2018	104.80%	99.59%	-0.74%	± 5.00%
2320 B	Alkalinity - Total	8/9/2018	103.60%	104.27%	0.85%	± 5.00%
2320 B	Alkalinity - Total	8/16/2018	102.40%	104.27%	-4.65%	± 5.00%
2320 B	Alkalinity - Total	8/27/2018	101.60%	100.89%	1.02%	± 5.00%
4500 NH_3 D	Ammonia	8/2/2017	101.40%	99.54%	2.09%	± 10.00%
4500 NH_3 D	Ammonia	8/9/2017	101.40%	99.02%	0.00%	± 10.00%
4500 NH_3 D	Ammonia	8/16/2018	98.00%	101.74%	-4.44%	± 10.00%
4500 NH_3 D	Ammonia	8/27/2018	103.80%	98.29%	4.40%	± 10.00%
4500 Cl D	Chlorine	8/30/2018	103.03%	96.97%	0.00%	± 5.00, ± 20.00%
2340 B	Hardness - Total	8/9/2018	101.75%	95.96%	2.99%	± 5.00%
2340 B	Hardness - Total	8/17/2018	101.75%	98.50%	3.64%	± 5.00%
2340 B	Hardness - Total	8/25/2018	98.25%	100.05%	4.28%	± 5.00%
2340 B	Hardness - Total	8/30/2018	105.26%	102.32%	-3.28%	± 5.00%

		Date	LCS (rec)	%REC M1	%REC M2	QC Limits
4500 O	DO - Winkler	8/2/2018	N/A	96.97%	100.00%	± 5.00%
4500 O	DO - Winkler	8/9/2018	N/A	97.06%	100.00%	± 5.00%
4500 O	DO - Winkler	8/16/2018	N/A	98.51%	101.54%	± 5.00%
4500 O	DO - Winkler	8/24/2018	N/A	101.56%	98.48%	± 5.00%
4500 O	DO - Winkler	8/30/2018	N/A	100.00%	103.03%	± 5.00%

		Date	Blank	%RPD	%REC MRS	QC Limits
2540 C	Dissolved Solids (TTL)	8/29/2018	99.9964%	0.38%	103.90%	±20%; ± 15%
2540 D	Suspended Solids (TTL)	8/24/2018	100.00022%	0.00%	90.77%	±20%, ± 15%

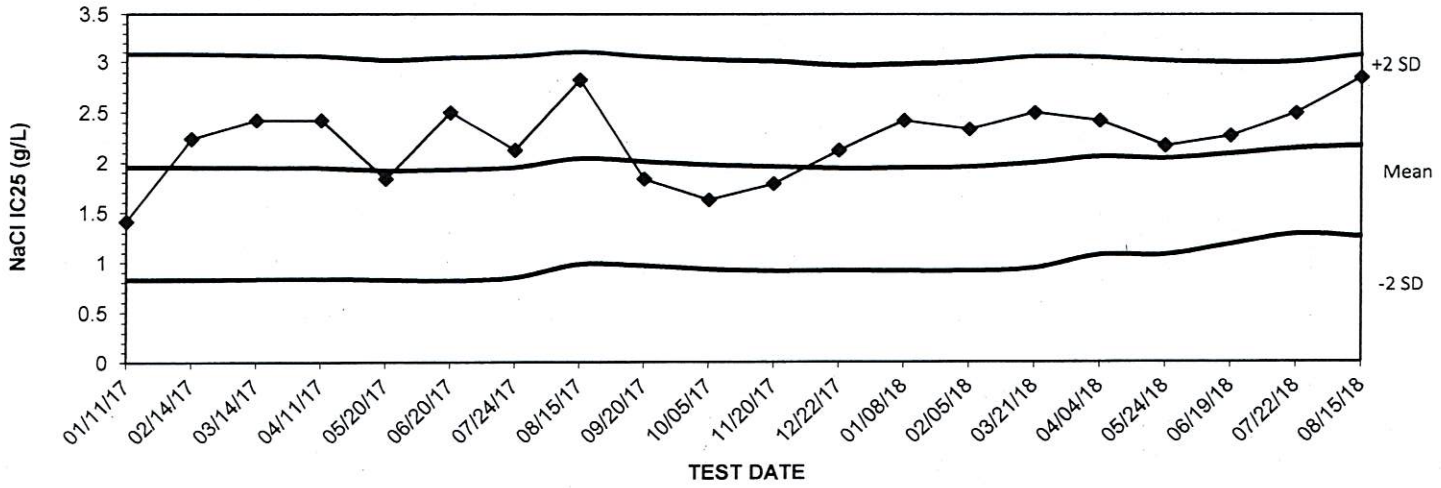
Signature: MPelse

Date: 09/05/18

Signature: [signature]

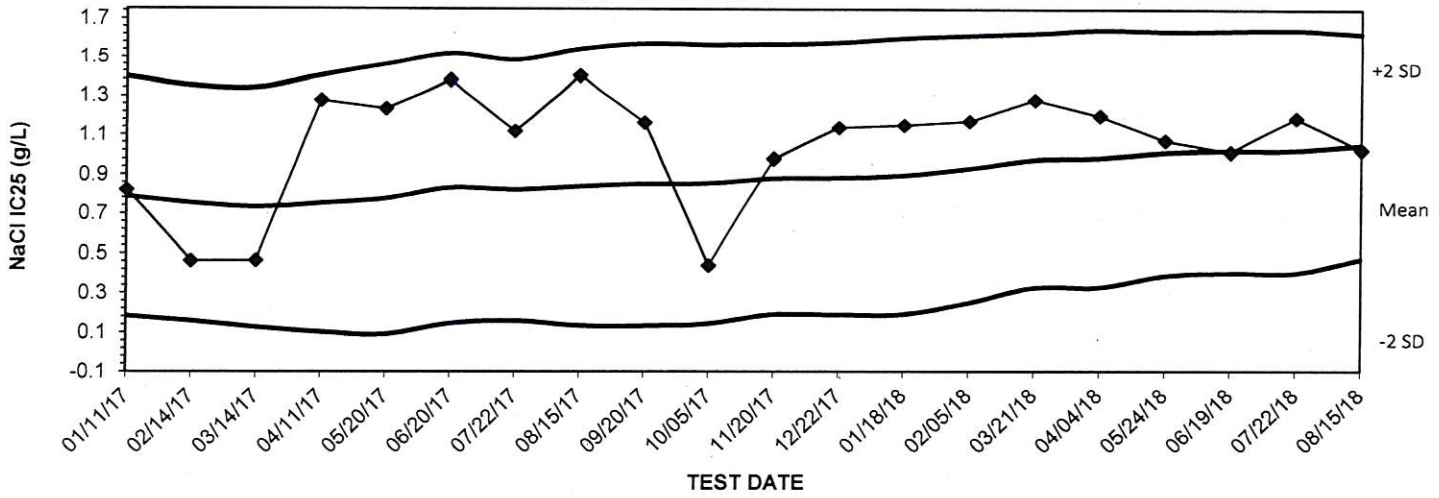
Date: 09/05/18

CERIODAPHNIA SURVIVAL IC25 NaCl REFTOX



Date	IC25	Mean	-2 SD	+2 SD
01/11/17	1.4100	1.9548	0.8280	3.0815
02/14/17	2.2353	1.9524	0.8284	3.0763
03/14/17	2.4211	1.9486	0.8318	3.0654
04/11/17	2.4211	1.9448	0.8353	3.0543
05/20/17	1.8333	1.9210	0.8252	3.0169
06/20/17	2.5000	1.9290	0.8182	3.0397
07/24/17	2.1250	1.9528	0.8480	3.0576
08/15/17	2.8284	2.0438	0.9834	3.1042
09/20/17	1.8333	2.0121	0.9693	3.0548
10/05/17	1.6250	1.9783	0.9334	3.0233
11/20/17	1.7857	1.9622	0.9162	3.0081
12/22/17	2.1250	1.9462	0.9223	2.9700
01/08/18	2.4211	1.9503	0.9191	2.9815
02/05/18	2.3333	1.9609	0.9183	3.0035
03/21/18	2.5000	2.0023	0.9459	3.0587
04/04/18	2.4211	2.0652	1.0795	3.0510
05/24/18	2.1719	2.0499	1.0827	3.0171
06/19/18	2.2692	2.0957	1.1883	3.0032
07/22/18	2.5000	2.1497	1.2907	3.0086
08/15/18	2.8571	2.1726	1.2640	3.0812

CERIODAPHNIA REPRODUCTION IC25 NaCl
REFTOX



Date	IC25	Mean	-2 SD	+2 SD
01/11/17	0.8196	0.7868	0.1816	1.3920
02/14/17	0.4621	0.7522	0.1564	1.3480
03/14/17	0.4637	0.7320	0.1254	1.3387
04/11/17	1.2762	0.7495	0.1006	1.3985
05/20/17	1.2328	0.7742	0.0902	1.4581
06/20/17	1.3758	0.8305	0.1432	1.5178
07/22/17	1.1166	0.8209	0.1569	1.4848
08/15/17	1.3990	0.8376	0.1322	1.5430
09/20/17	1.1638	0.8516	0.1313	1.5718
10/05/17	0.4375	0.8546	0.1423	1.5669
11/20/17	0.9798	0.8800	0.1893	1.5707
12/22/17	1.1379	0.8836	0.1881	1.5791
01/18/18	1.1509	0.8962	0.1904	1.6019
02/05/18	1.1717	0.9318	0.2475	1.6161
03/21/18	1.2810	0.9761	0.3242	1.6281
04/04/18	1.1996	0.9861	0.3266	1.6456
05/24/18	1.0705	1.0122	0.3852	1.6393
06/19/18	1.0137	1.0217	0.4008	1.6425
07/22/18	1.1886	1.0234	0.4008	1.6460
08/15/18	1.0246	1.0483	0.4711	1.6255

Appendix 5 – Analytical Data

Table 6. Analytical Data.

	Effluent	Filtrate	Precipitate	Desired Mock Conc. Based on Precipitate Concentration	Mock 1: CaCO3 Concentration	Mock 2: CaSO4 Concentration
Calcium	470	97.5	330	330	87.75	303.04
Iron	1.24	ND	1.3	1.3	0	0
Magnesium	197	181	12	12	10.84	8.51
Manganese	0.057	0.004	1.2	1.2	0.043	0.46
Strontium	NA	NA	5.5	5.5	2.7	2.9

SeaCrestGroup
AN ENVIRONMENTAL SERVICES COMPANY

September 25, 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 Mr. Fry and Ms. Johnson:

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 updated baseline test was intended to re-assess the potential toxicity of samples initially taken in March 2018. This report represents an updated baseline test as part of Phase II of the TIE protocol. The TIE was performed in accordance with EPA protocols for the conduct of such investigations.

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): Invoice
Report

SeaCrestGroup
AN ENVIRONMENTAL SERVICES COMPANY

500 S Arthur Ave. Suite 450
Louisville, CO 80027-3065
(303) 661-9324 Phone
(303) 661-9325 Fax

Invoice

Invoice Number:
418208.B_Base_Re-run
Invoice Date:
September 25, 2018

BILL TO:
Elk Ridge Mining and Rec. 27646 W. 5th Ave. Nucla, CO 81424

Customer Contact	Customer PO#	Terms	Customer ID
Mr. Thomas Fry	0212454	Payable Upon Receipt	Elk Ridge

QTY	Description	Unit Price	Extended Price
1	Chronic biomonitoring tests conducted on effluent from the New Horizon Mine 013 discharge using *Ceriodaphnia dubia* TIE updated baseline	$678.00	$678.00

Total: $678.00

All invoices are due and payable upon receipt.
Outstanding balances over 30-days are subject to a finance charge of 1.5% per month.

THANK YOU FOR YOUR BUSINESS!

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

Prepared for:

Ms. 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

September 25, 2018

TABLE OF CONTENTS

LIST OF TABLES

Chronic Toxicity Test Summary

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

Client: Elk Ridge Mining and Reclamation

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

Sample Number: 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

	Ceriodaphnia dubia
Test Initiation Time	1345
Test Initiation Date	08-02-2018
Test Completion Time	1408
Test Completion Date	08-08-2018

Dilution Water: • Moderately hard laboratory reconstituted water

Test Organism Source: • *Ceriodaphnia dubia*
SeaCrest Group

Reference Toxicant: • Sodium Chloride

Abstract of Results

Test Concentrations:	Control (0%), 40%, 60%, 80%, 100%
Number of Organisms/Concentration:	10 for *Ceriodaphnia dubia*
Replicates at each Concentration:	10 for *Ceriodaphnia dubia*

	Ceriodaphnia dubia
Test vessel size	30ml
Exposure volume	15ml
Pass/Fail Status	**N/A**
Temperature Range (°C)	24.2 – 25.9
Dissolved Oxygen Range (mg/L)	6.3 – 7.0
pH Range	7.6 – 8.2

	CONTROL	**100%**
Alkalinity (mg/L as $CaCO_3$)	58	511
Hardness (mg/L as $CaCO_3$)	80	1998
Total residual chlorine (mg/L)	<0.01	<0.01
Total ammonia (mg/L as NH_3)	<0.03	2.29

INTRODUCTION

Biomonitoring provides an effective means by which the toxicity of discharges from municipal, industrial and mining operations can be tested. Among the advantages of biomonitoring is the ability to test complex effluents containing a broad range of contaminants. Biomonitoring, when used in conjunction with chemical analyses, can generate data capable of identifying a much wider range of contaminants.

The Colorado Water Quality Control Division requires certain NPDES permittees to perform acute and/or chronic biomonitoring tests. The chronic test measures significant differences in lethality and in reproduction (*Ceriodaphnia dubia*) or growth (Fathead minnow – *Pimephales promelas*) between control and exposed organisms.

The present report discusses the results of chronic TIE Phase II Baseline Update test conducted on effluent from Elk Ridge Mining and Reclamation New Horizon Mine 013 discharge. These tests were conducted in August 2018.

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 Baseline Update test was started on 08-02-2018. The test was performed according to the procedures outlined in USEPA[3] and the Colorado Department of Public Health and Environment[4].

Individual *Ceriodaphnia dubia* were placed in 30ml plastic containers containing approximately 15ml of exposure medium. Ten replicates at each concentration were used. The animals were fed daily with the YCT mixture and an equal volume of the green algae *(Selenastrum capricornutum)*. The exposure medium was changed daily in each container and the number of young released overnight were counted and recorded. Young were removed from the containers daily and discarded. Routine measurements were made each day of temperature, dissolved oxygen and pH before and after the water changes.

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]).

Table 1. Statistics methods used in testing for significant differences in test parameters.

Species	Normality	Homogeneity
Ceriodaphnia dubia	Kolmogorov Test	Bartlett's Test
Statistical Difference		

	Survival	Reproduction	IC_{25}
Ceriodaphnia dubia	Fisher's Exact Test	Steel's Many-One Rank Test	ICp

RESULTS

Ceriodaphnia dubia Test Results

Test results for the *Ceriodaphnia dubia* are summarized in Table 2 and provided on the data sheets located in Appendix 2. Survival was 80% in the 100% effluent concentration and ranged from 80% - 100% in the remaining effluent concentrations. Control survival was 90%. No statistically significant mortality was measured in any effluent concentration when compared to the control. The No Observable Effect Concentration (NOEC) for lethality was 100%. The IC_{25} for lethality was >100%.

Table 2. Summary of *Ceriodaphnia dubia* test results. Ten animals were exposed at each concentration. An asterisk (*) denotes a statistically significant difference from the control.

Concentration	No. Surviving	Mean Births	Min.	Max.	Significant Difference	
					Lethality	Reprod.
Control (0%)	9	19.2	11	27		
40%	8	11.8	2	22		*
60%	10	8.0	4	13		*
80%	8	3.3	0	12		*
100%	8	0.7	0	3		*

Average numbers of neonates was 0.7 in the 100% effluent concentration and ranged from 3.3 – 11.8 in the remaining effluent concentrations. Average number of neonates in the control was 19.2 for statistical analyses and 20.1 for test acceptability criteria. A statistically significant difference in the number of neonates was found between the control and the 40%, 60%, 80%, and 100% effluent concentrations. The NOEC for reproduction was <40%. The IC_{25} for reproduction was estimated to be 25.9%.

Test Acceptability

Acceptable control survival was achieved in the test. Similarly, *Ceriodaphnia dubia* reproduction in control organisms met required levels. PMSD was within the required limits for an acceptable test (Table 4).

Table 4. PMSD for chronic test parameters.

	Survival		Reproduction		Result
	Ceriodaphnia dubia	Fathead Minnow	Lower bound	Upper bound	Test parameters deemed to be acceptable if numbers fall within limits for the test.
PMSD (% Minimum significant difference)	N/A	N/A	13	47	
			22.0		

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].

A baseline update toxicity test with the following dilution series, 0%, 40%, 60%, 80%, and 100%, was initiated on August 2, 2018. This test was performed to evaluate the persistence of toxicity to the *C. dubia* test species from the sample collected on March 7, 2018. This baseline updated test exhibited statistically significant sublethal toxicity, resulting in an estimated IC_{25} of 25.9%.

REFERENCES

1. **Hach Chemical Company.** 2008. *Hach's Water Analysis Handbook.* Fifth Edition. Hach Chemical Company, Loveland, Colorado. Digital Medium.
2. **APHA/AWWA/WEF.** 1998. *Standard Methods for the Examination of Water and Wastewater.* 20th Edition. American Public Health Association, Washington, D.C.
3. **USEPA.** 2002. *Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms.* EPA-821-R-02-013. 335 pp.
4. **CDPHE (Colorado Department of Public Health and Environment).** 1998. *Laboratory Guidelines for Conducting Whole Effluent Toxicity Tests.* Water Quality Control Division.
5. **USEPA.** 2000. *Method of Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing* (40 CFR Part 136). EPA/821/B-00/004.
6. **USEPA**. 2000. *Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications under the National Pollutant Discharge Elimination System Program.* EPA/833/R-00/003.

Appendix 1 – Data Sheets for the *Ceriodaphnia dubia* Test

WET TEST REPORT FORM – CHRONIC

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

Permit No.: CO-0000213

Test Type: Routine ☒ TIE ☒

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

Test Start Time	Test Start Date	Test End Time	Test End Date
1345	08-02-2018	1408	08-08-2018

Test Results	Lethality	Reproduction
NOEC	100%	<40%
	N/A	**N/A**
IC_{25}	>100%	25.9%
	N/A	**N/A**

Dilution(s) - % Effluent

Measurements	Control (0%)	40%	60%	80%	100%
% Survival for day 1	100	100	100	80	90
% Survival for day 2	100	100	100	80	90
% Survival for day 3	100	90	100	80	90
% Survival for day 4	100	90	100	80	90
% Survival for day 5	90	90	100	80	90
% Survival for day 6	90	80	100	80	80
Mean 3 Brood Total	19.2	11.8	8.0	3.3	0.7

Hardness (mg/L) – Receiving Water: N/A Effluent: 1998 Recon Water: 80
Alkalinity (mg/L) – Receiving Water: N/A Effluent: 511 Recon Water: 58
Chlorine (mg/L) – Effluent: <0.01 pH (initial/final) – Control: 8.0/8.0 100%: 7.8/7.8
Total Ammonia as NH_3 (mg/L) - Effluent: 2.29

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 Corey Sutton

Signature _____ *Date* 9/25/18

SeaCrest Group
Louisville, CO

418208C(U8

Ceriodaphnia Chronic Benchsheet

Form #: 101a
Effective: January 2009

Permittee: Baseline - Elk Ridge Lab #: 418 208 Site: NPDES 013
IWC %: 100 Template #: 5 Dilution Water: MH 18-015 Sample Date: 030718
Age & Source: cerio 2016 080218 Test Start: 080218 ~~1550~~ 1345 Test End: 080818 1408
Test Conditions:

	0	1	2	3	4	5	6	7	8	Total
(C) 0	0	0	0	0	6	10	8			24
	0	0	0	4	8	12	0			24
	0	0	0	0	7	8	0			15
	0	0	0	0	6	11	10			27
	0	0	0	4	5	12	0			21
	0	0	0	0	6	8	0			14
	0	0	0	0	4	3	5			12
	0	0	0	5	8	7	0			20
	0	0	0	0	5	10	9			24
	0	0	0	3 +1	0	7 D				11 ▷
DO	6.9	6.8 6.9	6.8 6.8	6.4 6.9	6.7 6.8	6.6 7.0	6.4			STAT
Temp	24.2	25.9 24.6	25.8 24.4	24.5 25.1	24.7 24.9	24.8 24.6	25.9			19.2
pH	8.0	8.1 8.0	7.9 8.0	8.2 8.0	8.2 8.1	7.9 8.0	8.0			ACCP
Cond	300	~~300~~ 303	307	306	307	310				20.11
(1) 40	0	0	0	0	4	7	3			14
	0	0	0	0	3	0	7			10
	0	0	0	4	3	0	00			7 ▷
	0	0	0	0	0	4	5			9
	0	0	0	0	6	9	7			22
	0	0	0	2 D						2 ▷
	0	0	0	3	3	0	9			15
	0	0	0	0	0	3	5			8
	0	0	0	0	0	7	11			18
	0	0	0	0	0	6	7			13
DO	6.9	6.8 6.9	6.8 6.8	6.4 6.9	6.7 6.8	6.6 7.3	6.4			
Temp	24.2	25.9 24.6	25.8 24.4	24.5 25.2	24.7 25.0	24.6 24.8	25.9			
pH	7.8	8.1 7.7	7.9 7.9	8.1 7.8	8.1 7.9	7.9 6.9	8.0			
Cond	1547	1591	1607	1560	1588	1596				118
	0	1	2	3	4	5	6	7	8	Total
(2) 00	0	0	0	0	2	0	4			6
	0	0	0	0	6	0	4			10
	0	0	0	0	3	0	5			8
	0	0	0	0	2	4	6			12
	0	0	0	0	4	3	0			7
	0	0	0	0	0	0	4			4
	0	0	0	0	2	0	6			8
	0	0	0	2	3	3	5			13
	0	0	0	0	4	0	4			8
	0	0	0	0	0	4	0			4
DO	6.8	6.8 6.8	6.8 6.8	6.7 7.0	6.8 6.8	6.5 7.5	6.5			
Temp	24.2	25.9 24.6	25.8 24.3	24.5 25.3	24.7 25.0	24.6 24.9	25.9			
pH	7.8	8.0 7.7	7.8 7.8	8.0 7.8	8.0 7.9	7.9 6.8	7.9			
Cond	1956	1997	1983	1962	1943	1975				8.0
(3) 30	0	~~0~~ D	0							0 ▷
	0	0	0	0	3	2	0			5
	0	~~0~~ D	0							0 ▷
	0	0	0	0	0	3	0			3
	0	0	0		0	2	0			2
	0	0	0	0	4	0	0			4
	0	0	0	2	3	4	3			12
	0	0	0	0	0	0	0			0
	0	0	0	0	4	0	0			4
	0	0	0	0	3	0	0			3
DO	6.6	6.9 6.8	6.9 6.9	6.8 7.0	6.8 6.7	6.5 7.8	6.5			
Temp	24.3	25.9 24.5	25.8 24.3	24.5 25.4	24.7 25.1	24.6 25.1	25.9			
pH	7.8	7.8 7.7	7.7 7.7	7.9 7.8	7.9 7.9	7.9 6.8	7.9			
Cond	2530	2610	2620	2590	2560	2640				3.3

	0	1	2	3	4	5	6	7	8	Total
(4) 100	0	0	0	0	0	0	0			0
	0	0	0	0	0	1	00			1
	0	~~0~~D	0							0
	0	0	0	0	0	0	0			0
	0	0	0	0	0	0	0			0
	0	0	0	0	0	0	3			3
	0	0	0	0	0	0	3			3
	0	0	0	0	0	0	0			0
	0	0	0	0	0	0	0			0
	0	0	0	0	0	0 10.7	0			0
DO	6.3	6.9 6.8	6.9 6.9	6.8 7.0	6.9 6.7	6.5 ~~6.0~~	6.5			
Temp	24.3	25.9 24.5	25.8 24.3	24.5 25.5	24.7 ~~25~~.1	24.6 25.1	25.9			
pH	7.8	7.7 7.7	7.6 7.7	7.8 7.7	7.8 7.9	7.9 ~~6.4~~	7.8			
Cond	3050	3090	3060	3040	3050	3180 80 EE				0.7
(5)	0	0	0							
	0	0	0							
	0	0	0							
	0	0	0							
	0	0	0							
	0	0	0							
	0	0	0							
	0	0	0							
	0	0	0							
	0	0	0							
DO										
Temp										
pH										
Cond										
Algae	ABS/ARO	ABS/ARO	ABS/ARO	ABS/ARO	ABS/ARO	ABS/ARO				
YCT	1805	1805	1805	1805	1805	1805				
H_2O	—	—	—	—	—	—	—			
Initials	THW	THW	THW	CS	CS	CS	CS			

	Eff #1	Eff #2	Eff #3	Rec'g #1	Rec'g #2	Rec'g #3	Recon #1	Recon #2	Recon #3
Hardness							80		
Alkalinity							58		
Chlorine							<0.01		
Ammonia							<0.03		

1. Exposure Chamber
   Total Capacity: 30 ml
   Test Solution Surface Area: _____ cm^2
   Test Solution Volume: 15 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: _____ cm diameter

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

6. Comments: Active & Mobile

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

1	2	3	4	5	6	7	8	9	10
A4	A5	A6	~~A10~~ EE B10	B6	B3	C1	C10	D1	E8

Ceriodaphnia Survival and Reproduction Test-7 Day Survival

Start Date:	8/2/2018	Test ID:	418208cd08	Sample ID:	
End Date:	8/8/2018	Lab ID:		Sample Type:	EFF1-POTW
Sample Date:		Protocol:	EPAF 94-EPA/600/4-91/002	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
ON-CONTROL	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
SN-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000
40	1.0000	1.0000	0.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000
60	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
80	0.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
100	1.0000	0.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Conc-%	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical
ON-CONTROL	1.0000	1.1111	0	10	10	10	0.5619	
SN-Control	0.9000	1.0000	1	9	10	10	*	
40	0.8000	0.8889	2	8	10	10	0.5000	0.0500
60	1.0000	1.1111	0	10	10	10	0.5000	0.0500
80	0.8000	0.8889	2	8	10	10	0.5000	0.0500
100	0.8000	0.8889	2	8	10	10	0.5000	0.0500

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	100	>100		1
Treatments vs SN-Control				

Reviewed by:

Ceriodaphnia Survival and Reproduction Test-7 Day Survival

Start Date:	8/2/2018	Test ID:	418208cd08	Sample ID:	
End Date:	8/8/2018	Lab ID:		Sample Type:	EFF1-POTW
Sample Date:		Protocol:	EPAF 94-EPA/600/4-91/002	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
)N-CONTROL	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
SN-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000
40	1.0000	1.0000	0.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000
60	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
80	0.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
100	1.0000	0.0000	0.0000	1.0000	1.0000	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
)N-CONTROL	1.0000	1.1111	1.0472	1.0472	1.0472	0.000	10		
SN-Control	0.9000	1.0000	0.9948	0.5236	1.0472	16.644	10	0.9000	1.0000
40	0.8000	0.8889	0.9425	0.5236	1.0472	23.424	10	0.9000	1.0000
60	1.0000	1.1111	1.0472	1.0472	1.0472	0.000	10	0.9000	1.0000
80	0.8000	0.8889	0.9425	0.5236	1.0472	23.424	10	0.8000	0.8889
100	0.8000	0.8889	0.9425	0.5236	1.0472	23.424	10	0.8000	0.8889

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.58694	0.947	-1.9289	2.18209
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.33)	1	2.10092		

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL	Skew
IC05	69.000			
IC10	78.000			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	8/2/2018	Test ID:	418208cd08	Sample ID:	
End Date:	8/8/2018	Lab ID:		Sample Type:	EFF1-POTW
Sample Date:		Protocol:	EPAF 94-EPA/600/4-91/002	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
ON-CONTROL	24.000	24.000	15.000	27.000	21.000	14.000	12.000	20.000	24.000	11.000
SN-Control	24.000	24.000	15.000	27.000	21.000	14.000	12.000	20.000	24.000	
40	14.000	10.000	7.000	9.000	22.000	2.000	15.000	8.000	18.000	13.000
60	6.000	10.000	8.000	12.000	7.000	4.000	8.000	13.000	8.000	4.000
80	0.000	5.000	0.000	3.000	2.000	4.000	12.000	0.000	4.000	3.000
100	0.000	1.000	0.000	0.000	0.000	3.000	3.000	0.000	0.000	0.000

			Transform: Untransformed					Rank	1-Tailed
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
ON-CONTROL	19.200	0.9547	19.200	11.000	27.000	29.950	10	*	
SN-Control	20.111	1.0000	20.111	12.000	27.000	26.246	9		
*40	11.800	0.5867	11.800	2.000	22.000	49.221	10	73.00	76.00
*60	8.000	0.3978	8.000	4.000	13.000	37.731	10	58.50	76.00
*80	3.300	0.1641	3.300	0.000	12.000	107.897	10	56.50	76.00
*100	0.700	0.0348	0.700	0.000	3.000	178.809	10	55.00	76.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.98083	0.947	0.16392	0.47533
Bartlett's Test indicates unequal variances (p = 6.72E-04)	19.3468	13.2767		
The control means are not significantly different (p = 0.72)	0.35837	2.10982		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	<40	40		
Treatments vs ON-CONTROL				

Reviewed by: KB

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	8/2/2018	Test ID:	418208cd08	Sample ID:	
End Date:	8/8/2018	Lab ID:		Sample Type:	EFF1-POTW
Sample Date:		Protocol:	EPAF 94-EPA/600/4-91/002	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
ON-CONTROL	24.000	24.000	15.000	27.000	21.000	14.000	12.000	20.000	24.000	11.000
SN-Control	24.000	24.000	15.000	27.000	21.000	14.000	12.000	20.000	24.000	
40	14.000	10.000	7.000	9.000	22.000	2.000	15.000	8.000	18.000	13.000
60	6.000	10.000	8.000	12.000	7.000	4.000	8.000	13.000	8.000	4.000
80	0.000	5.000	0.000	3.000	2.000	4.000	12.000	0.000	4.000	3.000
100	0.000	1.000	0.000	0.000	0.000	3.000	3.000	0.000	0.000	0.000

			Transform: Untransformed					1-Tailed		
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
ON-CONTROL	19.200	0.9547	19.200	11.000	27.000	29.950	10	*		
SN-Control	20.111	1.0000	20.111	12.000	27.000	26.246	9			
*40	11.800	0.5867	11.800	2.000	22.000	49.221	10	3.897	2.223	4.222
*60	8.000	0.3978	8.000	4.000	13.000	37.731	10	5.898	2.223	4.222
*80	3.300	0.1641	3.300	0.000	12.000	107.897	10	8.373	2.223	4.222
*100	0.700	0.0348	0.700	0.000	3.000	178.809	10	9.742	2.223	4.222

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.98083	0.947	0.16392	0.47533
Bartlett's Test indicates unequal variances (p = 6.72E-04)	19.3468	13.2767		
The control means are not significantly different (p = 0.72)	0.35837	2.10982		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs ON-CONTROL	<40	40			4.22212	0.2199	533.65	18.0311	4.3E-12	4, 45

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	8/2/2018	Test ID:	418208cd08	Sample ID:	
End Date:	8/8/2018	Lab ID:		Sample Type:	EFF1-POTW
Sample Date:		Protocol:	EPAF 94-EPA/600/4-91/002	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
)N-CONTROL	24.000	24.000	15.000	27.000	21.000	14.000	12.000	20.000	24.000	11.000
SN-Control	24.000	24.000	15.000	27.000	21.000	14.000	12.000	20.000	24.000	
40	14.000	10.000	7.000	9.000	22.000	2.000	15.000	8.000	18.000	13.000
60	6.000	10.000	8.000	12.000	7.000	4.000	8.000	13.000	8.000	4.000
80	0.000	5.000	0.000	3.000	2.000	4.000	12.000	0.000	4.000	3.000
100	0.000	1.000	0.000	0.000	0.000	3.000	3.000	0.000	0.000	0.000

			Transform: Untransformed					Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
)N-CONTROL	19.200	0.9547	19.200	11.000	27.000	29.950	10	19.200	1.0000
SN-Control	20.111	1.0000	20.111	12.000	27.000	26.246	9		
40	11.800	0.5867	11.800	2.000	22.000	49.221	10	11.800	0.6146
60	8.000	0.3978	8.000	4.000	13.000	37.731	10	8.000	0.4167
80	3.300	0.1641	3.300	0.000	12.000	107.897	10	3.300	0.1719
100	0.700	0.0348	0.700	0.000	3.000	178.809	10	0.700	0.0365

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.98083	0.947	0.16392	0.47533
Bartlett's Test indicates unequal variances (p = 6.72E-04)	19.3468	13.2767		
The control means are not significantly different (p = 0.72)	0.35837	2.10982		

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL		Skew
IC05*	5.189	3.716	3.581	14.008	5.9323
IC10*	10.378	5.352	7.162	28.016	3.4381
IC15*	15.568	6.474	10.744	40.343	2.2678
IC20*	20.757	7.206	14.325	42.713	1.5331
IC25*	25.946	7.545	17.906	45.073	1.0094
IC40	41.474	7.498	28.650	54.124	0.2130
IC50	51.579	7.538	35.812	62.433	-0.1329

* indicates IC estimate less than the lowest concentration

Appendix 2 – QA/QC and Reference Toxicant Test Chart

Quality Assurance Check List – Chronic Whole Effluent Toxicity Test

Client: Elk Ridge Mining and Reclamation **SeaCrest Sample No.:** 418208.B

Species Tested: *Ceriodaphnia dubia*

Start Date of Test
(*Ceriodaphnia dubia*)

08-02-2018

Sample received in lab properly preserved (0-6°C)?	**Y**
Sample delivered on ice or equivalent?	**Y**
Test initiated within 36-hours of collection?	**Y**
Test protocol conforms to CDPHE guidelines (*Ceriodaphnia dubia)*?	**Y**
Average test temp. ±1°C (*Ceriodaphnia dubia*)?	**Y**
DO level ≥4.0mg/L; no super-saturation (*Ceriodaphnia dubia*)?	**Y**
Survival in control ≥90%, ≥80% for chronic (*Ceriodaphnia dubia*)?	**Y**
Ceriodaphnia dubia neonates <24-hours old?	**Y**
Appropriate reference toxicity test conducted?	**Y**
Lab. Ref. Tox. test results within the confidence limits for the lab?	**Y**

Signature _____ *Date* 09/25/18
Position: Laboratory Director

SeaCrestGroup
AN ENVIRONMENTAL SERVICES COMPANY

METHOD QC

Method	Analyte	Date	LCS (rec)	%REC	%RPD	QC LIMITS
2320 B	Alkalinity - Total	7/7/2018	100.80%	102.91%	-0.58%	± 5.00%
2320 B	Alkalinity - Total	7/14/2018	100.80%	98.28%	0.40%	± 5.00%
2320 B	Alkalinity - Total	7/25/2018	102.40%	96.49%	4.48%	± 5.00%
2320 B	Alkalinity - Total	7/29/2018	101.60%	102.41%	-0.69%	± 5.00%
4500 NH_3 D	Ammonia	7/5/2018	103.80%	98.84%	1.58%	± 10.00%
4500 NH_3 D	Ammonia	7/12/2018	98.00%	103.59%	1.63%	± 10.00%
4500 NH_3 D	Ammonia	7/18/2018	97.60%	104.29%	1.91%	± 10.00%
4500 NH_3 D	Ammonia	7/25/2018	97.40%	102.93%	4.08%	± 10.00%
4500 Cl D	Chlorine	7/5/2018	96.97%	96.97%	0.00%	± 5.00, ± 20.00%
2340 B	Hardness - Total	7/6/2018	101.75%	96.29%	-3.92%	± 5.00%
2340 B	Hardness - Total	7/12/2018	101.75%	98.86%	4.88%	± 5.00%
2340 B	Hardness - Total	7/18/2018	96.49%	103.84%	0.58%	± 5.00%
2340 B	Hardness - Total	7/25/2018	96.49%	104.17%	-4.00%	± 5.00%

		Date	LCS (rec)	%REC M1	%REC M2	QC Limits
4500 O	DO - Winkler	7/5/2018	N/A	97.06%	100.00%	± 5.00%
4500 O	DO - Winkler	7/12/2018	N/A	98.41%	101.64%	± 5.00%
4500 O	DO - Winkler	7/19/2018	N/A	98.36%	101.69%	± 5.00%
4500 O	DO - Winkler	7/26/2018	N/A	98.53%	101.52%	± 5.00%

		Date	Blank	%RPD	%REC MRS	QC Limits
2540 C	Dissolved Solids (TTL)	7/26/2018	99.9940%	0.85%	105.45%	±20%, ± 15%
2540 D	Suspended Solids (TTL)	7/26/2018	100.00037%	0.00%	89.40%	±20%, ± 15%

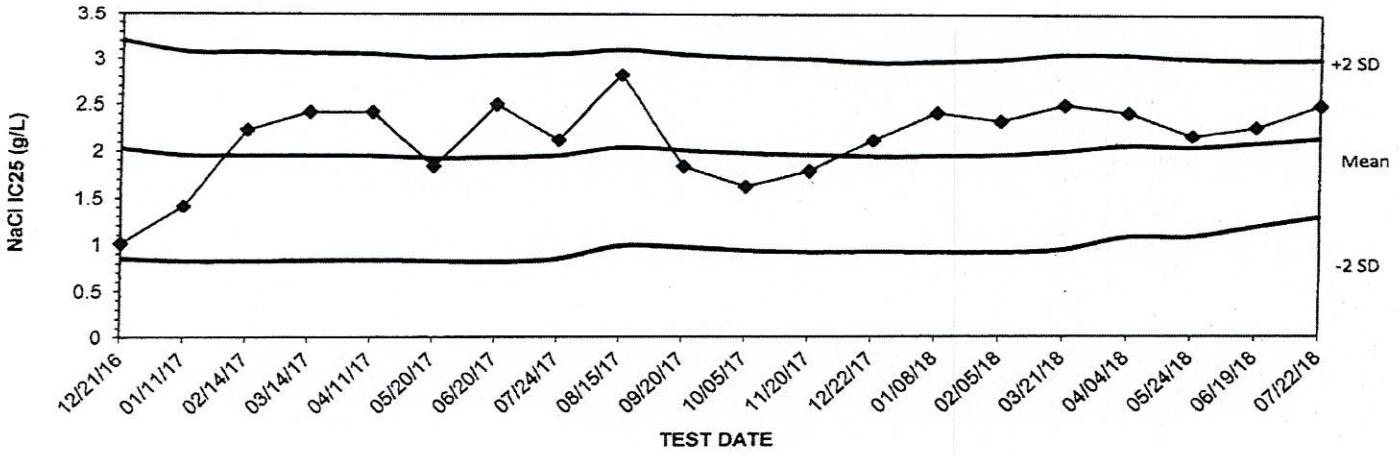
Signature: _____

Date: 07/31/18

Signature: _____

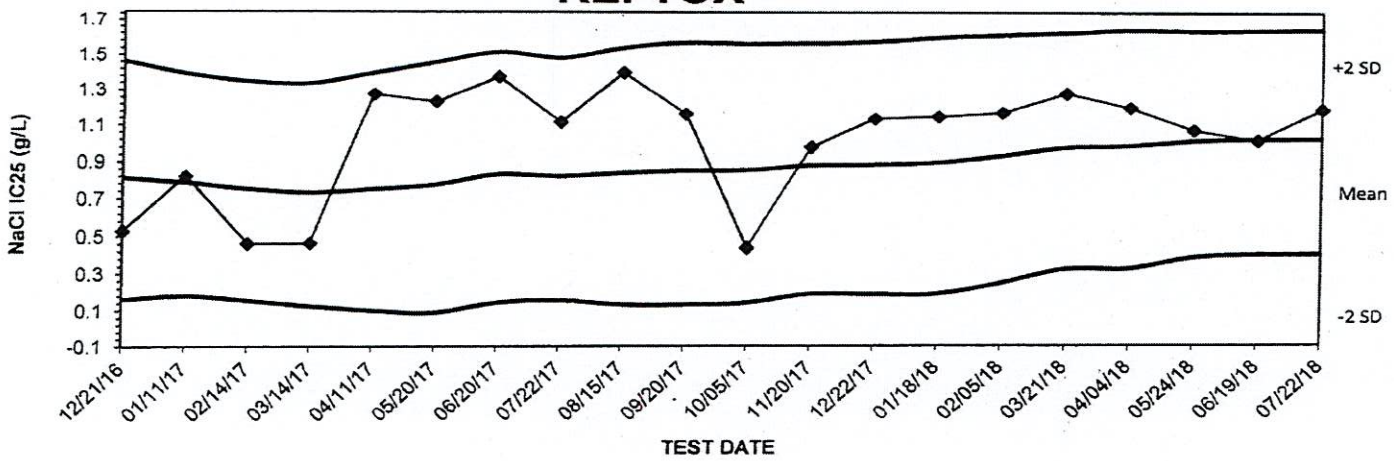
Date: 08/01/2018

CERIODAPHNIA SURVIVAL IC25 NaCl REFTOX



Date	IC25	Mean	-2 SD	+2 SD
12/21/16	1.0083	2.0278	0.8515	3.2041
01/11/17	1.4100	1.9548	0.8280	3.0815
02/14/17	2.2353	1.9524	0.8284	3.0763
03/14/17	2.4211	1.9486	0.8318	3.0654
04/11/17	2.4211	1.9448	0.8353	3.0543
05/20/17	1.8333	1.9210	0.8252	3.0169
06/20/17	2.5000	1.9290	0.8182	3.0397
07/24/17	2.1250	1.9528	0.8480	3.0576
08/15/17	2.8284	2.0438	0.9834	3.1042
09/20/17	1.8333	2.0121	0.9693	3.0548
10/05/17	1.6250	1.9783	0.9334	3.0233
11/20/17	1.7857	1.9622	0.9162	3.0081
12/22/17	2.1250	1.9462	0.9223	2.9700
01/08/18	2.4211	1.9503	0.9191	2.9815
02/05/18	2.3333	1.9609	0.9183	3.0035
03/21/18	2.5000	2.0023	0.9459	3.0587
04/04/18	2.4211	2.0652	1.0795	3.0510
05/24/18	2.1719	2.0499	1.0827	3.0171
06/19/18	2.2692	2.0957	1.1883	3.0032
07/22/18	2.5000	2.1497	1.2907	3.0086

CERIODAPHNIA REPRODUCTION IC25 NaCl
REFTOX



Date	IC25	Mean	-2 SD	+2 SD
12/21/16	0.5270	0.8117	0.1615	1.4619
01/11/17	0.8196	0.7868	0.1816	1.3920
02/14/17	0.4621	0.7522	0.1564	1.3480
03/14/17	0.4637	0.7320	0.1254	1.3387
04/11/17	1.2762	0.7495	0.1006	1.3985
05/20/17	1.2328	0.7742	0.0902	1.4581
06/20/17	1.3758	0.8305	0.1432	1.5178
07/22/17	1.1166	0.8209	0.1569	1.4848
08/15/17	1.3990	0.8376	0.1322	1.5430
09/20/17	1.1638	0.8516	0.1313	1.5718
10/05/17	0.4375	0.8546	0.1423	1.5669
11/20/17	0.9798	0.8800	0.1893	1.5707
12/22/17	1.1379	0.8836	0.1881	1.5791
01/18/18	1.1509	0.8962	0.1904	1.6019
02/05/18	1.1717	0.9318	0.2475	1.6161
03/21/18	1.2810	0.9761	0.3242	1.6281
04/04/18	1.1996	0.9861	0.3266	1.6456
05/24/18	1.0705	1.0122	0.3852	1.6393
06/19/18	1.0137	1.0217	0.4008	1.6425
07/22/18	1.1886	1.0234	0.4008	1.6460