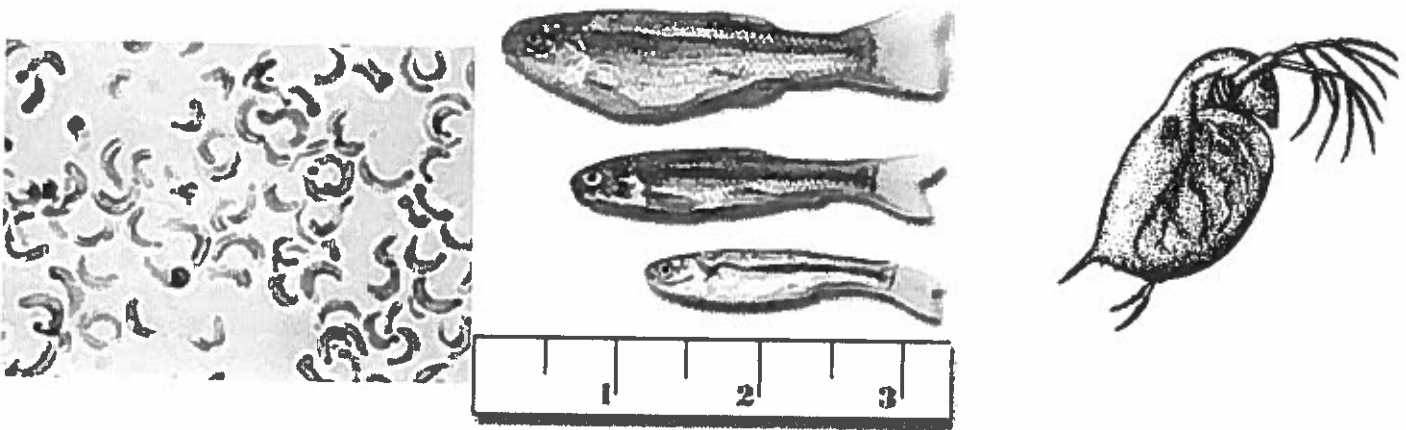


BIOMONITORING REPORT
FOR
CITY OF CALDWELL WWTP

LAB #1802032
PERMIT # ID0021504



January 2018

PREPARED BY:

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SUMMARY OF ANALYSES
CITY OF CALDWELL WWTP
JANUARY 2018
PERMIT # ID0021504

The results for the *Ceriodaphnia dubia* reproduction study:

NOEC:	100%
LOEC:	>100%
IC25:	>100%
TUc:	1

The results for the *Ceriodaphnia dubia* survival study:

NOEC:	100%
LOEC:	>100%
IC25:	>100%
TUc:	1

Statistical analyses of survival and reproduction data for test method 1002.0 demonstrated that all concentrations tested were not significantly different from the controls and displayed no chronic toxicity.

Introduction

Toxicity analysis consisting of chronic bioassay EPA Test Method 1002.0 was conducted on effluent samples collected by the City of Caldwell WWTP. Samples were collected January 16, January 18, and January 19, 2018, as 24-hour effluent composites. Once collected, samples were sent immediately to Analytical Laboratories, Inc. for analyses. Effluent composites were collected in one-gallon jugs for solution renewal water and in one liter cubitainers for water chemistries testing. Samples were chilled during transport by the addition of cold packs to the coolers. Method 1002.0, utilizing the freshwater flea *Ceriodaphnia dubia*, was conducted on January 16, 2018 and completed on January 23, 2018. Testing was conducted according to Short-Term Methods for Estimating the Chronic Toxicity of Effluents and receiving Waters to Freshwater Organisms, Fourth Edition October 2002 EPA-821-R-02-013 and Standard Methods for the Examination of Water and Wastewater, 19th Edition.

Methods and Materials

Test methods are designed to estimate and measure chronic toxicity of whole and partial effluents to the model freshwater aquatic organisms, freshwater flea *Ceriodaphnia dubia* in a 7-day static renewal test. Test water was collected as 24-hour effluent composites using mechanical sampling equipment. Samples were then transported to the laboratory for analyses. Effluent was used; whole or combined, with artificially prepared dilution water to prepare dilution series. Dilution water was prepared (20% v/v Perrier Mineral Water in deionized water) to produce a moderately hard dilution of control water. Water was prepared in bulk 24 hours prior to analyses and was aerated continuously to increase dissolved oxygen.

For Test Method 1002.0, less than 24hr neonate *Ceriodaphnia dubia* were sent from Aquatic Biosystems Inc., Fort Collins, Colorado. Neonates were selected from a composite pool, inspected, and arranged in five dilutions and a control. Analyses at a static renewal were performed over seven consecutive days. Data obtained was used to determine NOEC, LOEC, IC25 and TUC for survival and reproduction (see Appendix I - Definition of Terms).

Test Design/Standard Conditions Method 1002.0

1. Test Type - static renewal (daily)
Collection #1 – Renewal Day 1 and 2 – January 16, 2018
Collection #2 – Renewal Day 3 and 4 – January 18, 2018
Collection #3 – Renewal Day 5 and 6 – January 19, 2018
Day 7 – Final counts and statistical review
2. Temperature - 25 +/- 1 degree Celsius.
3. Light Quality - Environmental Chamber Fisher/11-67966
4. Light Intensity - Incubation chamber (as above)
5. Photoperiod - 16 hours light; 8 hours dark
6. Test Chamber - 30 ml Comet Heavyweight Plastic Portion Cups
7. Renewal - All dilutions daily
8. Age - Neonates/less than 24 hours
9. Organisms per chamber - One
10. Replicates - Ten chambers/control and each dilution
11. Feeding - 0.1 ml YTC; 0.1 ml *Selenastrum capricornutum* suspension - once daily
12. Dilution water - 20% v/v Perrier Mineral Water in deionized water
13. Concentrations used - 100%, 69.5%, 39%, 19.5%, 9.75 % and Control
14. Duration - Seven days
15. Endpoint - Survival/reproduction
16. Acceptability - 80% or greater of control survival / 60% of control produce 3rd brood / Average of 15 young/surviving female
17. Source of organisms - Aquatic Biosystems, Inc., Fort Collins, Colorado

Interpretation - Statistical Review

Statistical endpoints of data from Method 1002.0 were determined by the use of WET Analysis Spreadsheet v1.6.1. The EPA uses this spreadsheet to analyze valid WET test data to obtain acute and chronic test endpoints identified in EPA's WET test methods under the NPDES program. The test analyses performed by this statistical software compare the raw data of test and control concentrations and determine if there are any statistically significant differences. The software infers normality and variance from the raw data, and chooses the appropriate analytical methodology. This minimizes the effect that extraneous circumstances may have on the NOEC, LOEC, and IC25. TUc (Chronic Toxicity Units) values are calculated by the following formulas:

For survival endpoints: $100/\text{NOEC}$

For all other test endpoints: $100/\text{IC25}$

Results - Method 1002.0

During EPA Method 1002.0, survival and reproduction test using *Ceriodaphnia dubia*, survival and reproduction values from specific dilutions of collected effluent are measured and compared to values obtained from control individuals.

Statistical analyses of survival data for test method 1002.0 demonstrated that all concentrations tested were not significantly different from the controls and displayed no chronic toxicity.

Endpoints Determined - Method 1002.0

		<u>NOEC</u>	<u>LOEC</u>	<u>IC25</u>
<i>Ceriodaphnia dubia</i>	Survival	100%	>100%	>100%
	Reproduction	100%	>100%	>100%

The mortality was less than twenty percent (<20%) in controls. An average of at least 15 young per surviving female within three broods was established. Reproduction test was declared valid.

Test Quality Control

Quality control practices for effluent toxicity tests include certain precautions at each of the following steps:

1. Effluent sampling and handling. Sampling containers prepared as per section 7 of Methods for Measuring and Chronic Toxicity of Effluent to Freshwater and Marine Organisms were provided to client. Insulated transportation containers with cooling packs to chill samples were provided.
2. Condition of test organisms. Test organisms for Method 1002.0 are purchased from Aquatic Biosystems, Inc. in Fort Collins, Colorado, a state and federally approved aquatic test organism supplier.
3. Conditions of test equipment. All test equipment used is maintained according to manufacturer's specifications. Equipment such as balances, thermometers, etc. is calibrated annually by outside sources and certificates are maintained. All equipment maintenance and calibrations are recorded and archived.
4. Test conditions. Only test methods directly from EPA references or methodologies provided are used. Any deviations or alterations from these procedures are documented and approved prior to use.
5. Reference toxicants. Reference toxicants are used for both Methods 1000.0 and 1002.0. Sodium chloride is made up in dilution control water at prescribed concentrations and is used to determine toxicity for each method. Reference toxicants are run once per month to ensure consistency in test methodology. Quality control data is provided and a graphical representation over time is attached.
6. Record Keeping. All raw data, data evaluation, and statistical analysis are included in report to client. Original hardcopies along with all test records are maintained at laboratory for client or future reference.

LIST OF TABLES AND APPENDICES

Table I	-	<i>Ceriodaphnia dubia</i> Survival and Reproduction Summary Method 1002.0
Table II	-	<i>Ceriodaphnia dubia</i> Water Renewal Chemistries - Old pH and Dissolved Oxygen
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CITY OF CALDWELL WWTP

LAB ID #1802032

JANUARY 2018

METHOD 1002.0

Concentration	Initial Count	48-hour Count	96-hour Count	Final Count	Percent Survival	Average Remaining Young/Female
Control	10	10	10	10	100%	25.1
9.75%	10	10	10	10	100%	22.4
19.5%	10	10	10	10	100%	23.5
39%	10	10	10	10	100%	23.0
69.5%	10	10	10	8	80%	22.0
100%	10	10	10	9	90%	20.4

Table I: *Ceriodaphnia dubia* Survival And Reproduction Summary

Concentration Day	Control		9.75%		19.5%		39%		69.5%		100%	
	DO	pH	DO	pH	DO	pH	DO	pH	DO	pH	DO	pH
1	7.7	8.1	7.8	8.2	7.7	8.3	7.5	8.3	7.6	8.4	7.8	8.5
2	7.5	8.2	7.4	8.2	7.2	8.2	6.8	8.3	7.5	8.3	7.0	8.4
3	7.6	8.3	7.3	8.3	7.3	8.3	7.3	8.3	7.3	8.4	7.3	8.4
4	7.7	8.4	7.4	8.4	7.4	8.4	7.5	8.4	7.6	8.5	7.7	8.5
5	7.9	8.1	7.8	8.3	7.7	8.3	7.8	8.4	7.9	8.5	7.9	8.5
6	8.2	8.0	8.2	8.2	8.2	8.3	8.3	8.3	8.3	8.4	8.4	8.5
7	7.6	8.0	7.3	8.2	7.4	8.1	7.3	8.2	7.7	8.3	7.7	8.4

Table II: Water Chemistries, Daily Renewals – Old Water pH & Dissolved Oxygen Values

Concentration	CHLORINE RESIDUAL	ALKALINITY	CONDUCTIVITY	HARDNESS	AMMONIA	pH
	(mg/L)	(mg/L)	(umhos)	(mg/L)	(mg/L)	S.U.
1/16/2018	<0.10	194	780	153	0.05	7.2
1/18/2018	<0.10	187	756	153	<0.04	7.0
1/19/2018	<0.10	190	773	152	<0.04	7.4

Table III: Dilution Chemistries Summary

Definition of Terms

1. Safe Concentration. The highest concentrations of toxicant that will permit normal propagation of fish and other aquatic life in receiving waters, biologically defined rather than statistically.

2. NOEC (No-Observed Effect Concentration) - The highest concentration of toxicant in which the values for the observed parameters (survival, growth, reproduction) in which there is no statistically significant difference from controls and no observable effect on organism behavior or health.

3. LOEC (Lowest-Observed Effect Concentration) - The lowest concentration of toxicant in which the values for the observed parameters (survival, growth, reproduction) do have a statistical significant difference from controls. At this concentration there is evidence of toxicity.

4. TU_c (chronic toxicity units) –100/IC25

5. IC25 (Inhibition concentration - 25%) – Concentration where at least 25% of the organisms have some statistically significant effect.

Taken from: Short-Term methods for Estimating the Chronic Toxicity of Effluents and receiving Waters to Freshwater Organisms, Fourth Edition. October 2002. EPA-821-R-02-013.

Summary Sheet

Facility	Analytical Laboratories	Analyst	Chris Pate
Test ID	1802032 City of Caldwell WWTP	Species	Ceriodaphnia dubia (water flea)
Date	1/24/2018	Test Type	Reproduction
IWC Conc.			

Input

Replicate	Concentrations					
	<u>0</u>	<u>9.75</u>	<u>19.5</u>	<u>39</u>	<u>69.5</u>	<u>100</u>
1	29	17	27	12	7	19
2	22	21	19	23	19	23
3	25	25	30	24	21	24
4	15	24	20	26	25	14
5	24	26	23	28	29	20
6	30	25	20	27	24	15
7	25	17	24	23	28	22
8	25	23	20	22	23	24
9	25	24	21	23		23
10	31		31	22		

Mean	25.100	22.444	23.500	23.000	22.000	20.444
Stdev	4.557	3.395	4.403	4.397	6.908	3.779

Output

Statistical Data	Conc.	Mean	Stdev	CV	T-test
	0	25.100	4.557	0.182	
	9.75	22.444	3.395	0.151	NS
	19.5	23.500	4.403	0.187	NS
	39	23.000	4.397	0.191	NS
	69.5	22.000	6.908	0.314	NS
	100	20.444	3.779	0.185	NS

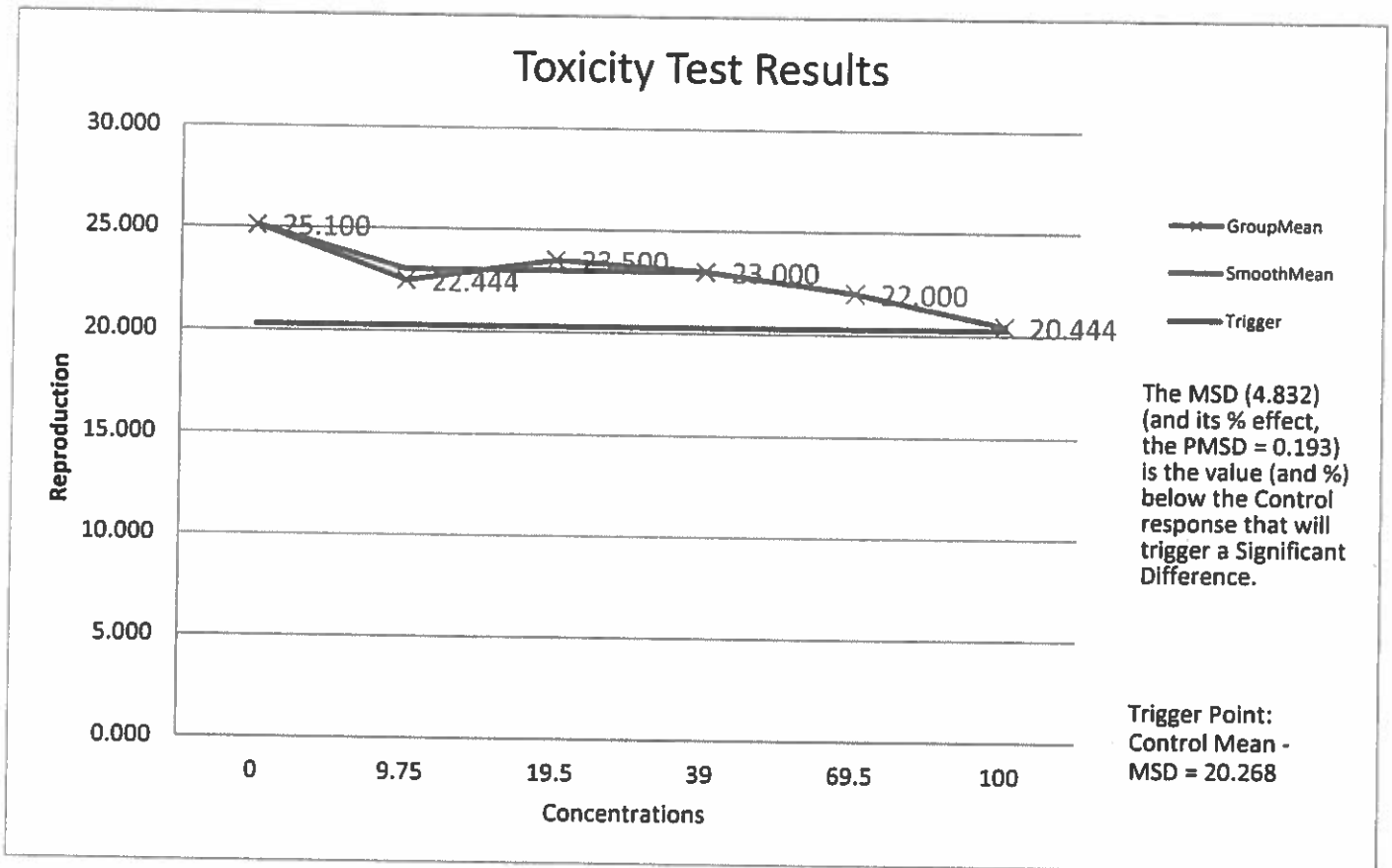
NOEC	LOEC	IC25	95% Confidence Intervals	
100	>100	>100	N/A	N/A

TST	Calculated t-value	Table t-value	Relative % Effect at IWC

MSD	PMSD
4.832	19.3%

Summary Sheet

Note - For statistical tests, "NS" indicates that the concentration is not statistically different from the control, while "Y" indicates that the concentration is statistically different from the control.



NOTICE

The United States Environmental Protection Agency (EPA), through its Office of Wastewater Management, funded and managed the development of the whole effluent toxicity (WET) Tool described here. This is a tool that calculates WET test endpoints for the EPA-approved WET test methods and is used by EPA internally for analyzing valid WET test data. Neither the EPA nor any of their employees, assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information or process disclosed. Furthermore, the WET Tool is supplied "as-is" without guarantee or warranty, expressed or implied, including without limitation, any warranty of merchantability or fitness for a specific purpose.

Summary Sheet

Facility	Analytical Laboratories	Analyst	Chris Pate
Test ID	1802032 City of Caldwell WWTP	Species	Ceriodaphnia dubia (water flea)
Date	1/24/2018	Test Type	Chronic Survival
IWC Conc.			

Input

Number of Organisms Exposed or Counted

Replicate	Concentrations					
	<u>0</u>	<u>9.75</u>	<u>19.5</u>	<u>39</u>	<u>69.5</u>	<u>100</u>
1	1	1	1	1	1	1
2	1	1	1	1	1	1
3	1	1	1	1	1	1
4	1	1	1	1	1	1
5	1	1	1	1	1	1
6	1	1	1	1	1	1
7	1	1	1	1	1	1
8	1	1	1	1	1	1
9	1	1	1	1	1	1
10	1	1	1	1	1	1

Number of Organisms Surviving or Responding

Replicate	Concentrations					
	<u>0</u>	<u>9.75</u>	<u>19.5</u>	<u>39</u>	<u>69.5</u>	<u>100</u>
1	1	1	1	1	1	1
2	1	1	1	1	1	1
3	1	1	1	1	1	1
4	1	1	1	1	1	1
5	1	1	1	1	0	1
6	1	1	1	1	1	1
7	1	1	1	1	1	1
8	1	1	1	1	1	1
9	1	1	1	1	1	0
10	1	1	1	1	0	1

Total Organisms	10	10	10	10	10	10
Total Responding	10	10	10	10	8	9
% Responding	100.0%	100.0%	100.0%	100.0%	80.0%	90.0%

Output

Summary Sheet

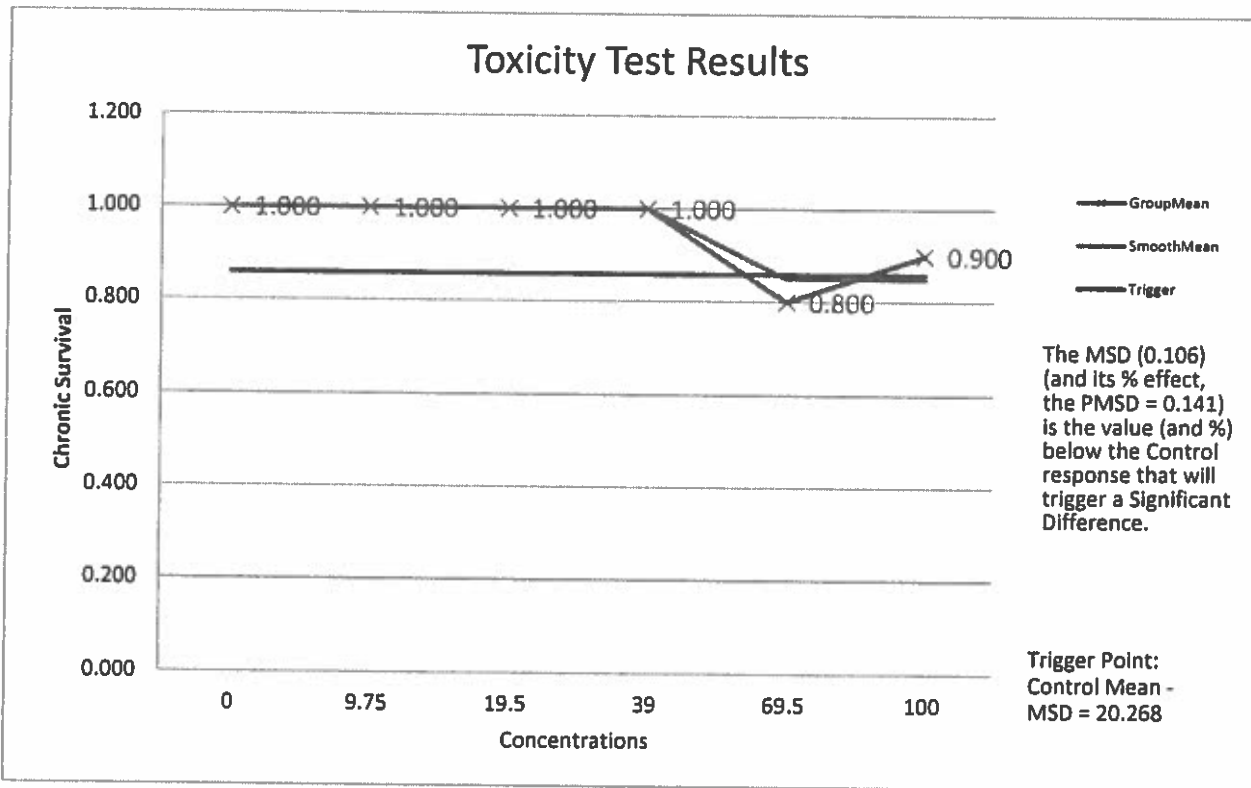
Statistical Data	Conc.	Mean	Stdev	CV	Steel test
	0	1.047	0.000	0.000	
Statistics are based on the transformed data used for endpoint calculations	9.75	1.047	0.000	0.000	NS
	19.5	1.047	0.000	0.000	NS
	39	1.047	0.000	0.000	NS
	69.5	0.942	0.221	0.234	NS
	100	0.995	0.166	0.166	NS

NOEC	LOEC	IC25	95% Confidence Intervals	
100	>100	>100	N/A	N/A

TST	Calculated t-value	Table t-value	Relative % Effect at IWC

MSD	PMSD
0.106	14.1%

Note - For statistical tests, "NS" indicates that the concentration is not statistically different from the control, while "Y" indicates that the concentration is statistically different from the control.



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Analytical Laboratories, Inc.

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Boise, Idaho 83703
Phone (208) 342-5515

Date Report Printed: 1/25/2018 7:08:10 AM
<http://www.analyticallaboratories.com>
These test results relate only to the items tested.

Laboratory Analysis Report

Sample Number: 1802032

Attn: SALVADOR ARREOLA
CALDWELL WASTEWATER
PO BOX 1179
CALDWELL, ID 83607

Collected By: R. HAWKER
Submitted By: B. MILLER

Source of Sample:
FE-C BIO-MONITORING DAY 1

Time of Collection: 8:13
Date of Collection: 1/16/2018
Date Received: 1/16/2018
Report Date: 1/25/2018

Field pH: Lab pH:
Field Temp: Temp Recvd in Lab: 7.3 °C

PWS#:
PWS Name:

Test Requested	MCL	Analysis Result	Units	MDL	Method	Date Completed	Analyst
Ceriodaphnia dubia		*			EPA 1002.0	1/24/2018	CP
Ammonia Direct (as N)		0.05	mg/L	0.04	EPA 350.1	1/22/2018	SMC
Alkalinity		194	mg/L		EPA 310.1	1/23/2018	SMC
Chlorine Residual, Cl2		<0.10	mg/L	0.10	EPA 330.5	1/16/2018	JD
Conductivity		780	umhos	2	EPA 120.1	1/16/2018	JMS
Hardness		153	mg/L	5.0	SM 2340	1/23/2018	SMC
pH		7.2	S.U.		SM 4500-H B	1/16/2018	JMS

Email: sarreola@ci.caldwell.id.us

Spencer Curtis / C.O. JAMES HIBBS

MCL = Maximum Contamination Level
MDL = Method/Minimum Detection Limit
UR = Unregulated

Thank you for choosing Analytical Laboratories for your testing needs.
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James Hibbs



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Date Report Printed: 1/25/2018 7:14:34 AM
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Laboratory Analysis Report

Sample Number: 1802540

Attn: SALVADOR ARREOLA
CALDWELL WASTEWATER
PO BOX 1179
CALDWELL, ID 83607

Collected By: R. HAWKER
Submitted By: B. MILLER

Source of Sample:
FE-C BIO-MONITORING DAY 2

Time of Collection: 7:32
Date of Collection: 1/18/2018
Date Received: 1/18/2018
Report Date: 1/25/2018

Field pH: Lab pH:
Field Temp: Temp Rcvd in Lab: 5.5 °C

PWS#:
PWS Name:

Test Requested	MCL	Analysis Result	Units	MDL	Method	Date Completed	Analyst
Ammonia Direct (as N)		<0.04	mg/L	0.04	EPA 350.1	1/22/2018	SMC
Alkalinity		187	mg/L		EPA 310.1	1/23/2018	SMC
Chlorine Residual, Cl2		<0.10	mg/L	0.10	EPA 330.5	1/18/2018	RME
Conductivity		756	umhos	2	EPA 120.1	1/18/2018	RME
Hardness		153	mg/L	5.0	SM 2340	1/23/2018	SMC
pH		7.0	S.U.		SM 4500-H B	1/18/2018	RME

Email: sarreola@ci.caldwell.id.us

James Hibbs / C.O. JAMES HIBBS

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MDL = Method/Minimum Detection Limit
UR = Unregulated



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Date Report Printed: 1/30/2018 8:06:40 AM
<http://www.analyticallaboratories.com>
These test results relate only to the items tested.

Laboratory Analysis Report

Sample Number: 1802819

Attn: SALVADOR ARREOLA
CALDWELL WASTEWATER
PO BOX 1179
CALDWELL, ID 83607

Collected By: R. HAWKER
Submitted By: C. PATE

Source of Sample:
FE-C BIO MONITORING DAY 3

Time of Collection: 7:47

Date of Collection: 1/19/2018

Date Received: 1/19/2018

Report Date: 1/30/2018

Field pH:

Lab pH:

PWS#:

Field Temp:

Temp Revd in Lab: 3.9 °C

PWS Name:

Test Requested	MCL	Analysis Result	Units	MDL	Method	Date Completed	Analyst
Ammonia Direct (as N)		<0.04	mg/L	0.04	EPA 350.1	1/29/2018	SMC
Alkalinity	190		mg/L		EPA 310.1	1/23/2018	SMC
Chlorine Residual, Cl2		<0.10	mg/L	0.10	EPA 330.5	1/19/2018	JH
Conductivity	773		umhos	2	EPA 120.1	1/19/2018	JH
Hardness	152		mg/L	5.0	SM 2340	1/23/2018	SMC
pH		7.4	S.U.		SM 4500-H B	1/19/2018	JH

Email: sarreola@ci.caldwell.id.us

SPEXEL CONTESTES | C.O. JAMES HIBBS

MCL = Maximum Contamination Level
MDL = Method/Minimum Detection Limit
UR = Unregulated

Thank you for choosing Analytical Laboratories for your testing needs.

If you have any questions about this report, or any future analytical needs, please contact your client manager:

James Hibbs

6	January 31, 2024	Complete Bidding Deliverable: The permittee will provide DEQ and EPA with written notice that the Bid has been awarded.
7	April 30, 2024	Start Construction Deliverable: The permittee will provide DEQ and EPA with a copy of the Notice to Proceed with construction.
8	April 30, 2026	Complete Construction Deliverable: The permittee will provide DEQ and EPA with written notice that the construction is completed.
9	September 30, 2026	Process Optimization and Achieve Final Effluent Limitation Deliverable: The permittee must achieve compliance with the final effluent limitations and provide DEQ and EPA with written notice of compliance with final effluent limitations.
Notes:		
1. The annual average total phosphorus concentration and load must be calculated as the sum of all daily discharges measured for total phosphorus during a calendar year, divided by the number of daily discharges measured for total phosphorus during that year.		
2. The annual average total phosphorus concentration and load must be reported on the December DMR.		

D. Whole Effluent Toxicity Testing Requirements

The permittee must conduct chronic toxicity tests on effluent samples from outfall 001. Testing must be conducted in accordance with subsections 1 through 7, below.

1. Toxicity testing must be conducted on 24-hour composite samples of effluent. In addition, a split of each sample collected must be analyzed for the chemical and physical parameters required in Part I.B, above, with a required effluent sampling frequency of once per month or more frequently, using the sample type required in Part I.B. For parameters for which grab samples are required in Part I.B, grab samples must be taken during the same 24-hour period as the 24-hour composite sample used for the toxicity tests. When the timing of sample collection coincides with that of the sampling required in Part I.B, analysis of the split sample will fulfill the requirements of Part I.B as well.
2. Chronic Test Species and Methods
 - a) For outfall 001, chronic tests must be conducted once per quarter. Quarters are defined as January – March, April through June, July – September, and October – December.
 - b) The permittee must conduct short-term tests with the water flea, *Ceriodaphnia dubia* (survival and reproduction test), the fathead minnow, *Pimephales promelas* (larval survival and growth test), and a green alga, *Selenastrum capricornutum* (growth test) for the first three suites of tests. After this screening period, monitoring must be conducted using the most sensitive species, which is defined below.

- (i) The most sensitive species is the species which, during the screening period, produces the greatest maximum toxicity result in chronic toxic units (TU_c), which is defined in Part I.D.2.d, below.
 - (ii) If all three species produce the identical maximum toxicity result (including no toxicity in 100% effluent) the permittee must use *Ceriodaphnia dubia* for subsequent tests.
 - (iii) If two species produce the identical maximum toxicity result, which is greater than 1.0 TU_c and also greater than the maximum toxicity result of the third species, the permittee may use either of the two species producing the greater maximum toxicity result for subsequent tests.
- c) The presence of chronic toxicity must be determined as specified in *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, Fourth Edition, EPA/821-R-02-013, October 2002.
- d) Results must be reported in TU_c (chronic toxic units), which is defined as follows:
- (i) For survival endpoints, $TU_c = 100/NOEC$.
 - (ii) For all other test endpoints, $TU_c = 100/IC_{25}$.
 - (iii) IC_{25} means "25% inhibition concentration." The IC_{25} is a point estimate of the toxicant concentration, expressed in percent effluent, that causes a 25% reduction in a non-quantal biological measurement (e.g., reproduction or growth) calculated from a continuous model (e.g., Interpolation Method).
 - (iv) $NOEC$ means "no observed effect concentration." The $NOEC$ is the highest concentration of toxicant, expressed in percent effluent, to which organisms are exposed in a chronic toxicity test [full life-cycle or partial life-cycle (short term) test], that causes no observable adverse effects on the test organisms (i.e., the highest concentration of effluent in which the values for the observed responses are not statistically significantly different from the controls).

3. Quality Assurance

- a) The toxicity testing on each organism must include a series of five test dilutions and a control. The dilution series must include the receiving water concentration (RWC), which is the dilution associated with the average monthly whole effluent toxicity limits, two dilutions above the RWC, and two dilutions below the RWC. The RWCs are:
 - (i) 62% effluent for April – June
 - (ii) 39% effluent for July – March Control, 9.75%, 19.5%, 39%, 69.5%, 100%
- b) All quality assurance criteria and statistical analyses used for chronic tests and reference toxicant tests must be in accordance with *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to*

Freshwater Organisms, Fourth Edition, EPA/821-R-02-013, October 2002, and individual test protocols.

- c) In addition to those quality assurance measures specified in the methodology, the following quality assurance procedures must be followed:
 - (i) If organisms are not cultured in-house, concurrent testing with reference toxicants must be conducted. If organisms are cultured in-house, monthly reference toxicant testing is sufficient. Reference toxicant tests must be conducted using the same test conditions as the effluent toxicity tests.
 - (ii) If either of the reference toxicant tests or the effluent tests do not meet all test acceptability criteria as specified in the test methods manual, the permittee must re-sample and re-test within 14 days of receipt of the test results.
 - (iii) Control and dilution water must be receiving water or lab water, as appropriate, as described in the manual. If the dilution water used is different from the culture water, a second control, using culture water must also be used. Receiving water may be used as control and dilution water upon notification of EPA and IDEQ. In no case shall water that has not met test acceptability criteria be used for either dilution or control.

4. Reporting

- a) The permittee must submit the results of the toxicity tests with the discharge monitoring reports (DMRs). Results must be reported on the DMRs for the last month of the quarter in which the samples were taken.
 - b) The report of toxicity test results must include all relevant information outlined in Section 10, Report Preparation, of *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, Fourth Edition, EPA/821-R-02-013, October 2002. In addition to toxicity test results, the permittee must report: dates of sample collection and initiation of each test; flow rate at the time of sample collection; and the results of the monitoring required in Part I.B of this permit, for parameters with a required monitoring frequency of once per month or more frequently.
5. Preparation of initial investigation toxicity reduction evaluation (TRE) workplan: By January 31, 2017, the permittee must submit to EPA a copy of the permittee's initial investigation TRE workplan. This plan shall describe the steps the permittee intends to follow in the event that chronic toxicity is detected above the applicable effluent limits in Table 1 of this permit, and must include at a minimum:
- a) A description of the investigation and evaluation techniques that would be used to identify potential causes/sources of toxicity, effluent variability, treatment system efficiency;

- b) A description of the facility's method of maximizing in-house treatment efficiency, good housekeeping practices, and a list of all chemicals used in operation of the facility; and
 - c) If a toxicity identification evaluation (TIE) is necessary, who will conduct it (i.e., in-house or other).
 - d) The initial investigation TRE workplan must be sent to the following address:
 - US EPA Region 10
 - Attn: NPDES WET Coordinator
 - 1200 Sixth Avenue
 - Suite 900 OWW-191
 - Seattle, WA 98101-3140
6. Accelerated testing: If chronic toxicity is detected above the applicable average monthly limit for whole effluent toxicity in Part I.B or I.C of this permit, the permittee must comply with the following:
- a) The permittee must conduct six more bi-weekly (every two weeks) chronic toxicity tests, over a 12-week period. This accelerated testing shall be initiated within 10 calendar days of receipt of the test results indicating the initial exceedance.
 - b) The permittee must notify EPA of the exceedance in writing at the address in Part I.C.5.d, above, within 5 calendar days of receipt of the test results indicating the exceedance. The notification must include the following information:
 - (i) A status report on any actions required by the permit, with a schedule for actions not yet completed.
 - (ii) A description of any additional actions the permittee has taken or will take to investigate and correct the cause(s) of the toxicity.
 - (iii) Where no actions have been taken, a discussion of the reasons for not taking action.
 - c) If none of the six accelerated chronic toxicity tests required under Part I.C.6.a are above the applicable average monthly limit in Part I.B or I.C of this permit, the permittee may return to the regular chronic toxicity testing cycle specified in Part I.D.2.a.
 - d) If any of the six accelerated chronic toxicity tests required under Part I.C.6.a are above the applicable average monthly limit in Part I.B or I.C of this permit, then the permittee must implement the initial investigation TRE workplan as described in Part I.D.7.
7. Implementation of Initial Investigation TRE Workplan
- a) The permittee must implement the initial investigation TRE workplan within 48 hours of the permittee's receipt of the accelerated toxicity test result demonstrating an exceedance of the applicable average monthly limit in Part I.B or I.C of this permit.

- (i) If implementation of the initial investigation workplan clearly identifies the source of toxicity to the satisfaction of EPA (e.g., a temporary plant upset), the permittee may return to the regular chronic toxicity testing cycle specified in Part I.D.2.a.
- (ii) If implementation of the initial investigation workplan does not clearly identify the source of toxicity to the satisfaction of EPA, then the permittee must begin implementation of further toxicity reduction evaluation (TRE) requirements in part I.D.8 below.

8. Detailed TRE/TIE

- a) If implementation of the initial investigation workplan does not clearly identify the source of toxicity to the satisfaction of EPA, then, in accordance with the permittee's initial investigation workplan and EPA manual EPA 833-B-99-002 (*Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants*), the permittee must develop as expeditiously as possible a more detailed TRE workplan, which includes:
 - (i) Further actions to investigate and identify the cause of toxicity;
 - (ii) Actions the permittee will take to mitigate the impact of the discharge and to prevent the recurrence of toxicity; and
 - (iii) A schedule for these actions.
- b) The permittee may initiate a TIE as part of the overall TRE process described in the EPA acute and chronic TIE manuals EPA/600/6-91/005F (Phase I), EPA/600/R-92/080 (Phase II), and EPA-600/R-92/081 (Phase III).
- c) If the detailed TRE/TIE clearly identifies the source of toxicity to the satisfaction of EPA, the permittee may return to the regular chronic toxicity testing cycle specified in Part I.D.2.a.

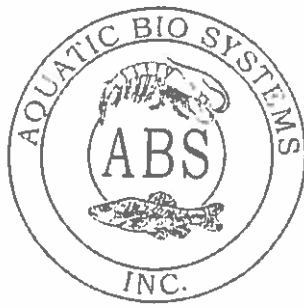
9. Inconclusive TRE/TIE

- a) If the detailed TRE described in Part I.D.8 is inconclusive, the permittee must conduct six bi-weekly (every two weeks) chronic toxicity tests, over a 12-week period. This accelerated testing shall be initiated within 10 calendar days of completing the detailed TRE/TIE.
- b) If none of the six accelerated chronic toxicity tests required under Part I.D.9.a exceed the applicable average monthly limit in Part I.B or I.C of this permit, the permittee may return to the regular chronic toxicity testing cycle specified in Part I.D.2.a.
- c) If any of the six accelerated chronic toxicity tests required under Part I.D.9.a exceed the applicable chronic toxicity trigger in Part I.D.6 of this permit, then the permittee must repeat the TRE/TIE process described in Part I.D.8.

E. Surface Water Monitoring

The permittee must conduct surface water monitoring. The program must meet the following requirements:

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524



Toll Free: 800/331-5916
Tel: 970/484-5091 Fax: 970/484-2514

ORGANISM HISTORY

DATE: 1/15/2018

SPECIES: Ceriodaphnia dubia

AGE: < 24 hour

LIFE STAGE: Neonate

HATCH DATE: 1/15/2018

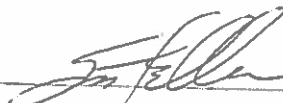
BEGAN FEEDING: Immediately

FOOD: YTC, Raphidocelis subcapitata*

Water Chemistry Record:

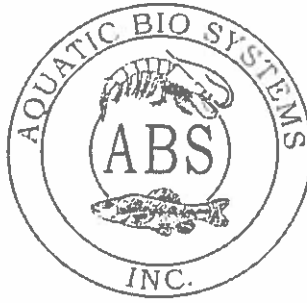
	Current	Range
TEMPERATURE:	<u>22°C</u>	<u>--</u>
SALINITY/CONDUCTIVITY:	<u>--</u>	<u>--</u>
TOTAL HARDNESS (as CaCO ₃):	<u>100 mg/l</u>	<u>--</u>
TOTAL ALKALINITY (as CaCO ₃):	<u>80 mg/l</u>	<u>--</u>
pH:	<u>8.20</u>	<u>--</u>

Comments: * Formerly known as *Pseudokirschneriella subcapitata* and *Selenastrum capricornutum*



Facility Supervisor

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524



Toll Free: 800/331-5916
Tel: 970/484-5091 Fax: 970/484-2514

Algae Preparation History

DATE: 1/15/2018

SPECIES: *Raphidocelis subcapitata**

INOCULATION DATE: 1/2/2018

HARVEST DATE: 1/8/2018

CONCENTRATION DATE: 1/10/2018

CELL COUNT (/ml): 3.0×10^7 cells/ml

Comments:

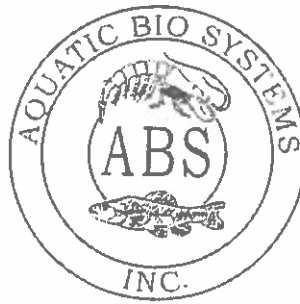
* Formerly known as *Pseudokirschneriella subcapitata* and *Selenastrum capricornutum*

** All concentrated algae diluted to proper cell count with reconstituted moderately hard DI water.



Supervisor

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524



Toll Free: 800/331-5916
Tel: 970/484-5091 Fax: 970/484-2514

YTC TOTAL SOLIDS MEASUREMENT
(Method from EPA/505/8-89-002a)

YTC Process Date: 1/10/2018; Best if used by 4/30/2018
Average Total Solids: 1810 mg/l

Ingredient Lot Numbers

Pines International® Wheat Grass: COCDW12S50; Zeigler Finfish Starter #1 (Lot 07/17/2017); Fleischmanns Yeast: G-3

EPA Required Toxic Metals and Pesticide Analyses*

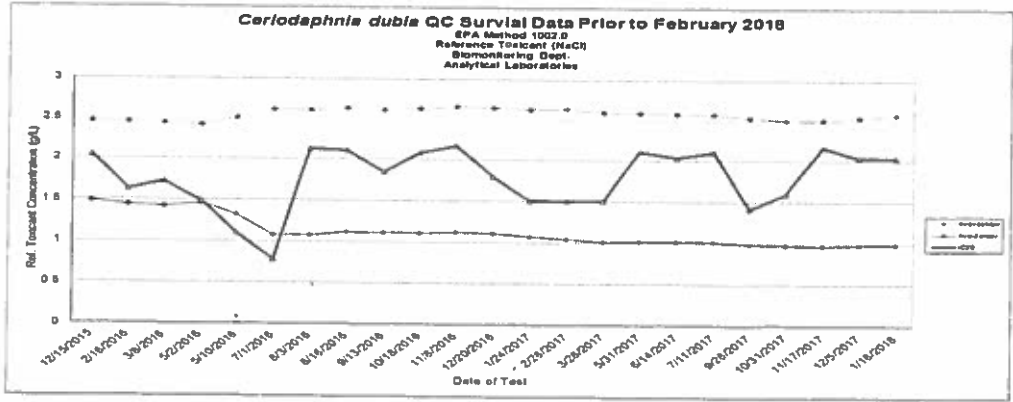
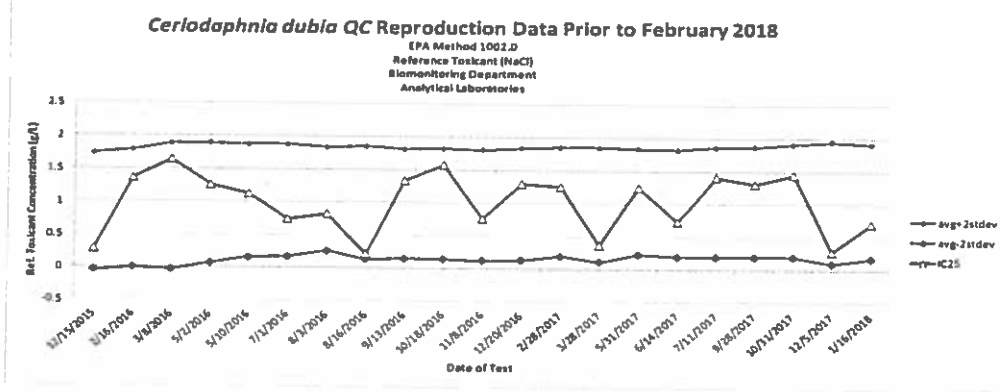
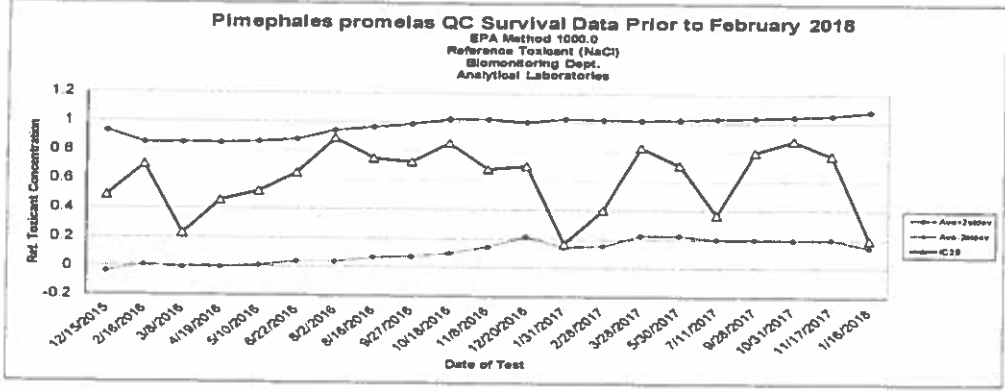
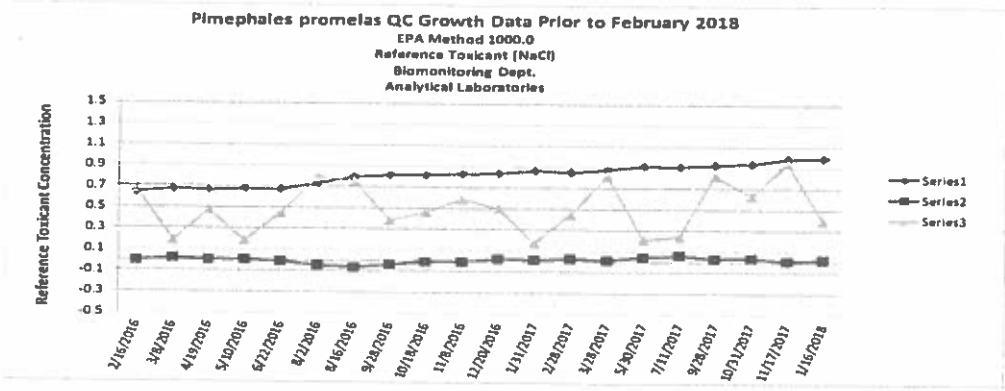
Analyzed Metals	Report Limits	Results (mg/L)
Aluminum	0.03	0.12
Arsenic	0.001	0.002
Cadmium	0.001	U
Chromium	0.005	.006
Copper	0.005	0.047
Iron	0.02	0.32
Lead	0.001	U
Mercury	0.001	U
Nickel	0.005	0.005
Silver	0.001	U
Zinc	0.01	0.18

Compounds	Report Limits	Results (ug/L)
Aldrin	0.5	U
alpha-BHC	0.5	U
beta-BHC	0.5	U
delta-BHC	0.5	U
gamma-BHC (Lindane)	0.5	U
alpha-Chlordane	0.5	U
gamma-Chlordane	0.5	U
4,4' - DDD	0.5	U
4,4' - DDE	0.5	U
4,4' - DDT	0.5	U
Dieldrin	0.5	U
Endosulfan I	0.5	U
Endosulfan II	0.5	U
Endosulfan sulfate	0.5	U
Endrin	0.5	U
Endrin aldehyde	0.5	U
Endrin ketone	0.5	U
Heptachlor	0.5	U
Heptachlor epoxide	0.5	U
Methoxychlor	0.5	U
Chlordane (technical)	5.0	U
Toxaphene	25	U
Aroclor-1016	5.0	U
Aroclor-1221	5.0	U
Aroclor-1232	5.0	U
Aroclor-1242	5.0	U
Aroclor-1248	5.0	U
Aroclor-1254	5.0	U
Aroclor-1260	5.0	U
Aroclor-1262	5.0	U
Aroclor-1268	5.0	U

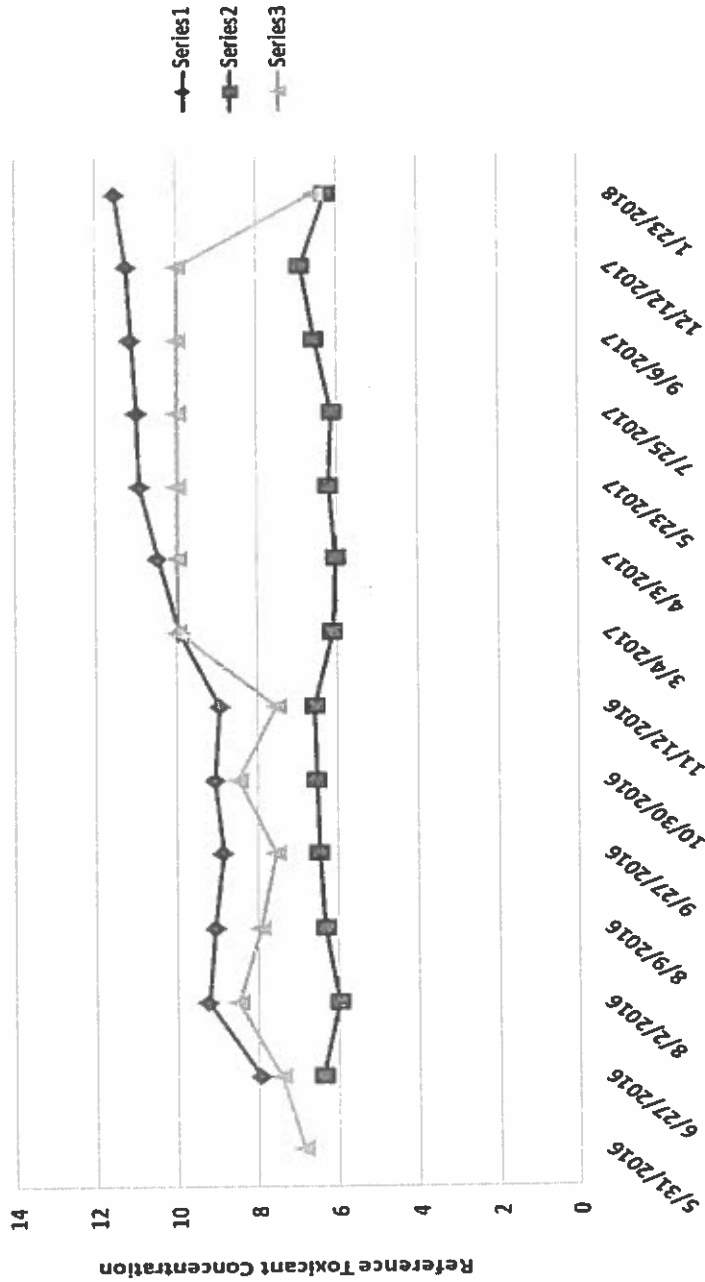
U - Indicates compound was analyzed for but not detected.
*Testing performed by Energy Labs, Billings, Montana

Literature Cited

1. Short-Term methods for Estimating the Chronic Toxicity of Effluents and receiving Waters to Freshwater Organisms, Fourth Edition. October 2002. EPA-821-R-02-013.
2. Methods for Measuring the Chronic Toxicity of Effluents to Freshwater and Marine Organisms, EPA/600/4-85/013, US EPA.
3. Standard Methods for the Examination of Water and Wastewater, 19 Edition, 1995, APHA, AWWA, WPCF.
4. Handbook for Analytical Quality Control in Water and Wastewater Laboratories, Environmental Monitoring and Support Laboratory, Cincinnati, EPA/600/4-79/019, US EPA



Selenastrum Capricornutum QC Data Prior to February 2018
EPA Method 1003.0
Reference Toxicant (NaCl)
Biomonitoring Department
Analytical Laboratories



BENCH SHEET FOR FATHEAD MINNOW INITIAL WEIGHT DATA EPA METHOD 1000.0

LAB ID#: January QC 2018 Test Start Date: 1/9/18

Drying Temp: 100°C

Weighing Date: 1/10/18 Test End Date: 1/16/18

Drying Time: 20 hrs

Location/Client: ALJ QC Jan. 2018

	Rep No.	Weight of Boat (g)	Boat and Dry Larvae (g)	Dry Weight of Larvae (g)	No. of Larvae	Mean Dry Weight of Larvae (mg)	Average
Initial	I1	1.2854	1.2862	0.0008	10	0.08	0.09 mg
	I2	1.2897	1.2908	0.0011	10	0.11	
	I3	1.2889	1.2898	0.0009	10	0.09	
	I4	1.2893	1.2902	0.0009	10	0.09	

Reviewed By: SC

Fathead Minnow QC Weight Data

Analyst: GP Test Month/Year: January 2018 Drying Temp: 100°C
 Weighing Date: 1/17/18 Drying Time: 18 hours

Conc.	Rep No.	Weight of Boat (g)	Boat and Dry Larvae (g)	Dry Weight of Larvae (g)	No. of Larvae	Mean Dry Weight of Larvae (mg)	Avg.-Init. = Avg. Wt. Gain (mg)
CONTROL	1	1.2777	1.2843	0.0046	10	0.46	
	2	1.2760	1.2813	0.0053		0.53	0.48mg - 0.09mg = 0.39mg
	3	1.2742	1.2787	0.0045		0.45	
	4	1.2690	1.2737	0.0047		0.47	
0.25g/L	X5	1.2944	1.2973	0.0029		0.29	
	X6	1.2637	1.2668	0.0031		0.31	0.38mg - 0.09mg = 0.29mg
	X7	1.2963	1.3010	0.0047		0.47	
	X8	1.2881	1.2927	0.0046		0.46	
1.5g/L	X9	1.2904	1.2933	0.0029		0.29	
	X10	1.2961	1.2987	0.0026		0.26	0.22mg - 0.09mg = 0.13mg
	X11	1.2982	1.2997	0.0015		0.15	
	X12	1.2864	1.2883	0.0019		0.19	
2.5g/L	X13	1.2944	1.2962	0.0018		0.18	
	X14	1.2972	1.2982	0.0010		0.10	0.20mg - 0.09mg = 0.11mg
	X15	1.2905	1.2933	0.0028		0.28	
	X16	1.2864	1.2887	0.0023		0.23	
3.5g/L	X17	1.2921	1.2945	0.0024		0.24	
	X18	1.2975	1.2999	0.0024		0.24	0.24mg - 0.09mg = 0.15mg
	X19	1.2929	1.2953	0.0024		0.24	
	X20	1.2885	1.2910	0.0025		0.25	
8.5g/L	X21	—					
	X22	—					
	X23	—					
	X24	—					

Reviewed By: SC

Summary Sheet

Facility Analytical Laboratories **Analyst** Chris Pate
Test ID Analytical Labs January QC 2018 **Species** Pimephales promelas (fathead minnow)
Date 1/18/2018 **Test Type** Growth
IWC Conc.

Input

Replicate	Concentrations					
	0	0.25	1.5	2.5	3.5	8.5
1	0.46	0.29	0.29	0.18	0.24	0
2	0.53	0.31	0.26	0.1	0.24	0
3	0.45	0.47	0.15	0.28	0.24	0
4	0.47	0.46	0.19	0.23	0.25	0

Mean	0.478	0.383	0.223	0.198	0.243	0.000
Stdev	0.036	0.096	0.064	0.077	0.005	0.000

Output

Statistical Data	Conc.	Mean	Stdev	CV	Steel test
	0	0.478	0.036	0.075	
	0.25	0.383	0.096	0.250	NS
	1.5	0.223	0.064	0.287	Y
	2.5	0.198	0.077	0.389	Y
	3.5	0.243	0.005	0.021	Y
	8.5	0.000			Y

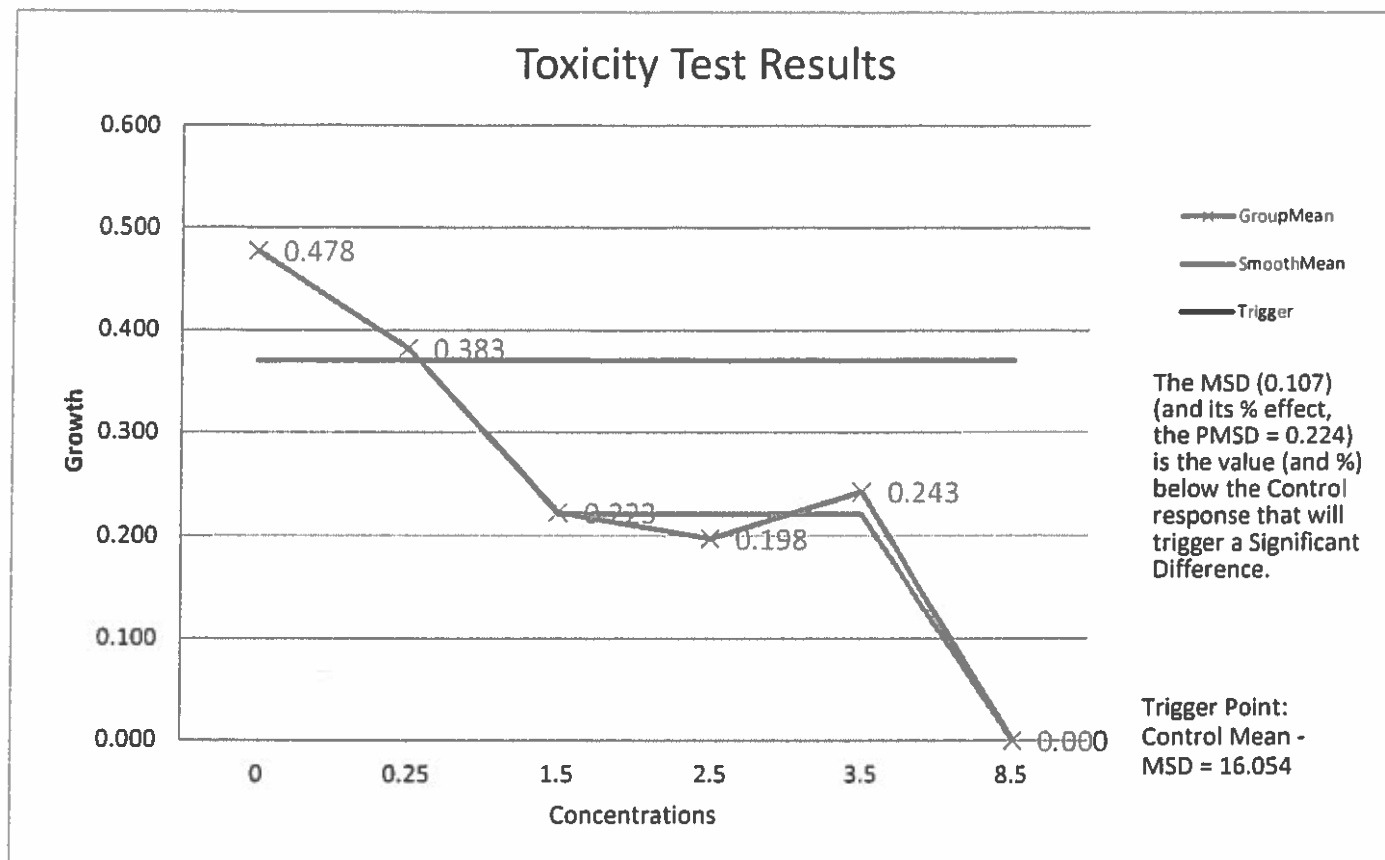
NOEC	LOEC	IC25	95% Confidence Intervals	
0.25	1.5	0.39	0.17	0.82

TST	Calculated t-value	Table t-value	Relative % Effect at IWC

MSD	PMSD
0.107	22.4%

Summary Sheet

Note - For statistical tests, "NS" indicates that the concentration is not statistically different from the control, while "Y" indicates that the concentration is statistically different from the control.



NOTICE

The United States Environmental Protection Agency (EPA), through its Office of Wastewater Management, funded and managed the development of the whole effluent toxicity (WET) Tool described here. This is a tool that calculates WET test endpoints for the EPA-approved WET test methods and is used by EPA internally for analyzing valid WET test data. Neither the EPA nor any of their employees, assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information or process disclosed. Furthermore, the WET Tool is supplied "as-is" without guarantee or warranty, expressed or implied, including without limitation, any warranty of merchantability or fitness for a specific purpose.

Summary Sheet

Facility	Analytical Laboratories	Analyst	Chris Pate
Test ID	Analytical Labs January QC 2018	Species	Pimephales promelas (fathead minnow)
Date	1/18/2018	Test Type	Chronic Survival
IWC Conc.			

Input

Number of Organisms Exposed or Counted

Replicate	Concentrations					
	<u>0</u>	<u>0.25</u>	<u>1.5</u>	<u>2.5</u>	<u>3.5</u>	<u>8.5</u>
1	10	10	10	10	10	10
2	10	10	10	10	10	10
3	10	10	10	10	10	10
4	10	10	10	10	10	10

Number of Organisms Surviving or Responding

Replicate	Concentrations					
	<u>0</u>	<u>0.25</u>	<u>1.5</u>	<u>2.5</u>	<u>3.5</u>	<u>8.5</u>
1	10	5	6	3	5	0
2	10	6	6	2	5	0
3	10	9	4	6	5	0
4	10	8	4	6	6	0

Total Organisms	40	40	40	40	40	40
Total Responding	40	28	20	17	21	0
% Responding	100.0%	70.0%	50.0%	42.5%	52.5%	0.0%

Output

Summary Sheet

Statistical Data	Conc.	Mean	Stdev	CV	Dunnett test
	0	1.412	0.000	0.000	
Statistics are based on the transformed data used for endpoint calculations	0.25	1.007	0.210	0.209	Y
	1.5	0.785	0.116	0.148	Y
	2.5	0.704	0.216	0.306	Y
	3.5	0.811	0.050	0.062	Y
	8.5				Y

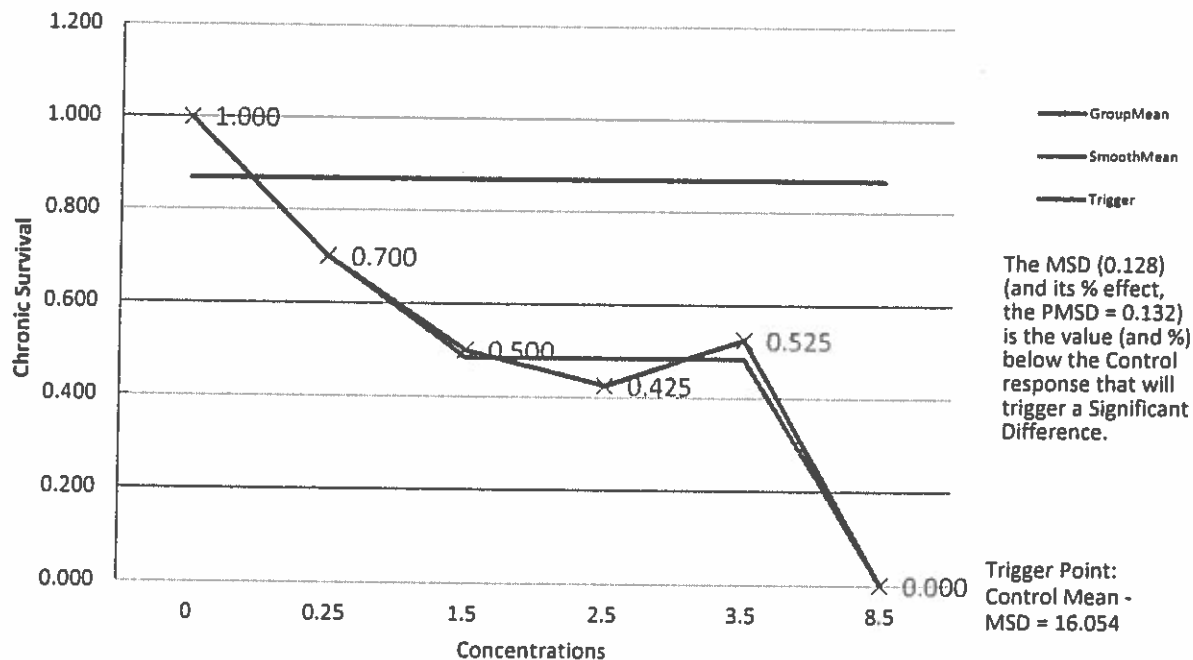
NOEC	LOEC	IC25	95% Confidence Intervals	
<0.25	0.25	0.20	0.13	0.65

TST	Calculated t-value	Table t-value	Relative % Effect at IWC

MSD	PMSD
0.128	13.2%

Note - For statistical tests, "NS" indicates that the concentration is not statistically different from the control, while "Y" indicates that the concentration is statistically different from the control.

Toxicity Test Results



NOTICE

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BENCH SHEET FOR QC CERIODAPHNIA SURVIVAL/REPRODUCTION TEST.

TEST MONTH January 2018

Analyst: CP BMC

Test Start Date/Time: 1/9/18, 1420

Test Stop Date/Time: 1/16/18, 1626

Young New D.O. New pH Old D.O. Old pH Daily Temp

Conc. **CONTROL**

Day-Lab #	1	2	3	4	5	6	7	8	9	10	XXX	XXX	XXX	XXX	XXX	XXX
0	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		8.1	7.9	XXX	XXX	22.8
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		7.6	8.2	8.1	8.1	23.8
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		7.7	8.2	8.0	8.4	23.6
3	1/1	1/1	1/2	1/1	1/2	1/3	1/2	1/2	1/2	1/2	18	7.6	8.2	8.0	8.5	24.8
4	✓	✓	2/4	✓	2/9	2/8	✓	✓	2/5	✓	26	7.7	8.2	8.1	8.5	23.7
5	2/4	2/7	3/2	2/7	✓	✓	2/8	2/9	✓	2/7	54	8.0	7.9	8.3	8.6	23.9
6	3/1	3/10	✓	3/9	3/12	3/12	3/10	3/11	3/9	3/11	95	8.0	7.8	8.4	8.1	20.8
7	✓	✓	4/10	✓	4/12	4/10	✓	✓	4/10	4/15	0			8.4	8.3	
Total	21	18	13	17	23	23	20	22	16	20	193					

Young New D.O. New pH Old D.O. Old pH Daily Temp

Conc. **0.50 g/L**

Day-Lab #	1	2	3	4	5	6	7	8	9	10	XXX	XXX	XXX	XXX	XXX	XXX
0	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		8.1	7.9	XXX	XXX	23.1
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		7.7	8.0	8.1	8.3	23.6
2	✓	✓	1/2	✓	✓	✓	✓	✓	✓	✓	2	7.7	7.9	7.9	8.4	23.6
3	1/3	1/3	✓	1/3	1/2	✓	✓	1/1	1/2	✓	14	7.6	8.0	8.0	8.4	24.5
4	2/8	✓	2/4	✓	2/3	1/4	1/2	✓	✓	✓	21	7.8	8.2	8.1	8.5	23.6
5	✓	2/3	3/6	2/7	✓	2/8	2/9	2/6	2/3	1/6	48	7.8	8.0	8.4	8.5	23.5
6	3/10	✓	✓	3/6	3/12	✓	✓	2/4	✓	2/8	45	7.9	7.9	8.4	8.2	23.2
7	✓	3/11	4/10	4/6	✓	3/11	3/9	✓	3/9	✓	40			8.3	8.3	
Total	21	17	12	16	17	23	20	16	14	14	170					

Young New D.O. New pH Old D.O. Old pH Daily Temp

Conc. **1.25 g/L**

Day-Lab #	1	2	3	4	5	6	7	8	9	10	XXX	XXX	XXX	XXX	XXX	XXX
0	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		8.1	7.9	XXX	XXX	23.0
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		7.7	8.0	7.9	8.3	23.5
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		7.7	7.9	7.9	8.3	23.6
3	✓	✓	✓	✓	✓	✓	1/1	1/2	1/1	1/3	7	7.6	8.1	8.2	8.3	24.5
4	✓	1/2	✓	✓	✓	1/3	✓	2/3	✓	2/4	12	7.8	8.2	NA	NA	23.5
5	1/2	2/1	1/2	1/1	1/2	2/1	2/3	✓	2/5	✓	17	7.9	8.1	8.4	8.5	23.4
6	✓	2/2	✓	✓	2/6	✓	3/2	3/4	2/7	3/2	28	8.0	7.9	8.5	8.2	23.0
7	2/6	3/5	2/6	2/3	✓	3/9	✓	✓	✓	✓	29			8.4	8.3	
Total	8	10	8	4	8	13	11	9	13	9	93					

BENCH SHEET QC CERIODAPHNIA SURVIVAL/REPRODUCTION TEST.

TEST MONTH January 2018

Analyst: CP Bon

Test Start Date/Time: 1/9/18, 1420

Test Stop Date/Time: 1/16/18, 1626

Young New D.O. New pH Old D.O. Old pH Daily Temp

Conc. **2.00 g/L**

Day-Lab #	1	2	3	4	5	6	7	8	9	10	XXX	XXX	XXX	XXX	XXX	XXX
0	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		8.1	7.9	XXX	XXX	22.9
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		7.7	8.0	8.0	8.3	23.4
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		7.8	8.0	7.9	8.3	23.5
3	✓	✓	✓	✓	✓	✓	✓	0	✓	0		7.7	8.0	8.1	8.3	24.7
4	✓	✓	✓	✓	✓	✓	✓	↓	✓	↓		7.8	8.1	8.1	8.4	23.5
5	✓	1/1	✓	✓	✓	✓	✓	↓	✓	↓	1	7.9	8.0	8.4	8.5	23.6
6	1/1	✓	1/1	1/1	✓	✓	1/1	↓	✓	↓	4	8.0	7.9	8.6	8.3	23.8
7	✓	✓	✓	✓	1/2	1/1	✓	↓	1/2	↓	5			8.3	8.2	
Total	1	1	1	1	2	1	1	0	2	0	10					

Young New D.O. New pH Old D.O. Old pH Daily Temp

Conc. **2.75 g/L**

Day-Lab #	1	2	3	4	5	6	7	8	9	10	XXX	XXX	XXX	XXX	XXX	XXX
0	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		8.1	7.9	XXX	XXX	22.8
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		7.7	8.0	7.9	8.2	23.3
2	0	0	0	0	0	✓	✓	0	0	0		7.7	7.9	8.1	8.2	23.5
3	↓	↓	↓	↓	↓	0	0	↓	↓	↓		7.7	8.0	8.3	8.4	24.6
4	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓						
5	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓						
6	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓						
7	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓						
Total	0	0	0	0	0	0	0	0	0	0						

Young New D.O. New pH Old D.O. Old pH Daily Temp

Conc. **3.50 g/L**

Day-Lab #	1	2	3	4	5	6	7	8	9	10	XXX	XXX	XXX	XXX	XXX	XXX
0	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		8.0	7.9	XXX	XXX	23.0
1	0	✓	0	✓	✓	0	0	0	0	0		7.7	8.0	8.0	8.2	23.1
2	↓	0	↓	0	0	↓	↓	↓	↓	↓		7.7	7.9	8.0	8.2	23.3
3	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓						
4	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓						
5	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓						
6	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓						
7	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓						
Total	0	0	0	0	0	0	0	0	0	0						

Summary Sheet

Facility	Analytical Laboratories	Analyst	Chris Pate
Test ID	Analytical Labs January 2018 QC	Species	Ceriodaphnia dubia (water flea)
Date	1/18/2018	Test Type	Chronic Survival
IWC Conc.			

Input

Number of Organisms Exposed or Counted

Replicate	Concentrations					
	<u>0</u>	<u>0.5</u>	<u>1.25</u>	<u>2</u>	<u>2.75</u>	<u>3.5</u>
1	1	1	1	1	1	1
2	1	1	1	1	1	1
3	1	1	1	1	1	1
4	1	1	1	1	1	1
5	1	1	1	1	1	1
6	1	1	1	1	1	1
7	1	1	1	1	1	1
8	1	1	1	1	1	1
9	1	1	1	1	1	1
10	1	1	1	1	1	1

Number of Organisms Surviving or Responding

Replicate	Concentrations					
	<u>0</u>	<u>0.5</u>	<u>1.25</u>	<u>2</u>	<u>2.75</u>	<u>3.5</u>
1	1	1	1	1	0	0
2	1	1	1	1	0	0
3	1	1	1	1	0	0
4	1	1	1	1	0	0
5	1	1	1	1	0	0
6	1	1	1	1	0	0
7	1	1	1	1	0	0
8	1	1	1	0	0	0
9	1	1	1	1	0	0
10	1	1	1	0	0	0

Total Organisms	10	10	10	10	10	10
Total Responding	10	10	10	8	0	0
% Responding	100.0%	100.0%	100.0%	80.0%	0.0%	0.0%

Output

Summary Sheet

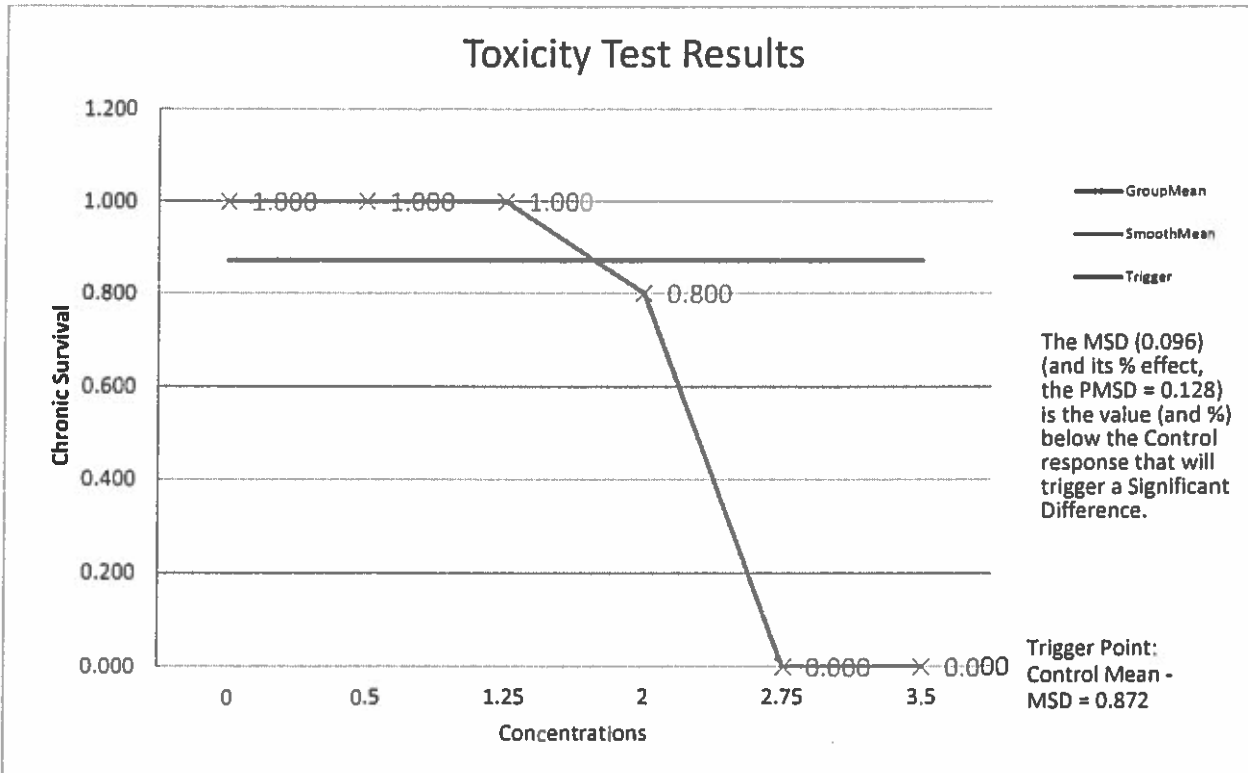
Statistical Data	Conc.	Mean	Stdev	CV	Steel test
	0	1.047	0.000	0.000	
Statistics are based on the transformed data used for endpoint calculations	0.5	1.047	0.000	0.000	NS
	1.25	1.047	0.000	0.000	NS
	2	0.942	0.221	0.234	NS
	2.75				Y
	3.5				Y

NOEC	LOEC	IC25	95% Confidence Intervals	
2	2.75	2.04	1.69	2.17

TST	Calculated t-value	Table t-value	Relative % Effect at IWC

MSD	PMSD
0.096	12.8%

Note - For statistical tests, "NS" indicates that the concentration is not statistically different from the control, while "Y" indicates that the concentration is statistically different from the control.



NOTICE

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Summary Sheet

Facility	Analytical Laboratories	Analyst	Chris Pate
Test ID	Analytical Labs January 2018 QC	Species	Ceriodaphnia dubia (water flea)
Date	1/18/2018	Test Type	Reproduction
IWC Conc.			

Input

Replicate	Concentrations					
	0	0.5	1.25	2	2.75	3.5
1	21	21	8	1	0	0
2	18	17	10	1	0	0
3	13	12	8	1	0	0
4	17	16	4	1	0	0
5	23	17	8	2	0	0
6	23	23	13	1	0	0
7	20	20	11	1	0	0
8	22	16	9	0	0	0
9	16	14	13	2	0	0
10	20	14	9	0	0	0

Mean	19.300	17.000	9.300	1.000	0.000	0.000
Stdev	3.268	3.432	2.669	0.667	0.000	0.000

Output

Statistical Data	Conc.	Mean	Stdev	CV	Steel test
	0	19.300	3.268	0.169	
	0.5	17.000	3.432	0.202	NS
	1.25	9.300	2.669	0.287	Y
	2	1.000	0.667	0.667	Y
	2.75	0.000			Y
	3.5	0.000			Y

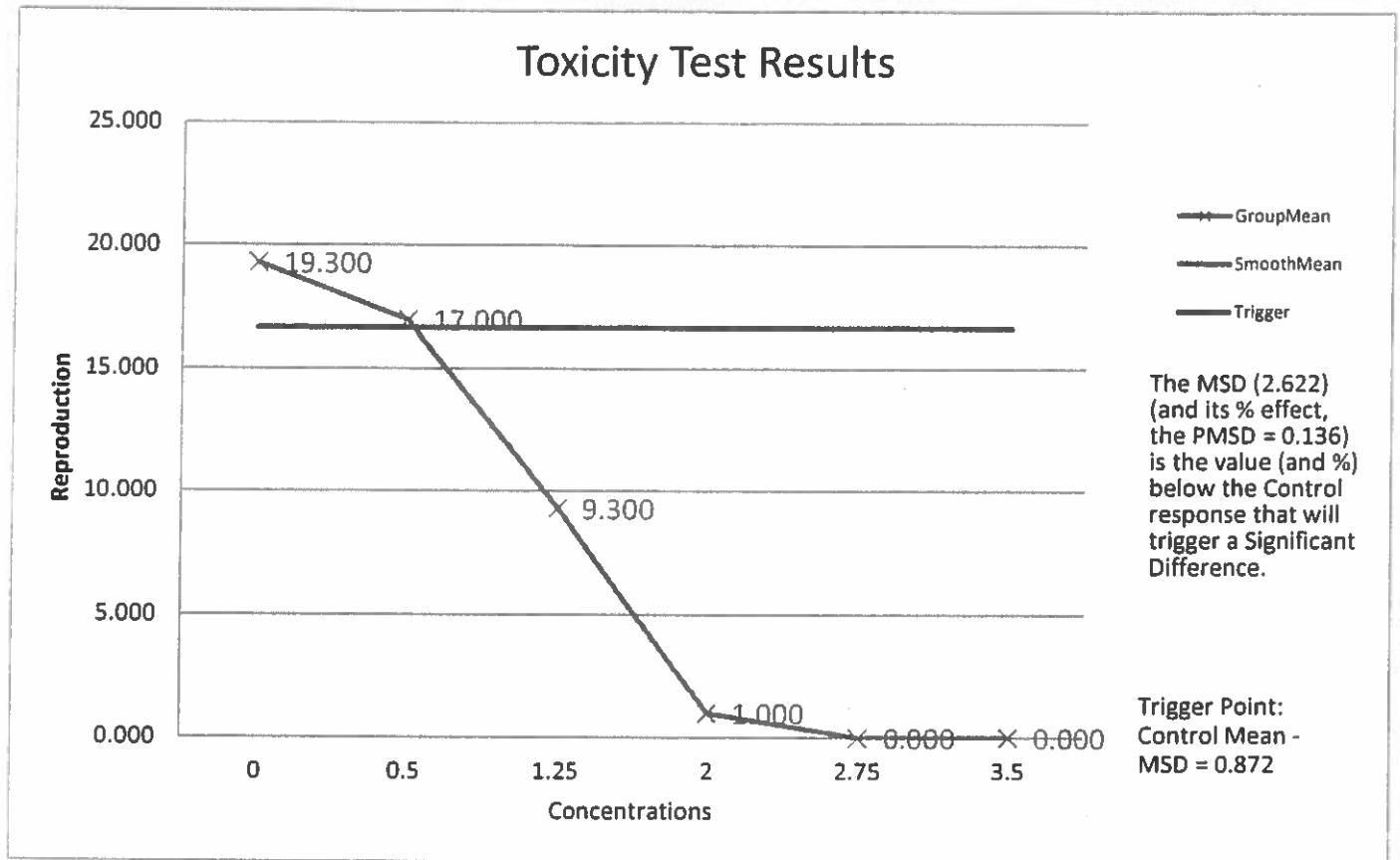
NOEC	LOEC	IC25	95% Confidence Intervals	
0.5	1.25	0.71	0.47	0.89

TST	Calculated t-value	Table t-value	Relative % Effect at IWC

MSD	PMSD
2.622	13.6%

Summary Sheet

Note - For statistical tests, "NS" indicates that the concentration is not statistically different from the control, while "Y" indicates that the concentration is statistically different from the control.



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**BENCH SHEET FOR *S. capicornutum* ALGAL QC GROWTH TEST.
EPA METHOD 1003.0**

Test Month/Year Jan. 2018 Analyst: cp, Bmc Final Report Review: SC

Test Start Date/Time: 1/17/18, 1400

Test Stop Date/Time: 1/21/18, 1430

Daily pH and Temp.

CONCENTRATION	Day 0		Day 1		Day 2		Day 3		Day 4		Comments
	pH	Temp	pH	Temp	pH	Temp	pH	Temp	pH	Temp	
Control	8.2	24.8	9.7	24.7	10.5	24.9	10.8	23.8	10.7	23.9	
0.50 g/L	8.1	24.4	9.6	24.4	10.5	24.5	10.7	24.7	10.8	23.1	
1.5 g/L	8.1	24.2	9.6	24.2	10.5	24.2	10.7	24.2	10.7	24.7	
5.5 g/L	8.0	24.1	9.4	25.0	9.9	23.7	10.1	24.2	10.2	24.2	
8.5 g/L	8.0	24.2	9.2	24.9	9.8	23.9	9.9	24.0	9.8	24.1	
10 g/L	8.0	24.3	9.1	24.6	9.7	23.8	9.9	23.8	10.0	24.5	

BENCH SHEET FOR *S. capricornutum* ALGAL QC GROWTH TEST

EPA TEST METHOD 1003.0

TEST MONTH/YEAR# Jan, 2018 ANALYST: BYL FINAL REPORT REVIEW: GC
 TEST START DATE/TIME: 1/17/18 1400
 TEST END DATE/TIME: 1/21/18 1430

Initial Algae Count (cells/mL)

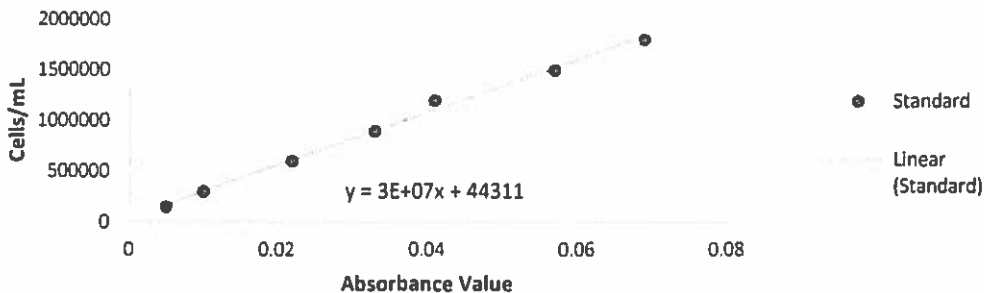
	Random Sample #1	Random Sample #2	Random Sample #3	Random Sample #4	Initial Average
	Absorbance Value: 0.011 0.37	Absorbance Value: 0.010 0.34	Absorbance Value: 0.010 0.34	Absorbance Value: 0.009 0.31	Absorbance Value: 0.010 Cells/mL: 0.344

Final Algae Count (cells/mL)

CONCENTRATION	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average
CONTROL	Absorbance Value: 0.084 2.56	Absorbance Value: 0.084 2.56	Absorbance Value: 0.073 2.23	Absorbance Value: 0.073 2.23	Absorbance Value: 0.079 Cells/mL: 2.40
0.5	Absorbance Value: 0.091 2.77	Absorbance Value: 0.096 2.92	Absorbance Value: 0.092 2.80	Absorbance Value: 0.092 2.80	Absorbance Value: 0.093 Cells/mL: 2.83
1.5	Absorbance Value: 0.099 3.01	Absorbance Value: 0.099 3.01	Absorbance Value: 0.100 3.04	Absorbance Value: 0.097 2.95	Absorbance Value: 0.099 Cells/mL: 3.01
5.5	Absorbance Value: 0.073 2.23	Absorbance Value: 0.072 2.20	Absorbance Value: 0.077 2.35	Absorbance Value: 0.072 2.20	Absorbance Value: 0.074 Cells/mL: 2.25
8.5	Absorbance Value: 0.054 1.66	Absorbance Value: 0.059 1.81	Absorbance Value: 0.056 1.72	Absorbance Value: 0.057 1.75	Absorbance Value: 0.057 Cells/mL: 1.74
10	Absorbance Value: 0.057 1.75	Absorbance Value: 0.062 1.90	Absorbance Value: 0.061 1.87	Absorbance Value: 0.059 1.81	Absorbance Value: 0.060 Cells/mL: 1.84

*Absorbance values (AV) obtained from Spectronic 601 spectrophotometer are used to determine cells/mL based on a standardized linear relationship $((3 \times 10^7)(AV) + 44311)$.

***Selenastrum capricornutum* Conversion Chart**



Summary Sheet

Facility	Analytical Laboratories	Analyst	Chris Pate
Test ID	Analytical Labs January 2018 QC	Species	Selenastrum capricornutum (green algae)
Date	1/23/2018	Test Type	Growth
IWC Conc.			

Input

Replicate	Concentrations					
	0	0.5	1.5	5.5	8.5	10
1	2.56	2.77	3.01	2.23	1.66	1.75
2	2.56	2.92	3.01	2.2	1.81	1.9
3	2.23	2.8	3.04	2.35	1.72	1.87
4	2.23	2.8	2.95	2.2	1.75	1.81

Mean	2.395	2.823	3.003	2.245	1.735	1.833
Stdev	0.191	0.067	0.038	0.071	0.062	0.067

Output

Statistical Data	Conc.	Mean	Stdev	CV	Dunnett test
	0	2.395	0.191	0.080	
	0.5	2.823	0.067	0.024	NS
	1.5	3.003	0.038	0.013	NS
	5.5	2.245	0.071	0.032	NS
	8.5	1.735	0.062	0.036	Y
	10	1.833	0.067	0.036	Y

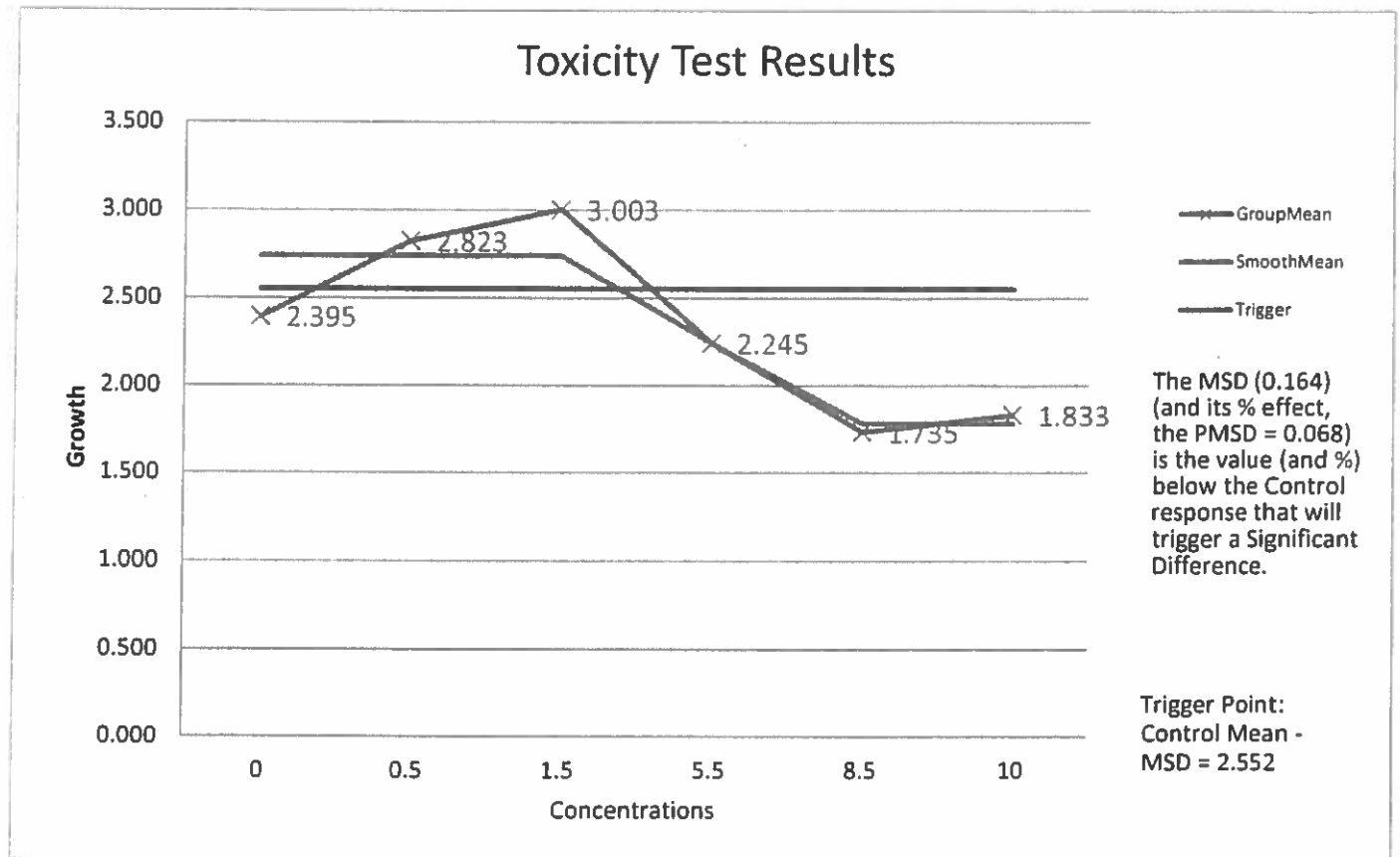
NOEC	LOEC	IC25	95% Confidence Intervals	
5.5	8.5	6.60	6.22	6.93

TST	Calculated t-value	Table t-value	Relative % Effect at IWC

MSD	PMSD
0.164	6.8%

Summary Sheet

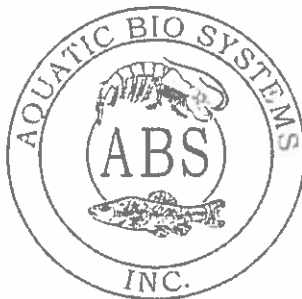
Note - For statistical tests, "NS" indicates that the concentration is not statistically different from the control, while "Y" indicates that the concentration is statistically different from the control.



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1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524



Toll Free: 800/331-5916
Tel: 970/484-5091 Fax: 970/484-2514

ORGANISM HISTORY

DATE: 1/8/2018

SPECIES: *Ceriodaphnia dubia*

AGE: < 24 hour

LIFE STAGE: Neonate

HATCH DATE: 1/8/2018

BEGAN FEEDING: Immediately

FOOD: YTC, *Raphidocelis subcapitata**

Water Chemistry Record:

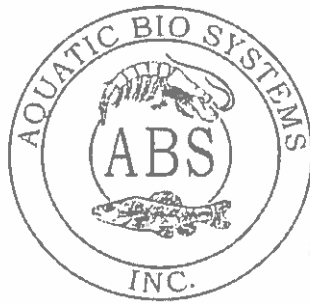
	Current	Range
TEMPERATURE:	<u>22°C</u>	<u>--</u>
SALINITY/CONDUCTIVITY:	<u>--</u>	<u>--</u>
TOTAL HARDNESS (as CaCO ₃):	<u>110 mg/l</u>	<u>--</u>
TOTAL ALKALINITY (as CaCO ₃):	<u>90 mg/l</u>	<u>--</u>
pH:	<u>8.12</u>	<u>--</u>

Comments: * Formerly known as *Pseudokirschneriella subcapitata* and *Selenastrum capricornutum*



Facility Supervisor

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ORGANISM HISTORY

DATE: 1/8/2018

SPECIES: *Pimephales promelas*

AGE: N/A

LIFE STAGE: Embryo

HATCH DATE: 1/8/2018 between 10:30 am - 11:30 am MST

BEGAN FEEDING: N/A

FOOD: N/A

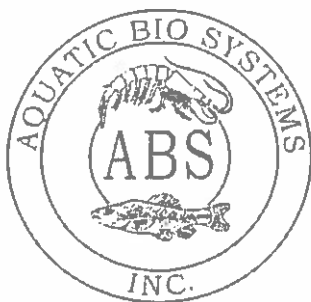
Water Chemistry Record:	Current	Range
TEMPERATURE:	<u>24°C</u>	<u>--</u>
SALINITY/CONDUCTIVITY:	<u>--</u>	<u>--</u>
TOTAL HARDNESS (as CaCO ₃):	<u>120 mg/l</u>	<u>--</u>
TOTAL ALKALINITY (as CaCO ₃):	<u>105 mg/l</u>	<u>--</u>
pH:	<u>7.96</u>	<u>--</u>

Comments:



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Tel: 970/484-5091 Fax: 970/484-2514

Algae Preparation History

DATE: 1/8/2018

SPECIES: *Raphidocelis subcapitata**

INOCULATION DATE: 12/26/2017

HARVEST DATE: 1/2/2018

CONCENTRATION DATE: 1/4/2018

CELL COUNT (/ml): 3.0×10^7 cells/ml

Comments:

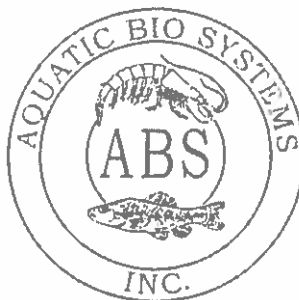
* Formerly known as *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*

** All concentrated algae diluted to proper cell count with reconstituted moderately hard DI water.



Supervisor

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STOCK SOLUTIONS FOR MEDIUM PREPARATION OF

*Raphidocelis subcapitata**

Formerly known as *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*

Compound**

Amount Dissolved in 4L Deionized H₂O

Solution #1

MgCl ₂ • 6H ₂ O	48.64g
CaCl ₂ • 2H ₂ O	17.60g
H ₃ BO ₃	0.7424g
MnCl ₂ • 4H ₂ O	1.6640g
ZnCl ₂	0.01312g
FeCl ₃ • 6H ₂ O	0.6392g
CoCl ₂ • 6H ₂ O	0.005712g
Na ₂ MoO ₄ • 2H ₂ O	0.02904g
CuCl ₂ • 2H ₂ O	0.000048g <u>OR</u> 0.06g in 1L H ₂ O Dilute 1 ml of this to 10 mls and take 8ml of this into 4L.
NaEDTA • 2H ₂ O	1.20g
Na ₂ SeO ₄	0.01g

Solution #2

NaNO₃ 102.0g

Solution #3

MgSO₄ • 7H₂O 58.80g

Solution #4

K₂HPO₄ 4.1760g

Solution #5

NaHCO₃ 60.0g

Preparation Date: 11/28/2017

* Adapted from USEPA/600/4-90/027 September, 1991 page 143 for *Selenastrum capricornutum*

**Purchased from Sigma-Aldrich Chemical, St. Louis, MO