



Environment Testing
TestAmerica

AQUATIC TOXICOLOGY REPORT

Project Name: SPOKANE COUNTY REGIONAL WRF

Location: SPOKANE, WASHINGTON

c/o Jacobs

Prepared by: Eurofins TestAmerica - Corvallis

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INTRODUCTION

Eurofins TestAmerica – Corvallis (ET-C) Aquatic Toxicology Laboratory conducted toxicity testing on samples from the Jacobs - Spokane County Regional Water Reclamation Facility, Spokane, Washington.

Testing was conducted on behalf of: Jacobs

The Project Name was: Spokane County Regional Water Reclamation facility

Testing was initiated on: January 9, 2020

The test was conducted using:

- the green algae (*Selenastrum capricornutum*, aka *Raphidocelis subcapitata*, aka *Pseudokirchneriella subcapitata*).

OVERVIEW OF REGULATORY GUIDANCE

The following provides an overview and excerpts of applicable permit specifics, regulatory guidance, and other relevant information. This is intended only as a helpful guide, from a laboratory perspective, for understanding test outcomes. The final responsibility for interpretation of results remains with the client and/or regulatory agency.

The following guidance is taken from ET-C's reading of the NPDES permit for Spokane County Regional Water Reclamation Facility (permit #WA0093317, effective Dec 1, 2011, expired Nov 31, 2016). At the time of testing, no additional permit information was available.

Chronic toxicity:

- *Effluent Limit for Chronic Toxicity:*
 - "No toxicity detected in a test concentration representing the chronic critical effluent concentration (CCEC)."
 - "The CCEC equals 8.4% effluent."
- *Compliance with the Effluent Limit for Chronic Toxicity:*
 - "Compliance with the effluent limit for chronic toxicity means the results ... show no statistically significant difference in response between the control and the CCEC."
 - "The Permittee must determine the statistical significance by conducting a hypothesis test at the 0.05 level of significance ..." (i.e. $\alpha = 0.05$)
 - "If the difference in survival between the control and the CCEC is less than 20 percent, ... must conduct the hypothesis test at the 0.01 level of significance."
- *Compliance Testing for Chronic Toxicity:*
 - "Conduct quarterly chronic toxicity testing on the final effluent ..."
 - "... using the following species on a rotating basis ..."
- *Response to Noncompliance with the Effluent Limit for Chronic Toxicity:*
 - "If a toxicity test ... determines a statistically significant difference in response between the CCEC and the control ... the Permittee must begin

additional compliance monitoring within one week of receiving the test results”.

The following is taken from the WDOE guidance (WQ-R-95-80, June 2016 revision):

- “To reduce WET limit violations due to statistically significance that is a Type I error (false positive), we lower the alpha for hypothesis testing when differences in test organisms response are small.”
- “Alpha will be lowered from 0.05 to 0.01 if a 10% difference in an acute test is significant or a 20% difference in a chronic test is significant.”

SUMMARY OF TEST RESULTS

Exhibit 1 provides a summary of the final test results.

EXHIBIT 1

Summary of Chronic Test Results

Species	NOEC (%)	LOEC (%)	IC ₂₅ (%)	Was a statistically significant difference in response shown between control and the CCEC?
<i>S. capricornutum</i> (all data)	56.5 ^a	100 ^a	9.44	No
<i>S. capricornutum</i> (outliers removed)	8.40 ^b	25.0 ^b	17.6 ^b	No

^a Indicates that the PMSD is above the upper bound listed in the protocol. As such, the hypothesis testing endpoints (NOEC/LOEC) should be considered questionable. See Table 1 for further discussion.

^b Indicates data with the identified statistical outliers removed. See Tables 1 and 2 for further discussion.

Note: acronyms are as defined below.

From the NPDES permit: “Compliance with the effluent limit for chronic toxicity means the results ... show no statistically significant difference in response between the control and the CCEC (8.4% effluent).”

More detailed information is provided in the Results and Discussion section.

ACRONYM DEFINITIONS (from EPA guidance):

NOEC = No Observed Effect Concentration: The highest test concentration that causes no observable adverse effects on the test organisms (i.e. no statistically significant reduction from the control).

LOEC = Low Observed Effect Concentration: The lowest test concentration that does cause an observable adverse effect on the test organisms (i.e. is statistically significant reduction from the control).

IC₂₅ = Inhibition Concentration (25%): A point estimate of the test concentration that would cause a 25 percent reduction of a non-quantal biological measurement (i.e. growth, reproduction, etc.) for the test population.

SAMPLE INFORMATION

Exhibit 2 provides a summary of the sample conditions as received.

EXHIBIT 2

Sample Conditions on Receipt

Sample ID	BIO20010705
ET-C SDG	B4584-01
Collection - Date and Time	01/07/2020 09:30
Receipt - Date and Time	01/08/2020 10:30
Temperature (°C)	- 0.6 (not frozen)
Dissolved Oxygen (mg/L)	10.5
pH	7.3
Conductivity (µS/cm)	881
Total Residual Chlorine (mg/L)	< 0.02
Ammonia (mg/L as NH ₃ -N)	< 0.10
Total Hardness (mg/L as CaCO ₃)	147
Total Alkalinity (mg/L as CaCO ₃)	110

Water quality measurements during testing remained within test design limits as prescribed by EPA and WDOE, except as noted with the individual test results. (see the Results and Discussion section)

METHODS AND MATERIALS

TEST METHODS

The chronic test methods were performed according to: *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, Fourth Edition, (2002), EPA-821-R-02-013.

Additional guidance was provided by:

- *Whole Effluent Toxicity Testing Guidance and Test Review Criteria*, Washington State Department of Ecology (revised June 2016) Pub# WQ-R-95-80.
- *Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing* (40 CFR Part 136), (EPA August 2000), EPA 821-B-00-004.

DEVIATIONS FROM PROTOCOLS

Deviations from required procedures in the test methods:

- None noted.

Deviations from recommended procedures in the test methods:

- None noted.

Note: While the test met Test Acceptability Criteria (TAC) for both growth rates and coefficient of variability limits in the control and is valid. However, while the test showed no statistically significant toxicity at the level of regulatory concern, the test showed an overall Percent Minimum Significant Difference (PMSD) above the bounds listed in the EPA manual. This is indicative of an unacceptably insensitive test. This is discussed in further detail under Table 1.

TEST DESIGN

The following summarizes the conditions used for both overall testing and the specifics for each test (observations and notations can be found on the datasheets in Appendix A):

Overall Test Design:

- Chronic test: 6.25, 8.4, 25, 56.5, and 100 percent sample + dilution water for the control.

Test Organism Conditions:

- All organisms tested were fed and maintained during culturing, acclimation, and testing as prescribed by the EPA (2002).
- The test organisms appeared vigorous and in good condition prior to testing.

S. capricornutum chronic test:

- Source: ET-C's in-house cultures
- Age: Culture acclimated to test conditions for 4 to 7 days.

- Design: Four test vessels per concentration, 10,000 cells/ml density at initiation
- Test Solution Renewal: None (static)
- Monitoring:
 - Initiation: DO, pH, Temperature, and Conductivity, all concentrations
 - Initiation: Hardness and Alkalinity, control + low, middle and high sample concentrations
 - Initiation: Light Intensity and Cell Density confirming counts, in triplicate
 - Daily: Temperature and pH, all concentrations
- Termination: 96 hours.
- Endpoints: Cell Density (at termination)

DILUTION WATER

The dilution water used was the standard culture water used by ET-C:

- Reconstituted, moderately hard water (as per EPA protocol) with a total hardness of 75 to 105 mg/L as CaCO₃ and an alkalinity of 50 to 75 mg/L as CaCO₃.
- For the *S. capricornutum* test: Reconstituted, moderately hard water with nutrients added, including EDTA, that was passed through a 0.45 micron filter prior to use.

SAMPLE COLLECTION AND STORAGE

Sample was collected by Jacobs – Spokane County personnel. The sample was accepted as scheduled by ET-C. Chain of Custody and Sample Receipt Records are provided in Appendix C.

- All samples were received within the EPA recommended 0 to 6 °C range.
- All samples were received within the WDOE required 0 to 6 °C range.
- All samples were initially used for test initiation or test solution renewal within the EPA recommended maximum holding time of 36 hours of sample collection.
- All subsequent uses of a sample occurred within the EPA recommended maximum holding time of 72 hours past the time of initial use of that sample.
- All subsequent uses of a sample occurred within the WDOE recommended maximum holding time of 72 hours past the time of sample collection.
- Following receipt, the samples were stored in the dark at 0 to 6 °C until test solutions were prepared and tested.

SAMPLE PREPARATION

Sample used during this test were:

- Temperature adjusted prior to test initiation and each daily renewal.
- The subsample used for the *S. capricornutum* test had nutrients, including EDTA, added, and was 1.60 micron filtered prior to use.

DATA ANALYSIS

The statistical analyses performed for the chronic tests were those outlined in *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to*

Freshwater Organisms, USEPA Office of Water, Fourth Edition (EPA 2002), EPA-821-R-02-013, using CETIS.

- The specific statistical analysis and CETIS version used for each endpoint evaluation is listed with the statistical outputs included with each test in Appendix A.
- If any additional analysis methods were also used, an explanation of the rationale and reference to the source method is included with the presentation of those results below.

RESULTS AND DISCUSSION

The raw data sheets for all tests are presented in Appendix A.

CHRONIC BIOASSAY

Table 1 summarizes the survival and growth data for the *S. capricornutum* chronic test.

Table 1 Summary of Chronic Results <i>S. capricornutum</i>	
Sample Concentration (%)	Growth (Cells/ml x 10⁶)
Control	3.379
6.25	3.149
8.40	2.558
25.0	1.906
56.5	2.645
100	2.422 ^a
^a Indicates a statistically significant difference from the control at alpha = 0.05.	

Statistical analysis in accordance with the EPA protocol and WDOE guidance results in:

- NOEC = 56.5 %
- LOEC = 100 %
- IC₂₅ = 9.44 %

From the NPDES permit: “Compliance with the effluent limit for chronic toxicity means the results ... show no statistically significant difference in response between the control and the CCEC (8.4% effluent).”

- No statistically significant difference between control and CCEC was shown.

The initial dissolved oxygen levels in the chronic test remained was at or above 4.0 mg/L. Test temperatures remained at 25±1°C.

The *S. capricornutum* chronic test meets Test Acceptability Criteria (TAC) for a minimum 1.0 x 10⁶ cells/ml control growth and a maximum coefficient of variance (CV%) of 20% in the controls. Unless referenced above, the test proceeded without any noted deviations or interruptions that could have affected test results. The testing should be considered “valid”.

However, as noted above, the test showed an overall Percent Minimum Significant Difference (PMSD = 38.7%) above the bounds listed in the EPA manual (9.1% to 29%). This is indicative of an unacceptably insensitive test. As part of the Corrective Action process, the data set was evaluated for statistical outliers.

As per EPA 821-R-02-012, Appendix A, Section 3: “An outlier is an inconsistent or questionable data point that appears unrepresentative of the general trend exhibited by the majority of the data. Outliers may be detected by tabulation of the data, plotting, and by an analysis of the residuals. An explanation should be sought for any questionable data points. Without an explanation, data points should be discarded only with extreme caution. If there is no explanation, the analysis should be reported both with and without the outlier, and the results of both analyses should be reported.”

Grubb’s Test identified the data at 8.4% Replicate A and 25% Replicate C as being statistical outliers. No explanation or reason was found in the performance of the test for this.

As per EPA 821-R-02-012, Appendix A, Section 3, the data with the outliers removed is also being presented.

Table 2 summarizes the survival and growth data for the *S. capricornutum* chronic test with the statistical outliers removed.

Table 1 Summary of Chronic Results <i>S. capricornutum</i>	
Sample Concentration (%)	Growth (Cells/ml x 10⁶)
Control	3.379
6.25	3.149
8.40	3.317
25.0	1.498 ^a
56.5	2.645 ^a
100	2.422 ^a
^a Indicates a statistically significant difference from the control at alpha = 0.05.	

Statistical analysis in accordance with the EPA protocol and WDOE guidance results in:

- NOEC = 8.40 %
- LOEC = 25.0 %
- IC₂₅ = 17.6 %

From the NPDES permit: “Compliance with the effluent limit for chronic toxicity means the results ... show no statistically significant difference in response between the control and the CCEC (8.4% effluent).”

- No statistically significant difference between control and CCEC was shown.

Note: With the statistical outliers removed, the test showed an overall Percent Minimum Significant Difference (PMSD = 17.7%) within the bounds listed in the EPA manual (9.1% to 29%). This is indicative of an acceptably sensitive test.

REFERENCE TOXICANT TEST

Reference toxicant (reftox) testing is performed to document both initial and ongoing laboratory performance of the test method(s). While the health of the test organisms is primarily evaluated by the performance of the laboratory control, reftox test results also may be used to assess the health and sensitivity of the test organisms. Reftox test results within their respective cumulative summary (Cusum) chart limits are indicative of consistent laboratory performance and normal test organism sensitivity.

In accordance with WDOE guidance, the *S. capricornutum* reftox test was performed concurrently with the client testing.

The results of the reftox test indicate that the test organisms were within their respective cusum chart limits based on EPA guidelines. This demonstrates ongoing laboratory proficiency of the test methods and suggests normal test organism sensitivity in the associated client testing.

The *S. capricornutum* reftox test was conducted using sodium chloride.

The data sheets for the reference toxicant test are provided in Appendix B.

Table 3 summarizes the reference toxicant test results and Cusum chart limits.

Table 3		
Chronic Reference Toxicant Test (g/L)		
Species	IC₂₅	Cusum Chart Limits
<i>S. capricornutum</i> (growth)	2.10	0.49 to 2.30

APPENDIX A
RAW DATA SHEETS

Client

Jacobs - Spokane County RWRF

SDG# B4524

Test Initiation: Date

1-B-20

Contact

Neil DeJonge (509) 536-3710 x20710

Test Termination: Date

1-12-20

Sample ID Number	Field ID	Collected		Received		Temp (°C) as Rcv'd	Total Residual Chlorine (mg/l) <input type="checkbox"/> Dechlorination allowed as Rcv'd / after Dechlor.	Ammonia NH ₃ -N mg/l as Rcv'd	Hardness mg/l as CaCO ₃ as Rcv'd	Alkalinity mg/l as CaCO ₃ as Rcv'd	DO (mg/L) as Rcv'd	pH as Rcv'd	Cond. (uS) as Rcv'd	60 um filtered? (organisms noted)
		Date (mm/dd/yy)	Time (Pacific Zone)	Date (mm/dd/yy)	Time (Pacific Zone)									
B4584-01	B1020010705	01/07/20	04:30	01/08/20	10:30	-0.6	20.02 / -	<0.10	147	110	10.5	7.3	881	<input type="checkbox"/>
							/							
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							/							
							/							
		Reporting Limits:				na	0.02 mg/L	0.10 mg/L	5 mg/L	5 mg/L	na	na	na	na

Note: "-" Indicates data collection or dechlorination not needed. Any other adjustments to samples prior to use are documented in Comments below or on Dilutions page.

Dilution Water	ID#	Hardness mg/l as CaCO ₃	Alkalinity mg/l as CaCO ₃	Comments: <input checked="" type="checkbox"/> Indicates the action was taken, (<input type="checkbox"/> = action not taken):	" - " = sample not dechlorinated, or analyte not collected/needed.
Recon MH (Algae)	5026	152	70	<input checked="" type="checkbox"/> Subsample for algae test had nutrients added and was 1.60 um filtered	

Water Quality Meters Used/TD#: Dissolved Oxygen # 4 pH # 1 Conductivity # 2

FRESHWATER TOXICITY TEST: TEST ORGANISM INFORMATION

Client Jacobs - Spokane County RWRf

Sample Designation (SDG): B 4584

Test Species Information	Sc # <u>569</u> <i>Selenastrum capricornutum</i> Chronic				
Organism Age at Initiation	Acclimated for <u>5</u> Days				
Test Container Size	125 ml				
Test Volume	50 ml				
Feeding: Type and Amount	1 ml/L Nutrients, incl. EDTA, added				
Aeration: In Test Chambers via Slow Bubble :	<input checked="" type="checkbox"/> None <input type="checkbox"/> Prior to use				
Acclimation Period	<u>5</u> Days				
Organism Source	In-House				
Size	-				
Loading Rate	-				

Dissolved Oxygen aeration justifications (in test chambers):

Test(s): ☐ All ☐ _____
 Date:

Comments:

Test Solution Preparation and Dilution Record

Client: Jacobs - Spokane County RWRF

Note: ☐ Indicates task not done, ☒ Indicates task was done. Temp adj. = Temperature adjusted to ambient or test temp
Ditto marks (' ') indicate that the same SDG, batch of dilution water, or food as the previous day's entry was used.

Algae - Chronic

Test Concentration (%)	Sample Volume (mls)	Final Volume (mls)
Control	0.00	→ 500
6.25	31.3	→ 500
8.4	42.0	→ 500
25.0	125	→ 500
56.5	283	→ 500
100	500	→ 500

Test Day 0 (Initiation)

Sample ID Used 4584-01

Daily Sample Preparation (prior to dilution)

☒ Temperature adjusted

☐ Aerated

☒ Nutrients added (incl. EDTA)

☐ 0.45-um filtered - Lot#

☒ 1.60 mm filtered (WDOE clients only) Lot# C1178340

Dilution Water Used ID # 5026 * 18/2020 12:30 Initials SD

Total Sample volume needed = 831 mls

* 5026 was slightly short. Approximately 0.75 L #5027 also used. 517 1-8-20

GREEN ALGAE (*Selenastrum capricornutum*) TEST CONDITIONS

Client: Jacobs - Spokane County RWRP

Sample Description: _____

Sample ID: B 4584 - 01

Test Start Date: 1 / 8 / 20 20

Time: 13 : 35

Initials: JD

Test End Date: 1 / 12 / 20 20

Time: 13 : 10

Initials: TA

TEST CONDITIONS AT INITIATION:

Species ID#: Rob 330 569
RS

Culturing period : 5 days

Incubator ID#: 2

INOCULUM (Axenic Culture)		Culture Density = Mean # of Cells/Field x 250,000	
# Cells	# Fields	Culture Density	
390	200		
MEAN	1.95	x 250,000 =	487500

Test Start Density x Test Volume / Culture Density = Inoculum Volume
10,000 x 50 ml / 487500 = 1.03 ml

Filtration	0.45 um filter	
	1.6 um filter	X

Test Vol. / Replicate	50 mls
No. of Reps	4
Nutrient Dosage	1 ml per L

Confirming Counts			
	# Cells	# Fields	Cell Conc. x10 ⁶
Flask #1	12	250	0.048 x 10 ⁶
Flask #2	10*	250	0.04 x 10 ⁶
Flask #3	13*	250	0.052 x 10 ⁶

Light Intensity at Test Initiation (foot-candles)		
Location #1	Location #2	Location #3
376	414	399

Technician 0 hr JD 24 hr ABC 48 hr ABC 72 hr TA 96 hr TA
Time 0 hr 13:25 24 hr 15:05 48 hr 15:05 72 hr 11:07 96 hr 13:10
Therm. ID# 0 hr #266 24 hr #266 48 hr #266 72 hr #266 96 hr #266

Test Concn.	D.O. (mg/L)	pH					Temperature (°C)					Conductivity (umohs/cm)	Hard.	Alk.
	0 hr	0 hr	24	48	72	96	0 hr	24	48	72	96	0 hr	0 hr	0 hr
Control	8.0	8.2	8.4	8.1	8.2	9.0	25.4	24.8	25.1	25.1	25.2	407	102	70
6.25 %	7.7	8.2	8.3	8.1	8.3	8.9	25.9	24.7	25.1	25.3	25.3	700 703 866 X	107	77
8.4 %	7.7	8.2	8.3	8.1	8.3	8.9	25.4	24.6	25.2	25.3	25.3	500		
25.0 %	7.8	8.1	8.3	8.1	8.3	8.9	25.0	24.7	25.3	25.3	25.3	546	140	90
56.5 %	8.0	8.1	8.4	8.2	8.4	9.0	25.6	24.7	25.0	25.5	25.4	699		
100 %	7.9	7.9	8.5	8.3	8.5	9.2	25.9	24.7	24.9	25.8	25.7	929	153	120

Comments: Flasks positioned randomly in incubator. Positions changed every day. Temp and pH taken in surrogate flasks.

* Rob JD 1-8-20
Conductivity also comes in at ~ 700 consistently. See attached (23.8) = Temp. out of recommended range



Analytical Comments Form

Date: 1-8-20
Initials: JD

Analytical Batch:
Or SDG: B4584-01

Comments

6.25% concentration for Spokane algae ^{in subsample}
test came in at around 700 μ S for conductivity.
When re-tested out of flask directly,
conductivity came in at 423 μ S.
There was likely an interference
with the cerid cup used to subsample.

Please use this form to record any hand notes, calculations, reminders, or other transcriptions. These comments will be kept as part of ASL's permanent records.

Raphidocelis subcapitata COULTER COUNTER WORKSHEET

As of Aug 2016, all data is
typed directly into form
by analyst. No review needed.

Client Jacobs-Spokane County RWRP

Sample Description _____

Lab ID B4584

Test Start Date 1/8/2020

Test Count Date 1/22/2020 Initials JK/BC

Instrument Settings:

Counter ID: PCOUNT3

As of: 30-Aug-16

the settings were as listed below

Aperture size 100 um
Aperture Kd value: 59.94
Set size 3.2 um
Gain: 256
Current: 1.414
Preamp Gain: 179.20

Dilution factor (DF) Default value: 51

Output: Concentration

Metered volume (ml): 0.5

Blank counts (use ISOTONE II):

Run	BLANK
DF	51
1	26418
2	15300
3	12750
4	9690
5	6222
6	5916

Note: Blank concentrations are Cells per ml (not x 10⁶)

Dilutions: DF = 11 (1.0 ml sample to 10mL Isotone II)

DF = 51 (0.20 ml sample to 10mL Isotone II)

DF = 101 (0.20 ml sample to 20mL Isotone II)

Where : * = Concentrations are in Cells per mL x 10⁶

COMMENTS

Isoton II Diluent ASL# 16462C expired 1/20/20.
QC standards and blanks passed requirements.
Counting processed as normal 1/22/2020 JK
Additional counts for 8.4% Rep A and 25% on
additional counts sheet

TEST CONCENTRATION		Control		
Run	REP A	REP B	REP C	REP D
DF (if other than default)				
1	2.708	3.508	3.714	3.654
2	2.717	3.451	3.642	3.629
3	2.718	3.477	3.684	3.640
Replicate Average	2.714	3.479	3.680	3.641
CONCENTRATION MEAN # Cells / mL* =		3.379	CV % =	13.4%

TEST CONCENTRATION		6.25 %		
Run	REP A	REP B	REP C	REP D
DF (if other than default)				
1	3.075	2.870	3.587	3.227
2	2.940	2.708	3.614	3.233
3	2.969	2.738	3.600	3.221
Replicate Average	2.995	2.772	3.600	3.227
CONCENTRATION MEAN # Cells / mL* =		3.149	CV % =	11.2%

TEST CONCENTRATION		8.4 %		
Run	REP A	REP B	REP C	REP D
DF (if other than default)				
1	0.275	3.123	3.523	3.312
2	0.270	3.109	3.477	3.304
3	0.300	3.153	3.525	3.324
Replicate Average	0.282	3.128	3.508	3.313
CONCENTRATION MEAN # Cells / mL* =		2.558	CV % =	59.6%

TEST CONCENTRATION		25.0 %		
Run	REP A	REP B	REP C	REP D
DF (if other than default)				
1	1.281	1.895	3.166	1.245
2	1.325	1.926	3.109	1.224
3	1.410	1.939	3.118	1.238
Replicate Average	1.339	1.920	3.131	1.236
CONCENTRATION MEAN # Cells / mL* =		1.906	CV % =	45.7%

TEST CONCENTRATION		56.5 %		
Run	REP A	REP B	REP C	REP D
DF (if other than default)				
1	2.656	2.365	2.468	3.118
2	2.669	2.379	2.427	3.024
3	2.685	2.374	2.462	3.115
Replicate Average	2.670	2.373	2.452	3.086
CONCENTRATION MEAN # Cells / mL* =		2.645	CV % =	12.1%

TEST CONCENTRATION		100 %		
Run	REP A	REP B	REP C	REP D
DF (if other than default)				
1	2.292	2.426	2.639	2.317
2	2.252	2.436	2.401	2.355
3	2.272	2.427	2.873	2.371
Replicate Average	2.272	2.430	2.638	2.348
CONCENTRATION MEAN # Cells / mL* =		2.422	CV % =	6.5%

Raphidocelis subcapitata COULTER COUNTER WORKSHEET

Client Jacobs Spokane

Sample Description _____

Lab ID B4584

Test Start Date 1/8/2020

Test Count Date 1/22/2020 Initials JK

Instrument Settings:

Counter ID: PCOUNT3

As of: 30-Aug-16

the settings were as listed below

Aperture size 100 um

Aperture Kd value: 59.94

Set size 3.2 um

Gain: 256

Current: 1.414

Preamp Gain: 179.20

Dilution factor (DF) Default value: 51

Output: Concentration

Metered volume (ml): 0.5

Blank counts (use ISOTONE II):

Run	BLANK
DF	51
1	26418
2	15300
3	12750
4	9690
5	6222
6	5916

Note: Blank concentrations are Cells per ml (not x 10⁶)

Dilutions: DF = 11 (1.0 ml sample to 10mL Isotone II)

DF = 51 (0.20 ml sample to 10mL Isotone II)

DF = 101 (0.20 ml sample to 20mL Isotone II)

Where : * = Concentrations are in Cells per mL x 10⁶

COMMENTS

TEST CONCENTRATION		Control		
Run	REP A	REP B	REP C	REP D
DF (if other than default)				
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
Replicate Average				
CONCENTRATION MEAN # Cells / mL* =			CV % =	

TEST CONCENTRATION		8.4		
Run	REP A	REP B	REP C	REP D
DF (if other than default)				
1	0.401			
2	0.351			
3	0.367			
Replicate Average	0.373			
CONCENTRATION MEAN # Cells / mL* =	0.373		CV % =	#DIV/0!

TEST CONCENTRATION		25		
Run	REP A	REP B	REP C	REP D
DF (if other than default)				
1	1.605	2.167	3.228	1.427
2	1.597	2.103	3.234	1.426
3	1.589	2.110	3.238	1.445
Replicate Average	1.597	2.127	3.233	1.433
CONCENTRATION MEAN # Cells / mL* =	2.097		CV % =	38.8%

TEST CONCENTRATION				
Run	REP A	REP B	REP C	REP D
DF (if other than default)				
1				
2				
3				
Replicate Average				
CONCENTRATION MEAN # Cells / mL* =			CV % =	

CETIS Summary Report

Report Date: 07 Feb-20 12:35 (p 1 of 1)
Test Code: B458401scc | 04-1694-1172

Green Algae Growth Test

Eurofins TestAmerica - Corvallis

Batch ID:	14-8403-3055	Test Type:	Cell Growth	Analyst:	Brett Muckey
Start Date:	08 Jan-20 13:35	Protocol:	EPA/821/R-02-013 (2002)	Diluent:	Mod-Hard Synthetic Water
Ending Date:	12 Jan-20 13:10	Species:	Selenastrum capricornutum	Brine:	
Duration:	96h	Source:	In-House Culture	Age:	
Sample ID:	08-6835-1458	Code:	B4584-01	Client:	
Sample Date:	07 Jan-20 09:30	Material:	POTW Effluent	Project:	
Receive Date:	08 Jan-20 10:30	Source:	Jacobs - Spokane County Regional Water (
Sample Age:	28h	Station:			

Test Note: 8.4% Rep A and 25% Rep C were identified via Grubb's test as statistical outliers. No explanation for these was found in the analytical process. Statistical analysis performed both with and without outliers.

Sample Note: Note: Temp on receipt observed @ -0.6 oC. However, sample was checked for ice formation and the sample was not frozen or partially frozen

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
20-0974-7024	Cell Density	56.5	100	75.17	38.7%	1.77	Steel Many-One Rank Sum Test <i>all data</i>
17-1134-7805	Cell Density	8.4	25	14.49	17.7%	11.9	Bonferroni Adj t Test <i>outliers removed</i>

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
07-2425-9405	Cell Density	IC25	17.57	11.25	25.42	5.692	Linear Interpolation (ICPIN) <i>outliers removed</i>
09-0454-9324	Cell Density	IC25	9.437	5.696	N/A	10.6	Linear Interpolation (ICPIN) <i>all data</i>

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
07-2425-9405	Cell Density	Control CV	0.1336	NL - 0.2	Yes	Passes Acceptability Criteria
09-0454-9324	Cell Density	Control CV	0.1336	NL - 0.2	Yes	Passes Acceptability Criteria
17-1134-7805	Cell Density	Control CV	0.1336	NL - 0.2	Yes	Passes Acceptability Criteria
20-0974-7024	Cell Density	Control CV	0.1336	NL - 0.2	Yes	Passes Acceptability Criteria
07-2425-9405	Cell Density	Control Resp	3.38E+6	1.00E+6 - NL	Yes	Passes Acceptability Criteria
09-0454-9324	Cell Density	Control Resp	3.38E+6	1.00E+6 - NL	Yes	Passes Acceptability Criteria
17-1134-7805	Cell Density	Control Resp	3.38E+6	1.00E+6 - NL	Yes	Passes Acceptability Criteria
20-0974-7024	Cell Density	Control Resp	3.38E+6	1.00E+6 - NL	Yes	Passes Acceptability Criteria
17-1134-7805	Cell Density	PMSD	0.1769	0.091 - 0.29	Yes	Passes Acceptability Criteria <i>outliers removed</i>
20-0974-7024	Cell Density	PMSD	0.387	0.091 - 0.29	Yes	Above Acceptability Criteria <i>all data</i>

Cell Density Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	3.378E+6	2.660E+6	4.097E+6	2.714E+6	3.680E+6	2.256E+5	4.513E+5	13.36%	0.0%
6.25		4	3.148E+6	2.585E+6	3.712E+6	2.772E+6	3.600E+6	1.769E+5	3.539E+5	11.24%	6.81%
8.4		4	2.558E+6	1.305E+5	4.985E+6	2.815E+5	3.508E+6	7.627E+5	1.525E+6	59.64%	24.29%
25		4	1.906E+6	5.216E+5	3.291E+6	1.236E+6	3.131E+6	4.351E+5	8.703E+5	45.65%	43.57%
56.5		4	2.645E+6	2.137E+6	3.153E+6	2.373E+6	3.086E+6	1.597E+5	3.194E+5	12.08%	21.71%
100		4	2.422E+6	2.171E+6	2.673E+6	2.272E+6	2.638E+6	7.884E+4	1.577E+5	6.51%	28.32%

Cell Density Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	2.714E+6	3.479E+6	3.680E+6	3.641E+6
6.25		2.995E+6	2.772E+6	3.600E+6	3.227E+6
8.4		2.815E+5	3.128E+6	3.508E+6	3.313E+6
25		1.339E+6	1.920E+6	3.131E+6	1.236E+6
56.5		2.670E+6	2.373E+6	2.452E+6	3.086E+6
100		2.272E+6	2.430E+6	2.638E+6	2.348E+6

CETIS Analytical Report

Report Date: 07 Feb-20 12:35 (p 1 of 4)
Test Code: B458401scc | 04-1694-1172

Green Algae Growth Test				Eurofins TestAmerica - Corvallis	
Analysis ID:	20-0974-7024	Endpoint:	Cell Density	CETIS Version:	CETISv1.8.8
Analyzed:	07 Feb-20 12:29	Analysis:	Nonparametric-Control vs Treatments	Official Results:	Yes
Batch ID:	14-8403-3055	Test Type:	Cell Growth	Analyst:	Brett Muckey
Start Date:	08 Jan-20 13:35	Protocol:	EPA/821/R-02-013 (2002)	Diluent:	Mod-Hard Synthetic Water
Ending Date:	12 Jan-20 13:10	Species:	Selenastrum capricornutum	Brine:	
Duration:	96h	Source:	In-House Culture	Age:	
Sample ID:	08-6835-1458	Code:	B4584-01	Client:	
Sample Date:	07 Jan-20 09:30	Material:	POTW Effluent	Project:	
Receive Date:	08 Jan-20 10:30	Source:	Jacobs - Spokane County Regional Water (
Sample Age:	28h	Station:			

Test Note: 8.4% Rep A and 25% Rep C were identified via Grubb's test as statistical outliers. No explanation for these was found in the analytical process. Statistical analysis performed both with and without outliers.

Sample Note: Note: Temp on receipt observed @ -0.6 oC. However, sample was checked for ice formation and the sample was not frozen or partially frozen

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	38.7%	56.5	100	75.17	1.77

Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		6.25	15	10	0	6	0.4761	Asymp	Non-Significant Effect
		8.4	14	10	0	6	0.3451	Asymp	Non-Significant Effect
		25	11	10	0	6	0.0805	Asymp	Non-Significant Effect
		56.5	11	10	0	6	0.0805	Asymp	Non-Significant Effect
		100*	10	10	0	6	0.0417	Asymp	Significant Effect

ALL DATA

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.554777E+12	1.110955E+12	5	1.883	0.1475	Non-Significant Effect
Error	1.062072E+13	5.900401E+11	18			
Total	1.61755E+13		23			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	15.7	15.09	0.0077	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8934	0.884	0.0156	Normal Distribution

Cell Density Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	3.378E+6	2.660E+6	4.097E+6	#Error	2.714E+6	3.680E+6	2.256E+5	13.36%	0.0%
6.25		4	3.148E+6	2.585E+6	3.712E+6	#Error	2.772E+6	3.600E+6	1.769E+5	11.24%	6.81%
8.4		4	2.558E+6	1.305E+5	4.985E+6	#Error	2.815E+5	3.508E+6	7.627E+5	59.64%	24.29%
25		4	1.906E+6	5.216E+5	3.291E+6	#Error	1.236E+6	3.131E+6	4.351E+5	45.65%	43.57%
56.5		4	2.645E+6	2.137E+6	3.153E+6	#Error	2.373E+6	3.086E+6	1.597E+5	12.08%	21.71%
100		4	2.422E+6	2.171E+6	2.673E+6	#Error	2.272E+6	2.638E+6	7.884E+4	6.51%	28.32%

Cell Density Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	D	2.714E+6	3.479E+6	3.680E+6	3.641E+6
6.25		2.995E+6	2.772E+6	3.600E+6	3.227E+6
8.4		2.815E+5	3.128E+6	3.508E+6	3.313E+6
25		1.339E+6	1.920E+6	3.131E+6	1.236E+6
56.5		2.670E+6	2.373E+6	2.452E+6	3.086E+6
100		2.272E+6	2.430E+6	2.638E+6	2.348E+6

CETIS Analytical Report

Report Date: 07 Feb-20 12:35 (p 2 of 4)
Test Code: B458401scc | 04-1694-1172

Green Algae Growth Test		Eurofins TestAmerica - Corvallis	
Analysis ID:	20-0974-7024	Endpoint:	Cell Density
Analized:	07 Feb-20 12:29	Analysis:	Nonparametric-Control vs Treatments
		CETIS Version:	CETISv1.8.8
		Official Results:	Yes

CETIS Analytical Report

Report Date: 07 Feb-20 12:35 (p 1 of 4)

Test Code: B458401sc | 04-1694-1172

Green Algae Growth Test

Eurofins TestAmerica - Corvallis

Analysis ID: 09-0454-9324	Endpoint: Cell Density	CETIS Version: CETISv1.8.8
Analyzed: 07 Feb-20 12:29	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 14-8403-3055	Test Type: Cell Growth	Analyst: Brett Muckey
Start Date: 08 Jan-20 13:35	Protocol: EPA/821/R-02-013 (2002)	Diluent: Mod-Hard Synthetic Water
Ending Date: 12 Jan-20 13:10	Species: Selenastrum capricornutum	Brine:
Duration: 96h	Source: In-House Culture	Age:
Sample ID: 08-6835-1458	Code: B4584-01	Client:
Sample Date: 07 Jan-20 09:30	Material: POTW Effluent	Project:
Receive Date: 08 Jan-20 10:30	Source: Jacobs - Spokane County Regional Water (
Sample Age: 28h	Station:	

Test Note: 8.4% Rep A and 25% Rep C were identified via Grubb's test as statistical outliers. No explanation for these was found in the analytical process. Statistical analysis performed both with and without outliers.

Sample Note: Note: Temp on receipt observed @ -0.6 oC. However, sample was check for ice formation and the sample was not frozen or partially frozen

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	749777	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC25	9.437	5.696	N/A	10.6	NA	17.56

Cell Density Summary

Calculated Variate

C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	3.378E+6	2.714E+6	3.680E+6	2.256E+5	4.513E+5	13.36%	0.0%
6.25		4	3.148E+6	2.772E+6	3.600E+6	1.769E+5	3.539E+5	11.24%	6.81%
8.4		4	2.558E+6	2.815E+5	3.508E+6	7.627E+5	1.525E+6	59.64%	24.29%
25		4	1.906E+6	1.236E+6	3.131E+6	4.351E+5	8.703E+5	45.65%	43.57%
56.5		4	2.645E+6	2.373E+6	3.086E+6	1.597E+5	3.194E+5	12.08%	21.71%
100		4	2.422E+6	2.272E+6	2.638E+6	7.884E+4	1.577E+5	6.51%	28.32%

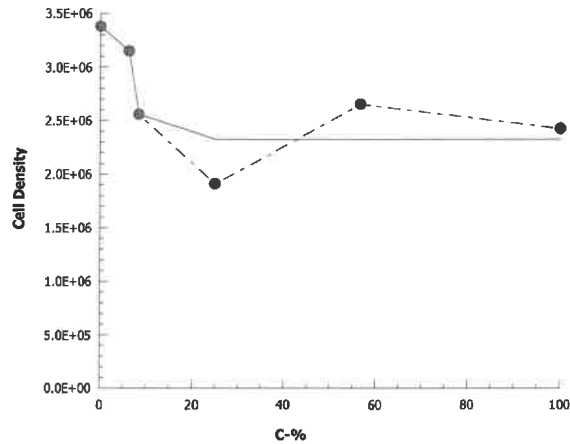
ALL DATA

Cell Density Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	2.714E+6	3.479E+6	3.680E+6	3.641E+6
6.25		2.995E+6	2.772E+6	3.600E+6	3.227E+6
8.4		2.815E+5	3.128E+6	3.508E+6	3.313E+6
25		1.339E+6	1.920E+6	3.131E+6	1.236E+6
56.5		2.670E+6	2.373E+6	2.452E+6	3.086E+6
100		2.272E+6	2.430E+6	2.638E+6	2.348E+6

Green Algae Growth Test		Eurofins TestAmerica - Corvallis	
Analysis ID:	09-0454-9324	Endpoint:	Cell Density
Analized:	07 Feb-20 12:29	Analysis:	Linear Interpolation (ICPIN)
		CETIS Version:	CETISv1.8.8
		Official Results:	Yes

Graphics



CETIS Analytical Report

Report Date: 07 Feb-20 12:35 (p 3 of 4)
Test Code: B458401scc | 04-1694-1172

Green Algae Growth Test

Eurofins TestAmerica - Corvallis

Analysis ID:	17-1134-7805	Endpoint:	Cell Density	CETIS Version:	CETISv1.8.8
Analyzed:	07 Feb-20 12:31	Analysis:	Parametric-Multiple Comparison	Official Results:	Yes
Batch ID:	14-8403-3055	Test Type:	Cell Growth	Analyst:	Brett Muckey
Start Date:	08 Jan-20 13:35	Protocol:	EPA/821/R-02-013 (2002)	Diluent:	Mod-Hard Synthetic Water
Ending Date:	12 Jan-20 13:10	Species:	Selenastrum capricornutum	Brine:	
Duration:	96h	Source:	In-House Culture	Age:	
Sample ID:	08-6835-1458	Code:	B4584-01	Client:	
Sample Date:	07 Jan-20 09:30	Material:	POTW Effluent	Project:	
Receive Date:	08 Jan-20 10:30	Source:	Jacobs - Spokane County Regional Water (
Sample Age:	28h	Station:			

Test Note: 8.4% Rep A and 25% Rep C were identified via Grubb's test as statistical outliers. No explanation for these was found in the analytical process. Statistical analysis performed both with and without outliers.

Sample Note: Note: Temp on receipt observed @ -0.6 oC. However, sample was checked for ice formation and the sample was not frozen or partially frozen

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	17.7%	8.4	25	14.49	11.9

Bonferroni Adj t Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		6.25	0.9944	2.583	6E+05	6	0.8371	CDF	Non-Significant Effect
		8.4	0.2475	2.583	6E+05	5	1.0000	CDF	Non-Significant Effect
		25*	7.527	2.583	6E+05	5	<0.0001	CDF	Significant Effect
		56.5*	3.171	2.583	6E+05	6	0.0148	CDF	Significant Effect
		100*	4.137	2.583	6E+05	6	0.0019	CDF	Significant Effect

OUTLIERS
REMOVED

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.326	2.758	0.2905	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	8.350563E+12	1.670113E+12	5	15.61	<0.0001	Significant Effect
Error	1.711892E+12	1.069933E+11	16			
Total	1.006246E+13		21			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.307	15.09	0.6528	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9688	0.8757	0.6836	Normal Distribution

Cell Density Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	3.378E+6	2.660E+6	4.097E+6	#Error	2.714E+6	3.680E+6	2.256E+5	13.36%	0.0%
6.25		4	3.148E+6	2.585E+6	3.712E+6	#Error	2.772E+6	3.600E+6	1.769E+5	11.24%	6.81%
8.4		3	3.317E+6	2.845E+6	3.789E+6	#Error	3.128E+6	3.508E+6	1.097E+5	5.73%	1.83%
25		3	1.498E+6	5.815E+5	2.415E+6	#Error	1.236E+6	1.920E+6	2.130E+5	24.63%	55.66%
56.5		4	2.645E+6	2.137E+6	3.153E+6	#Error	2.373E+6	3.086E+6	1.597E+5	12.08%	21.71%
100		4	2.422E+6	2.171E+6	2.673E+6	#Error	2.272E+6	2.638E+6	7.884E+4	6.51%	28.32%

CETIS Analytical Report

Report Date: 07 Feb-20 12:35 (p 4 of 4)
Test Code: B458401scc | 04-1694-1172

Green Algae Growth Test

Eurofins TestAmerica - Corvallis

Analysis ID: 17-1134-7805 Endpoint: Cell Density
Analyzed: 07 Feb-20 12:31 Analysis: Parametric-Multiple Comparison

CETIS Version: CETISv1.8.8
Official Results: Yes

Cell Density Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	D	2.714E+6	3.479E+6	3.680E+6	3.641E+6
6.25		2.995E+6	2.772E+6	3.600E+6	3.227E+6
8.4		3.128E+6	3.508E+6	3.313E+6	
25		1.339E+6	1.920E+6	1.236E+6	
56.5		2.670E+6	2.373E+6	2.452E+6	3.086E+6
100		2.272E+6	2.430E+6	2.638E+6	2.348E+6

CETIS Analytical Report

Report Date: 07 Feb-20 12:35 (p 3 of 4)

Test Code: B458401scc | 04-1694-1172

Green Algae Growth Test

Eurofins TestAmerica - Corvallis

Analysis ID: 07-2425-9405	Endpoint: Cell Density	CETIS Version: CETISv1.8.8
Analyzed: 07 Feb-20 12:33	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes
Batch ID: 14-8403-3055	Test Type: Cell Growth	Analyst: Brett Muckey
Start Date: 08 Jan-20 13:35	Protocol: EPA/821/R-02-013 (2002)	Diluent: Mod-Hard Synthetic Water
Ending Date: 12 Jan-20 13:10	Species: Selenastrum capricornutum	Brine:
Duration: 96h	Source: In-House Culture	Age:
Sample ID: 08-6835-1458	Code: B4584-01	Client:
Sample Date: 07 Jan-20 09:30	Material: POTW Effluent	Project:
Receive Date: 08 Jan-20 10:30	Source: Jacobs - Spokane County Regional Water (
Sample Age: 28h	Station:	

Test Note: 8.4% Rep A and 25% Rep C were identified via Grubb's test as statistical outliers. No explanation for these was found in the analytical process. Statistical analysis performed both with and without outliers.

Sample Note: Note: Temp on receipt observed @ -0.6 oC. However, sample was check for ice formation and the sample was not frozen or partially frozen

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	939716	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.326	2.758	0.2905	No Outliers Detected

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC25	17.57	11.25	25.42	5.692	3.935	8.888

Cell Density Summary

Calculated Variate

C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	3.378E+6	2.714E+6	3.680E+6	2.256E+5	4.513E+5	13.36%	0.0%
6.25		4	3.148E+6	2.772E+6	3.600E+6	1.769E+5	3.539E+5	11.24%	6.81%
8.4		3	3.317E+6	3.128E+6	3.508E+6	1.097E+5	1.900E+5	5.73%	1.83%
25		3	1.498E+6	1.236E+6	1.920E+6	2.130E+5	3.690E+5	24.63%	55.66%
56.5		4	2.645E+6	2.373E+6	3.086E+6	1.597E+5	3.194E+5	12.08%	21.71%
100		4	2.422E+6	2.272E+6	2.638E+6	7.884E+4	1.577E+5	6.51%	28.32%

OUTLIERS
REMOVED

Cell Density Detail

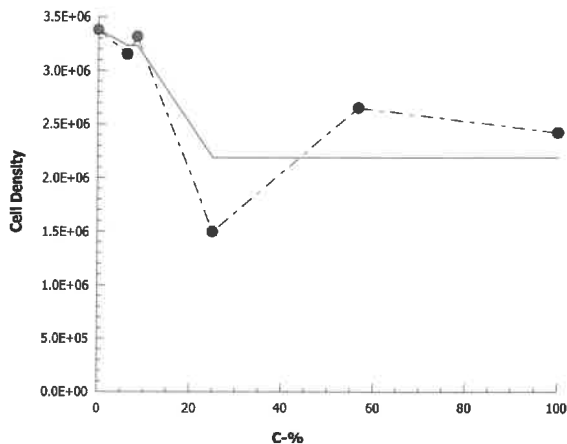
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	2.714E+6	3.479E+6	3.680E+6	3.641E+6
6.25		2.995E+6	2.772E+6	3.600E+6	3.227E+6
8.4		3.128E+6	3.508E+6	3.313E+6	
25		1.339E+6	1.920E+6	1.236E+6	
56.5		2.670E+6	2.373E+6	2.452E+6	3.086E+6
100		2.272E+6	2.430E+6	2.638E+6	2.348E+6

CETIS Analytical Report

Report Date: 07 Feb-20 12:35 (p 4 of 4)
Test Code: B458401scc | 04-1694-1172

Green Algae Growth Test		Eurofins TestAmerica - Corvallis	
Analysis ID:	07-2425-9405	Endpoint:	Cell Density
Analized:	07 Feb-20 12:33	Analysis:	Linear Interpolation (ICPIN)
		CETIS Version:	CETISv1.8.8
		Official Results:	Yes

Graphics



APPENDIX B

REFERENCE TOXICANT DATA SHEETS

Client:

QA / QC

Sample Description:

NaCl

Reagent Log #2B 075-02

Test Start Date: 1-8-20

Time: 1330

Initials: JD

Test End Date: 1/12/20

Time: 1318

Initials: TA

TEST CONDITIONS AT INITIATION:

Species ID#: RS969

Age: 5 days

Dilution Water ID #: 5026

Note: includes EDTA

INOCULUM (Axenic Culture)		Culture Density = Mean # of Cells/Field x 250,000	
# Cells	# Fields	Culture Density	
390	200	x 250,000 = 487500	
MEAN	1.95		

Filtration	None
Note: 50 g/L NaCl stock based off of DI water (0.2 um filtered)	

Test Start Density x Test Volume / Culture Density = Inoculum Volume
10,000 x 50 ml / 487500 = 1.03 ml

Test Vol. / Replicate	50 mls
No. of Reps	4
Nutrient Dosage	1 ml per L in Dilution water only. NaCl stock not nutrient dosed

Confirming Counts			
	# Cells	# Fields	Cell Conc. x 10 ⁶ / cells/ml
Flask #1	12	250	0.064 x 10 ⁶
Flask #2	10	250	0.04 x 10 ⁶
Flask #3	13	250	0.052 x 10 ⁶

Light Intensity at Test Initiation (foot-candles)		
Location #1	Location #2	Location #3
376	414	399

Technician 0 hr JD 24 hr JD 48 hr JD 72 hr TA 96 hr TA
Time 0 hr 1320 24 hr 1307 48 hr 1307 72 hr 11:15 96 hr 13:18
Thermometer ID: 0 hr #266 24 hr #266 48 hr #266 72 hr #266 96 hr #266

Test Concn. (g/L)	D.O. (mg/L)	pH					Temperature (°C)					Conductivity (umohs/cm)	Hard. 0 hr	Alk. 0 hr
		0 hr	24	48	72	96	0 hr	24	48	72	96			
Control	7.8	8.0	8.3	8.1	8.6	9.2	24.2	25.2	25.3	25.1	24.9	407	102	70
0.25	8.0	8.1	8.3	8.2	8.5	9.1	24.0	25.0	25.4	24.8	24.7	930	100	73
0.50	7.9	8.1	8.3	8.2	8.4	9.0	24.3	24.8	25.4	24.5	24.6	1451		
1.0	7.7	8.1	8.3	8.2	8.5	9.1	24.6	24.9	25.5	24.6	24.5	2420	100	67
2.0	8.1	8.0	8.3	8.2	8.4	8.8	24.9	25.0	25.5	24.4	24.6	4710		
4.0	8.0	8.0	8.3	8.2	8.3	8.7	25.0	25.1	25.9	24.6	24.7	7880	100	70

Task Manager

Endpoint : Growth

Project Manager

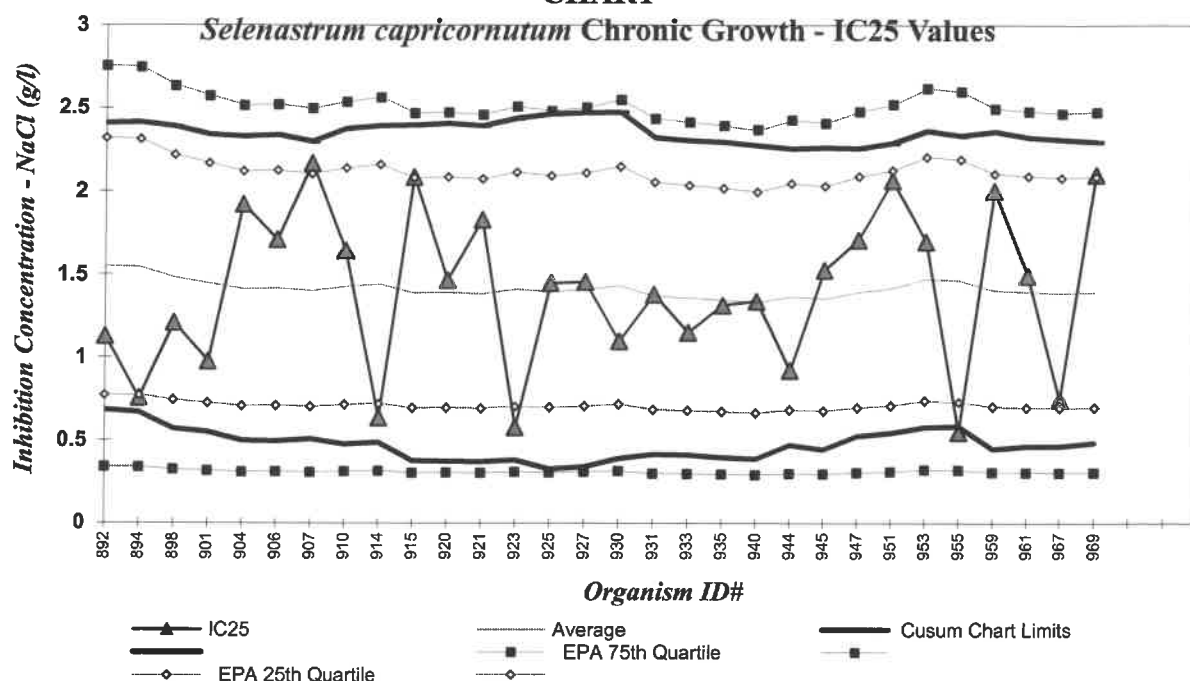
IC25 : 2.10

QA Officer

Cusum Chart Limits : 0.49 L 2.30

Comments:

REFERENCE TOXICANT CUMULATIVE SUMMARY (CUSUM) CHART



Selenastrum capricornutum - Green Algae - Chronic (EPA Test Method 1003.0)

SODIUM CHLORIDE (g/L)

Endpoint: Chronic Growth

Stats Method: Linear Interpolation

Test Conditions: Recon MH, 25 oC

From EPA 833-R-00-003:

10th Quartile CV (control limit) = 0.02

25th Quartile CV (warning limit) = 0.25

75th Quartile CV (warning limit) = 0.39

90th Quartile CV (control limit) = 0.51

As per EPA 833-R-00-003, section B.2.1, the quartiles listed above are from just a few labs (6) and therefore not to be considered typical or representative. Cusum limits are based on ASL data only.

Event #	Algae ID #	Test Start Date	IC25	Running Average	Running SD	Cusum Chart Limits		Intralab CV
						AVG-2SD	AVG+2SD	
186	930	2/19/2019	1.10	1.43	0.52	0.39	2.48	0.35
187	931	3/5/2019	1.38	1.37	0.48	0.42	2.32	0.35
188	933	4/11/2019	1.15	1.36	0.47	0.41	2.31	0.35
189	935	5/8/2019	1.31	1.35	0.48	0.40	2.30	0.35
190	940	7/2/2019	1.34	1.33	0.47	0.39	2.28	0.33
191	944	7/30/2019	0.92	1.37	0.45	0.47	2.26	0.34
192	945	8/1/2019	1.53	1.35	0.45	0.45	2.26	0.31
193	947	8/8/2019	1.71	1.39	0.43	0.53	2.26	0.31
194	951	9/3/2019	2.07	1.42	0.44	0.54	2.29	0.30
195	953	9/11/2019	1.70	1.47	0.45	0.58	2.37	0.30
196	955	9/26/2019	0.55	1.46	0.44	0.59	2.34	0.34
197	959	11/7/2019	2.00	1.40	0.48	0.45	2.36	0.33
198	961	11/19/2019	1.49	1.40	0.47	0.46	2.33	0.33
199	967	12/30/2019	0.74	1.39	0.46	0.46	2.31	0.33
200	969	1/8/2020	2.10	1.39	0.45	0.49	2.30	0.33
201								
202								

APPENDIX C
CHAIN OF CUSTODY



Environment Testing
TestAmerica

Sample Receipt Record

Batch Number: B4584-01
Client/Project: JACOBS-SPOKANE

Date Received: 1-8-20
Received By: JK

Were custody seals intact?

☒ Yes ☐ No ☐ N/A

Packing Material:

☐ Ice ☐ Blue Ice ☐ Box

Temp OK? ($\leq 6^{\circ}\text{C}$) Therm ID: TH169 Expires: 1/14/2020 Observed: -0.6 $^{\circ}\text{C}$, Actual Temp: 0.6 $^{\circ}\text{C}$ ☒ Yes ☐ No ☐ N/A

If sample is noted @ $\leq 0.0^{\circ}\text{C}$, is the sample frozen or partially frozen?

☐ Yes ☒ No ☐ N/A

Was a Chain of Custody (CoC) Provided?

☒ Yes ☐ No ☐ N/A

Was the CoC correctly filled out? (If No, document below)

☒ Yes ☐ No ☐ N/A

Were the sample containers in good condition (not broken or leaking)?

☒ Yes ☐ No ☐ N/A

Are all samples within 36 hours of collection?

☒ Yes ☐ No ☐ N/A

Method of Shipment: ☐ Hand Delivered, ☐ FedEx, ☒ UPS, ☐ Greyhound, ☐ Other: _____ ☐ N/A

Sample Exception Report (The following exceptions were noted)

Client was	Resolution
<div><div>NEIL DEJONGE (509) 536-3710 JACOBS 1004 N FREYA ST SPOKANE WA 99202-6004</div><div>SHIP MICHELLE BENNETT TO: (541) 243-6137 EUROFINS TESTAMERICA STE 310 1100 NE CIRCLE BLVD CORVALLIS OR 97330-4741</div><div>33 LBS 1 OF 1 SHIP WT: 33 LBS DATE: 07 JAN 2020 AH</div><div>OR 973 1-01</div><div>UPS NEXT DAY AIR TRACKING #: 1Z 940 X50 01 5170 8335</div><div>BILLING: P/P</div><div>ISH 13.00H Z2P 450 30.5U 10/2019</div><div>SEE INVOICE ON REVERSE regarding UPS terms and notice of limitation of liability. Where allowed by law, shipper authorizes UPS to act as forwarding agent for export control and to file and process the necessary documentation for the shipment. The shipper warrants that the commodity, technology or software is not subject to export control and that the shipment is in compliance with the applicable export control regulations. Shipment contrary to law is prohibited. RSD 882 1119</div></div>	

Client SPOKANE COUNTY / JACOBS NPDES# WA-0093317
Address 1000 N FRANK ST

Spokane WA 99202

Contact Person: Mr. DeTong
Phone: 509 536 3710

#0#

Ship Samples to:

Eurofins TestAmerica - Corvallis
Attention: Aquatic Toxicology Laboratory
1100 NE Circle Blvd, Suite 310
Corvallis, OR 97330
Phone: 541.243.6137

Composite Sample Information

Samples/Hour	<u>4</u>	Volume/Sample	<u>100 mL</u>
Total Hours	<u>29</u>	Total Volume	<u>9600 mL</u>
Initiated:	Date <u>1/6/2020</u>	Time	<u>0950</u>
Ended:	Date <u>1/7/2020</u>	Time	<u>0930</u>
Chilled During Collection	<u>yes</u>		

Analysis Required / Comments

[illegible]

Sampled By & Title <i>Benel's Delap</i>	(Please sign and print name)	Date/Time 1/7/2020 1030	Relinquished By <i>Benel's Delap</i>	(Please sign and print name)	Date/Time 1/7/2020 1100
Received By <i>John R. G. G.</i>	(Please sign and print name)	Date/Time 1-8-20 1030	Relinquished By	(Please sign and print name)	Date/Time
Received By	(Please sign and print name)	Date/Time	Relinquished By	(Please sign and print name)	Date/Time
Received By	(Please sign and print name)	Date/Time	Shipped Via UPS Bus Fed-Ex Hand Other	Shipping #	
Work Authorized By	(Please sign and print name)	Remarks	COC Bioassay.xls Doc Control ID: ASL612-0519		