PACIFIC ECORISK ENVIRONMENTAL CONSULTING & TESTING

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Keith Green
Los Alamos National Laboratory
Bldg 1237, Drop Point O3U
Los Alamos, NM 87545

September 20, 2005

3554 S
3555-S
3728S

Dear Mr. Green:

I have enclosed two copies of our report "An Evaluation of the Toxicity of Los Alamos National Laboratory Ambient Waters to *Selenastrum capricornutum*" for two sets of ambient water samples, the first set of samples being collected July 26, 2005, and the second set of samples being collected August 18, 2005. Briefly, none of the Los Alamos ambient water samples exhibited a significant reduction in *Selenastrum* growth relative to the Lab Control treatments.

If you have any questions regarding the performance and interpretation of these tests, feel free to contact my colleague Dr. Christopher Pincetich or myself at (925) 313-8080.

Sincerely,

R. Scott Ogle, Ph.D.
Principal & Special Projects Director

RECEIVED
OCT 25 2006
BY ML

835 Arnold Drive, Suite 104 Martinez, CA 94553 Phone: 925.313.8080 Fax: 925.313.8089

14463

An Evaluation of the Toxicity of Los Alamos National Laboratory Ambient Waters to *Selenastrum capricornutum*

Samples collected July 26 and August 18, 2005

Submitted To:

Los Alamos National Laboratory
Bldg 1237, Drop Point 03U
Los Alamos, NM 87545

Prepared By:

Pacific EcoRisk
835 Arnold Drive, Suite 104
Martinez, CA 94553

September 2005

PACIFIC ECORISK
ENVIRONMENTAL CONSULTING & TESTING

An Evaluation of the Toxicity of Los Alamos National Laboratory Ambient Waters to *Selenastrum capricornutum*

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An Evaluation of the Toxicity of Los Alamos National Laboratory Ambient Waters to *Selenastrum capricornutum*

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Appendix D Test Data and Summary of Statistics for the Reference Toxicant Evaluation of the *Selenastrum capricornutum* for the Testing of the July 26 Ambient Water Samples

Appendix E Test Data and Summary of Statistics for the Reference Toxicant Evaluation of the *Selenastrum capricornutum* for the Testing of the August 18 Ambient Water Samples

1. INTRODUCTION

The Los Alamos National Laboratory (LANL) has contracted Pacific EcoRisk (PER) to perform chronic toxicity evaluations of ambient water samples collected from watersheds that may have received surface water runoff or groundwater from historical LANL operations. These chronic toxicity evaluations consist of performing the US EPA short-term (96-hr) chronic toxicity algal growth test with the green alga *Selenastrum capricornutum.*

These tests were performed on two sets of ambient water samples, the first set of samples being collected on July 26, 2005, and the second set of samples being collected on August 18, 2005. In order to assess the sensitivity of the algal test organisms to toxic stress, reference toxicant tests were also performed. This report describes the performance and results of these tests.

2. CHRONIC TOXICITY TEST PROCEDURES

The methods used in conducting these tests followed the guidelines established by the EPA manual "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms, Third Edition" (EPA/600/4-91/002).

2.1 Receipt and Handling of Ambient Water Samples

On July 26, 2005, LANL staff collected 5 ambient water samples for toxicity testing; an additional set of 3 ambient water samples was similarly collected on August 18, 2005 (Table 1). Within 24 hours of collection, the samples were transported, on ice and under chain-of-custody, to the PER laboratory facility in Martinez, CA. Upon receipt at the testing laboratory, an aliquot of each water sample was removed for analysis of initial water quality characteristics (Table 1). The water samples were then stored at 4°C, until being used to initiate testing. The chain-of-custody records for the collection and delivery of these samples are provided in Appendix A.

Table 1. Initial water quality characteristics of the Los Alamos ambient water samples.

Sample ID	Sample Date	Temp (°C)	pH	D.O. (mg/L)	Alkalinity (mg/L)	Hardness (mg/L)	Conductivity (µS/cm)	Total Ammonia (mg/L N)
CAMO-05-61170	7/26/05	10.8	7.72	10.7	119	111	357	<1.0
CAMO-05-61172	7/26/05	9.7	7.13	10.2	170	159	422	<1.0
CAMO-05-61174	7/26/05	7.9	7.24	11.3	138	117	423	<1.0
CAMO-05-61176	7/26/05	6.7	7.66	12.2	130	90	483	<1.0
CALA-05-61185	7/26/05	6.9	7.64	10.9	64	93	199	<1.0
CAMO-05-61166	8/18/05	6.0	7.05	10.2	90	99	244	<1.0
CAMO-05-61178	8/18/05	8.9	7.33	9.8	78	52	265	<1.0
CAMO-05-61180	8/18/05	8.9	7.80	11.1	92	82	271	<1.0

2.2 Algal Growth Toxicity Testing with *Selenastrum capricornutum*

The short-term chronic toxicity algal test consists of exposing *Selenastrum capricornutum* to the ambient waters for 96-hrs, after which the effects on cell growth are evaluated. The specific procedures used in these tests are described below.

Each ambient water was pre-filtered (using sterile 0.45 μm filters) before use in the algal tests and each was tested at the 100% concentration only. The Lab Control water consisted of reverse osmosis, de-ionized (RO/DI) water. The Lab Control water and the pre-filtered ambient site waters were spiked with nutrients and then filtered through sterile 0.45 μm filters, as per EPA guidelines. "New" water quality characteristics (pH, dissolved oxygen [D.O.], and conductivity) were measured on the resulting test solutions prior to use in tests.

There were 4 replicates at each test treatment, each replicate consisting of a 250-mL glass Erlenmeyer flask containing 100 mL of test solution. Each flask was inoculated to an initial algal cell density of 10,000 cells/mL *S. capricornutum* from an ongoing laboratory culture of *Selenastrum* that is maintained in log growth phase. Each flask was loosely-capped and randomly positioned within a temperature-controlled water bath at 25°C, under continuous cool-white fluorescent illumination. Each day, temperature and pH were measured and recorded from a randomly-selected replicate flask at each test treatment; all replicate flasks were gently shaken in the morning and in the afternoon and re-positioned within the water bath.

After ~ 96-hrs exposure, the flasks were removed from the water bath and the algal cell density in each was determined by spectrophotometric analysis. The resulting cell density data at each ambient site water treatment were analyzed and statistically compared to the appropriate Lab Control treatment to determine any growth impairment, or toxicity, caused by the site water; all statistical analyses were performed using the CETIS® statistical software (Version 1.1.1revC, TidePool Scientific, McKinleyville, CA).

2.2.1 Reference Toxicant Testing of the *Selenastrum capricornutum*

Reference toxicant tests are performed in order to assess the sensitivity of the *Selenastrum* to toxic stress. The reference toxicant tests were performed similarly to the ambient water tests except that test solutions consisting of "Lab Control" media spiked with zinc (as $ZnCl_2$) at concentrations of 0.5, 1.0, 2.5, 5, 10, and 20 μg/L were used instead of the ambient water treatments. The resulting test response data were statistically analyzed to determine key dose-response point estimates (e.g., IC_{25}); all statistical analyses were made using the CETIS® software. These response endpoints were then compared to the 'typical response' range established by the mean ± 2 SD of the point estimates generated by the 20 most recent previous reference toxicant tests performed by this lab.

3. RESULTS

3.1 Effects of Los Alamos Ambient Waters on *Selenastrum capricornutum*

The results of the algal tests of the ambient water samples collected on July 26 are summarized below in Table 2. Briefly, there was a mean final algal cell density of 721,000 cells/mL at the Lab Control treatment. The mean algal cell densities were ≥1,350,000 cells/mL in each of the 5 ambient waters, none of which was significantly less than the Lab Control.

The results of the algal tests of the ambient water samples collected on August 18 are also summarized below in Table 2. Briefly, there was a mean final algal cell density of 244,000 cells/mL at the Lab Control treatment. The mean algal cell densities were ≥499,000 cells/mL in each of the 3 ambient waters, none of which was significantly less than the Lab Control.

The test data and summary of statistical analyses for the tests of the ambient water samples collected on July 26 are presented in Appendix B; the test data and summary of statistical analyses for the tests of the ambient water samples collected on August 18 are presented in Appendix C.

Table 2. Effect of Los Alamos ambient waters on *Selenastrum capricornutum* growth.

Treatment/Sample ID	Mean Algal Cell Density (cells/mL x 10^6)
Lab Control (for samples collected July 26)	0.721
CAMO-05-61170	1.860
CAMO-05-61172	2.890
CAMO-05-61174	2.110
CAMO-05-61176	1.350
CALA-05-61185	1.720
Lab Control (for samples collected August 18)	0.244
CAMO-05-61166	2.120
CAMO-05-61178	0.499
CAMO-05-61180	0.972

3.1.1 Reference Toxicant Toxicity to *Selenastrum capricornutum*

The results of the reference toxicant test that was performed concurrently with testing of the ambient water samples collected July 26 and August 18 are summarized below in Table 3a and Table 3b, respectively. For the samples collected on July 26, there was a mean of 721,000 cells/mL at the Lab Control treatment. The IC_{25} was 8.8 μg/L Zn. For the samples collected on August 18, there was a mean of 478,000 cells/mL at the Lab Control treatment. The IC_{25} was 13.2 μg/L Zn.

These reference toxicant test results are consistent with previous *Selenastrum* reference toxicant tests, indicating that these organisms were responding to toxic stress in a typical fashion.

The test data and summary of statistical analyses for this test are presented in Appendix C.

Table 3a. Reference toxicant testing: Effects of zinc on *Selenastrum capricornutum* growth.

Zinc Treatment (μg/L)	Mean Algal Cell Density (cells/mL x 10^6)
Lab Control	0.721
0.5	0.749
1	0.679
2.5*	**0.625***
5*	**0.606***
10*	**0.535***
20*	**0.502***
Summary of Statistics	
IC_{25} = 8.8 μg/L Zn	

* - Significantly less than the Control treatment response at $p < 0.05$.

Table 3b. Reference toxicant testing: Effects of zinc on *Selenastrum capricornutum* growth.

Zinc Treatment (μg/L)	Mean Algal Cell Density (cells/mL x 10^6)
Lab Control	0.478
0.5	0.370
1	0.487
2.5	0.582
5	0.427
10	0.387
20*	**0.302***
Summary of Statistics	
IC_{25} = 13.2 μg/L Zn	

* - Significantly less than the Control treatment response at $p < 0.05$.

4. SUMMARY AND CONCLUSIONS

4.1 Chronic Toxicity of Los Alamos Ambient Waters to *Selenastrum capricornutum*

None of the Los Alamos ambient water samples exhibited a significant reduction in *Selenastrum* growth relative to the Lab Control treatment.

4.2 QA/QC Summary

Test Conditions - Test conditions (pH, D.O., temperature, etc.) were all within acceptable limits for the algal growth test with *Selenastrum capricornutum*. All analyses were performed according the laboratory Standard Operating Procedures.

Test conditions (pH, D.O., temperature, etc.) were all within acceptable limits for the algal growth test with *Selenastrum capricornutum*. Due to the elevated variability between the Control treatment test replicates (CV >20%) in the initial testing of the August 18 ambient water samples, these samples were re-tested immediately; the re-test of the August 18 samples were very similar to the initial test results, and the inter-replicate variability at the Control treatment was well within acceptable levels (Table 4) and it is the results of this re-test that were reported in Section 3.1 of this report.

Table 4. Comparative test results for the initial test and re-test of the August 18 ambient water samples

Test Treatment	Mean Algal Cell Density (cells/mL x 10^6)	
	Initial Test	Re-Test
Lab Control	0.206 (CV = 26%)	0.244 (CV = 7%)
CAMO-05-61166	1.275	2.120
CAMO-05-61178	0.656	0.499
CAMO-05-61180	0.622	0.972

Negative Control – Except as noted above. the biological responses for the test organisms at the Lab Control treatments were within acceptable limits.

Positive Control - The results for the concurrent reference toxicant tests were consistent with the reference toxicant test database, indicating that these test organisms were responding to toxic stress in a typical fashion.

Appendix A

Chain-of-Custody Record for the Collection and Delivery of the Los Alamos Ambient Water Samples

Tuesday, July 26, 2005

CHAIN OF CUSTODY DOCUMENT NUMBER: 3555SC

Los Alamos
NATIONAL LABORATORY

REQUEST NUMBER: 3555S

ANALYSIS TYPE: OTHER

ATTN: Scott Olge
Pacific Ecorisk Laboratories
835 Arnold Dr., Suite 104
Martinez, CA 94553

SAMPLE ID	CONT ID	CONTAINER DESCRIPTION	ORDER TEMPLATE CODE	PRESERVATIVE	MATRIX
CAMO-05-61170	15	2 L WIDE MOUTH POLY	Bioassay+ Algae	NONE	W
CAMO-05-61172	15	2 L WIDE MOUTH POLY	Bioassay+ Algae	NONE	W
CAMO-05-61174	15	2 L WIDE MOUTH POLY	Bioassay+ Algae	NONE	W
CAMO-05-61176	15	2 L WIDE MOUTH POLY	Bioassay+ Algae	NONE	W

Final Page of CHAIN OF CUSTODY DOCUMENT FOR REQUEST NUMBER 3555S Page 1

Relinquished By:	Date	Time	Received By:	Date	Time
PRINTED NAME / SIGNATURE	7/26/05	1430	Raffaele Perrotta / Raffaele Perrotta	7/26/05	9:15
PRINTED NAME / SIGNATURE			PRINTED NAME / SIGNATURE		
PRINTED NAME / SIGNATURE			PRINTED NAME / SIGNATURE		

Received for DISPOSAL By:	Date	Time	Remarks:
PRINTED NAME / SIGNATURE			

Tuesday, July 26, 2005

CHAIN OF CUSTODY DOCUMENT NUMBER: 3554SC

Los Alamos
NATIONAL LABORATORY

REQUEST NUMBER: 3554S

ANALYSIS TYPE: OTHER

ATTN: Scott Olge
Pacific Ecorisk Laboratories
835 Arnold Dr., Suite 104
Martinez, CA 94553

SAMPLE ID	CONT ID	CONTAINER DESCRIPTION	ORDER TEMPLATE CODE	PRESERVATIVE	MATRIX
CALA-05-61185	17	2 L WIDE MOUTH POLY	Bioassay+ Algae	NONE	W

Final Page of CHAIN OF CUSTODY DOCUMENT FOR REQUEST NUMBER 3554S

Relinquished By:		Date	Time	Received By:		Date	Time
[illegible]		7/26/05	1437	Raffaele Pecoraro	Raffaele Pecoraro	7/27/05	9:05
PRINTED NAME	SIGNATURE			PRINTED NAME	SIGNATURE		
PRINTED NAME	SIGNATURE			PRINTED NAME	SIGNATURE		
PRINTED NAME	SIGNATURE			PRINTED NAME	SIGNATURE		

Received for DISPOSAL By:		Date	Time	Remarks:
PRINTED NAME	SIGNATURE			

Tuesday, July 26, 2005

REQUEST NUMBER: 3555S

ANALYSIS TYPE: OTHER

Los Alamos
NATIONAL LABORATORY

ATTN: Scott Olge
Pacific Ecorisk Laboratories
835 Arnold Dr., Suite 104
Martinez, CA 94553

Please analyze the enclosed samples according to the schedule indicated:

SHIP DATE: 07/26/2005
REPORT DUE: 08/25/2005
TURN AROUND REQ'D: 30

RAD SCREENING: NOT REQ
COMMENTS:

These samples are on:

LANL Request Number: 3555S
Per Agreement Number:
Project Cost Code: MRAA0550P7D0

LANL ER SMO CONTACT:
Signature:

METHOD CODE	SAMPLE ID	CONT ID	SAMPLE MATRIX	DATE SAMPLED	COMMENTS
EPA:100.2	CAMO-05-61170	15	W	07/26/2005	
EPA:1003.0	CAMO-05-61170	15	W	07/26/2005	
EPA:100.2	CAMO-05-61172	15	W	07/26/2005	
EPA:1003.0	CAMO-05-61172	15	W	07/26/2005	
EPA:100.2	CAMO-05-61174	15	W	07/26/2005	
EPA:1003.0	CAMO-05-61174	15	W	07/26/2005	
EPA:100.2	CAMO-05-61176	15	W	07/26/2005	
EPA:1003.0	CAMO-05-61176	15	W	07/26/2005	

Final Page of REQUEST NUMBER 3555S

Tuesday, July 26, 2005

REQUEST NUMBER: 3554S

ANALYSIS TYPE: OTHER

Los Alamos
NATIONAL LABORATORY

ATTN: Scott Olge
Pacific Ecorisk Laboratories
835 Arnold Dr., Suite 104
Martinez, CA 94553

Please analyze the enclosed samples according to the schedule indicated:

SHIP DATE: 07/26/2005
REPORT DUE: 08/25/2005
TURN AROUND REQ'D: 30

RAD SCREENING: NOT REQ
COMMENTS:

These samples are on:

LANL Request Number: 3554S
Per Agreement Number:
Project Cost Code: MRAA0550P7D0

LANL ER SMO CONTACT:
Signature:

METHOD CODE	SAMPLE ID	CONT ID	SAMPLE MATRIX	DATE SAMPLED	COMMENTS
EPA:100.2	CALA-05-61185	17	W	07/26/2005	
EPA:1003.0	CALA-05-61185	17	W	07/26/2005	

Final Page of REQUEST NUMBER 3554S

Thursday, August 18, 2005

CHAIN OF CUSTODY DOCUMENT NUMBER: 3728SC

Los Alamos
NATIONAL LABORATORY

REQUEST NUMBER: 3728S

ANALYSIS TYPE: OTHER

ATTN: Scott Olge
Pacific Ecorisk Laboratories
835 Arnold Dr., Suite 104
Martinez, CA 94553

SAMPLE ID	CONT ID	CONTAINER DESCRIPTION	ORDER TEMPLATE CODE	PRESERVATIVE	MATRIX
CAMO-05-61166	15	2 L WIDE MOUTH POLY	Bioassay+ Algae	NONE	W
CAMO-05-61178	15	2 L WIDE MOUTH POLY	Bioassay+ Algae	NONE	W
CAMO-05-61180	15	2 L WIDE MOUTH POLY	Bioassay+ Algae	NONE	W

Final Page of CHAIN OF CUSTODY DOCUMENT FOR REQUEST NUMBER 3728S

Relinquished By:		Date	Time	Received By:		Date	Time
M Rivera	M Rivera	8-18-05	1400	Natasha Jeter	Natasha Jeter	8-19-05	930
PRINTED NAME	SIGNATURE			PRINTED NAME	SIGNATURE		
PRINTED NAME	SIGNATURE			PRINTED NAME	SIGNATURE		
PRINTED NAME	SIGNATURE			PRINTED NAME	SIGNATURE		

Received for DISPOSAL By:		Date	Time	Remarks:
PRINTED NAME	SIGNATURE			

Thursday, August 18, 2005

REQUEST NUMBER: 3728S

ANALYSIS TYPE: OTHER

Los Alamos
NATIONAL LABORATORY

ATTN: Scott Olge
Pacific Ecorisk Laboratories
835 Arnold Dr., Suite 104
Martinez, CA 94553

Please analyze the enclosed samples according to the schedule indicated:

SHIP DATE: 08/18/2005
REPORT DUE: 09/17/2005
TURN AROUND REQ'D: 30

RAD SCREENING: NOT REQ
COMMENTS:

These samples are on:

LANL Request Number: 3728S
Per Agreement Number:
Project Cost Code: MRAA0550P7D0

LANL ER SMO CONTACT:
Signature:

METHOD CODE	SAMPLE ID	CONT ID	SAMPLE MATRIX	DATE SAMPLED	COMMENTS
EPA:100.2	CAMO-05-61178	15	W	08/18/2005	
EPA:1003.0	CAMO-05-61180	15	W	08/18/2005	
EPA:100.2	CAMO-05-61166	15	W	08/18/2005	
EPA:100.2	CAMO-05-61180	15	W	08/18/2005	
EPA:1003.0	CAMO-05-61166	15	W	08/18/2005	
EPA:1003.0	CAMO-05-61178	15	W	08/18/2005	

Final Page of REQUEST NUMBER 3728S

Appendix B

Test Data and Summary of Statistics for the Evaluation of the Chronic Toxicity of the Los Alamos Ambient Waters to *Selenastrum capricornutum*

Appendix B

Test Data and Summary of Statistics for the Evaluation of the Chronic Toxicity of Los Alamos Ambient Water Samples to *Selenastrum capricornutum* – Samples Collected August 18, 2005

CETIS Test Summary

Report Date: 01 Aug-05 11:55 AM
Test Link: 15-6553-7063/14104

Selenastrum Growth Test — **Pacific EcoRisk**

Test No:	17-1144-4405	**Test Type:**	Cell Growth	**Duration:**	94h
Start Date:	27 Jul-05 12:30 PM	**Protocol:**	EPA/600/4-91/002 (1994)	**Species:**	Selenastrum capricornutum
Ending Date:	31 Jul-05 10:30 AM	**Dil Water:**	Not Applicable	**Source:**	In-House Culture
Setup Date:	27 Jul-05 12:30 PM	**Brine:**	Not Applicable		

Sample No:	06-6329-1920	**Material:**	Ambient Water	**Client:**	Los Alamos National Laboratory
Sample Date:	26 Jul-05 02:30 PM	**Code:**	10419	**Project:**	
Receive Date:	27 Jul-05 09:05 AM	**Source:**	Los Alamos National Laboratory		
Sample Age:	22h (10.8 °C)	**Station:**	CAMO-05-61170		

Comparison Summary

Analysis	Endpoint	NOEL	LOEL	ChV	PMSD	Method
12-8180-5201	Cell Density	100	> 100	N/A	25.67%	Equal Variance t Two-Sample

Cell Density Summary

Conc-%	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Lab Water	4	7.21E+5	6.86E+5	7.69E+5	1.74E+4	3.47E-4	4.82%
100		4	1.86E+6	1.62E+6	2.04E+6	9.37E+4	1.87E+5	10.07%

Cell Density Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water	7.69E+5	7.12E+5	6.86E+5	7.18E+5
100		1.98E+6	1.81E+6	2.04E+6	1.62E+6

Selenastrum capricornutum Cell Density Enumeration Data Sheet

Client: Neptune & Co
Test Material: CAMO-05-61170
Test Start Date: 7-27-05
Test ID #: 14104
Project #: 10019
Test End Date: 7/31/05
Control/Diluent: Algal Medium

Test Treatment	Temp (°C)	pH	D.O. (mg/L)	Conductivity (µS/cm)	Sign-Off
Lab Water Control	24.8	7.52	8.8	119	Inoculation Time: 1230
100%	24.8	JC ~~7.55~~ 7.82	9.5	473	Test Solution Prep: GP
					New WQ: dC
Lab Water Control	25.0	8.39			Date: 7/28/05
100%	25.0	8.31			WQ Time: 15.25
					WQ Signoff: RP
Lab Water Control	24.6	8.00			Date: 7.29.05
100%	24.6	8.48			WQ Time: 1200
					WQ Signoff: SL
Lab Water Control	25.0	9.58			Date: 7/30/05
100%	25.0	8.92			WQ Time: 1030
					WQ Signoff: MW
Lab Water Control	24.9	9.69	9.6	100	Date: 7-31-05
100%	24.9	9.52	10.5	419	WQ Time: 1200
					WQ Signoff: JC

Initial Count: 10,000 cells/mL Termination Time: 1030 Enumerating Scientist: MW

Treatment	Rep.	Cell Density (cells/mL x 10^6) Count 1	Count 2	Count 3	Mean Cell Density (cells/mL x 10^6)
Lab Water Control	A	0.758	0.781	0.767	0.769
	B	0.715	0.709	0.713	0.712
	C	0.680	0.690	0.684	0.686
	D	0.717	0.712	0.726	0.718
100%	A	1.994	2.006	1.931	1.975
	B	1.739	1.838	1.858	1.812
	C	2.027	2.009	2.077	2.038
	D	1.583	1.635	1.638	1.619

Initial Test Conditions	
Alkalinity	118
Hardness	98
Light Intensity (ftc)	520

This datasheet has been reviewed for completeness and consistency with identification of any water quality measures or other issues of concern.

Signoff: JS
Date: 7/31/05
Time: 1425

CETIS Test Summary

Report Date: 01 Aug-05 11:57 AM
Test Link: 15-3990-7210/14105

Selenastrum Growth Test — **Pacific EcoRisk**

Test No:	17-1144-4405	**Test Type:**	Cell Growth	**Duration:**	94h
Start Date:	27 Jul-05 12:30 PM	**Protocol:**	EPA/600/4-91/002 (1994)	**Species:**	Selenastrum capricornutum
Ending Date:	31 Jul-05 10:30 AM	**Dil Water:**	Not Applicable	**Source:**	In-House Culture
Setup Date:	27 Jul-05 12:30 PM	**Brine:**	Not Applicable		

Sample No:	05-2127-1193	**Material:**	Ambient Water	**Client:**	Los Alamos National Laboratory
Sample Date:	26 Jul-05 02:30 PM	**Code:**	10419	**Project:**	
Receive Date:	27 Jul-05 09:05 AM	**Source:**	Los Alamos National Laboratory		
Sample Age:	22h (9.7 °C)	**Station:**	CAMO-05-61172		

Comparison Summary

Analysis	Endpoint	NOEL	LOEL	ChV	PMSD	Method
04-4641-8616	Cell Density	100	> 100	N/A	9.87%	Equal Variance t Two-Sample

Cell Density Summary

Conc-%	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Lab Water	4	7.21E+5	6.86E+5	7.69E+5	1.74E+4	3.47E+4	4.82%
100		4	2.89E+6	2.84E+6	2.97E+6	3.23E+4	6.45E+4	2.23%

Cell Density Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water	7.69E+5	7.12E+5	6.86E+5	7.18E+5
100		2.92E+6	2.97E+6	2.84E+6	2.84E+6

Selenastrum capricornutum Cell Density Enumeration Data Sheet

Client: Neptune & Co
Test Material: CAMG-05-01172
Test Start Date: 7-27-05
Test ID #: 14105
Project #: 10419
Test End Date: 7/31/05
Control/Diluent: Algal Medium

Test Treatment	Temp (°C)	pH	D.O. (mg/L)	Conductivity (µS/cm)	Sign-Off
Lab Water Control	24.8	7.52	8.8	119	Inoculation Time: 1230
100%	24.8	7.24	9.4	519	Test Solution Prep: [illegible]
					New WQ: JC
Lab Water Control	25.0	8.39			Date: 7/28/05
100%	25.0	8.17			WQ Time: [illegible]
					WQ Signoff: RP
Lab Water Control	24.6	8.00			Date: 7.29.05
100%	24.6	8.51			WQ Time: 1800
					WQ Signoff: SL
Lab Water Control	25.0	9.58			Date: 7/30/05
100%	25.0	9.00			WQ Time: 0300
					WQ Signoff: MW
Lab Water Control	24.9	9.69	9.6	100	Date: 7-31-05
100%	24.9	~~9.52~~ 9.8	10.9	471	WQ Time: ~~[illegible]~~ 1200
					WQ Signoff: JC

Initial Count: 10,000 cells/mL Termination Time: 1030 Enumerating Scientist: MW

Treatment	Rep.	Cell Density (cells/mL x 10^6) Count 1	Count 2	Count 3	Mean Cell Density (cells/mL x 10^6)
Lab Water Control	A	0.755	0.781	0.767	0.769
	B	0.715	0.709	0.713	0.712
	C	0.680	0.690	0.689	0.686
	D	0.717	0.712	0.726	0.718
100%	A	2.881	2.926	2.956	2.921
	B	3.008	2.956	2.955	2.973
	C	2.758	2.871	2.891	2.840
	D	2.810	2.847	2.872	2.843

Initial Test Conditions	
Alkalinity	174
Hardness	208
Light Intensity (ftc)	520

This datasheet has been reviewed for completeness and consistency with identification of any water quality measures or other issues of concern.

Signoff: JS
Date: 7/31/05
Time: 1425

CETIS Test Summary

Report Date: 01 Aug-05 11:59 AM
Test Link: 06-6156-3355/14106

Selenastrum Growth Test — **Pacific EcoRisk**

Test No:	17-1144-4405	**Test Type:**	Cell Growth	**Duration:**	94h
Start Date:	27 Jul-05 12:30 PM	**Protocol:**	EPA/600/4-91/002 (1994)	**Species:**	Selenastrum capricornutum
Ending Date:	31 Jul-05 10:30 AM	**Dil Water:**	Not Applicable	**Source:**	In-House Culture
Setup Date:	27 Jul-05 12:30 PM	**Brine:**	Not Applicable		

Sample No:	05-5859-6538	**Material:**	Ambient Water	**Client:**	Los Alamos National Laboratory
Sample Date:	26 Jul-05 02:30 PM	**Code:**	10419	**Project:**	.
Receive Date:	27 Jul-05 09:05 AM	**Source:**	Los Alamos National Laboratory		
Sample Age:	22h (7.9 °C)	**Station:**	CAMO-05-61174		

Comparison Summary

Analysis	Endpoint	NOEL	LOEL	ChV	PMSD	Method
06-1168-3989	Cell Density	100	> 100	N/A	23.51%	Equal Variance t Two-Sample

Cell Density Summary

Conc-%	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Lab Water	4	7.21E+5	6.86E+5	7.69E+5	1.74E+4	3.47E+4	4.82%
100		4	2.11E+6	1.89E+6	2.27E+6	8.55E+4	1.71E+5	8.11%

Cell Density Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water	7.69E+5	7.12E+5	6.86E+5	7.18E+5
100		2.27E+6	2.06E+6	2.22E+6	1.89E+6

Selenastrum capricornutum Cell Density Enumeration Data Sheet

Client: Neptune & Co
Test Material: CAMO-05-61174
Test Start Date: 7-27-05
Test ID #: 14106
Project #: 10919
Test End Date: 7/31/05
Control/Diluent: Algal Medium

Test Treatment	Temp (°C)	pH	D.O. (mg/L)	Conductivity (µS/cm)	Sign-Off
Lab Water Control	24.8	7.52	8.8	119	Inoculation Time: 12:30
100%	24.8	7.44	9.3	528	Test Solution Prep: RP
					New WQ: JC
Lab Water Control	25.0	8.39			Date: 7/28/05
100%	25.0	8.30			WQ Time: 15:35
					WQ Signoff: RP
Lab Water Control	24.6	8.00			Date: 7.29.05
100%	24.6	8.61			WQ Time: 1200
					WQ Signoff: SL
Lab Water Control	25.8	9.58			Date: 7/30/05
100%	23.0	9.15			WQ Time: 1030
					WQ Signoff: MW
Lab Water Control	24.9	9.69	9.6	100	Date: 7-31-05
100%	24.9	9.85	11.2	486	WQ Time: 12:00
					WQ Signoff: JC

Initial Count: 10,000 cells/mL Termination Time: 1030 Enumerating Scientist: MW

Treatment	Rep.	Cell Density (cells/mL x 10^6) Count 1	Count 2	Count 3	Mean Cell Density (cells/mL x 10^6)
Lab Water Control	A	0.758	0.781	0.767	0.769
	B	0.715	0.709	0.713	0.712
	C	0.650	0.690	0.659	0.666
	D	0.717	0.712	0.720	0.718
100%	A	2.257	2.265	2.283	2.268
	B	2.023	2.059	2.091	2.058
	C	2.207	2.214	2.233	2.218
	D	1.856	1.930	1.887	1.890

Initial Test Conditions	
Alkalinity	✓ 140
Hardness	✓ 86
Light Intensity (ftc)	520

This datasheet has been reviewed for completeness and consistency with identification of any water quality measures or other issues of concern.	Signoff: JS
	Date: 7/31/05
	Time: 1425

CETIS Test Summary

Report Date: 01 Aug-05 12:01 PM
Test Link: 04-0457-9358/14107

Selenastrum Growth Test — **Pacific EcoRisk**

Test No:	17-1144-4405	**Test Type:**	Cell Growth	**Duration:**	94h
Start Date:	27 Jul-05 12:30 PM	**Protocol:**	EPA/600/4-91/002 (1994)	**Species:**	Selenastrum capricornutum
Ending Date:	31 Jul-05 10:30 AM	**Dil Water:**	Not Applicable	**Source:**	In-House Culture
Setup Date:	27 Jul-05 12:30 PM	**Brine:**	Not Applicable		

Sample No:	18-0153-0911	**Material:**	Ambient Water	**Client:**	Los Alamos National Laboratory
Sample Date:	26 Jul-05 02:30 PM	**Code:**	10419	**Project:**	
Receive Date:	27 Jul-05 09:05 AM	**Source:**	Los Alamos National Laboratory		
Sample Age:	22h (6.7 °C)	**Station:**	CAMO-05-61176		

Comparison Summary

Analysis	Endpoint	NOEL	LOEL	ChV	PMSD	Method
03-0553-9345	Cell Density	100	> 100	N/A	5.09%	Equal Variance t Two-Sample

Cell Density Summary

Conc-%	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Lab Water	4	7.21E+5	6.86E+5	7.69E+5	1.74E+4	3.47E+4	4.82%
100		4	1.35E+6	1.33E+6	1.37E+6	7.43E+3	1.49E+4	1.10%

Cell Density Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water	7.69E+5	7.12E+5	6.86E+5	7.18E+5
100		1.37E+6	1.33E+6	1.36E+6	1.36E+6

Selenastrum capricornutum Cell Density Enumeration Data Sheet

Client: Neptune & Co. Test Material: CAMO-05-61176

Test Start Date: 7-27-05 Test ID #: 1407 Project #: 10419

Test End Date: 7/31/05 Control/Diluent: Algal Medium

Test Treatment	Temp (°C)	pH	D.O. (mg/L)	Conductivity (µS/cm)	Sign-Off
Lab Water Control	24.8	7.52	8.8	119	Inoculation Time: 12:30
100%	24.8	7.88	9.4	585	Test Solution Prep: [illegible]
					New WQ: [illegible]
Lab Water Control	25.0	8.39			Date: 7/28/05
100%	25.0	8.35			WQ Time: 12:25
					WQ Signoff: RP
Lab Water Control	24.6	8.00			Date: 7.29.05
100%	24.6	8.51			WQ Time: 1200
					WQ Signoff: SC
Lab Water Control	25.0	9.58			Date: 7/30/05
100%	25.0	8.13			WQ Time: 1030
					WQ Signoff: MC
Lab Water Control	24.9	9.89	9.6	100	Date: 7-31-05
100%	24.9	9.32	10.4	566	WQ Time: 1200
					WQ Signoff: JC

Initial Count: 10,000 cells/mL Termination Time: 1030 Enumerating Scientist: ML

Treatment	Rep.	Cell Density (cells/mL x 10^6) Count 1	Count 2	Count 3	Mean Cell Density (cells/mL x 10^6)
Lab Water Control	A	0.758	0.781	0.767	0.769
	B	0.715	0.709	0.713	0.712
	C	0.650	0.690	0.659	0.650
	D	0.717	0.712	0.726	0.718
100%	A	1.351	1.371	1.364	1.369
	B	1.337	1.339	1.326	1.334
	C	1.333	1.375	1.372	1.360
	D	1.344	1.361	1.362	1.356

Initial Test Conditions	
Alkalinity	131
Hardness	56
Light Intensity (ftc)	520

This datasheet has been reviewed for completeness and consistency with identification of any water quality measures or other issues of concern.

Signoff: JS

Date: 7/31/05

Time: 1425

CETIS Test Summary

Report Date: 01 Aug-05 12:03 PM
Test Link: 07-0222-1699/14103

Selenastrum Growth Test — **Pacific EcoRisk**

Test No:	17-1144-4405	**Test Type:**	Cell Growth	**Duration:**	94h
Start Date:	27 Jul-05 12:30 PM	**Protocol:**	EPA/600/4-91/002 (1994)	**Species:**	Selenastrum capricornutum
Ending Date:	31 Jul-05 10:30 AM	**Dil Water:**	Not Applicable	**Source:**	In-House Culture
Setup Date:	27 Jul-05 12:30 PM	**Brine:**	Not Applicable		

Sample No:	03-3971-2984	**Material:**	Ambient Water	**Client:**	Los Alamos National Laboratory
Sample Date:	26 Jul-05 02:30 PM	**Code:**	10419	**Project:**	
Receive Date:	27 Jul-05 09:05 AM	**Source:**	Los Alamos National Laboratory		
Sample Age:	22h (6.9 °C)	**Station:**	CALA-05-61185		

Comparison Summary

Analysis	Endpoint	NOEL	LOEL	ChV	PMSD	Method
04-6280-6903	Cell Density	100	> 100	N/A	11.16%	Equal Variance t Two-Sample

Cell Density Summary

Conc-%	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Lab Water	4	7.21E+5	6.86E+5	7.69E+5	1.74E+4	3.47E+4	4.82%
100		4	1.72E+6	1.62E+6	1.79E+6	3.76E+4	7.53E+4	4.38%

Cell Density Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water	7.69E+5	7.12E+5	6.86E+5	7.18E+5
100		1.76E+6	1.79E+6	1.62E+6	1.70E+6

Selenastrum capricornutum Cell Density Enumeration Data Sheet

Client: Neptune & Co
Test Material: CALA-05-61185
Test Start Date: 7-27-05
Test ID #: 14103
Project #: 10419
Test End Date: 7/31/05
Control/Diluent: Algal Medium

Test Treatment	Temp (°C)	pH	D.O. (mg/L)	Conductivity (µS/cm)	Sign-Off
Lab Water Control	24.8	7.52	8.8	119	Inoculation Time: 1730
100%	24.8	7.94	9.4	300	Test Solution Prep: JC
					New WQ: JC
Lab Water Control	25.0	8.39			Date: 7/28/05
100%	25.0	8.20			WQ Time: 15-25
					WQ Signoff: RJ
Lab Water Control	24.6	8.00			Date: 7.29.05
100%	24.6	8.58			WQ Time: 1200
					WQ Signoff: SL
Lab Water Control	25.0	9.58			Date: 7/30/05
100%	25.0	9.05			WQ Time: 1030
					WQ Signoff: ML
Lab Water Control	24.9	9.69	9.6	100	Date: 7-31-05
100%	24.9	9.73	10.2	280	WQ Time: 1200
					WQ Signoff: JC

Initial Count: 10,000 cells/mL Termination Time: 1030 Enumerating Scientist: ML

Treatment	Rep.	Cell Density (cells/mL x 10^6) Count 1	Count 2	Count 3	Mean Cell Density (cells/mL x 10^6)
Lab Water Control	A	0.758	0.781	0.767	0.769
	B	0.715	0.704	0.713	0.712
	C	0.680	0.690	0.688	0.686
	D	0.717	0.712	0.726	0.718
100%	A	1.766	1.745	1.773	1.761
	B	1.752	1.801	1.829	1.794
	C	1.598	1.628	1.646	1.624
	D	1.697	1.685	1.702	1.695

Initial Test Conditions	
Alkalinity	67
Hardness	71
Light Intensity (ftc)	520

This datasheet has been reviewed for completeness and consistency with identification of any water quality measures or other issues of concern.	Signoff: JS
	Date: 7/31/05
	Time: 1425

Appendix C

Test Data and Summary of Statistics for the Evaluation of the Chronic Toxicity of Los Alamos Ambient Water Samples to *Selenastrum capricornutum* – Samples Collected August 18, 2005

CETIS Test Summary

Report Date: 30 Aug-05 9:31 AM
Test Link: 15-2518-0839/14616

Selenastrum Growth Test — **Pacific EcoRisk**

Test No:	07-1886-6704	**Test Type:**	Cell Growth	**Duration:**	95h
Start Date:	24 Aug-05 01:45 PM	**Protocol:**	EPA/600/4-91/002 (1994)	**Species:**	Selenastrum capricornutum
Ending Date:	28 Aug-05 01:00 PM	**Dil Water:**	Not Applicable	**Source:**	In-House Culture
Setup Date:	24 Aug-05 01:45 PM	**Brine:**	Not Applicable		

Sample No:	08-0754-9803	**Material:**	Ambient Water	**Client:**	Los Alamos National Laboratory
Sample Date:	18 Aug-05 02:00 PM	**Code:**	10481	**Project:**	
Receive Date:	19 Aug-05 09:30 AM	**Source:**	Los Alamos National Laboratory		
Sample Age:	5d 23h (6 °C)	**Station:**	CAMO-05-61166		

Comparison Summary

Analysis	Endpoint	NOEL	LOEL	ChV	PMSD	Method
10-6495-5137	Cell Density	100	> 100	N/A	32.51%	Equal Variance t Two-Sample

Cell Density Summary

Conc-%	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Lab Water	4	2.44E+5	2.20E+5	2.57E+5	8.51E+3	1.70E+4	6.99%
100		4	2.12E+6	2.04E+6	2.21E+6	3.98E+4	7.97E+4	3.76%

Cell Density Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water	2.57E+5	2.55E+5	2.42E+5	2.20E+5
100		2.15E+6	2.21E+6	2.07E+6	2.04E+6

Selenastrum capricornutum Cell Density Enumeration Data Sheet

Client: Los Alamos (retest) Test Material: CAMO-05-61166

Test Start Date: 8-24-05 Test ID #: 14616 Project #: 10781

Test End Date: 8-28-05 Control/Diluent: Algal Medium

Test Treatment	Temp (°C)	pH	D.O. (mg/L)	Conductivity (µS/cm)	Sign-Off
Lab Water Control	24.4	7.51	8.9	95.6	Inoculation Time: 1345
100%	24.4	7.65	8.8	328	Test Solution Prep QD
					New WQ: YK
Lab Water Control	24.4	9.20			Date: 8-25-05
100%	24.4	8.10			WQ Time: 10:30
					WQ Signoff: YK
Lab Water Control	24.8	8.11			Date: 8.26.05
100%	24.8	8.41			WQ Time: 1120
					WQ Signoff: PG
Lab Water Control	24.3	9.42			Date: 8/27/05
100%	24.3	9.41			WQ Time: 14:35
					WQ Signoff: K
Lab Water Control	24.4	8.68	7.7	166	Date: 8-28-05
100%	24.4	10.24	9.2	331	WQ Time: 1050
					WQ Signoff: CS

Initial Count: 10,000 cells/mL Termination Time: 113:00 Enumerating Scientist: YK

Treatment	Rep.	Cell Density (cells/mL x 10^6) Count 1	Count 2	Count 3	Mean Cell Density (cells/mL x 10^6)
Lab Water Control	A	0.258	0.257	0.257	0.257
	B	0.257	0.260	0.253	0.255
	C	0.249	0.241	0.237	0.242
	D	0.199	0.213	0.247	0.220
100%	A	2.171	2.152	2.140	2.154
	B	2.226	2.184	2.231	2.214
	C	2.092	2.060	2.058	2.070
	D	2.074	2.019	2.024	2.039

Initial Test Conditions	
Alkalinity	✓ ~~108~~ 108
Hardness	✓ 99
Light Intensity (ftc)	530

This datasheet has been reviewed for completeness and consistency with identification of any water quality measures or other issues of concern.	Signoff: CS
	Date: 8/28/05
	Time: 14:00

CETIS Test Summary

Report Date: 30 Aug-05 9:33 AM
Test Link: 18-0870-7439/14617

Selenastrum Growth Test — **Pacific EcoRisk**

Test No:	07-1886-6704	**Test Type:**	Cell Growth	**Duration:**	95h
Start Date:	24 Aug-05 01:45 PM	**Protocol:**	EPA/600/4-91/002 (1994)	**Species:**	Selenastrum capricornutum
Ending Date:	28 Aug-05 01:00 PM	**Dil Water:**	Not Applicable	**Source:**	In-House Culture
Setup Date:	24 Aug-05 01:45 PM	**Brine:**	Not Applicable		

Sample No:	06-9178-4076	**Material:**	Ambient Water	**Client:**	Los Alamos National Laboratory
Sample Date:	18 Aug-05 02:00 PM	**Code:**	10481	**Project:**	
Receive Date:	19 Aug-05 09:30 AM	**Source:**	Los Alamos National Laboratory		
Sample Age:	5d 23h (8.9 °C)	**Station:**	CAMO-05-61178		

Comparison Summary

Analysis	Endpoint	NOEL	LOEL	ChV	PMSD	Method
12-0620-2480	Cell Density	100	> 100	N/A	48.36%	Equal Variance t Two-Sample

Cell Density Summary

Conc-%	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Lab Water	4	2.44E+5	2.20E+5	2.57E+5	8.51E+3	1.70E+4	6.99%
100		4	4.99E+5	3.60E+5	6.43E+5	6.00E+4	1.20E+5	24.07%

Cell Density Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water	2.57E+5	2.55E+5	2.42E+5	2.20E+5
100		6.43E+5	4.56E+5	5.35E+5	3.60E+5

Analyst:_____ Approval:_____

Selenastrum capricornutum Cell Density Enumeration Data Sheet

Client: Los Alamos (retest) Test Material: CAMO-05-61178

Test Start Date: 8-24-05 Test ID #: 14617 Project #: 10481

Test End Date: 8-28-05 Control/Diluent: Algal Medium

Test Treatment	Temp (°C)	pH	D.O. (mg/L)	Conductivity (µS/cm)	Sign-Off
Lab Water Control	24.4	7.51	8.9	95.6	Inoculation Time: 1345
100%	24.4	7.77	8.8	348	Test Solution Prep: QD
					New WQ: YK
Lab Water Control	24.4	9.20			Date: 8-25-05
100%	24.4	8.19			WQ Time: 10:30
					WQ Signoff: YK
Lab Water Control	24.8	8.11			Date: 8.26.05
100%	24.8	8.59			WQ Time: 1120
					WQ Signoff: PG
Lab Water Control	24.3	9.42			Date: 8/27/05
100%	24.3	9.24			WQ Time: 14:25
					WQ Signoff: KF
Lab Water Control	24.4	8.68	7.7	166	Date: 8.28.05
100%	24.4	9.63	8.6	357	WQ Time: 1050
					WQ Signoff: LS

Initial Count: 10,000 cells/mL Termination Time: 13:00 Enumerating Scientist: YK

Treatment	Rep.	Cell Density (cells/mL x 10^6) Count 1	Count 2	Count 3	Mean Cell Density (cells/mL x 10^6)
Lab Water Control	A	0.258	0.257	0.257	0.257
	B	0.257	0.260	0.253	0.255
	C	0.249	0.241	0.237	0.242
	D	0.199	0.213	0.247	0.220
100%	A	0.647	0.631	0.651	0.643
	B	0.473	0.442	0.454	0.456
	C	0.535	0.529	0.541	0.535
	D	0.357	0.362	0.360	0.360

Initial Test Conditions	
Alkalinity	✓ ~~84~~ ~~86~~ 84
Hardness	✓ 92
Light Intensity (ftc)	530

This datasheet has been reviewed for completeness and consistency with identification of any water quality measures or other issues of concern.	Signoff: CAF
	Date: 8/29/05
	Time: 14:00

CETIS Test Summary

Report Date: 30 Aug-05 9:35 AM
Test Link: 02-2985-6169/14618

Selenastrum Growth Test — **Pacific EcoRisk**

Test No:	07-1886-6704	**Test Type:**	Cell Growth	**Duration:**	95h
Start Date:	24 Aug-05 01:45 PM	**Protocol:**	EPA/600/4-91/002 (1994)	**Species:**	Selenastrum capricornutum
Ending Date:	28 Aug-05 01:00 PM	**Dil Water:**	Not Applicable	**Source:**	In-House Culture
Setup Date:	24 Aug-05 01:45 PM	**Brine:**	Not Applicable		

Sample No:	05-1760-6863	**Material:**	Ambient Water	**Client:**	Los Alamos National Laboratory
Sample Date:	18 Aug-05 02:00 PM	**Code:**	10481	**Project:**	
Receive Date:	19 Aug-05 09:30 AM	**Source:**	Los Alamos National Laboratory		
Sample Age:	5d 23h (8.9 °C)	**Station:**	CAMO-05-61180		

Comparison Summary

Analysis	Endpoint	NOEL	LOEL	ChV	PMSD	Method
08-6019-9453	Cell Density	100	> 100	N/A	36.50%	Equal Variance t Two-Sample

Cell Density Summary

Conc-%	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Lab Water	4	2.44E+5	2.20E+5	2.57E+5	8.51E+3	1.70E+4	6.99%
100		4	9.72E+5	8.84E+5	1.09E+6	4.49E+4	8.99E+4	9.25%

Cell Density Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water	2.57E+5	2.55E+5	2.42E+5	2.20E+5
100		8.84E+5	9.93E+5	1.09E+6	9.21E+5

Analyst:_____ Approval:_____

Selenastrum capricornutum Cell Density Enumeration Data Sheet

Client: Los Alamos (w/c,t°) Test Material: CAMO-05-61180

Test Start Date: 8-24-05 Test ID #: 14614 Project #: 10781

Test End Date: 8/28/05 Control/Diluent: Algal Medium

Test Treatment	Temp (°C)	pH	D.O. (mg/L)	Conductivity (µS/cm)	Sign-Off
Lab Water Control	24.4	7.51	8.9	95.6	Inoculation Time: 1345
100%	24.4	7.74	9.0	338	Test Solution Prep: QN
					New WQ: YK
Lab Water Control	24.4	9.20			Date: 8-25-05
100%	24.4	8.25			WQ Time: 10:30
					WQ Signoff: YK
Lab Water Control	24.8	8.11			Date: 8.26.05
100%	24.8	8.63			WQ Time: 1120
					WQ Signoff: PG
Lab Water Control	24.3	9.41			Date: 8/27/05
100%	24.3	9.15			WQ Time: 14:25
					WQ Signoff: [illegible]
Lab Water Control	24.4	8.68	7.7	166	Date: 8.28.05
100%	24.4	9.62	8.6	339	WQ Time: 1050
					WQ Signoff: CS

Initial Count: 10,000 cells/mL Termination Time: 13:00 Enumerating Scientist: YK

Treatment	Rep.	Cell Density (cells/mL x 10^6) Count 1	Count 2	Count 3	Mean Cell Density (cells/mL x 10^6)
Lab Water Control	A	0.258	0.257	0.257	0.257
	B	0.251	0.260	0.253	0.255
	C	0.249	0.241	0.237	0.242
	D	0.199	0.213	0.247	0.220
100%	A	0.880	0.888	0.885	0.884
	B	0.998	1.009	0.972	0.993
	C	1.116	1.092	1.055	1.088
	D	0.942	0.908	0.913	0.921

Initial Test Conditions	
Alkalinity	✓ ~~103~~ 104
Hardness	✓ 98
Light Intensity (ftc)	530

This datasheet has been reviewed for completeness and consistency with identification of any water quality measures or other issues of concern.	Signoff: CAP
	Date: 8/28/05
	Time: 14:00

Appendix D

Test Data and Summary of Statistics for the Reference Toxicant Evaluation of the *Selenastrum capricornutum* for the Testing of the July 26 Ambient Water Samples

CETIS Test Summary

Report Date: 01 Aug-05 10:13 AM
Test Link: 16-5216-7554/13997

Phytoplankton Growth Inhibition Test — **Pacific EcoRisk**

Test No:	02-7549-9782	**Test Type:**	Cell Growth	**Duration:**	94h
Start Date:	27 Jul-05 11:15 AM	**Protocol:**	EPA/600/4-91/002 (1994)	**Species:**	Selenastrum capricornutum
Ending Date:	31 Jul-05 09:25 AM	**Dil Water:**	Deionized Water	**Source:**	In-House Culture
Setup Date:	27 Jul-05 11:15 AM	**Brine:**	Not Applicable		

Sample No:	15-7040-2810	**Material:**	Zinc chloride	**Client:**	
Sample Date:	27 Jul-05	**Code:**	10410	**Project:**	
Receive Date:	27 Jul-05	**Source:**	Reference Toxicant		
Sample Age:	11h	**Station:**			

Comparison Summary

Analysis	Endpoint	NOEL	LOEL	ChV	PMSD	Method
03-9857-7892	Cell Density	1	2.5	1.5811	11.98%	Dunnett's Multiple Comparison

Point Estimate Summary

Analysis	Endpoint	% Effect	Conc-µg/L	95% LCL	95% UCL	Method
00-6506-7501	Cell Density	5	0.8258869	0.3102551	1.866468	Linear Interpolation
		10	1.47979	0.4348803	4.53633	
		15	2.545673	0.07133384	8.231207	
		20	6.243816	N/A	8.919359	
		25	8.840548	3.815924	13.7483	
		40	> 20	N/A	N/A	
		50	> 20	N/A	N/A	

Cell Density Summary

Conc-µg/L	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Lab Water	4	7.21E+5	6.86E+5	7.69E+5	1.74E+4	3.47E+4	4.82%
0.5		4	7.49E+5	6.70E+5	8.29E+5	3.45E+4	6.90E+4	9.21%
1		4	6.79E+5	6.26E+5	7.55E+5	3.05E+4	6.09E+4	8.98%
2.5		4	6.25E+5	5.33E+5	6.99E+5	3.60E+4	7.20E+4	11.52%
5		4	6.06E+5	5.55E+5	6.52E+5	2.30E+4	4.60E+4	7.60%
10		4	5.35E+5	5.27E+5	5.53E+5	6.14E+3	1.23E+4	2.30%
20		4	5.02E+5	4.86E+5	5.25E+5	9.03E+3	1.81E+4	3.60%

Cell Density Detail

Conc-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water	7.69E+5	7.12E+5	6.86E+5	7.18E+5
0.5		8.29E+5	7.76E+5	7.19E+5	6.70E+5
1		6.26E+5	7.00E+5	7.55E+5	6.33E+5
2.5		6.61E+5	6.99E+5	5.33E+5	6.07E+5
5		6.36E+5	6.52E+5	5.55E+5	5.79E+5
10		5.53E+5	5.31E+5	5.28E+5	5.27E+5
20		5.07E+5	4.89E+5	5.25E+5	4.86E+5

Selenastrum capricornutum Cell Density Enumeration Data

Client: **Reference Toxicant** Initial Count: 10,000 cells/mL

Test Material: $ZnCl_2$ Enumerating Scientist: MW

Test Start Date: 7-27-05 Start Time: 1015 Project #: 10910

Test End Date: 7/31/05 End Time: 9:25 Test ID #: 13997

Ref Tox Test Treatment	Rep.	Cell Density (cells/mL x 10^6) Count 1	Count 2	Count 3	Mean Cell Density (cells/mL x 10^6)
Lab Water Control	A	0.758	0.781	0.767	0.769
	B	0.715	0.704	0.713	0.712
	C	0.661	0.646	0.654	0.656
	D	0.717	0.712	0.726	0.718
0.5 µg/L Zn	A	0.813	0.824	0.849	0.829
	B	0.765	0.780	0.786	0.776
	C	0.713	0.731	0.712	0.719
	D	0.669	0.666	0.674	0.670
1 µg/L Zn	A	0.622	0.627	0.629	0.626
	B	0.695	0.701	0.704	0.700
	C	0.761	0.748	0.755	0.755
	D	0.630	0.635	0.634	0.633
2.5 µg/L Zn	A	0.657	0.665	0.661	0.661
	B	0.699	0.694	0.704	0.699
	C	0.529	0.535	0.534	0.533
	D	0.600	0.605	0.616	0.607
5 µg/L Zn	A	0.634	0.640	0.633	0.636
	B	0.653	0.665	0.638	0.652
	C	0.556	0.555	0.553	0.555
	D	0.570	0.579	0.589	0.579
10 µg/L Zn	A	0.555	0.557	0.564	[illegible]
	B	0.533	0.530	0.529	0.531
	C	0.520	0.529	0.536	0.528
	D	0.517	0.526	0.539	0.527
20 µg/L Zn	A	0.506	0.509	0.505	0.507
	B	0.492	0.484	0.490	0.489
	C	0.521	0.536	0.518	0.525
	D	0.502	0.468	0.489	0.486

This datasheet has been reviewed for completeness and consistency with identification of any water quality measures or other issues of concern.	Signoff:	Date:	Time:
	JS	7/31/05	1920

Selenastrum capricornutum Algal Toxicity Test Water Quality Data

Client: **Reference Toxicant** Test ID #: 13597 Test Date: 7-27-05

Test Material: $ZnCl_2$ Project #: 10 4 16 Control/Diluent: Algal Medium

Reference Toxicant Test Treatment (µg/L Zn)	Temp (°C)	pH	D.O. (mg/L)	Conductivity (µS/cm)	Sign-Off
Lab Water Control	24.8	7.52	9.3	112	Test Solution Prep: GP
0.5	24.8	8.00	8.7	99	New WQ: TR
1	24.8	7.03	8.6	97	Innoculation Date: 7-27-05
2.5	24.8	7.70	8.7	94	Innoculation Time: 1115
5	24.8	7.66	8.8	95	Innoculation Signoff: GP
10	24.8	7.63	8.8	95	
20	24.8	7.60	9.1	94	
Lab Water Control	[illegible]	8.46			Date: 7/28/05
0.5	[illegible]	8.24			WQ Time: [illegible]
1	[illegible]	8.09			WQ Signoff: [illegible]
2.5	[illegible]	8.07			
5	[illegible]	7.92			
10	[illegible]	7.94			
20	[illegible]	7.85			
Lab Water Control	24.6	8.52			Date: 7.29.05
0.5	24.6	8.30			WQ Time: 1145
1	24.6	8.18			WQ Signoff: SL
2.5	24.6	8.06			
5	24.6	7.98			
10	24.6	7.91			
20	24.6	7.79			
Lab Water Control	25.0	9.57			Date: 7/30/05
0.5	25.0	9.36			WQ Time: 1025
1	25.0	9.35			WQ Signoff: MW
2.5	25.0	9.61			
5	25.0	9.60			
10	25.0	9.61			
20	25.0	9.52			
Lab Water Control	24.9	9.78	9.3	108	Date: 7/31/05
0.5	24.9	10.14	8.8	108	Termination Time: 925
1	24.9	9.76	9.1	100	Termination Signoff: MW
2.5	24.9	10.03	9.2	~~9.20~~ 101	WQ Time: 1030
5	24.9	9.95	8.8	100	WQ Signoff: JC
10	24.9	10.05	9.7	104	
20	24.9	10.17	9.3	103	

Initial Test Conditions	Alkalinity	Hardness	Light Intensity (ftc)
	15	15	520

Appendix E

Test Data and Summary of Statistics for the Reference Toxicant Evaluation of the *Selenastrum capricornutum* for the Testing of the August 18 Ambient Water Samples

CETIS Test Summary

Report Date: 29 Aug-05 5:04 PM
Test Link: 10-9124-5243/14613

Selenastrum Growth Test — **Pacific EcoRisk**

Test No:	03-4925-8106	**Test Type:**	Cell Growth	**Duration:**	96h
Start Date:	25 Aug-05 11:30 AM	**Protocol:**	EPA/600/4-91/002 (1994)	**Species:**	Selenastrum capricornutum
Ending Date:	29 Aug-05 11:00 AM	**Dil Water:**	Deionized Water	**Source:**	In-House Culture
Setup Date:	25 Aug-05 11:30 AM	**Brine:**	Not Applicable		

Sample No:	10-0665-4058	**Material:**	Zinc chloride	**Client:**	
Sample Date:	25 Aug-05	**Code:**	10487	**Project:**	
Receive Date:	25 Aug-05	**Source:**	Reference Toxicant		
Sample Age:	11h	**Station:**			

Comparison Summary

Analysis	Endpoint	NOEL	LOEL	ChV	PMSD	Method
08-4701-5179	Cell Density	10	20	14.142	16.91%	Steel Many-One Rank

Point Estimate Summary

Analysis	Endpoint	% Effect	Conc-µg/L	95% LCL	95% UCL	Method
05-5975-8055	Cell Density	5	3.65021	N/A	6.024928	Linear Interpolation
		10	4.80042	2.864137	13.48435	
		15	7.444058	2.502503	15.02421	
		20	10.38576	2.433162	16.38809	
		25	13.22886	4.744736	17.691	
		40	> 20	N/A	N/A	
		50	> 20	N/A	N/A	

Cell Density Summary

Conc-µg/L	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Lab Water	4	4.78E+5	4.42E+5	5.09E+5	1.38E+4	2.76E+4	5.78%
0.5		4	3.70E+5	3.01E+5	4.59E+5	3.96E+4	7.92E+4	21.38%
1		4	4.87E+5	4.69E+5	5.02E+5	7.79E+3	1.56E+4	3.20%
2.5		4	5.82E+5	5.72E+5	5.99E+5	5.90E+3	1.18E+4	2.03%
5		4	4.27E+5	3.76E+5	4.55E+5	1.86E+4	3.71E+4	8.69%
10		4	3.87E+5	3.37E+5	5.05E+5	3.97E+4	7.94E+4	20.55%
20		4	3.02E+5	2.92E+5	3.17E+5	5.99E+3	1.20E+4	3.96%

Cell Density Detail

Conc-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water	4.76E+5	4.42E+5	4.83E+5	5.09E+5
0.5		4.59E+5	4.15E+5	3.06E+5	3.01E+5
1		4.97E+5	4.69E+5	5.02E+5	4.78E+5
2.5		5.80E+5	5.72E+5	5.99E+5	5.77E+5
5		4.54E+5	4.23E+5	4.55E+5	3.76E+5
10		3.57E+5	5.05E+5	3.37E+5	3.47E+5
20		2.92E+5	2.93E+5	3.07E+5	3.17E+5

Selenastrum capricornutum Cell Density Enumeration Data

Client: **Reference Toxicant** Initial Count: 10,000 cells/mL

Test Material: $ZnCl_2$ Enumerating Scientist: MC / ZD

Test Start Date: 8-25-05 Start Time: 1130 Project #: 10487

Test End Date: 8-29-05 End Time: 1100 Test ID #: 14613

Ref Tox Test Treatment	Rep.	Cell Density (cells/mL x 10^6) Count 1	Count 2	Count 3	Mean Cell Density (cells/mL x 10^6)
Lab Water Control	A	0.467	0.472	0.489	0.476
	B	0.424	0.450	0.453	0.442
	C	0.502	0.480	0.466	0.483
	D	0.516	0.500	0.512	0.509
0.5 µg/L Zn	A	0.452	0.455	0.471	0.459
	B	0.416	0.405	0.424	0.415
	C	0.314	0.300	0.303	0.306
	D	0.318	0.291	0.294	0.301
1 µg/L Zn	A	0.504	0.499	0.490	0.497
	B	0.460	0.474	0.472	0.469
	C	0.497	0.502	0.506	0.502
	D	0.488	0.473	0.473	0.478
2.5 µg/L Zn	A	0.577	0.583	0.579	0.580
	B	0.569	0.573	0.573	0.572
	C	0.599	0.601	0.598	0.599
	D	0.577	0.579	0.575	0.577
5 µg/L Zn	A	0.455	0.455	0.453	0.454
	B	0.421	0.423	0.425	0.423
	C	0.448	0.460	0.457	0.455
	D	0.374	0.378	0.377	0.376
10 µg/L Zn	A	0.355	0.348	0.367	0.357
	B	0.495	0.510	0.510	0.505
	C	0.334	0.337	0.340	0.337
	D	0.336	0.349	0.356	0.347
20 µg/L Zn	A	0.287	0.295	0.295	0.292
	B	0.279	0.299	0.301	0.293
	C	0.302	0.310	0.309	0.307
	D	0.313	0.317	0.322	0.317

This datasheet has been reviewed for completeness and consistency with identification of any water quality measures or other issues of concern.	Signoff:	Date:	Time:
	QD	8-29-05	1200

Selenastrum capricornutum Algal Toxicity Test Water Quality Data

Client: Reference Toxicant | Test ID #: 14613 | Test Date: 8/25/05
Test Material: $ZnCl_2$ | Project #: 10487 | Control/Diluent: Algal Medium

Reference Toxicant Test Treatment (µg/L Zn)	Temp (°C)	pH	D.O. (mg/L)	Conductivity (µS/cm)	Sign-Off
Lab Water Control	25.1	7.46	8.5	93.0	Test Solution Prep: QV
0.5	25.1	8.84	8.3	97.7	New WQ: YK
1	25.1	8.80	7.9	94.2	Innoculation Date: 8-25-05
2.5	25.1	8.74	8.0	97.0	Innoculation Time: 1130
5	25.1	8.70	7.8	93.0	Innoculation Signoff: QV
10	25.1	8.59	7.9	99.1	
20	25.1	8.56	7.8	93.4	
Lab Water Control	24.6	8.54			Date: 8.26.05
0.5	24.6	8.28			WQ Time: 1150
1	24.6	8.14			WQ Signoff: PG
2.5	24.6	8.09			
5	24.6	8.01			
10	24.6	7.96			
20	24.6	7.92			
Lab Water Control	25.1	8.30			Date: 8/27/05
0.5	25.1	8.33			WQ Time: 14:02
1	25.1	8.39			WQ Signoff: RP
2.5	25.1	8.53			
5	25.1	8.69			
10	25.1	8.57			
20	25.1	8.53			
Lab Water Control	25.0	9.40			Date: 8.28.05
0.5	25.0	9.28			WQ Time: 0205
1	25.0	9.70			WQ Signoff: CS
2.5	25.0	9.60			
5	25.0	9.36			
10	25.0	9.12			
20	25.0	8.70			
Lab Water Control	24.5	9.38	8.4	~~172~~ 135 NT	Date: 8-29-05
0.5	24.5	9.54	8.8	109	Termination Time:
1	24.5	9.41	8.5	113	Termination Signoff:
2.5	24.5	9.33	8.7	115	WQ Time: 1130
5	24.5	9.29	8.8	113	WQ Signoff: NT
10	24.5	9.17	8.7	114	
20	24.5	9.07	8.7	107	

Initial Test Conditions	Alkalinity	Hardness	Light Intensity (ftc)
	✓ 11	✓ 16	613