

Appendix D. Aquatic Toxicity



David Brown
CSU Chico
GEOS Dept.
Chico, CA 95929-0205

March 16, 2004

Dear David,

I have enclosed two (2) copies of our report "Evaluation of the Chronic Toxicity of an Ambient Water Sample to *Ceriodaphnia dubia*" for the sample collected February 27, 2004. The results of this test indicated that the "CWCC" sample was not toxic to *Ceriodaphnia* survival at the 100% concentration. However, the "CWCC" was toxic to *Ceriodaphnia* reproduction at the 100% effluent concentration.

If you have any questions regarding the report or the performance and interpretation of the test, please call my colleague Stephen Clark or myself at (925) 313-8080.

Sincerely,

Alison Torbitt
Senior Ecotoxicologist

**Evaluation of the Chronic Toxicity of
An Ambient Water Sample to *Ceriodaphnia dubia***

(Samples collected February 27, 2004)

Prepared For:

CSU Chico
GEOS Dept.
Chico, CA 95929-0205

Prepared By:

Pacific EcoRisk
835 Arnold Dr. Suite 104
Martinez, CA 94553

March 2004



PACIFIC ECORISK
ENVIRONMENTAL CONSULTING & TESTING

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1.0 INTRODUCTION

Under contract to CSU Chico (Chico), Pacific EcoRisk (PER) performed a "short-term" chronic toxicity evaluation of an ambient water sample using the U.S. EPA's 3-brood (6-8 day) survival and reproduction test with the crustacean *Ceriodaphnia dubia*. This test was conducted on the "CWCC" sample collected February 27, 2004. This report describes the performance and results of the effluent test.

2.0 CHRONIC TOXICITY TEST PROCEDURES

The methods used in conducting this test followed the guidelines established by the EPA manual "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" (EPA-600-4-91-002).

2.1 RECEIPT AND HANDLING OF SAMPLES

On February 27, 2004, CSU Chico staff collected an ambient water sample into appropriately-cleaned 10-L polyethylene cubitainers. The sample was transported, on ice and under chain-of-custody, to the PER testing laboratory in Martinez. Upon receipt at the testing laboratory, an aliquot of the sample was removed for analysis of initial water quality characteristics (Table 1). Additional aliquots were removed for use in setting up or maintaining the toxicity test, with the remainder of each sample being stored at 4°C. The chain-of-custody record for the collection and delivery of the sample is provided in Appendix A.

Sample Date	Temp. (°C)	pH	D.O. (mg/L)	Alkalinity (mg/L)	Hardness (mg/L)	Salinity (ppt)	Conductivity (µS/cm)	Total Ammonia (mg/L)
2/27/04	7.9	7.71	10.6	40	56	0.0	78.8	<0.01

2.2 TEST ORGANISMS

The *Ceriodaphnia dubia* used in these tests were obtained from in-house laboratory cultures.

2.3 SURVIVAL & REPRODUCTION TOXICITY TESTING WITH *Ceriodaphnia dubia*

The short-term chronic *Ceriodaphnia* test consists of exposing individual females to the ambient water sample for the length of time it takes for the Control treatment females to produce 3 broods of offspring (6-8 days), after which effects on survival and reproduction are evaluated. The specific procedures used in this test are described below.

The Control/dilution water for this test consisted of an 80%:20% mix of commercial spring waters (Arrowhead® spring water and Evian® spring water, respectively). The ambient water sample was used to prepare daily test solutions at the 100% concentration only. Each treatment consisted of 200 mL of test solution to which the alga *Selenastrum capricornutum* and Yeast-Cerophyll-Trout Food (YCT) had been added for food. “New” water quality characteristics (pH, dissolved oxygen [D.O.], and conductivity) were measured on these 200-mL aliquots prior to use of the test solutions in this test. There were 10 replicates for the Control and the 100% ambient water treatment, each replicate consisting of 15 mL of test media in a 30-mL plastic cup.

Neonate *Ceriodaphnia* (< 24 hours old) were used to start this “3-brood” test, which was initiated by allocating 1 neonate *Ceriodaphnia* into each of the replicate cups. The cups containing the Control and ambient water treatment were placed into foam boards which floated in a temperature-controlled water bath so as to maintain the water temperature in each replicate cup at 25°C, under fluorescent lighting on a 16L:8D photoperiod.

Each day of the test, fresh test solutions and a “new” set of replicate cups were prepared, as before. “New” water quality characteristics (pH, D.O., and conductivity) were measured on these solutions prior to use in the test. The foam boards containing the *Ceriodaphnia* exposure cups were removed from the water bath and then each replicate cup was examined, with surviving ‘original’ individual organisms being transferred to the corresponding new cup. The number of neonate offspring produced by each original organism was determined and then “old” water quality characteristics (pH and D.O.) were measured for the old media from one randomly-selected replicate for each treatment. The test boards, now carrying *Ceriodaphnia* in fresh media, were placed back into the water bath.

After 6 days of exposure, it was determined that ≥60% of the *Ceriodaphnia* in the Control treatment had produced their third brood of offspring, and the test was terminated. This test produced 2 types of endpoint data: percent survival and number of offspring. The data for the ambient water treatment were statistically analyzed and compared to the Control treatment to determine any survival or reproduction toxicity caused by the ambient water; all statistical analyses were performed using the CETIS® statistical package (Version 1.023, TidePool Scientific, McKinleyville, CA).

3.0 RESULTS

3.1 TOXICITY OF “CWCC” AMBIENT WATER TO *Ceriodaphnia dubia*

The survival and reproduction results of this test are both summarized below in Table 2. Briefly, there was 90% survival at the Control and 90% survival at the 100% ambient water treatment, which was not significantly less than the Control. The survival No Observable Effect Concentration (NOEC) was 100% ambient water, and the Lowest Observable Effect Concentration (LOEC) was >100% ambient water.

As for reproduction, there was a mean of 21.1 neonates per female at the Control, and 7.6 neonates per female at the 100% ambient water treatment, which was significantly less than the Control. The reproduction NOEC was <100% ambient water, and the LOEC was 100% ambient water.

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

Table 2. Effects of “CWCC” ambient water on survival and reproduction of <i>Ceriodaphnia dubia</i> .		
Effluent Treatment (%)	% Survival	Reproduction (# neonates/female)
Control	90	21.1
100*	90	7.6*
Summary of Key Statistics		
NOEC =	100% effluent	<100% effluent
LOEC =	>100% effluent	100% effluent

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

4.0 SUMMARY

The results of this test indicated that the “CWCC” ambient water was not toxic to *Ceriodaphnia* survival at the 100% treatment. However, the “CWCC” ambient water was toxic to *Ceriodaphnia* reproduction at the 100% treatment.

APPENDIX A

Chain-of-Custody Record for the Collection and Transport of an Ambient Water Sample

CHAIN OF CUSTODY RECORD

PACIFIC ECORISK

835 Arnold Drive, Suite 104
Martinez, CA 94553
(925)313-8080 fax: (925)313-8089

RESULTS TO:

CSU Chico
GEOS Dept
Chico CA 95929-0205
Attn: David L. Brown Tel: 530-898-4035

BILL TO:

CSU Chico Research Foundation
% GEOS Dept
Chico CA 95929-0205
Attn: David L. Brown Tel: 530-898-4035

PROJECT: Cherokee Watershed Water Quality

SAMPLE IDENTIFICATION	DATE	TIME	SAMPLE MATRIX	GRAB/COMP.	# CONTAINERS/TYP	ANALYSES REQUESTED				REMARKS	
CW CC	2-27-04	0830	Fresh Water Grab		4 12.5 gal						Analyzes per quote
					1						Bottles
					1						Marked
					1						FLPE
					1						
					1						
					1						
					1						
					1						

METHOD OF SHIPMENT: FED X _____ UPS _____ HAND OTHER _____

COMMENTS:

CODES:

RELINQUISHED BY: (SIGNATURE)	DATE	TIME	RECEIVED BY: (SIGNATURE)	DATE	TIME	PAGE #
David L. Brown	2-27-04	0810	William Davis	2-27-04	0840	1 OF 1
William Davis	2-27-04	1125	John Kelly	2-27-04	1125	

White - Return w/sample
Yellow - Keep for your records

APPENDIX B

Test Data and Summary of Statistics for the Chronic Toxicity Evaluation of the “CWCC” Ambient Water to *Ceriodaphnia dubia*

CETIS Report

Test Summary: Page 1 of 1
 Report Date: 10 Mar-04 12:09 PM
 Link: 10-2364-3425

Cladoceran Survival and Reproduction Test Pacific EcoRisk

Test: 03-6912-6921	Test Type: Reproduction-Survival (7d)	Species: Ceriodaphnia dubia
Start Date: 27 Feb-04 02:30 PM	Protocol: EPA/600/4-91/002 (1994)	Source: In-House Culture
End Date: 04 Mar-04 03:45 PM	Dil Water:	
Setup Date: 27 Feb-04 02:30 PM	Brine:	

Sample: 10-1072-4099	Material: Ambient Water	Client: CSU Chico
Sampled: 27 Feb-04 08:30 AM	Code: 9193	Project:
Received: 27 Feb-04 11:25 AM	Source: CSUC	Latitude:
Temp °C: 7.9	Station: CWCC	Longitude:

Comparison Summary						
Analysis	Endpoint	NOEL	LOEL	ChV	MSDp	Method
06-2921-8951	7d Proportion Survived	100	>100	N/A	N/A	Fisher's Exact
15-7665-2082	Reproduction	<100	100	N/A	24.80%	Mann-Whitney U

7d Proportion Survived Summary								
Conc-%	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Lab Water	10	0.9	0	1	0.1	0.3162278	35.14%
100		10	0.9	0	1	0.1	0.3162278	35.14%

Reproduction Summary								
Conc-%	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Lab Water	10	21.1	0	27	2.6350206	8.3326666	39.49%
100		10	7.6	0	14	1.4696938	4.6475800	61.15%

7d Proportion Survived Detail											
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Water	1	1	1	1	1	1	0	1	1	1
100		1	1	1	1	1	1	1	0	1	1

Reproduction Detail											
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Water	21	14	26	23	27	24	0	25	24	27
100		13	6	7	5	7	2	10	0	14	12

CETIS Report

Comparisons: Page 1 of 1

Report Date: 10 Mar-04 12:09 PM

Analysis: 06-2921-8951

Cladoceran Survival and Reproduction Test						Pacific EcoRisk					
Endpoint	Analysis Type	Sample Link	Control Link	Date Analyzed	Version						
7d Proportion Survived	Comparison	10-2364-3425	10-2364-3425	10 Mar-04 12:09 PM	CETISv1.023						
Method	Alt H	Data Transform	NOEL	LOEL	Toxic Units	ChV	MSDp				
Fisher's Exact	C > T	Untransformed	100	>100	1.00	N/A					
Group Comparisons											
Control	vs	Conc-%	Statistic	Critical	Decision(0.05)						
Lab Water		100	0.7631579	0.05	Non-Significant Effect						
Data Summary											
Conc-%	Control Type	Non-Responders	Responders	Total Observed							
0	Lab Water	9	1	10							
100		9	1	10							
Data Detail											
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Water	1	1	1	1	1	1	0	1	1	1
100		1	1	1	1	1	1	1	0	1	1

CETIS Report

Comparisons: Page 1 of 1
 Report Date: 10 Mar-04 12:09 PM
 Analysis: 15-7665-2082

Cladoceran Survival and Reproduction Test							Pacific EcoRisk				
Endpoint	Analysis Type	Sample Link	Control Link	Date Analyzed	Version						
Reproduction	Comparison	10-2364-3425	10-2364-3425	10 Mar-04 12:09 PM	CETISv1.023						
Method	Alt H	Data Transform	Z	NOEL	LOEL	Toxic Units	ChV	MSDp			
Mann-Whitney U	C > T	Untransformed		<100	100		N/A	24.80%			
ANOVA Assumptions											
Attribute	Test	Statistic	Critical	P Level	Decision(0.01)						
Variances	Variance Ratio	3.214506	6.54109	0.09692966	Equal Variances						
Distribution	Shapiro-Wilk W	0.8177834	0.868259	0.001103936	Non-normal Distribution						
ANOVA Table											
Source	Sum of Squares	Mean Square	DF	F Statistic	P Level	Decision(0.05)					
Between	911.25	911.25	1	20.02014	0.0002931042	Significant Effect					
Error	819.3	45.51667	18								
Total	1730.55	956.7667	19								
Group Comparisons											
Control	vs	Conc-%	Statistic	Critical	P Level	Ties	Decision(0.05)				
Lab Water		100	90		0.00075234	5	Significant Effect				
Data Summary											
			Original Data				Transformed Data				
Conc-%	Control Type	Count	Mean	Minimum	Maximum	SD	Mean	Minimum	Maximum	SD	
0	Lab Water	10	21.1	0	27	8.3326666					
100		10	7.6	0	14	4.6475800					
Data Detail											
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Water	21	14	26	23	27	24	0	25	24	27
100		13	6	7	5	7	2	10	0	14	12

Short-Term Chronic 3-Brood *Ceriodaphnia dubia* Survival & Reproduction Test Data

Client: CSA CHICO

Sample ID: CWC C

Test ID #: 9438

Test Date: 2-27-04

Control Water: 80:20 Spring Water

Day	pH		D.O.		Cond. (µS/cm)	Temp (°C)	Survival / Reproduction										SIGN-OFF	
	New	Old	New	Old			A	B	C	D	E	F	G	H	I	J		
0	7.77		10.7		207	25.0	0	0	0	0	0	0	0	0	0	0	0	Date: 2/27/04 Time: 14:30 WQ: JF Counts: 20
1	7.74	8.04	8.0	7.1	208	25.1	0	0	0	0	0	0	0	0	0	0	0	Date: 2/28/04 Time: 12:30 WQ: MT Counts: 23
2	7.64	7.87	8.8	8.1	206	25.1	0	0	0	0	0	0	0	0	0	0	0	Date: 2/29/04 Time: 10:30 WQ: KW Counts: 24
3	7.92	8.03	8.1	7.5	206	25.4	0	0	0	0	0	0	0	0	0	0	0	Date: 3/1/04 Time: 11:30 WQ: KW Counts: 20
4	8.12	7.76	8.8	6.4	201	25.7	4	4	5	3	5	3	3	4	4	4	4	Date: 3/2/04 Time: 10:15 WQ: JF Counts: 20
5	7.31	8.09	7.9	8.2	203	26.2	5	0	6	8	7	10	8	4	4	4	4	Date: 3/3/04 Time: 10:15 WQ: JF Counts: 20
6	7.85	8.11	8.39	6.4	216	24.8	12	10	15	11	12	12	13	19	19	19	19	Date: 3/4/04 Time: 15:45 WQ: JF Counts: 23
7																		Date: Time: Counts: 23
8																		Date: Time: Counts: 23
Total =							21	14	26	23	27	24	25	24	24	24	24	X = 261

Day	pH		D.O.		Cond. (µS/cm)	Temp (°C)	Survival / Reproduction										SIGN-OFF	
	New	Old	New	Old			A	B	C	D	E	F	G	H	I	J		
0	8.12		12.5		213	25.0	0	0	0	0	0	0	0	0	0	0	0	
1	7.97	8.02	8.2	7.0	107.6	25.0	0	0	0	0	0	0	0	0	0	0	0	
2	7.94	8.10	10.1	7.7	88.9	25.0	0	0	0	0	0	0	0	0	0	0	0	
3	8.17	8.05	9.0	6.1	90.9	25.0	0	0	0	0	0	0	0	0	0	0	0	
4	8.07	8.05	9.5	6.1	91.5	25.0	2	0	0	2	0	2	2	2	2	2	2	
5	8.05	8.46	9.5	8.1	91.8	25.0	3	0	1	3	2	5	4	4	0	0	0	
6	7.63	8.16	8.6	5.8	89.6	25.0	8	0	6	0	0	0	0	0	0	0	0	
7						25.0												
8						25.0												
Total =							13	6	7	5	7	2	10	14	12	12	12	X = 7.6

Lab Control

100% Site Water