

Final Technical Report

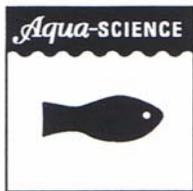
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Acute Toxicity of American River and Tributary Waters to *Ceriodaphnia dubia* and Fathead Minnows

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ENVIRONMENTAL TOXICOLOGY CONSULTANTS

***ACUTE TOXICITY OF AMERICAN RIVER AND TRIBUTARY
WATERS TO CERIODAPHNIA DUBIA AND FATHEAD
MINNOWS - PHASE I***

FINAL REPORT

Submitted to:

***Central Valley Regional Water
Quality Control Board
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Attention: Robert Holmes
Work Order No. SJS-04 5-L01-am1***

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TOXICITY OF AMERICAN RIVER AND TRIBUTARY WATERS TO *CERIODAPHNIA DUBIA* AND FATHEAD MINNOWS – PHASE I

1.0 EXECUTIVE SUMMARY

Water samples collected from the American River watershed upstream of Lake Natoma in May, September and November of 2006 were tested for 96 hr acute toxicity to *Ceriodaphnia dubia* and larval fathead minnows (*Pimephales promelas*). Each of the three sample events incorporated 10-11 samples. None of the 31 samples tested caused a statistically significant reduction in *C. dubia* or fathead minnow survival. However, four of the samples tested in the May event with fathead minnows exhibited unequal replicate mortality that is characteristic of pathogen-related toxicity (PRT). PRT is a test interference that occurs sporadically in fathead minnow tests with ambient samples and is believed to be caused by water-borne pathogen(s).

2.0 INTRODUCTION

AQUA-Science (A-S; Davis, CA) was retained by the Central Valley Regional Water Quality Control Board (CVRWQCB) under Work Order *SJS-04 5-L01-am1* to perform aquatic toxicity tests on water samples collected from American River tributaries and urban creeks. The toxicity tests were 96-hour acute *Ceriodaphnia dubia* survival and 96-hour acute fathead minnow (*Pimephales promelas*) survival. Toxicity test results of a total of 3 sample sets consisting of 10-11 samples each are reported here. A fourth event consisting of 14 samples that focused on urban creeks was also tested with *C. dubia* and will be reported separately.

3.0 MATERIALS AND METHODS

3.2 Sample Collection and Transport

Water column samples were collected by CVRWQCB personnel on 5/25/06 (10 samples), 9/27/06 (11 samples), and 11/13/06 (10 samples). Sample sites are shown in Table 1. Samples were collected in 1 gallon amber bottles (I-Chem™, VWR Scientific, Brisbane, CA) and transported in ice chests with wet ice to A-S. Field measurements included temperature, dissolved oxygen (DO), pH, and conductivity. Water quality measurements including temperature, DO, pH, alkalinity, hardness, conductivity and ammonia were made on water from each site at sample log-in. Samples were stored in the dark at 4 °C until tested, within 24 hrs of collection. Appropriate chain-of-custody procedures were employed during collection and transport.

Table 1. Test Sample Locations

Sample Code	Location	Longitude (°N) Latitude (°W)
514LSAC08	Bunch Creek @ Yankee Jim's Rd	39.06298 -120.94386
514LSAC09	Willow Creek @ Folsom Blvd.	38.6569 -121.1822
514LSAC10	Alder Creek @ Folsom Blvd.	38.6381 -121.1992
514LSAC11	Webber Creek @ Jurgens Rd.	38.7516 -120.987
514LSAC12	White Rock Creek @ Mosquito Rd.	38.7585 -120.7643
514LSAC13	Middle Fork American River u/s Hwy 49	38.9153 -121.0366
514LSAC14	North Fork American River u/s Hwy 49	38.9163 -121.0351
514LSAC15	South Fork American River @ Salmon Falls Rd.	38.7727 -121.0377
514LSAC16	South Fork American River d/s Aspen Creek @ Aspen Creek Tract Rd.	38.8125 -120.0871
514LSAC17	Hangman Creek @ Main St.	38.72795 -120.80817
500FDQ010	Field Duplicate 514LSAC16	38.8125 -120.0871
500FDQ011	Field Duplicate 514LSAC16	38.8125 -120.0871
500FEQ012	Field Duplicate 514LSAC08	39.06298 -120.94386

3.3 Water Quality Measurements

Meter calibration/verification and water quality measurements followed the procedures described by the SWRCB QAMP¹ for SWAMP-compliant data. Temperature was measured in initial and 48-hr test solutions at change-out with a calibrated digital thermometer (Central Co., Friendswood, TX). Temperature was continuously recorded in all environmental chambers with a Dickson pen recorder (Model ICT855, Addison, IL). DO (YSI Model 550A, Yellow Springs, OH), pH (Beckman 240, Fulton, CO), and conductivity (WTW Model 330, Ft. Myers, FL) were measured in the initial and 48-hr test solutions at change-out. Alkalinity (Hach Model AL-DT) and hardness (Hach HA-DT) were measured with Hach colorimetric tests (Hach Co., Loveland, CO). Ammonia was measured in the initial sample using a Hach DR-700 Colorimeter (Method 8038).

¹ SWRCB. 2002. Quality Assurance Management Plan for the State of California's Surface Water Ambient Monitoring Program. Division of Water Quality. Sacramento, CA.

3.4 Toxicity Test Protocols

The 96-hr acute *Ceriodaphnia dubia* and 96-hour acute fathead minnow bioassays were conducted in accordance with the U.S. Environmental Protection Agency (USEPA) 5th edition protocols². Tests were considered invalid if control survival was < 90%.

3.5 *Ceriodaphnia dubia* Toxicity Tests

C. dubia 96-hour acute toxicity tests were initiated with < 24 hr old neonates collected within an 8-hr period from in-house cultures. Each test sample was tested using four replicates of 5 neonates each in a 20-mL glass scintillation vials containing 18-mL of test solutions. Test duration was 96 hours, and test solutions were renewed at 48 hours. *C. dubia* were fed a mixture of green algae (*S. capricornutum*) and YTC (a mixture of yeast, organic alfalfa and trout chow) 4 hrs prior to 48-hr test solution renewal. Tests were conducted at 25 ± 2 °C with a 16 hour light:8 hour dark photoperiod. Mortality was noted daily.

3.6 Fathead Minnow Toxicity Tests

Fathead minnows were obtained from Aquatox, Inc. (Hot Springs, AK), and were maintained in EPA moderately hard (EPAMH) water until tested when 5-9 days old. Each test sample was tested using 4 replicates of 10 fish each in 400 mL glass beakers containing 250 mL of test solutions. Test duration was 96 hours and test solutions were renewed at 48 hours. Fish were fed *Artemia* nauplii 4 hours prior to sample renewal at 48 hours. Tests were conducted at 25 ± 2 °C with a 16 hour light:8 hour dark photoperiod. Mortality was noted daily.

4.0 RESULTS

4.1 Event 1 (5/25/06)

Results of *C. dubia* and larval fathead minnow 96-hour toxicity tests for Event 1 are summarized in Table 2. For *C. dubia*, control survival was 100%, while test samples had 95-100% survival. Fathead minnow control survival was 90%, while test samples ranged from 72.5-100% survival. Four test samples exhibited characteristics of pathogen-related toxicity (PRT), which included delayed onset of toxicity and unequal mortality among the replicates (Table 2). PRT occurs occasionally in fathead minnow tests with ambient samples, and is believed to be caused by bacterial pathogens³. The raw data for this event is found in Appendix I.

² Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fifth Edition. October 2002. EPA 821-R-02-012.

³ Setac.org; "Frequently Asked Questions".

Table 2. Summary of Acute Toxicity Tests on American River Watershed Ambient Samples (Event 06-01)

<i>Test Sample</i>	<i>Ceriodaphnia % Survival</i>	<i>Fathead Minnow % Survival</i>
Control	100	90
514LSAC08	100	95
514LSAC09	95	77.5 ^a
514LSAC10	100	100
514LSAC11	100	97.5
514LSAC12	95	90
514LSAC13	100	90
514LSAC14	100	80 ^b
514LSAC15	95	72.5 ^c
514LSAC16	100	85
500FDQ010	100	82.5 ^d

Sample Date: 5/25/06

Test Date: 5/26/06

a Rep B had 9/10 dead

b Rep B had 8/10 dead

c Rep A had 6/10 dead; Rep D had 4/10 dead

d Rep D had 6/10 dead

4.2 Event 2 (9/27/06)

Results of *C. dubia* and larval fathead minnow 96-hour toxicity tests for Event are summarized in Table 3. *C. dubia* control survival was 95%, while test samples ranged from 95-100% survival. Fathead minnow control survival was 100%, while test samples ranged from 97.5-100% survival. No instances of PRT were observed in this event. The raw data for this event is found in Appendix II.

Table 3. Summary of Acute Toxicity Tests on American River Watershed Ambient Samples (Event 06-02)

<i>Test Sample</i>	<i>Ceriodaphnia % Survival</i>	<i>Fathead Minnow % Survival</i>
Control	95	100
514LSAC08	100	97.7
514LSAC09	100	100
514LSAC10	100	92.5
514LSAC11	100	100
514LSAC12	100	97.5
514LSAC13	95	97.5
514LSAC14	100	97.5
514LSAC15	100	100
514LSAC16	100	100
514LSAC17	100	97.5
500FDQ011	95	97.5

Sample Date: 9/27/06

Test Date: 9/27/06

4.3 Event 3 (11/13/06)

Results of *C. dubia* and larval fathead minnow 96-hour toxicity tests for Event 3 are summarized in Table 4. *C. dubia* control survival was 90%, while test samples ranged from 85-100% survival. Fathead minnow control survival was 100%, while test samples ranged from 95-100% survival. No instances of PRT were observed in this event. The raw data for this event is found in Appendix III.

Table 4. Summary of Acute Toxicity Tests on American River Watershed Ambient Samples (Event 06-03)

<i>Test Sample</i>	<i>Ceriodaphnia % Survival</i>	<i>Fathead Minnow % Survival</i>
Control	90	100
514LSAC08	100	100
514LSAC09	90	100
514LSAC10	100	100
514LSAC11	100	95
514LSAC12	95	100
514LSAC13	100	100
514LSAC14	100	100
514LSAC15	85	100
514LSAC16	100	100
500FDQ012	100	100

Sample Date: 11/13/06

Test Date: 11/14/06

5.0 CONCLUSIONS

- A total of 31 test samples collected during three sampling events in May, September and November, 2006, were tested for acute 96-hour toxicity to *C. dubia* and fathead minnows.
- None of the samples tested resulted in acute toxicity to *C. dubia*.
- None of the test samples resulted in a statistically significant reduction in fathead minnow survival. However, four of the samples tested in Event 1 exhibited unequal replicate mortality that is a characteristic of pathogen-related toxicity (PRT). PRT is a test inference that is occasionally observed in fathead minnow tests with ambient samples.