SWAMP
Surface Water Ambient Monitoring Program

Aqua-SCIENCE

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Acute Toxicity of Sacramento Area Urban Creeks to *Ceriodaphnia dubia*

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ACUTE TOXICITY OF SACRAMENTO AREA URBAN CREEKS TO CERIODAPHNIA DUBIA

FINAL REPORT

Submitted to:

Central Valley Regional Water
Quality Control Board
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Work Order No. SJS-04 5-L01-am1

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ACUTE TOXICITY OF SACRAMENTO AREA URBAN CREEKS TO CERIODAPHNIA DUBIA

1.0 EXECUTIVE SUMMARY

A total of 14 water samples collected from Sacramento area urban creeks during an anticipated rainfall event in February 2007 were tested for acute (96-hour) toxicity to *Ceriodaphnia dubia*. The only sample that caused toxicity (Elk Grove Creek) was subjected to a Phase I Toxicity Identification Evaluation (TIE) to determine the chemical class(es) of the toxicant(s) responsible for the toxicity. The TIE baseline dilution series toxicity test detected 5.8 toxic units (TUa; $100/EC_{50}$). Toxicity was unaffected by EDTA, suggesting that cationic metals were not implicated in the toxicity. Solid phase extraction (SPE) column treatment removed the toxicity and it was recovered in the SPE column methanol eluate, suggesting the toxicity was caused by one or more non-polar organic chemical(s). PBO addition did not prevent or delay the toxicity. Chemical analysis detected four pyrethroid and seven organophosphate (OP) insecticides in the sample. The Phase III TIE analysis, designed to estimate the contribution of each of the toxicants to the total sample toxicity, indicated that the sample contained toxic concentrations of two pyrethroids, cyfluthrin (0.97 TUa) and permethrin (0.36 TUa) and three OPs, chlorpyrifos (0.09 TUa), diazinon (0.06 TUa), and malathion (2.4 TUa). However, the combined role of these chemicals in the sample toxicity could not be assessed due to the lack of information regarding the additivity of OP and pyrethroid toxicity to *C. dubia.* The toxic Elk Grove Creek sample was the only sample that was collected during a rain associated stormwater runoff event. Since the rainfall dissipated prior to sampling, the other samples tested were more characteristic of a late winter dry weather event. This study suggests that additional stormwater runoff sampling of Sacramento area urban creeks is warranted to identify the frequency, duration and intensity of toxicity. In addition, the interaction of OP and pyrethroid insecticide toxicity to *C. dubia* should be characterized.

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 96-hour acute toxicity tests with *Ceriodaphnia dubia* on water samples collected from American River tributaries and urban creeks. The toxicity testing, conducted in coordination with the CVRWQCB Surface Water Ambient Monitoring Program (SWAMP) was designed to identify potential impacts from urban, historical mining, agriculture, and other sources to the beneficial uses of the American River drainage and Sacramento area urban creeks of the lower Sacramento River basin. Sample collection, toxicity testing, water quality analyses, and data reporting were compliant with SWAMP quality assurance requirements.

3.0 MATERIALS AND METHODS

3.1 Sample Collection and Transport

CVRWQCB personnel collected the test samples on 2/7/07. 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, dissolved oxygen (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 hours of collection. Appropriate chain-of-custody procedures were employed during collection and transport.

Table 1. Test Sample Locations

Sample Code	Location	Longitude - Latitude
519SAC20	American River @ Discovery Park	38.60094 -121.50550
519LSAC59	Dry Creek @ Wallerga Rd	38.73699 -121.36481
519KSAC60	Dry Creek @ Eureka Rd	38.75913 -121.25665
519LSAC58	Arcade Creek @ Auburn Blvd	38.64331 -121.36755
519LSAC51	Steelhead Creek @ Beach Lake Rd	38.6079 -121.4908
514LSAC18	Willow Creek @ Prairie City/Blue Ravine	38.65706 -121.16907
514LSAC19	Buffalo Creek @ Gold River Rd	38.62415 -121.26205
532ELD006	Carson Creek @ Barranca St	38.64368 -121.07896
519LSAC56	Morrison Creek @ McRoberts St	38.54219 -121.27616
519LSAC57	Elk Grove Creek @ Emerald Vista St	38.41093 -121.38424
519LSAC35	Pleasant Grove Creek @ Crocker Ranch West	38.80276 -121.33842
519LSAC41	Kaseberg Creek @ Country Club Dr	38.76415 -121.32280
519LSAC45	South Branch Pleasant Grove Creek @ Painted Desert St	38.79047 -121.33415
500FDQ013	American River @ Discovery Park (Field Duplicate)	38.60094 -121.50550

3.2 Water Quality Measurements

Meter calibration/verification and water quality measurements followed the procedures described by the SWRCB QAMP[1] 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-

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

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).

3.3 *Ceriodaphnia dubia* Toxicity Tests

C. dubia 96-hour acute toxicity tests were conducted in accordance with the U.S. Environmental Protection Agency (USEPA) 5th edition protocols[2]. Tests were initiated with < 24 hr old neonates collected within an 8-hr period from in-house cultures. Tests were considered invalid if control survival was < 90%. Each 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. 4 Phase I Toxicity Identification Evaluation (TIE)

Phase I TIE procedures followed those described by USEPA[3]. TIE treatments included a baseline dilution series toxicity test, C-8 solid phase extraction (SPE) without pH adjustment, SPE column eluate add-back for non-polar organics (NPOs), ethylenediaminetetraacetic acid (EDTA) addition for cationic metals, and piperonyl butoxide (PBO) addition for metabolically-activated organophosphorous insecticides. If the TIE indicated NPO toxicity, the SPE column eluate was chemically analyzed to identify potential toxicant(s) by the California Department of Fish and Game Water Pollution Control Laboratory (CDFG-WPCL, Rancho Cordova, CA).

4.0 RESULTS AND DISCUSSION

4.1 *C. dubia* Toxicity Tests

Results of *C. dubia* toxicity tests are summarized in Table 2. With the exception of Elk Grove Creek, none of the other samples tested detected significant toxicity. The Elk Grove Creek sample resulted in 100% *C. dubia* mortality within 24 hours. This sample was subjected to a Phase I TIE to determine the chemical class(es) of the toxicant(s). The raw data for the toxicity tests 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]Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents. Phase I. EPA /600/6-91/005F

Table 2. Test Sample Locations

Sample Code	Location	*Ceriodaphnia* % Survival
Control #1	--	100
519SAC20	American River @ Discovery Park	100
519LSAC59	Dry Creek @ Wallerga Rd	100
519KSAC60	Dry Creek @ Eureka Rd	100
519LSAC58	Arcade Creek @ Auburn Blvd	100
519LSAC51	Steelhead Creek @ Beach Lake Rd	100
514LSAC18	Willow Creek @ Prairie City/Blue Ravine	100
514LSAC19	Buffalo Creek @ Gold River Rd	100
532ELD006	Carson Creek @ Barranca St	96
Control #2	--	100
519LSAC56	Morrison Creek @ McRoberts St	95
519LSAC57	Elk Grove Creek @ Emerald Vista St	0[a,b]
519LSAC35	Pleasant Grove Creek @ Crocker Ranch West	100
519LSAC41	Kaseberg Creek @ Country Club Dr	95
519LSAC45	South Branch Pleasant Grove Creek @ Painted Desert St	100
500FDQ013	American River @ Discovery Park (Field Duplicate)	100

Sample Date: 2/7/07
Test Date: 2/8/07
* Statistically different from control (p=0.05)
a 100% mortality observed with 24 hours
b A Phase I TIE was conducted on this sample

4.2 Phase I TIE

Results of the Phase I TIE are shown in Table 3.

Table 3. Results of *C. dubia* Phase I TIE with Elk Grove Creek Sample 519LSAC57

Treatment	*Survival (%)*						*Comments*
	Control /Blank	*5*	*10*	*25*	*50*	*100*	
Baseline	100	95	100	0*	0*	0*	NOEC = 10 EC_{25} = 13.5 EC_{50} = 17.3
C-8 SPE Through-Column	90	--	--	--	100	90	Toxicity removed by SPE and recovered in the MeOH eluate
2X add-back	100	--	--	--	--	0*	
PBO Treatment	100	--	--	--	0*	0*	Toxicity not prevented by PBO
EDTA Treatment	100	--	--	--	0*	0*	Treatment ineffective – no metals toxicity

Sample Date: 2/7/07
Test Date: 2/9/07
* = significantly different than control (p=0.05)

The TIE baseline dilution series detected 5.8 TUa. EDTA treatment did not affect sample toxicity, indicating that toxic concentrations of cationic metals were not present in this sample. SPE column treatment removed the majority of the toxicity, which was recovered in the SPE column eluate, suggesting that one or more NPO chemical(s) were responsible for the sample toxicity. PBO addition did not prevent or delay the toxicity. The SPE column methanol eluate and the raw water sample were sent to the CDFG-WPCL for chemical analysis. The TIE raw data is found in Appendix II.

4.3 Phase III TIE

The purpose of the Phase III TIE is to determine if the concentrations of the toxicants detected in the sample accounted for the total sample toxicity. Results of the chemical analyses are presented in Table 4. TUa values were calculated for each of the chemicals that could have contributed to the toxicity based on available *C. dubia* EC_{50} values as follows:

$$TUa = \text{sample concentration } (\mu g/L) / EC_{50} (\mu g/L)$$

Four pyrethroids were detected in the sample, but EC_{50} values were only available for cyfluthrin and permethrin which could have contributed 0.97 TUa and 0.36 TUa, respectively, to the sample toxicity. No *C. dubia* EC_{50} values were available in the USEPA ECOTOX database for deltamethrin and lambda cyhalothrin. Although the pyrethroids could have accounted for 1.3 TUa of the sample toxicity, we are uncertain of their actual contribution because we are not aware of any published information on the interaction of pyrethroids and OP insecticides with *C. dubia*.

The three OPs detected in the sample for which EC_{50} values were available could have accounted for 2.6 TUa or about 45% of the total TUa. Malathion contributed the majority of the OP-caused toxicity in the sample (2.4 TUa). Unfortunately, no EC_{50}

values were available for Dioxathion (Delnav®) or Disulfoton (Disyston®), which were present in the sample at potentially significant concentrations (0.948 µg/L and 1.27 µg/L, respectively). In the numerous TIEs conducted in this laboratory, we have never identified Dioxathion or Disulfoton as causes of toxicity in ambient samples, regardless of their source. Another interesting characteristic of the sample was the unusually large number and types of contaminants detected (4 pyrethroids and 7 OPs). This information, coupled with land-use information and pesticide application reports, may provide useful clues to identify practices that resulted in the discharge of these chemicals into Elk Grove Creek.

Table 4. Results of Elk Grove Creek Chemical Analyses [a,b]

Insecticide Class	*Chemical (µg/L)*	*Concentration (µg/L)*	*C. dubia EC_{50} (µg/mL)*	*TUa*	*Total TUa*
Pyrethroid	Cyfluthrin	0.136	0.140[c]	0.97	**1.3**
	Deltamethrin	0.023	n/a[d]	--	
	λ-cyhalothrin	0.008	n/a	--	
	Permethrin	0.027	0.075[c]	0.36	
OP	Chlorpyrifos	0.007	0.080[e]	0.09	**2.6**
	Diazinon	0.024	0.380[e]	0.06	
	Dimethoate	0.045	n/a	--	
	Dioxathion	0.948	n/a	--	
	Disulfoton	1.27	n/a	--	
	Malathion	2.40	1.0[e]	2.4	
	Metrication	0.033	n/a	--	

a Sample Code: 519LSAC57; Sample Date: 2/7/07
b Chemical analyses conducted by CDFG-WPCL
c Data from USEPA ECOTOX database
d Data not found on USEPA ECOTOX database
e Data from AQUA-Science

5.0 CONCLUSIONS

- A sample collected in February 2007 from Elk Grove Creek was highly toxic to *C. dubia*.
- The TIE detected 5.8 TUa. Three of the OPs detected in the sample for which EC_{50} values were available could have accounted for 2.6 TUa, about half of the sample toxicity. Malathion was the principle toxicant identified, contributing 2.4 TUa to the sample toxicity.
- Two pyrethroid insecticides detected in the sample (permethrin and cyfluthrin), could have accounted for a total of 1.3 TUa.

- However, the combined role of the OP and pyrethroid insecticides detected in the sample is unknown due to uncertainties about additive toxicity of these two different chemical classes to *C. dubia.*
- The sample contained an unusually large number of toxicants (4 pyrethroids and 7 OPs). This finding, in conjunction with land-use and pesticide application information may provide clues to the practices that resulted in discharge of these chemicals into Elk Grove Creek.
- The toxic Elk Grove Creek water sample was the only sample that was collected during rain associated stormwater runoff. Sampling during periods of rainfall driven stormwater runoff are important for characterizing winter wet season water quality in urban creeks.

6.0 RECOMMENDATIONS

- The high *C. dubia* toxicity detected in the Elk Grove Creek sample suggests that additional sampling should be conducted to determine the frequency, intensity and duration of toxicity in Sacramento urban creeks during stormwater runoff events.
- Toxicity monitoring conducted in this laboratory in conjunction with this and other monitoring projects (agricultural waiver) indicates that OP and pyrethroid insecticides sometimes co-occur in ambient runoff samples. Therefore, additional research should be conducted to characterize the interaction of OP and pyrethroid toxicity to *C. dubia.*