Final Report

Acute Toxicity of Ammonia, Copper, and Pesticides to *Eurytemora affinis*, of the San Francisco Estuary

Submitted to:

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Executive Summary

Toxicity testing (96-h) of ambient surface waters in April-May 2008 from several locations in the North and South Delta-San Francisco Estuary (SFE) was shown to significantly affect the survival of *Eurytemora affinis*. Although chemical contaminants such as ammonia, bifenthrin, copper diuron, lambda cyhalothrin, and polyaromatic hydrocarbons have been detected in ambient waters, the impacts of these contaminants to pelagic organisms in the SFE food web are critically unknown particularly to the dominant zooplankton, i.e., *E. affinis*. The acute toxicity of ammonia, bifenthrin, chlorpyrifos, copper, cyfluthrin and permethrin to *E. affinis* was addressed in the current study as shown by the results of 96hr-LC50 values of the different contaminants: 1) ammonia - 10.97 mg/L total ammonia or 0.78 mg/L unionized ammonia at pH 8.1, 7.56 mg/L total ammonia or 0.12 mg/L unionized ammonia at pH7.6, and 10.93 mg/L total ammonia or 0.068 mg/L unionized ammonia at pH7.2; 2) bifenthrin - 11.37 ng/L, 3) chlorpyrifos - 803.20 ng/L 4) copper - 3.48 μg/L, 5) cyfluthrin - 12.72 ng/L and 6) permethrin -158.08 ng/L. Current findings indicated that *E. affinis* were sensitive to ammonia, copper, and pyrethroid pesticides (bifenthrin, cyfluthrin, and permethrin) and organophosphate insecticide (chlorpyrifos). Based on the results of this study, it is likely that the toxicities observed in *E. affinis* in 2008 may have been due, in part, to the presence of some of these chemicals in examined ambient waters. The potential impact of one or additive effects of these chemicals pose serious implications to the health and survival of zooplankton as important components of the SFE food web.

Introduction

Eurytemora affinis is an important food source to higher trophic level pelagic fish such as delta smelt, threadfin shad, and longfin smelt in the San Francisco Estuary (SFE). Previous study in this laboratory revealed that ambient surface waters from several locations in the North and South Delta in April-May 2008 showed significant effects to *E. affinis* survival (Teh *et al*., 2008). The initial detection of several chemical contaminants including ammonia, bifenthrin, chlorpyrifos, copper, cyfluthrin and permethrin in ambient waters prompted the need to examine their acute toxicity to *E. affinis*. Assessing the 96-hour LC50 values to establish the toxicity of these contaminants to *E. affinis* under controlled laboratory conditions was the main objective of the current study.

Experimental Details

1. Copepods

Brood stock of *E. affinis* was grown in aerated 120 L tanks placed in an environmentally controlled room at 20 ± 1 ^{o}C. Water quality in the tank including dissolved oxygen (>8 mg/L), pH (8.0 ± 0.1), water hardness (100 mg/L), salinity (2.0 ppt), and ammonia (<1 µg/L) were monitored weekly. An equal biovolume of the Instant Algae (*Nannochloropsis* and *Pavlova*) mix was given as food at 400 µg C.L^{-1}.day-1.

2. *Chemicals*

Stock solutions of ammonium chloride (10.0 g/L), bifenthrin (8.0 mg/L), chlorpyrifos (4.0 mg/L) copper chloride (4.0 mg/L), cyfluthrin (4.0 mg/L), and permethrin (8.0 mg/L) were prepared by personnel of Aquatic Toxicology Laboratory at UC Davis. The concentrations of the chemical used were: 1) bifenthrin (methanol control, 4.0, 8.0, 16.0, 32.0, and 64.0 ng/L), 2) chlorpyrifos (methanol control, 300, 600, 900, 1200, 1500 ng/L), 3) cyfluthrin (methanol control, 1.0, 3.0, 5.0, 7.0, 9.0 ng/L), and 4) permethrin (methanol control, 150, 175, 200, 225, 250 ng/L). Methanol was used as solvent for these chemicals, and therefore served as control using the highest concentration in each of the chemical treatments. The concentrations used for ammonia were: 1) 0.0, 10.0, 15.0, 20.0, 25.0, and 30.0 mg/L at pH 8.1, 2) 0.0, 10.0, 15.0, 20.0, 25.0, and 30.0 mg/L at pH7.6, and 3) 0.0, 4.0, 6.0, 8.0, 10.0, and 12.0 mg/L at pH7.2 that were prepared by diluting the ammonium chloride stock solution with culture water and the pH adjusted with 1N HCl. The concentrations used for copper chloride were 0.0, 1.0, 2.0, 4.0, 6.0, and 8.0 µg/L. Graded concentrations of these chemicals were prepared by diluting the stock solution with culture water (same source of water as used for culturing the *E. affinis*) 30-45 minutes prior to the initiation of the 96-hour exposures.

3. *Acute Toxicity Test*

Groups of juvenile *E. affinis* (N = 20 per replicate; three replicates per concentration) were exposed separately to ammonia, bifenthrin, chlorpyrifos, copper, cyfluthrin and

permethrin using the standard static renewal method for acute toxicity testing (1993). The test conditions used for the acute toxicity tests for ammonia, bifenthrin, chlorpyrifos, copper, cyfluthrin and permethrin are shown in Table 1. Briefly, Copepods were fed with nutritious algae and 80% of the tested water was replaced at 24, 48, and 72 h with newly prepared corresponding treatment solutions previously acclimated to 20 ^{o}C. Mortalities were recorded daily for 4 days. At the end of 96 hr, the number of survivors in each beaker was counted to derive the mean percentage survival of *E. affinis* exposed to each chemical concentration. The estimated 96-hour LC50 values (Lethal Concentration causing 50% mortality of the *E. affinis*) were calculated using the U.S. Environmental Protection Agency Probit Analysis Program v1.5 (http://www.epa.gov/nerleerd/stat2.htm).

4. *Water parameters and chemical analysis*

Water quality was monitored and recorded daily for each of the acute toxicity trials. Unionized ammonia was calculated from total ammonia nitrogen using free ammonia calculator (http://cobweb.ecn.purdue.edu/~piwc/w3-research/free-ammonia/nh3.html). The concentrations of the chemicals used for the toxicity trials will be verified at the Aquatic Toxicology Laboratory at UC Davis by testing I L subsamples of each of the chemical concentrations prior to the exposure trials.

Results and Discussions

The mean survival (%) of *E. affinis* at the end of 96 hour of toxicity testing is given in Table 2. The 96hr-LC10 and 96hr-LC50 values with 95% confidence intervals as calculated using the USEPA Probit Analysis Program v1.5 are shown in Table 3.
The data demonstrates that juvenile *E. affinis* are sensitive to the ammonia, copper, pyrethroid pesticides (.bifenthrin, cyfluthrin and permethrin), and organophosphate insecticide (chlorpyrifos). This pilot study aimed to establish LC50 values for *E. affinis* to support the hypothesis that ambient water samples from certain locations in the SFE are toxic to *E. affinis.* Based on the results of this study, it is likely that the toxicities observed in *E. affinis* in 2008 may have been due, in part, to the presence of these chemicals in examined ambient waters. The potential impact of one or additive effects of these chemicals pose serious implications to the health and survival of zooplankton as important components of the SFE food web.

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References

US Environmental Protection Agency. 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms (Fourth Edition). Report EPA/600/4-90/027F/93. Environmental Monitoring Systems Laboratory, Office of Research and Development. Cincinnati, OH.
Teh SJ, Min L, Teh, FC Lesmeister, S, Werner,I, Krause, J, and Deanovic, L. 2008. Toxic effects of surface water in the upper San Francisco Estuary on *Eurytemora affinis*. Final report to San Luis and Delta-Mendota Water Authority.

Table 1 Test conditions used for *Eurytemora affinis*

Temperature (°C)	**20 ± 0.1**
Salinity (ppt)	**2**
pH	**8.0 ± 0.1**
Conductivity (µmhos)	**3000**
Hardness (mg/L)	**360**
Alkalinity (mg/L)	**60**
Acceptability in control survival	**≥80%**
Size of test beaker (mL)	**600**
Volume of test solution (mL)	**500**
Life stage of copepods	**Juvenile**
# of copepods	**20**
# of replicates per concentration	**3**
# of concentrations	**6**
Feeding regime	**Daily**
Static-renewal test Duration	**24-96 h**

Table 2 Mean % survivorship of *E. affinis* at the end of 96 hour exposure

Chemicals	Concentration	% Survivorship
Ammonia mg/L at pH 8.1	Control	96.66
	10	56.66
	15	20.00
	20	5.00
	25	0
	30	0
Ammonia mg/L at pH 7.6	Control	88.33
	10	16.66
	15	0
	20	0
	25	0
	30	0
Ammonia mg/L at pH 7.2	Control	88.33
	4	60.00
	6	56.66
	8	55.00
	10	46.66
	12	35.00
Bifenthrin ng/L (pptr)	Methanol control	85.00
	4	75.00
	8	43.33
	16	38.33
	32	16.67
	64	3.33
Chlorpyrifos ng/L (pptr)	Methanol control	83.33
	300	76.66
	600	65.00
	900	26.66
	1200	18.33
	1500	15.00
Copper µg/L (ppb)	Control	88.33
	1	88.33
	2	61.66
	4	23.33
	6	30.00
	8	13.33

Cyfluthrin ng/L (pptr)	Methanol control	88.33
	1	85.00
	3	68.33
	5	56.66
	7	68.33
	9	46.66
Permethrin ng/L (pptr)	methanol Control	88.33
	150	46.66
	175	35
	200	31.66
	225	25
	250	11.66

Table 3 Estimates LC 10 and 50 values of *E. affinis* calculated using Probit Analysis (95% confidence intervals are indicated in parentheses)

Chemicals	96hr-LC10	96hr-LC50
Total Ammonia (mg/L; pH8.1)	7.01 (5.50, 8.71)	10.97 (9.76, 11.96)
Unionized Ammonia (mg/L; pH8.1)	0.46 (0.35, 0.55)	0.78 (0.68, 0.86)
Total Ammonia (mg/L; pH7.6)	5.02 (1.42, 6.85)	7.56 (4.07, 8.95)
Unionized Ammonia (mg/L; pH7.6)	0.08 (0.02, 0.11)	0.12 (0.06, 0.14)
Total Ammonia (mg/L; pH7.2)	1.82 (0, 2.79)	10.93 (7.34,49.0)
Unionized Ammonia (mg/L; pH7.2)	0.011 (0.0, 0.017)	0.068 (0.046, 0.306)
Bifenthrin (ng/L; pptr)	2.76 (1.27, 4.43)	11.37 (8.04, 14.80)
Chlorpyrifos (ng/L; pptr)	384.49 (211.81, 515.58)	803.20 (640.17, 926.41)
Copper (µg/L; ppb)	1.42 (0.61, 1.45)	3.48 (2.85, 4.15)
Cyfluthrin (ng/L; pptr)	1.40 (0.05, 2.89)	12.72 (8.05, 55.55)
Permethrin (ng/L; pptr)	83.37 (38.71, 110.83)	158.08 (125.55, 175.99

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Pelagic Organism Decline (POD): Acute and Chronic Invertebrate and Fish Toxicity Testing in the Sacramento-San Joaquin Delta 2008-2010

Progress Report

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