


# UC Davis Aquatic Health Program Laboratory



	<b>Progress Report #</b>	1	
	<b>Reporting Period:</b>	Nov. 1, 2021	to Jan. 31, 2022
	<b>Submittal Date:</b>	February 10, 2022	
<b>Contract No:</b>	Department of Pesticide Regulation No. 21-C0044		
<b>Project Name:</b>	Evaluation of Pyrethroid Toxicity Removal in Agricultural Detention Basins using <i>Hyalella azteca</i>		
<b>Contractor Name:</b>	UC Davis Aquatic Health Program Laboratory		
<p>I certify under penalty of law that this document and all attachments were prepared by me or under my direction in accordance with the terms and conditions of each Research Agreement Task. Based on my inquiry of the persons or persons who manage the project or those directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete. All information submitted in this document and all attachments conform to and are in accordance with the state and federal laws and I so here certify with my signature. I am aware that there are significant penalties for submitting false or misleading information.</p>			
<b>Project Director:</b>	Dr. Swee Teh		
	Printed Name		Signature
<p>Funding for this project has been provided in full or in part through an agreement with the California Department of Pesticide Regulation. The contents of this document do not necessarily reflect the views and policies of the California Department of Pesticide Regulation, nor does mention of trade names or commercial products constitute endorsement or recommendation for use (Gov. Code, § 7550, 40 C.F.R. § 31.20).</p>			

## Report Narrative

### Introduction

Retention basins and other wetland-based management practices have been shown to be effective in removing pyrethroid pesticides from surface water. This water treatment practice is likely to be successful in removing pyrethroids from agricultural runoff, however, research on pyrethroid removal and mitigation of pyrethroid toxicity has been limited. The Department of Pesticide Regulation has contracted with Dr. Tom Young of UC Davis to determine the distribution and settling properties of particle suspensions of pyrethroids in field collected agricultural samples. This project will focus on quantifying pyrethroid toxicity removal in the process of sedimentation using pyrethroid sensitive testing organism *Hyalella azteca* (*H. azteca*).

### Summary of Activities

The contract was approved on November 5, 2021. All parties held a project kick-off meeting on November 10, 2021, to discuss the logistics of the project and to work out sampling plans. It was decided that half of the

samples would be collected at this time, in part due to the delay in getting the contract in place, which put us outside the irrigation season. It was also decided to do a partial collection in order to optimize the protocols to be used for the project and to ensure that the DOC/TOC were at the proper ratio for testing. Samples were collected from Sal\_Hartnel (Site 1) on November 29, 2021 and from Sal\_Tembl (Site 2) on November 30, 2021. Toxicity tests were initiated on December 1, 2021.

## Materials and Methods

### *Water Sample Collection*

For *H. azteca* toxicity tests, 3L of whole water (WW) was collected from each site as sub-surface grabs via a pump. Three (3)-L of clarified supernatant (CS) was collected via a flow-through centrifuge from each site. These samples were collected in pre-cleaned 1L amber bottles. An additional 240L of WW were collected in 12 -20L cubtainers for future settling tests. All containers used for water collections were labeled with the site ID, collection date and time, initials of the sampler and then rinsed three times with ambient water prior to filling. All samples were placed on wet ice for transport to the UCD AHP and kept between 0-6°C<sup>3</sup>. Upon receipt, samples were stored in the dark in an environmental chamber maintained between 0-4°C until their use in a test.

### *Water Quality*

Ammonia-nitrogen was measured at UCD AHPL within 24 hours of sample receipt using a HACH DR-3900 spectrophotometer and a HACH Am-Ver Low-Range Ammonia Reagent Set. Hardness and alkalinity were measured on all ambient samples (titrimetric methods) within 48-hours of sample receipt. These measurements were taken on the control and the 100% concentration of each ambient sample (whole water and clarified supernatant) only.

### *Toxicity Testing Methods – Acute Hyalella*

UCD AHP toxicity testing methods are based on protocols developed by UCD AHP SOPs<sup>1</sup>, SWAMP QAPrP<sup>2</sup>, and USEPA<sup>3</sup>. Acute toxicity testing for *H. azteca* followed protocols outlined in *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*<sup>3</sup> and SWAMP Method Quality Objectives<sup>2</sup>.

Before test initiation and water renewals, water samples were shaken thoroughly in their original sample containers for 60 seconds. Prior to test initiation and renewals, waters were warmed to test temperature ( $23 \pm 1^\circ\text{C}$ ) using a water bath maintained at  $25 \pm 2^\circ\text{C}$  and aerated at a rate of 100 bubbles per minute until the DO concentration fell below saturation. Water quality measurements including pH, Specific Conductivity (SC), Dissolved Oxygen (DO) and temperature were recorded for all treatments at test initiation, water renewals, and termination.

*H. azteca* were obtained from Aquatic Research Organisms (Hampton, NH) and were acclimated to laboratory conditions for 24-h prior to test initiation. Acute 96-h toxicity tests consisted of five 250 mL replicate glass beakers with 100 mL of sample, a 1-in<sup>2</sup> of nitex screen for artificial substrate, and 10 organisms each. Samples were tested in a dilution series consisting of five concentrations of either WW or CS, and a control. Reverse-Osmosis water amended with inorganic salts to USEPA moderately hard standards (ROEPAMHR) was used as the control and as dilution water. Eighty percent of the test solution was renewed at the 48-hr timepoint. Organisms were fed 1.5 mL of YCT (a mixture of yeast, trout chow, organic alfalfa and water) after test initiation and water renewal. Tests were conducted at  $23 \pm 1^\circ\text{C}$  with a 16-hr light: 8-hr dark photoperiod under

fluorescent light. Mortality was scored daily; at this time dead organisms and detritus were removed from test chambers if present. A reference toxicant test with sodium chloride as the toxicant, was conducted currently with each set of toxicity tests.

### Statistics

Each sample was characterized by descriptive statistics, including the mean response and variation among replicates. Toxicity is defined as a statistically significant reduction in test organism performance in an ambient sample compared to a laboratory control. Lethal effect concentrations were calculated using CETIS v. 1.8.7.2 (Tidepool Scientific Software, McKinleyville, CA, USA). NOEC and LOEC values were calculated using USEPA standard statistical protocols. LC50s were calculated using linear regression.

### Results

Ambient samples were collected on November 29 and 30, 2021 and applied in toxicity tests with *Hyalella azteca* on December 1, 2021. Statistically significant reductions in survival were observed in the 100%, 50%, and 25% dilutions of Sal\_Hartnel WW. The calculated LC50 was 26.29% (20.93, 32.04), with a NOEC of 12.5% and a LOEC of 25%. There were 3.8 Toxic Units in this sample. No significant reductions in survival were observed in any other treatment. Toxicity test results are presented below in Tables 1-4. Water quality measurements from these tests are outlined in Tables 5-8.

Table 1. Summary of results from a *H. azteca* toxicity test initiated on December 1, 2021 with whole water samples collected from Sal-Hartnel on November 29, 2021.

Sample	24-hr survival			48-hr survival			72-hr Survival			96-hr Survival		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
0% (Control)	100.0	0.00	0.00	100.0	0.00	0.00	100.0	0.00	0.00	100.0	0.00	0.00
6.25% Sal_Hartnel WW	100.0	0.00	0.00	98.0	4.47	2.00	98.0	4.47	2.00	96.0	5.48	2.45
12.5% Sal_Hartnel WW	100.0	0.00	0.00	98.0	4.47	2.00	92.0	17.89	8.00	88.0	21.68	9.70
25% Sal_Hartnel WW	98.0	4.47	2.00	90.0	12.25	5.48	82.0	13.04	5.83	62.0	8.37	3.74
50% Sal_Hartnel WW	36.0	11.40	5.10	16.0	8.94	4.00	4.0	5.48	2.45	4.0	5.48	2.45
100% Sal_Hartnel WW	26.0	15.17	6.78	14.0	8.94	4.00	2.0	4.47	2.00	2.0	4.47	2.00

1. Highlighted cells indicate a statistically significant reduction ( $P < 0.05$ ) in survival compared to the control.

Table 2. Summary of results from a *H. azteca* toxicity test initiated on December 1, 2021 with clarified supernatant samples collected from Sal-Hartnel on November 29, 2021.

Sample	24-hr survival			48-hr survival			72-hr Survival			96-hr Survival		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
0% (Control)	98.0	4.47	2.00	98.0	4.47	2.00	98.0	4.47	2.00	98.0	4.47	2.00
6.25% Sal_Hartnel CS	100.0	0.00	0.00	100.0	0.00	0.00	100.0	0.00	0.00	100.0	0.00	0.00
12.5% Sal_Hartnel CS	98.0	4.47	2.00	98.0	4.47	2.00	98.0	4.47	2.00	98.0	4.47	2.00
25% Sal_Hartnel CS	100.0	0.00	0.00	98.0	4.47	2.00	98.0	4.47	2.00	98.0	4.47	2.00
50% Sal_Hartnel CS	100.0	0.00	0.00	100.0	0.00	0.00	98.0	4.47	2.00	98.0	4.47	2.00
100% Sal_Hartnel CS	100.0	0.00	0.00	96.0	5.48	2.45	94.0	5.48	2.45	94.0	5.48	2.45

Table 3. Summary of results from a *H. azteca* toxicity test initiated on December 1, 2021 with whole water samples collected from Sal-Tembl on November 30, 2021.

Sample	24-hr survival			48-hr survival			72-hr Survival			96-hr Survival		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
0% (Control)	100.0	0.00	0.00	100.0	0.00	0.00	100.0	0.00	0.00	100.0	0.00	0.00
6.25% Sal_Tembl WW	100.0	0.00	0.00	98.0	4.47	2.00	98.0	4.47	2.00	98.0	4.47	2.00

Sample	24-hr survival			48-hr survival			72-hr Survival			96-hr Survival		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
12.5% Sal_Tembl WW	100.0	0.00	0.00	100.0	0.00	0.00	100.0	0.00	0.00	98.0	4.47	2.00
25% Sal_Tembl WW	100.0	0.00	0.00	100.0	0.00	0.00	100.0	0.00	0.00	100.0	0.00	0.00
50% Sal_Tembl WW	100.0	0.00	0.00	100.0	0.00	0.00	100.0	0.00	0.00	100.0	0.00	0.00
100% Sal_Tembl WW	100.0	0.00	0.00	100.0	0.00	0.00	94.0	13.42	6.00	94.0	13.42	6.00

Table 4. Summary of results from a *H. azteca* toxicity test initiated on December 1, 2021 with clarified supernatant samples collected from Sal-Tembl on November 30, 2021.

Sample	24-hr survival			48-hr survival			72-hr Survival			96-hr Survival		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
0% (Control)	100.0	0.00	0.00	100.0	0.00	0.00	100.0	0.00	0.00	100.0	0.00	0.00
6.25% Sal_Tembl CS	100.0	0.00	0.00	100.0	0.00	0.00	100.0	0.00	0.00	98.0	4.47	2.00
12.5% Sal_Tembl CS	100.0	0.00	0.00	100.0	0.00	0.00	96.0	5.48	2.45	98.0	4.47	2.00
25% Sal_Tembl CS	98.0	4.47	2.00	98.0	4.47	2.00	98.0	4.47	2.00	100.0	0.00	0.00
50% Sal_Tembl CS	100.0	0.00	0.00	96.0	5.48	2.45	96.0	5.48	2.45	100.0	0.00	0.00
100% Sal_Tembl CS	100.0	0.00	0.00	100.0	0.00	0.00	100.0	0.00	0.00	94.0	13.42	6.00

Table 5. Summary of water quality measurements from a *H. azteca* toxicity test initiated on December 1, 2021 with whole water samples collected from Sal\_Hartnel on November 29, 2021.

Sample	DO (mg/L)				Initial SC ( $\mu\text{S}/\text{cm}$ )	
	Initial	Final	Min	Max		
0% (Control)	8.78	7.67	7.67	8.78	358	
6.25% Sal_Hartnel WW	8.58	7.64	7.60	8.58	415	
12.5% Sal_Hartnel WW	8.88	7.54	7.54	8.88	450	
25% Sal_Hartnel WW	8.78	7.55	7.55	8.78	534	
50% Sal_Hartnel WW	8.98	7.23	7.23	8.98	699	
100% Sal_Hartnel WW	8.25	7.21	7.21	8.25	1034	
Sample	pH				Total Ammonia (mg/L)	Unionized Ammonia (mg/L) <sup>1</sup>
	Initial	Final	Min	Max		
0% (Control)	7.74	7.60	7.58	7.94	ND	ND
6.25% Sal_Hartnel WW	7.70	7.69	7.51	7.93	NR	NR
12.5% Sal_Hartnel WW	7.70	7.59	7.48	7.96	NR	NR
25% Sal_Hartnel WW	7.69	7.64	7.54	7.96	NR	NR
50% Sal_Hartnel WW	7.66	7.69	7.63	7.99	NR	NR
100% Sal_Hartnel WW	7.67	7.82	7.67	7.99	2.58	0.052
Sample	Temp ( $^{\circ}\text{C}$ )				Alkalinity (mg/L as $\text{CaCO}_3$ )	Hardness (mg/L as $\text{CaCO}_3$ )
	Initial	Final	Min	Max		
0% (Control)	23.4	22.2	22.2	24.3	54	100
6.25% Sal_Hartnel WW	23.5	22.0	22.0	24.3	NR	NR
12.5% Sal_Hartnel WW	23.4	22.2	22.2	24.5	NR	NR
25% Sal_Hartnel WW	23.4	22.1	22.1	24.3	NR	NR
50% Sal_Hartnel WW	23.3	22.2	22.2	24.3	NR	NR
100% Sal_Hartnel WW	23.4	22.1	21.4	24.3	150	376

1: This unionized ammonia reading is based on the total ammonia measured upon sample receipt and upon the water chemistry measured at test initiation. NR: Ammonia, hardness, and alkalinity were measured on the control and the 100% concentration of the ambient sample only. ND: Non-detect.

Table 6. Summary of water quality measurements from a *H. azteca* toxicity test initiated on December 1, 2021 with clarified supernatant samples collected from Sal\_Hartnel on November 29, 2021.

Sample	DO (mg/L)				Initial SC ( $\mu\text{S}/\text{cm}$ )	
	Initial	Final	Min	Max		
0% (Control)	8.83	7.72	7.72	8.83	355	
6.25% Sal_Hartnel CS	8.56	8.00	7.76	8.56	411	
12.5% Sal_Hartnel CS	8.83	7.83	7.77	8.83	461	
25% Sal_Hartnel CS	9.09	8.00	7.84	9.09	541	
50% Sal_Hartnel CS	9.15	8.11	7.77	9.15	750	
100% Sal_Hartnel CS	8.90	8.24	7.68	8.90	1054	
Sample	pH				Total Ammonia (mg/L)	Unionized Ammonia (mg/L) <sup>1</sup>
	Initial	Final	Min	Max		
0% (Control)	7.72	7.58	7.58	7.87	ND	ND
6.25% Sal_Hartnel CS	7.70	7.64	7.64	7.89	NR	NR
12.5% Sal_Hartnel CS	7.70	7.63	7.63	7.92	NR	NR
25% Sal_Hartnel CS	7.70	7.79	7.70	7.94	NR	NR
50% Sal_Hartnel CS	7.70	7.88	7.70	8.04	NR	NR
100% Sal_Hartnel CS	7.68	8.02	7.63	8.08	0.17	0.004
Sample	Temp ( $^{\circ}\text{C}$ )				Alkalinity (mg/L as $\text{CaCO}_3$ )	Hardness (mg/L as $\text{CaCO}_3$ )
	Initial	Final	Min	Max		
0% (Control)	23.4	22.1	22.1	24.3	54	100
6.25% Sal_Hartnel CS	23.6	21.9	21.9	24.3	NR	NR
12.5% Sal_Hartnel CS	23.5	22.0	21.7	24.2	NR	NR
25% Sal_Hartnel CS	23.6	22.0	21.7	24.1	NR	NR
50% Sal_Hartnel CS	23.5	21.9	21.9	24.0	NR	NR
100% Sal_Hartnel CS	23.6	21.8	21.8	24.0	134	384

1: This unionized ammonia reading is based on the total ammonia measured upon sample receipt and upon the water chemistry measured at test initiation. NR: Ammonia, hardness, and alkalinity were measured on the control and the 100% concentration of the ambient sample only. ND: Non-detect.

Table 7. Summary of water quality measurements from a *H. azteca* toxicity test initiated on December 1, 2021 with whole water samples collected from Sal\_Tembl on November 30, 2021.

Sample	DO (mg/L)				Initial SC ( $\mu\text{S}/\text{cm}$ )	
	Initial	Final	Min	Max		
0% (Control)	8.83	7.68	7.68	8.83	357.5	
6.25% Sal_Tembl WW	9.03	7.77	7.67	9.03		
12.5% Sal_Tembl WW	9.19	7.78	7.74	9.19	587	
25% Sal_Tembl WW	9.20	8.22	7.69	9.20	786	
50% Sal_Tembl WW	9.24	8.40	7.74	9.24	1197	
100% Sal_Tembl WW	9.29	8.66	7.70	9.29	1962	
Sample	pH				Total Ammonia (mg/L)	Unionized Ammonia (mg/L) <sup>1</sup>
	Initial	Final	Min	Max		
0% (Control)	7.69	7.59	7.56	7.94	ND	ND
6.25% Sal_Tembl WW	7.73	7.7	7.67	8.13	NR	NR
12.5% Sal_Tembl WW	7.79	7.81	7.72	8.05	NR	NR
25% Sal_Tembl WW	7.84	7.97	7.84	8.14	NR	NR
50% Sal_Tembl WW	7.87	8.23	7.87	8.23	NR	NR
100% Sal_Tembl WW	7.89	8.37	7.89	8.37	0.098	0.003
Sample	Temp ( $^{\circ}\text{C}$ )				Alkalinity (mg/L as $\text{CaCO}_3$ )	Hardness (mg/L as $\text{CaCO}_3$ )
	Initial	Final	Min	Max		
0% (Control)	23.6	22.1	22.1	24.1	54	100
6.25% Sal_Tembl WW	23.6	21.9	21.9	24.2	NR	NR
12.5% Sal_Tembl WW	23.6	22.1	22.1	24.1	NR	NR
25% Sal_Tembl WW	23.6	21.9	21.9	24.1	NR	NR
50% Sal_Tembl WW	23.6	22.2	22.2	24.0	NR	NR
100% Sal_Tembl WW	23.6	22.1	22.1	23.8	296	720

1: This unionized ammonia reading is based on the total ammonia measured upon sample receipt and upon the water chemistry measured at test initiation. NR: Ammonia, hardness, and alkalinity were measured on the control and the 100% concentration of the ambient sample only. ND: Non-detect.

Table 8. Summary of water quality measurements from a *H. azteca* toxicity test initiated on December 1, 2021 with clarified supernatant samples collected from Sal\_Tembl on November 30, 2021.

Sample	DO (mg/L)				Initial SC ( $\mu\text{S}/\text{cm}$ )	
	Initial	Final	Min	Max		
0% (Control)	8.89	7.66	7.66	8.89	354	
6.25% Sal_Tembl CS	8.82	8.86	7.53	8.86	479	
12.5% Sal_Tembl CS	9.00	7.78	7.60	9.00	574	
25% Sal_Tembl CS	9.11	7.90	7.66	9.11	794	
50% Sal_Tembl CS	8.95	7.75	7.68	8.95	1189	
100% Sal_Tembl CS	9.22	7.64	7.59	9.22	1961	
Sample	pH				Total Ammonia (mg/L)	Unionized Ammonia (mg/L) <sup>1</sup>
	Initial	Final	Min	Max		
0% (Control)	7.67	7.64	7.59	7.92	ND	ND
6.25% Sal_Tembl CS	7.76	7.70	7.66	8.04	NR	NR
12.5% Sal_Tembl CS	7.80	7.79	7.75	8.06	NR	NR
25% Sal_Tembl CS	7.87	7.91	7.85	8.15	NR	NR
50% Sal_Tembl CS	7.90	8.13	7.90	8.22	NR	NR
100% Sal_Tembl CS	7.91	8.27	7.91	8.32	ND	ND
Sample	Temp ( $^{\circ}\text{C}$ )				Alkalinity (mg/L as $\text{CaCO}_3$ )	Hardness (mg/L as $\text{CaCO}_3$ )
	Initial	Final	Min	Max		
0% (Control)	23.7	22.1	22.1	24.1	54	100
6.25% Sal_Tembl CS	23.7	22.2	22.2	24.3	NR	NR
12.5% Sal_Tembl CS	23.6	22.3	22.3	24.4	NR	NR
25% Sal_Tembl CS	23.6	22.4	22.4	24.4	NR	NR
50% Sal_Tembl CS	23.5	22.3	22.3	24.4	NR	NR
100% Sal_Tembl CS	23.5	22.0	22.0	24.4	286	720

1: This unionized ammonia reading is based on the total ammonia measured upon sample receipt and upon the water chemistry measured at test initiation. NR: Ammonia, hardness, and alkalinity were measured on the control and the 100% concentration of the ambient sample only. ND: Non-detect.

Table 9. Summary of Work Completed To Date.

Task	Items for Review #	% Complete	Estimated Due Date	Work Completed
Task 1	<b>1: Coordination of sample collection</b>	50	Ongoing	
	1.1 Collection of whole water	50	May 30, 2022	
	1.2 Collection of clarified supernatant	50	May 30, 2022	
Task 2	<b>2: <i>H. azteca</i> toxicity testing: WW + CS</b>	50	Ongoing	
	2.1 Central Coast Location 1	100	Nov. 2021	Dec. 1, 2021
	2.2 Central Coast Location 2	100	Nov. 2021	Dec. 1, 2021
	2.3 Central Coast Location 3	0	May 2022	
	2.3 Central Coast Location 4	0	May 2022	
Task 3	<b>3: <i>H. azteca</i> settling column tests</b>	0	Ongoing	
	3.1 Supernatant: Suspended solids	0	April 2022	
	3.2 Re-suspended supernatant: suspended solids	0	April 2022	
	3.3 Separated supernatant: suspended solids	0	April 2022	
	3.4 Post-test analytical chemistry	0	April 2022	
Task 4	<b>4: Toxicity information to mathematical model</b>	0	Ongoing	
Task 5	<b>5: Application of model to field scale sites</b>	0	Ongoing	
Task 6	<b>6: Administration and Reporting</b>	6	Ongoing	
	6.1 Quarterly Reports	6	Feb. 1, 2023	Feb. 15, 2022
	6.2 Quarterly Invoices	6	Jun. 30, 2023	Ongoing
	6.3 Draft Project Report	0	Apr. 15, 2023	
	6.6 Final Report	0	Jun. 30, 2023	

### Literature Cited

1. Aquatic Health Program. 2020. Standard Operating Procedures. University of California, Davis. Davis, CA.
2. Surface Water Ambient Monitoring Program. 2008. Quality Assurance Project Plan. For the State Water Resources Control Board. Sacramento, CA.
3. USEPA, 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. 4<sup>th</sup> ed. EPA/821/R-02/013. Office of Water. Washington, DC.