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The Toxicity and Interactions among Common Aquatic Contaminants in Binary Mixtures

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Aquatic Health Program
School of Veterinary Medicine
University of California
Davis, California

The Toxicity and Interactions among Common Aquatic Contaminants in Binary Mixtures
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FINAL REPORT

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1. EXECUTIVE SUMMARY

Mixtures of contaminants are ubiquitous in the aquatic environment, yet their toxic interactions are not well characterized. The current study used a robust experimental design and a two-tiered analytical approach to characterize and quantify the lethal and sublethal toxicity of binary combinations of aquatic contaminants on the model species, *Hyalella azteca.* Chemicals tested were selected based on their environmental prevalence and toxicity. These included the type I pyrethroid pesticides, bifenthrin and permethrin; type II pyrethroids, cyfluthrin and lambda cyhalothrin; the organophosphorous pesticide, chlorpyrifos; as well as copper and ammonia. Generalized Linear Models (GLM) were used to test for significant interactions between chemicals in a given mixture, while the additive models of Concentration Addition (CA) and Independent Action (IA) were compared to empirical data to further characterize mixture responses. Comparisons of mixture data to either of the two models were used to confirm concentration additive (CA) toxicity in mixtures with no significant interactions or to distinguish negative interactions as either additive by IA, or less than additive (antagonistic) (Figure 1).

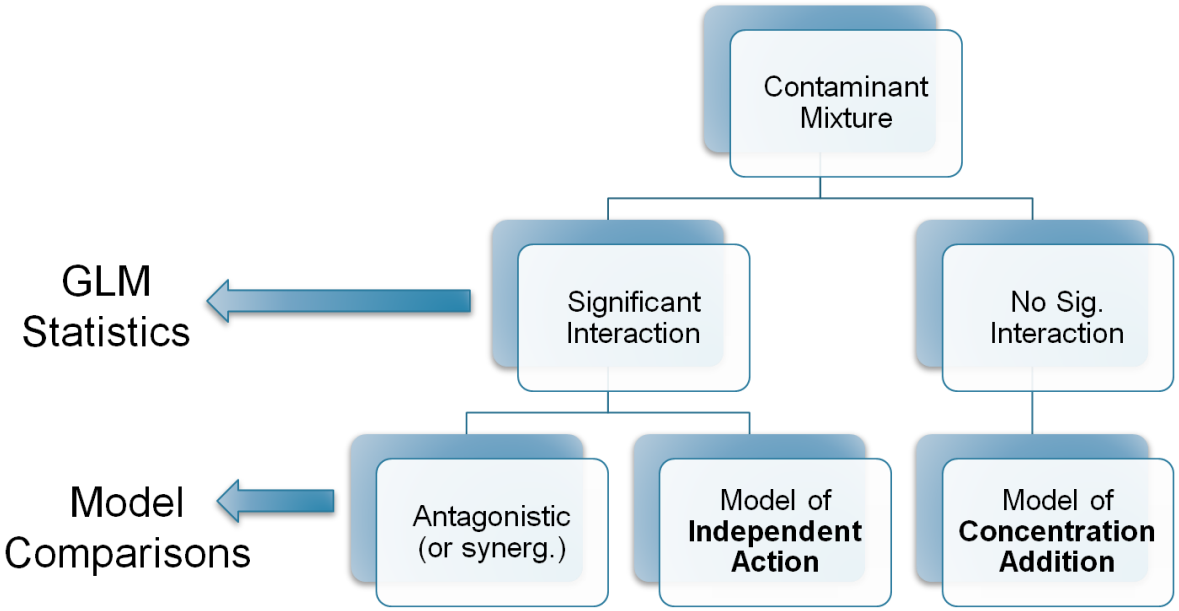


Figure 1. Diagram showing the structure of the two-tiered statistical approach used to analyze mixture effects.

Additive responses varied across the mixtures and endpoints tested, however all mixtures were close to additive by either the model of Concentration Addition or Independent Action (refer to Table 1 at the end of this section). Most mixtures were found to be more toxic than their component chemicals individually, including all combinations of type I and type II pyrethroid pesticides, bifenthrin x chlorpyrifos, and bifenthrin x copper. Bifenthrin and ammonia, on the other hand, were less than additive by either model and were no more toxic in combination than individually, suggesting an antagonistic response.

The acute 4-day toxicity among three of the seven neurotoxic pesticide mixtures, including bifenthrin x chlorpyrifos, bifenthrin x cyfluthrin and permethrin x cyfluthrin, were antagonistic according to GLM analyses. The mixtures of chemicals with different mechanisms of action, bifenthrin x copper and bifenthrin x ammonia, were antagonistic after both four and 10-day exposures. Qualitative comparisons of mixture data to the models of IA and CA indicate that antagonistic interactions between bifenthrin and copper were additive by the model of IA, while bifenthrin x ammonia were additive by IA at the 4-day endpoint, but less than additive (antagonistic) at the 10-day endpoint. Contrary to GLM results, all pesticides mixtures, including bifenthrin x chlorpyrifos, bifenthrin x cyfluthrin and permethrin x cyfluthrin, appeared to follow the model of CA after both four and 10-day exposures. All chemicals affected the endpoints of growth and swimming velocity in a concentration-dependant manner, with the exception of ammonia, which did not affect either sublethal endpoint; and chlorpyrifos, which did not affect growth. There were no interactions on the sublethal toxicity of any chemical mixture. In addition, there were no interactions among the pyrethroid and organophosphorous pesticides in mixtures at the 10-day mortality, swimming or growth endpoints.

In summary, mixtures of pyrethroids, chlorpyrifos and copper were more toxic on both lethal and sublethal endpoints than their component chemicals individually, while bifenthrin and ammonia were generally less than additive and not significantly more toxic in combination than alone. The additive effects or interactions among some mixtures, however varied across the endpoints measured. Antagonistic interactions were significant among three pesticide mixtures at the 4-day exposure endpoint that were not present after 10-day exposures. Additional investigations on the mechanisms of acute versus chronic effects of pesticides in mixtures on *H. azteca* would shed light on these anomalous findings. Because results from this study indicate that neurotoxic pesticides, even from different classes, are concentration additive on multiple endpoints, future work to evaluate additional neurotoxic chemicals commonly found in the aquatic environment is warranted. Results of this study substantiate the basic interactions of binary mixtures under laboratory conditions and set the stage for future studies to examine additional factors that bring test conditions closer to environmental relevance. Such directions could include testing more complex combinations of contaminants (ternary, quaternary, etc.) as well as testing for effects within the various components of environmentally representative matrix water, such as dissolved organic matter and salinity. The overall goal of this and future studies is to evaluate the ecological risk of aquatic contaminants, by accounting for factors and mixture interactions apparent in field matrices, for the conservation and management of resources in aquatic systems worldwide.

Table 1. A summary showing the model that most closely defines the additive response for each mixture tested.

Mixture	4-day Mortality	10-day Mortality	Growth	Swimming velocity
Bifenthrin x Copper	IA	IA	CA	CA
Bifenthrin x Ammonia	IA	<IA	CA*	CA*
Bifenthrin x Chlorpyrifos	IA	CA	CA*	CA
Bifenthrin x Permethrin	CA	CA	CA	CA
Permethrin x Cyfluthrin	IA	CA	CA	CA
Bifenthrin x Cyfluthrin	IA	--	--	--
Bifenthrin x λ-Cyhalothrin	CA	--	--	--
Permethrin x λ-Cyhalothrin	CA	--	--	--
Cyfluthrin x λ-Cyhalothrin	CA	--	--	--

*The secondary chemical did not cause an effect on the given endpoint, thus the additive response was not more severe than the effect of bifenthrin, alone.

2. INTRODUCTION

2.1 Purpose and Scope

Native fish populations in the Sacramento-San Joaquin Delta and the San Francisco Bay have recently experienced a significant decline (Werner et al. 2008, Sommer et al. 2007). These declines have been attributed to habitat degradation and loss, food limitation, and to the toxicity of legacy and contemporary environmental contaminants. Declines in both estuarine zooplankton and native fish suggest a potential trophic linkage. Furthermore, the conceptual model on the Pelagic Organism Decline (POD) proposed that bottom-up interactions from nutrient loading and contaminant inputs may be contributing to the profound decline in fisheries (Werner et al. 2008). Organisms lower in the food web are highly sensitive to many of the contaminants that are prominent in the Delta; therefore these chemicals may affect the availability of prey for native fish species (Werner et al. 2008). Water and sediment quality monitoring in the Delta and its tributaries has frequently resulted in toxicity to invertebrate species (e.g., Kuivila and Foe 1995, Werner et al. 2008, Weston et al. 2010). The Sacramento River, and upper and lower Delta have been listed as impaired due to "unknown toxicity" (2006 Clean Water Act, Section 303(d)). In addition, Delta waters and its tributaries are listed for ammonia, heavy metals and pesticides, among other pollutants. Results from studies on the interactions of pyrethroid pesticides in mixtures are variable in the literature, and especially few studies have investigated the interactions of pyrethroids with other aquatic contaminants, or the sublethal effects of mixtures. Because protective limits for pesticides and other chemicals are determined based on standard toxicity tests that analyze contaminants individually for their lethal effects, chemical interactions are not well accounted for in these figures. The toxicity of chemicals in mixtures could have major ecological consequences at concentrations below the current water quality protection limits.

This study will provide a better understanding of how common aquatic contaminants act in concert, as they occur in the environment. The toxicity and interaction of binary mixtures are analyzed to determine whether chemicals' known mechanisms of action accurately predict their toxicity when mixed together, and whether the mixtures act the same on a sublethal level. The characterization of physiological mechanisms of interaction will help to assign appropriate models for predicting toxicity in more complex mixtures (i.e. ternary, quaternary, etc.), as is necessary for the protection of natural waterways and their beneficial uses.

2.2 Models of Mixture Response

Toxicants in mixtures can be additive, antagonistic or synergistic. To test for interactions in a given mixture, various analytical approaches can be used including Generalized Linear Model statistics (as used in this study). Significant mixture interactions represent either antagonistic or synergistic effects, distinguishable by the sign of the interaction parameter estimate. No significant interaction indicates that the chemical effects are concentration additive (follow the model of CA), while significant antagonistic interactions could represent additive toxicity by IA, less than additive toxicity (but the mixture still more toxic that the component chemicals

individually), or an antagonistic response such that the mixture of chemicals is less additive than the component chemicals individually (Figure 2).

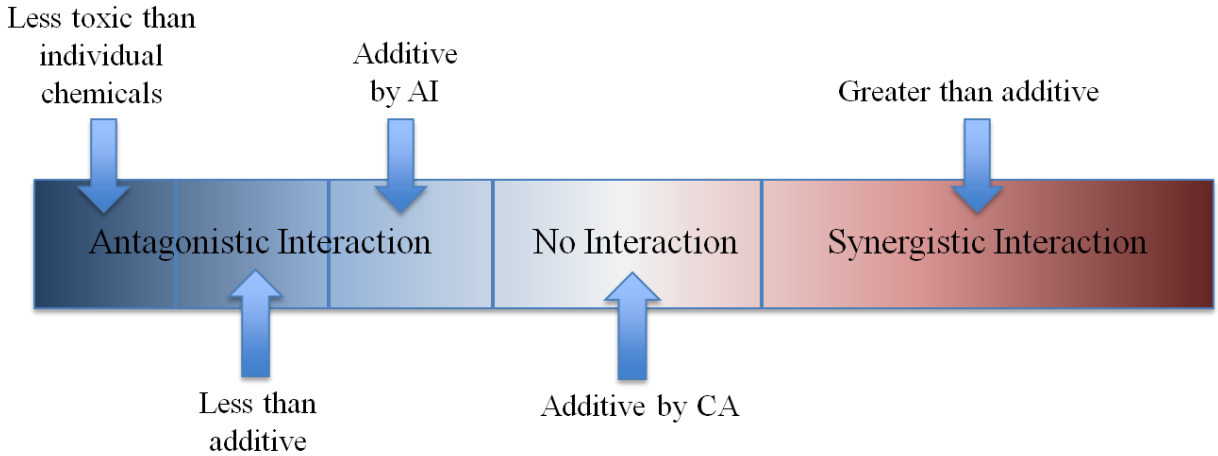


Figure 2. Interactions and additive effects delineated. Interactions defined within the shaded frame are based on statistical analyses that test for interactions, whereas effects designated with arrows are qualitatively interpreted by comparing mixture data to models of CA and IA.

The two widely employed models of additive toxicity are used to characterize mixtures as either with the same mechanisms of toxic action (CA) (Loewe and Muischnek 1926, Altenburger et al. 2000) or with different mechanisms of action (IA) (Bliss 1939, Backhaus et al. 2000). Theoretically, the fit of one model over another indicates that the chemicals target either similar or different biological sites. Because most toxicants act and compete on multiple target organ and tissue sites within an organism, it is possible that chemicals even from the same class may not be completely additive and chemicals with different mechanisms of action may act more additive than expected. This study compares binary mixtures of common aquatic contaminants against both the model of Concentration Addition (CA) and the model of Independent Action (IA). The model of CA calculates the concentration required to elicit a given response based on the proportional contribution from each chemical (p_i) in the mixture and their individual effects (ECx_i). The model of Independent Action estimates the effect expected in a mixture based on the product of the individual proportions of non-response ($1 - E(c_i)$) from each chemical. Models are provided below in Equations 1 (CA) and 2 (IA).

$$ECx_{mix} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right) \qquad \text{(Eq. 1)}$$

$$E(c_{Mix}) = 1 - \prod_{i=1}^{n} [1 - E(c_i)] \qquad \text{(Eq. 2)}$$

2.3 Mixture Experiments

Compounds selected for this study are both pervasive in the environment and of critical concern regarding their toxicity to aquatic organisms. Pesticides include bifenthrin, permethrin, cyfluthrin, lambda-cyhalothrin and chlorpyrifos, all of which are abundantly used and considered "high risk" chemicals (CEPA 2009, Trimble 2009). In this study, emphasis is placed on the analysis of mixtures containing bifenthrin, due to its high toxicity and prevalence in the environment (Weston 2010, Amweg 2006, CEDEN Database 1993-2008). Copper and ammonia are also included in this study because they commonly occur at toxic concentrations in ambient water samples from the Sacramento/San Joaquin Region (Ballard 2009, CEDEN Database 1993-2008).

2.3.1 Pyrethroid and contaminant mixtures – acute, chronic and sublethal endpoints

Five binary mixture experiments including combinations of bifenthrin, permethrin, cyfluthrin, chlorpyrifos, copper and ammonia were conducted (Table 2). Exposure treatments for all toxicants fell within the range of concentrations found in the Delta and its tributaries (CEDEN Database 1989-2010). Endpoints tested included 4-day and 10-day survival, growth and swimming behavior.

Table 2. Acute, chronic and sublethal mixture experiments and test initiation dates

Start Date	Contaminant 1	Contaminant 2
11-12-2010	Bifenthrin	Chlorpyrifos
12-13-2010	Bifenthrin	Ammonia
05-10-2011	Bifenthrin	Copper
05-27-2011	Bifenthrin	Permethrin
08-08-2011	Permethrin	Cyfluthrin

2.3.2 Additional acute toxicity testing of pyrethroid mixtures

Table 3 shows the additional four binary mixture experiments containing type I and II pyrethroids that were conducted to evaluate the toxic effects and potential interactions within this pesticide class. The type I pyrethroids, bifenthrin and permethrin, and the type II pyrethroids, cyfluthrin and lambda-cyhalothrin were tested in all possible combinations within this study (Tables 2 and 3). Mortality was analyzed for this set of mixtures after four days of exposure.

Table 3. Acute mixture experiments and test initiation dates

Start Date	Contaminant 1	Contaminant 2
06-21-2011	Bifenthrin	Cyfluthrin
09-12-2011	Cyfluthrin	Lambda-Cyhalothrin
09-16-2011	Permethrin	Lambda-Cyhalothrin
10-18-2011	Bifenthrin	Lambda-Cyhalothrin

3. MATERIALS AND METHODS

3.1 Experimental Design

Treatment concentrations were designed based on a modified dilution such that concentrations were more equally distributed across treatment levels. Individual chemical responses were tested within each mixture experiment using seven chemical concentrations selected around previously determined lethal concentrations. In this study, a toxic unit (TU) was defined as the concentration of chemical tested in solution that causes 50% response (mortality) after four days of exposure. Mixture treatments contained equipotent levels of each contaminant, and a three by three "partial factorial" encompassing non-equipotent mixture concentrations (Figure 2).

TU	Chemical 1							
Chemical 2	0	1/8	1/4	1/2	3/4	1	1.5	2
1/8	1/4							
1/4		1/2	3/4	1				
1/2		3/4	1	5/4				
3/4		1	5/4	1.5				
1					2			
1.5								
2								

Figure 3. Experimental design of binary mixtures. Values shown represent toxic units and are hypothetical for mixture treatments. Shading distinguishes treatment groups (dark shade = individual chemical exposures, white = equipotent mixtures, light shade = non-equipotent mixtures).

3.2 Toxicity Testing Methods

3.2.1 Mixture Exposures

University of California, Davis (UCD) Aquatic Health Program (AHP) toxicity testing methods were based on protocols developed by USEPA (2000, 2002) and UCD AHP (UCD AHP, 2009). Chronic 10-day water column toxicity testing for *Hyalella azteca* were modified from sediment protocols outlined in *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates* (USEPA, 2000) and based on protocols described in the Quality Assurance Management Plan for the State of California's Surface Water

Ambient Monitoring Program (California State Water Resources Control Board, http://www.waterboards.ca.gov/water_issues/programs/swamp/quamp.shtml#appendixf).

Pesticides were purchased from Sigma-Aldrich Company LLC and were used within three months of purchase. Control (matrix) water was prepared according to USEPA moderately hard guidelines (EPA/600/R-99/064) and used for all exposures. Stock solutions were prepared in methanol (vehicle control) and all treatments were spiked with a combination of stock and methanol as needed to attain 0.01% vehicle control.

Amphipods were purchased from Aquatic Research Organisms (New Hampshire, USA). All batches were acclimated for three to four days and exposures initiated with 10-14 day old *H. azteca*. Each 250 ml treatment beaker contained 100 ml of treatment water with an approximately 2.5 x 2.5 cm square piece of nitex screen (as substrate). Ten amphipods were tested per beaker, with five replicate beakers per treatment. The test was kept within two degrees of 23°C, with a 16:8-h light:dark photoperiod. Beakers were relocated by the investigator daily (haphazardly), to avoid spatial effects on treatments, and 75% of the treatment water was renewed on days 2, 4, 6 and 8 after which amphipods were fed 1 mL of YCT as a food source (a mixture of yeast, alfalfa and trout chow). Acute exposures lasted for 4 days and chronic exposures, 10 days.

3.2.2 Swimming Behavior

Individual amphipods were videotaped at the end of each 10-day exposure for the analysis of chemical and mixture effects on swimming behavior. Surviving organisms were transferred (along with their own treatment water) via pipette into a five-welled plate, placed on a light-board and recorded using a Panasonic black and white CCTV camera (12V DC) in MPEG-2 format at 30 frames/sec. Due to the substantial amount of time necessary to record swimming performance, non-equipotent treatments were omitted from swimming analysis in three of the five experiments tested. These tests included bifenthrin x ammonia, bifenthrin x permethrin and permethrin x cyfluthrin. Individual animal movement was tracked by the method of dynamic subtraction using Ethovision XT (version 6.1) software (Noldus Information Technology; Netherlands). Organisms were analyzed for the total distance traveled over a 90 second recording period (mean velocity).

3.2.3 Weight

Surviving amphipods were weighed at the end of each 10-day exposure to test for chemical and mixture effects on growth. A subsample of amphipods were weighed on day 0 (test set-up) to confirm growth across treatments. At test termination (day 10), amphipods were pooled per replicate beaker, transferred to an aluminum weigh vessel (~1.0 grams) and desiccated at 80°C overnight. Dry tare weights for individual vessels were collected prior to test termination. Total dry weight was measured the day following test termination along with a subsample of empty weigh vessels to determine the temporal variability in tare measurements. Mean dry weight per amphipod was calculated using the tare-subtracted total weight divided by the number of *H.*

azteca per vessel. Due to the limited scale precision, only vessels containing five or more amphipods were included in the analyses.

3.3 Statistical analyses

Lethal concentrations were analyzed using CETIS (version 1.7) statistical software. Linear interpolation methods were used to determine LC50 concentrations for individual chemicals and their mixture. Four and ten day mixture toxicity data were also analyzed for interactions using generalized linear models (GLM) with a quasibinomial distribution. Sub-lethal endpoints were also tested for interactions using GLM methods with either normal or lognormal distribution functions (based on the best-fit distribution for each dataset). All GLMs were analyzed using the program R (version 2.1). Mortality data were also qualitatively compared to two theoretical models of additivity, CA and IA (equations 1 and 2). Model curves were generated using a simple least squares fit to individual concentration-response curves for either chemical.

3.4 Analytical Chemistry

Samples for organic chemical analysis were preserved by the addition of 10 ml/L dichloromethylene immediately after treatment waters were prepared (day 0) and stored at 3°C (±3°C). Samples were submitted to the California Department of Fish and Game Water Pollution Control Laboratory (Rancho Cordova, CA) for organic chemical analysis. Pyrethroids (bifenthrin, cyfluthrin, lambda-cyhalothrin and cis/trans permethrin) and/or organophosphate pesticides (chlorpyrifos) were measured by gas chromatography (Agilent 6890 plus; Agilent Technologies Inc., Santa Clara, CA) with dual columns (DB5 and DB7) and dual flame photometric detectors in phosphorous mode (OP pesticides), or dual micro-electron capture detectors (pyrethroid pesticides). Pyrethroids were confirmed using GC-MS or GC-MSMS.

Water quality of control water and all treatments was measured upon test initiation, at each test renewal and at test termination. Both initial and final treatment water chemistry was recorded, including pH, specific conductance (SC), electrical conductivity (EC), dissolved oxygen (DO), and temperature. DO was measured using a YSI model 58 meter, SC/EC were measured using YSI model 30/10 FT and pH was measured with a Beckman Coulter IP67 pH meter. Meters were calibrated according to the manufacturer's specifications prior to any measurements and checked for instrument drift after every 20 samples. Ammonia-nitrogen, alkalinity and hardness were measured in control water for each experiment. Ammonia-nitrogen was tested using a Hach DR-890 portable colorimeter and Hach AmVer Ammonia Test'N Tube Reagent Set (low range). Alkalinity and hardness were analyzed using titrimetric methods.

4. RESULTS

4.1 Quality Assurance/Quality Control

Quality Assurance/Quality Control (QA/QC) measures were included in this project to assess the reliability of the data collected. These QA/QC procedures included test acceptability criteria and positive control tests (i.e., reference toxicant tests). The components of these QA/QC measures are outlined below. All data including toxicity results and water quality measurements are appended to this report.

4.1.1 Test Acceptability Criteria

Test acceptability criteria for the chronic *H. azteca* 10-day and acute 96-hr water column toxicity tests required 90% or greater control survival. To evaluate whether organism sensitivity was consistent throughout the project period, positive control reference toxicant tests were performed monthly using sodium chloride (NaCl) as the toxicant.

4.1.2 Reference Toxicant Tests

Reference toxicant (RT) tests were conducted to ascertain whether organism responses fell within the acceptable range as dictated by USEPA. The LC_{50} was plotted to determine whether it fell within the 95% confidence interval of the running mean. If an effect concentration (LC_{50}) was outside of the 95% confidence interval, test organism sensitivity was considered atypical and results of tests conducted during the month of reference toxicant outliers could be considered suspect.

RT control survival was greater than 93% throughout the study period. It should be noted that *H. azteca* survival in the months of May and June, 2011, fell below the 95% confidence interval, with average survival of 95% and 93%, respectively. Although these data points are out of range, survival for these tests met the minimum requirements for test acceptability. *H. azteca* survival in RT tests is fairly constant, and these data points fell out of range due to a large, precise data set rather than being indicative of a trend towards greater sensitivity. Therefore, it is the authors' opinion that *H. azteca* performance fell within acceptable limits during the project period.

4.1.3 H. azteca

Average control survival throughout the study period was 98%. Vehicle control survival averaged 99% while non-vehicle control was slightly lower at an average of 96%. One 10-day experiment initiated on May 10, 2011 experienced 87% survival in the non-vehicle control. In the same test, the vehicle control remained at 98% survival, and the LC50 concentration for bifenthrin was in close range (if not higher) relative to previous experiments, therefore we do not believe that *H. azteca* performance was compromised.

4.1.4 Completeness

UCD AHP strives for a minimum 90% completeness of work performed in accordance with SWAMP guidelines. All tests met test acceptability criteria.

Nine tests were conducted for all mixture combinations during the project period and all met the required test acceptability criteria. Completeness for the toxicity testing portion of this project is 100%. For all experiments in this study, one or more treatment samples from each chemical were submitted for chemical analysis. Ammonia concentrations were measured in-lab. Copper was not submitted for chemical analysis for the experiment initiated on May 10, 2011 due to an oversight by the author. As a result, chemical analyses conducted for this study are 94% complete.

Swimming analysis was conducted for *H. azteca* in both mixture and individual treatments at test termination for each chronic 10-day experiment. All experiments were successfully analyzed for swimming behavior. Due to the substantial amount of time necessary to record swimming performance, however, non-equipotent treatments were not analyzed in three of the five experiments tested. These tests included bifenthrin x ammonia, bifenthrin x permethrin and permethrin x cyfluthrin. Within the framework of contracts 09-093-150 and 10-067-150 non-equipotent treatments were not required, therefore completeness for the *H. azteca* swimming analysis portion of this project is 100%.

Dry weight of *H. azteca* with in each beaker was measured in all surviving treatments from chronic exposures and mean individual weight analyzed. Completeness for the *H. azteca* dry weight (growth) portion of this project is 100%.

4.1.5 Deviations

One deviation occurred over the project period. This deviation occurred on December 15, 2010 upon renewal of the bifenthrin x ammonia mixture study initiated on December 13, 2010. Due to a miscalculation, treatments were renewed with a concentration of bifenthrin approximately 1.25x the desired concentration. As a result (upon realization), treatments containing bifenthrin were renewed a second time. The second renewal occurred within a period of ~6 hours from the initial renewal. Because the resulting bifenthrin LC50 from this test was low relative to other tests conducted, it is possible that the second renewal could have had a significant effect on toxicity of those treatments, and thus this data should be interpreted with caution.

4.2 Test Water Parameters and Quality

Water quality measurements of all treatments in this study were within the physiologically optimal ranges of *H. azteca*. Temperature measures fell below optimal ranges in multiple samples however the drop in temperature is not representative of exposure temperatures, but rather occurred due to samples sitting in ambient temperatures prior to measuring, while tests were initiated or renewed. Exposure temperatures were continuously monitored throughout the experimental period and remained within 2° of 23°C at all times. We are confident that no

aspects of water quality contributed to the toxicity observed in this study. Maximum observed concentrations of total and unionized ammonia nitrogen were far below toxic levels in all treatments (except those spiked with ammonia). Among all treatment samples, specific conductivity measurements ranged from 287 - 736 uS/cm, temperatures ranged from 14.4 – 25.1 °C, pH ranged from 3.5 – 9.8, and dissolved oxygen levels ranged from 3.5 – 9.8 mg/L. Control water chemistry resulted in a hardness range of 98 – 116 mg/L as $CaCO_3$, alkalinity range of 50 – 64 mg/L as $CaCO_3$, and a total ammonia nitrogen range of 0.00 - 0.05 mg/L.

4.3 Mixture Responses and Interactions

Results from each mixture experiment varied depending on the endpoint measured and duration of chemical exposure. In three of the pesticide mixtures, responses differed between four-day and ten-day survival results. Four-day exposures of bifenthrin x chlorpyrifos, bifenthrin x cyfluthrin and permethrin x cyfluthrin resulted in significant antagonistic interactions, indicating that these mixtures were additive by the model of IA or were less than additive, while there were no significant interactions among these mixtures at the ten-day endpoint. Further, the interactions detected with GLM statistics were not visible when comparing the toxicity of equipotent treatments with models of mixture response, as all pesticide mixtures appeared to best fit the model of CA. GLM results and model comparisons for the remainder of the mixtures tested in this study concurred. Bifenthrin x ammonia and bifenthrin x copper resulted in significant antagonistic interactions and the mixtures most closely followed the model of IA or were less than additive by either model. Bifenthrin x permethrin, bifenthrin x lambda-cyhalothrin, permethrin x lambda-cyhalothrin and cyfluthrin x lambda-cyhalothrin did not interact significantly at either the four or 10-day mortality endpoint and most closely followed the model of CA.

A limited number of samples were submitted for chemical analysis to confirm measured treatment concentrations in each experiment. As a result, and due to the variability across treatments, the extrapolation of nominal to detected concentrations did not seem appropriate for this study. All results are therefore provided as nominal values. A discussion and analysis of actual to nominal ratios per chemical tested in this study is provided in the Discussion section of this report.

4.3.1 Lethal Concentrations

Lethal concentrations (LC50s) for each chemical alone and in an equipotent binary mixture for all experiments are summarized in Tables 4 and 5. The ratios of chemicals per binary mixture were set to be equipotent based on previously determined LC50s; however due to variability across experiments, toxic responses result in weighted potency from one chemical or the other in many of the mixture results. For example the bifenthrin x ammonia experiment resulted in an LC50 for bifenthrin alone that was less than the LC50 for bifenthrin in the mixture (though not significantly different). This is due to the fact that the potency of bifenthrin in the mixture was greater than that of ammonia. In this case, bifenthrin accounted for approximately all of the toxicity in the mixture, with ammonia only having a minor effect (even slightly antagonistic).

Upper and lower confidence intervals are also shown. Data summaries for all tests conducted under this study are provided in the Appendix.

Table 4. Acute 4-day LC50's for each chemical per experiment and their individual contribution in the mixture.

Mixture Experiment (Chemicals 1 and 2)	Chemical 1 (alone)			Chemical 2 (alone)			Chemical 1 (in mixture)			Chemical 2 (in mixture)		
	LC50	Lower CL	Upper CL	LC50	Lower CL	Upper CL	LC50	Lower CL	Upper CL	LC50	Lower CL	Upper CL
Bifenthrin(1) x Ammonia(2)	**1.95**	1.5	3.58	**22.7**	15.2	30.5	**2.35**	2.18	2.66	**14.69**	13.63	16.63
Bifenthrin(1) x Chlorpyrifos(2)	**5.47**	4.37	7.47	**114.4**	105.9	122.2	**3.52**	2.40	4.14	**88.0**	60.0	103.5
Bifenthrin(1) x Copper(2)	**7.36**	6.26	8.71	**554.7**	441.2	745.3	**3.67**	3.27	4.32	**550.5**	490.5	648.0
Bifenthrin(1) x Permethrin(2)	**5.58**	4.88	6.92	**55.0**	4.90	10.3	**3.63**	2.89	4.65	**36.30**	28.90	46.50
Permethrin(1) x Cyfluthrin(2)	**48.4**	24.8	69.6	**3.98**	3.29	NA	**46.6**	19.20	50.00	**2.33**	0.96	2.50
Bifenthrin(1) x Cyfluthrin(2)	**6.66**	5.74	7.20	**0.48**	0.39	0.76	**1.63**	1.20	2.67	**0.59**	0.44	0.97
Bifenthrin(1) x λ-Cyhalothrin(2)	**6.42**	5.25	7.67	**5.16**	1.87	5.74	**2.74**	2.38	3.32	**2.97**	2.58	3.60
Permethrin(1) x λ-Cyhalothrin(2)	**69.0**	38.9	82.3	**6.36**	5.33	8.05	**24.6**	16.36	32.36	**3.45**	2.29	4.53
Cyfluthrin(1) x λ-Cyhalothrin(2)	**6.18**	5.34	9.37	**7.21**	6.05	9.48	**2.14**	1.35	3.52	**2.94**	1.86	4.83

Note: Concentrations are shown in ng/L, with the exception of copper as μg/L, and ammonia as mg/L

Table 5. Chronic 10-day LC50's for each chemical per experiment and their individual contribution in the mixture.

Mixture Experiment (Chemicals 1 and 2)	Chemical 1 (alone)			Chemical 2 (alone)			Chemical 1 (in mixture)			Chemical 2 (in mixture)		
	LC50	Lower CL	Upper CL	LC50	Lower CL	Upper CL	LC50	Lower CL	Upper CL	LC50	Lower CL	Upper CL
Bifenthrin(1) x Ammonia(2)	**1.75**	1.46	2.18	**15.69**	10.07	17.02	**2.05**	1.30	2.40	**12.79**	8.12	15.0
Bifenthrin(1) x Chlorpyrifos(2)	**3.49**	2.80	4.05	**84.23**	62.37	101.2	**1.31**	1.03	1.99	**32.68**	25.68	49.85
Bifenthrin(1) x Copper(2)	**6.331**	5.497	7.732	**327.3**	286	387.6	**2.09**	1.78	2.52	**313.7**	2.66	3.78
Bifenthrin(1) x Permethrin(2)	**4.65**	3.46	6.07	**47.24**	42.2	60.5	**2.32**	2.04	2.68	**23.2**	20.39	26.76
Permethrin(1) x Cyfluthrin(2)	**38.6**	24.8	53.0	**3.27**	2.57	4.00	**28**	7.6	38.8	**1.40**	0.38	1.94

Note: Concentrations are shown as ng/L, with the exception of copper as μg/L, and ammonia as mg/L

4.3.2 Acute and Chronic Toxicity - Generalized Linear Model Statistics

Chemicals with the same mechanisms of action are theoretically expected to be concentration additive in toxicity. There were no significant interactions between four of the six pyrethroid pesticide mixtures tested, suggesting an additive response by IA (as expected). Two pyrethroid mixtures, however, permethrin x cyfluthrin and bifenthrin x cyfluthrin, were antagonistic at the 4-day exposure endpoint (Table 6), indicating that they were either additive by the model of IA or less than additive by either model. Bifenthrin and chlorpyrifos were also antagonistic after 4-days exposures. There were no significant interactions among any of the pesticide mixtures at the 10-day exposure endpoint (Table 7). Chronic mortality, therefore, appeared to be consistently additive across all pesticide mixtures, while interactions among pesticides on an acute level varied. Mixtures of bifenthrin with copper or ammonia resulted in antagonistic interactions on both the four and ten day mortality endpoints, indicating additivity by the model of IA or less than additive toxicity by either model.

Among the pesticide mixtures that tested antagonistic, the magnitude of the interaction was small, indicating that the antagonism may be representative of additive toxicity by the model of IA. The fit of the mixture responses to the additive models cannot be determined based on GLM analysis, thus qualitative comparisons of mixture data to the IA model were used to ascertain the function of the interaction (next section, Figures 3, 4, 5, 7 and 8a). Model comparisons also confirmed the concentration addition of toxicity among mixtures with no significant interactions.

Table 6. Generalized Linear Model tests for chemical interactions after acute, 4-day exposures. Low p-values indicate significant interactions, where negative parameter estimates = antagonistic interactions (i.e. less than additive by CA) and positive parameter estimates = synergistic.

Mixture	P-value	Parameter Estimate	Standard Error
Bifenthrin x Chlorpyrifos	0.0173*	-0.001985	0.0008244
Bifenthrin x Ammonia	<0.001**	-0.047387	0.006325
Bifenthrin x Copper	<0.001**	-5.16E-04	9.09E-05
Bifenthrin x Permethrin	0.108	-0.002052	0.00127
Permethrin x Cyfluthrin	0.00594*	-0.006058	0.002169
Bifenthrin x Cyfluthrin	<0.001**	-0.39338	0.09037
Lambda-Cyhalothrin x Cyfluthrin	0.174	0.02874	0.02105
Lambda-Cyhalothrin x Permethrin	0.156	-0.002135	0.001497
Lambda-Cyhalothrin x Bifenthrin	0.219	-0.02914	0.02361

*significant (p = 0.001 - 0.05), **highly significant (p < 0.001)

Table 7. Generalized Linear Model tests for chemical interactions after acute, 4-day exposures. Low p-values indicate significant interactions, where negative parameter estimates = antagonistic interactions (i.e. less than additive by CA) and positive parameter estimates = synergistic.

Mixture	P-value	Parameter Estimate	Standard Error
Bifenthrin x Chlorpyrifos	0.759	0.001426	0.00463
Bifenthrin x Ammonia	<0.001**	-0.061595	0.009738
Bifenthrin x Copper	<0.001**	-0.0007955	0.0002069
Bifenthrin x Permethrin	0.402	-0.002475	0.002942
Permethrin x Cyfluthrin	0.118	-0.005733	0.003649

**highly significant ($p < 0.001$)

4.3.3 Acute and Chronic Toxicity – Theoretical Model Fit

Model comparisons for each mixture experiment are shown in Figures 4 – 10. Only equipotent mixture treatments are included in these comparisons. All mixtures of pyrethroids as well as the mixture of bifenthrin and chlorpyrifos appear additive by the model of CA, while mixtures of bifenthrin with either copper or ammonia appear to follow the model of IA. Dose-dependant responses are visible across a number of the mixture experiments such that treatments appear to be greater than additive (synergistic) at low concentrations but more closely follow one of the two models towards the higher concentrations (Figures 4 and 8). These trends are most likely due to the fact that concentration-response models for the individual chemicals were fit assuming a y-intercept of zero, whereas some tests experienced mortality in the controls and low treatment levels. In these cases, model predictions, which were calculated based on the concentration-response curves of the individual chemicals, underestimated the toxicity at the low concentrations. The additive models (Concentration Addition, "CA" and Independent Action, "IA") compared to empirical mixture data ("Mixture") are shown in Figures 4 – 10.

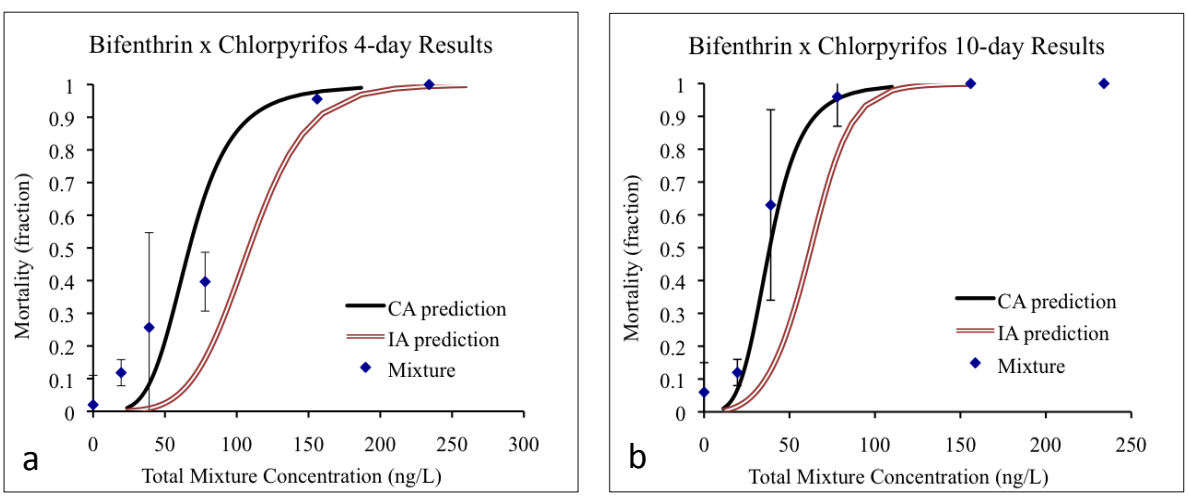


Figure 4a-4b. Acute and chronic toxicity results for the mixture of bifenthrin and chlorpyrifos compared to additive models of mixture toxicity.

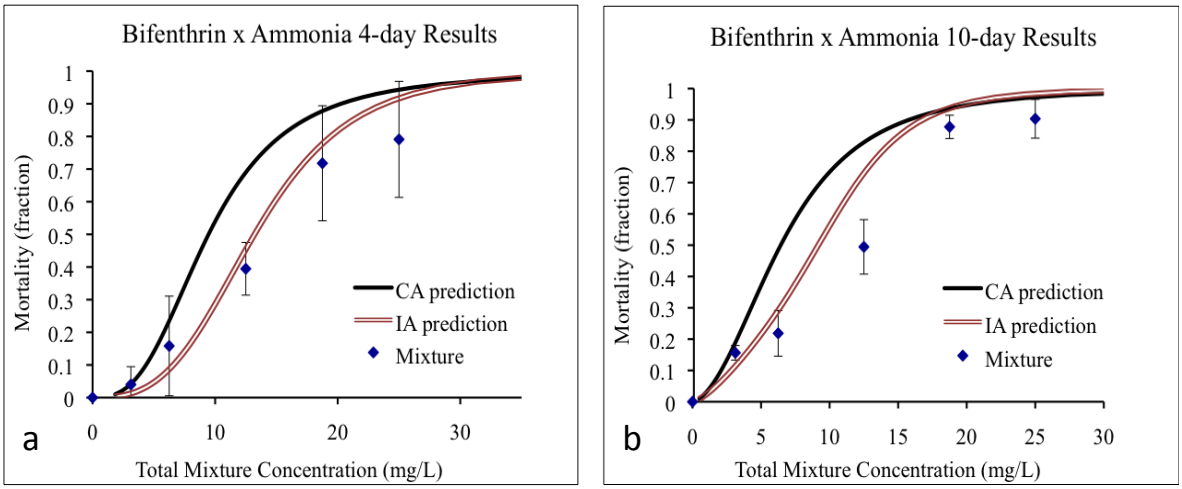


Figure 5a-5b. Acute and chronic toxicity results for the mixture of bifenthrin x ammonia compared to additive models of mixture toxicity.

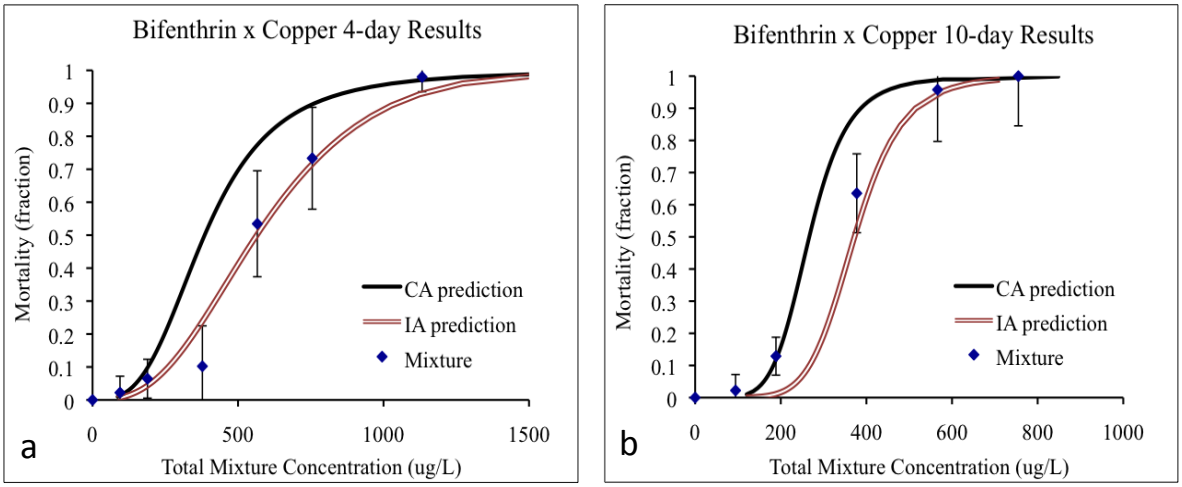


Figure 6a-6b. Acute and chronic toxicity results for the mixture of bifenthrin x copper compared to additive models of mixture toxicity.

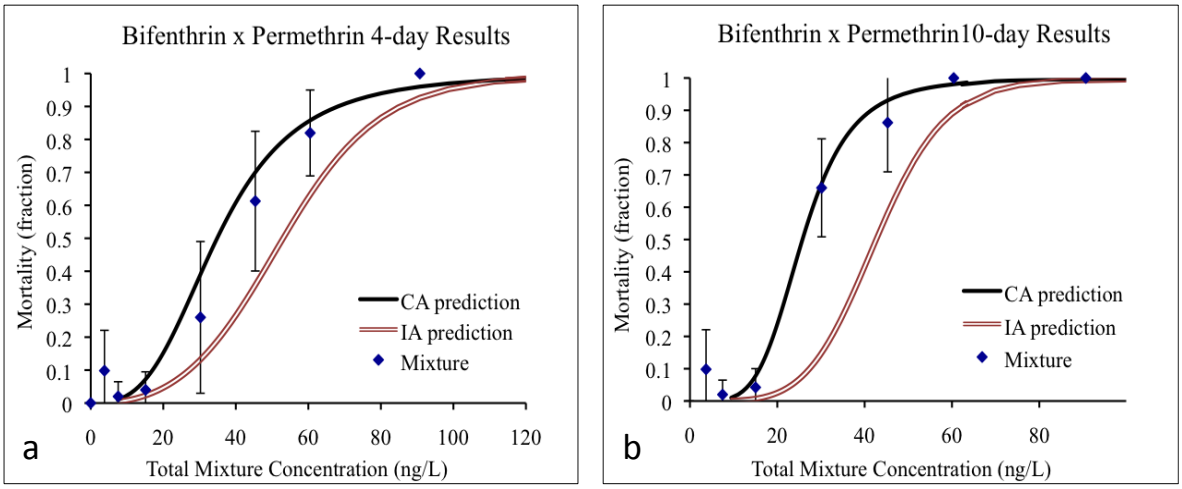


Figure 7a-7b. Acute and chronic toxicity results for the mixture of bifenthrin x permethrin compared to additive models of mixture toxicity.

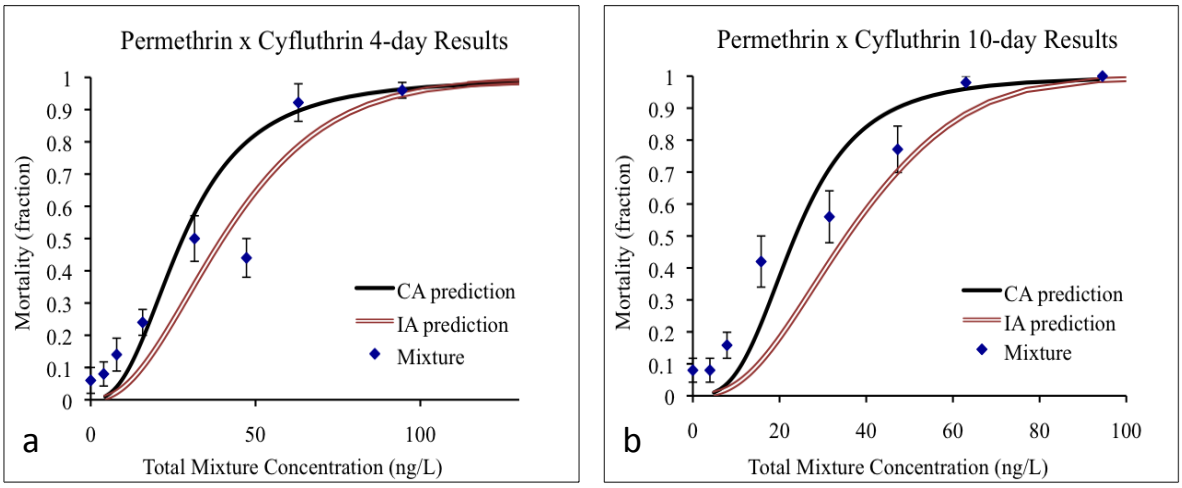


Figure 8a-8b. Acute and chronic toxicity results for the mixture of permethrin x cyfluthrin compared to additive models of mixture toxicity.

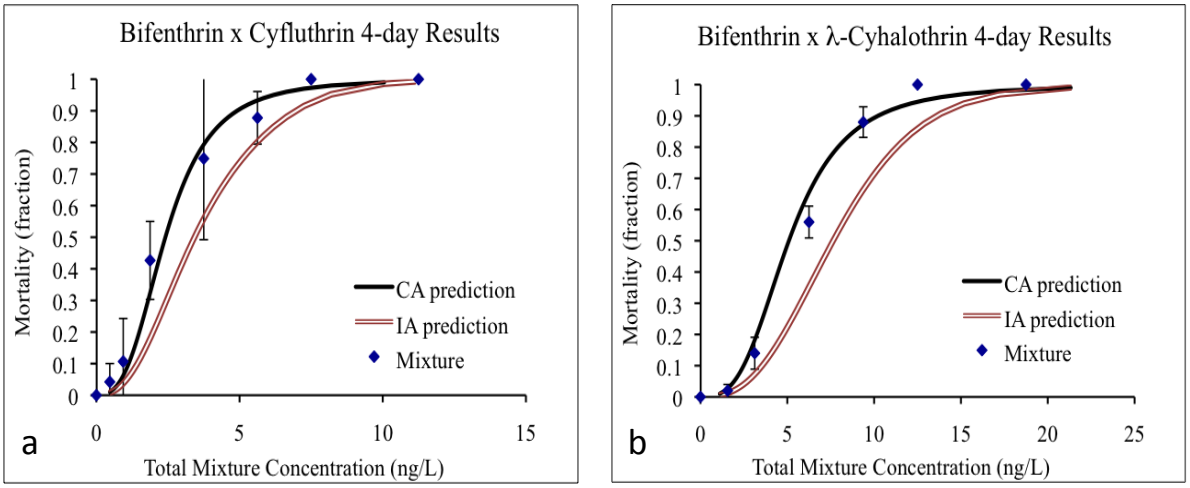


Figure 9a-9b. Acute toxicity results for the mixture of bifenthrin and cyfluthrin, and bifenthrin and lambda-cyhalothrin compared to additive models of mixture toxicity.

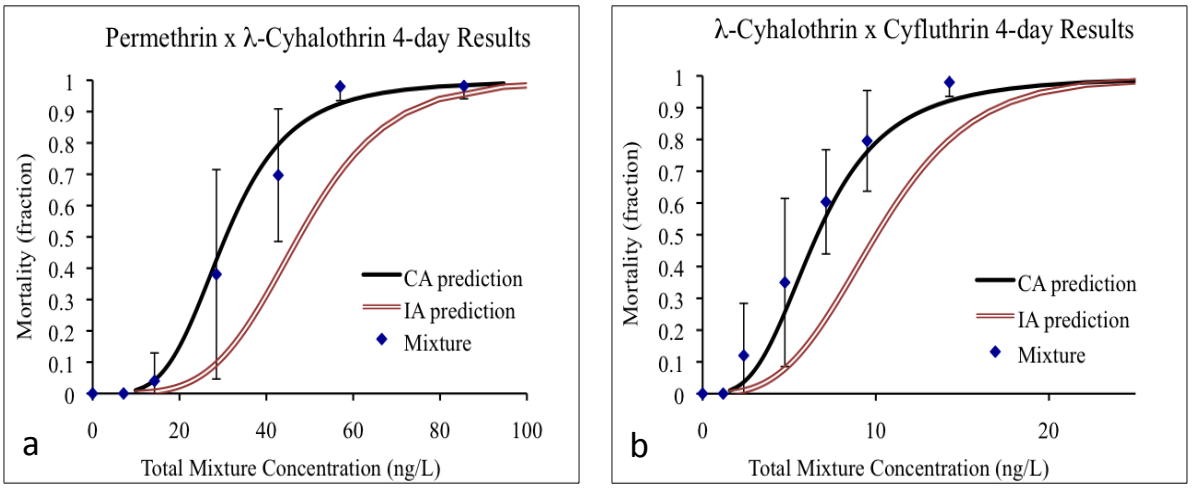


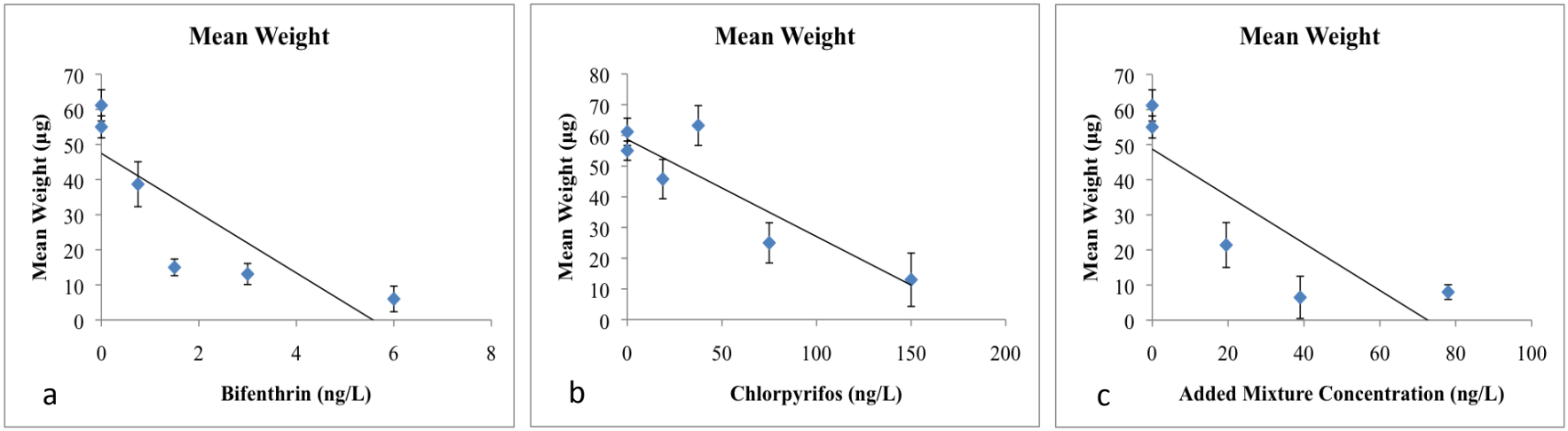
Figure 10a-10b. Acute toxicity results for the mixture of permethrin and lambda-cyhalothrin, and lambda-cyhalothrin and cyfluthrin compared to additive models of mixture toxicity.

4.3.4 Sub-lethal Effects – Generalized Linear Model Statistic

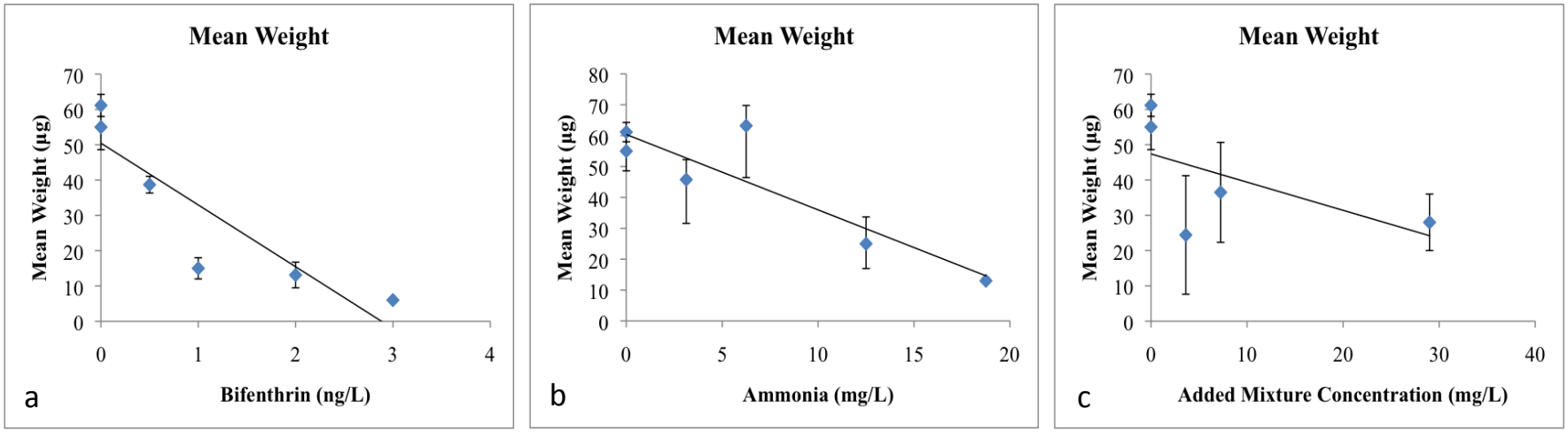
Growth and swimming behavior were affected in a concentration-dependant manner by most chemicals applied in this study, however there were no interactions found between chemicals on these endpoints (all chemicals appeared concentration-additive). Due to the high level of mortality by the end of each chronic exposure, there were few treatments and replicates available for sublethal testing. As a result, sublethal data are generally not as powerful as with tests of mortality, thus mixture effects or interactions may be less evident in terms of statistical significance.

Growth

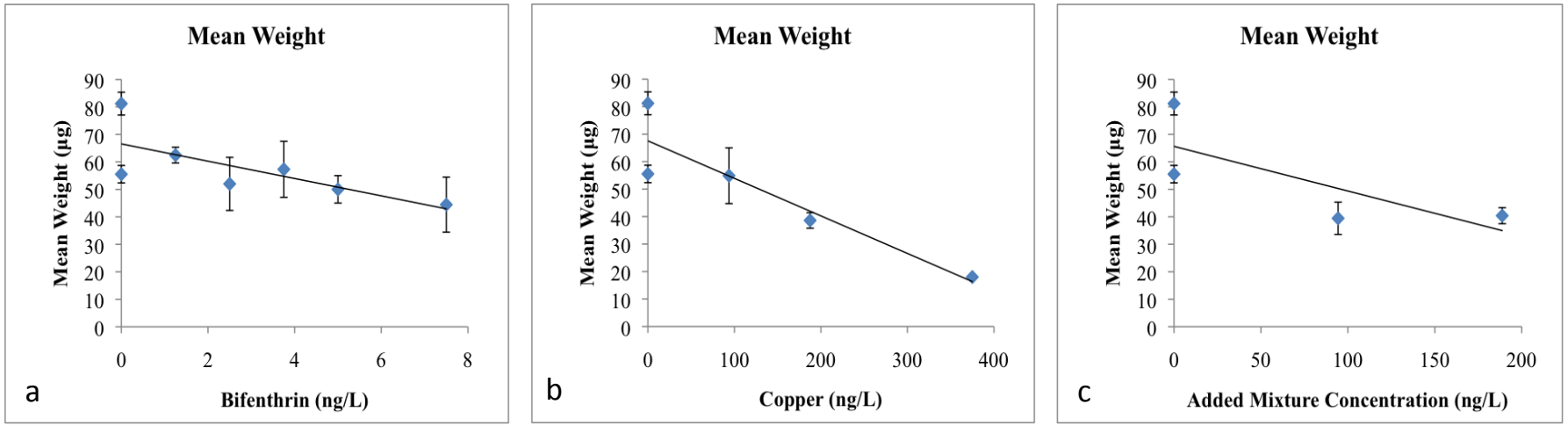
Figures 11 – 15 show concentration-response curves for all growth results. Treatments containing bifenthrin, permethrin, cyfluthrin, lambda-cyhalothrin (all of the pyrethroids), or copper caused a significant reduction in weight as indicated by the significant effect from generalized linear model analyses (Table 8). As mentioned, there were no significant interactions among any of the mixtures, therefore indicating that these mixtures are concentration additive in their effect on growth. Ammonia and chlorpyrifos did not significantly reduce growth, though there is a visible trend in growth in Figure 11b showing a concentration-response to chlorpyrifos. Because the generalized linear model approach includes all of the experimental data including mixture treatments in its analysis, this statistic is a more reliable measure of effect (or lack there of) than the apparent trend in Figure 11b. The authors concluded, then, that chlorpyrifos did not have a significant effect (or had variable effects) on growth in *H. azteca*.



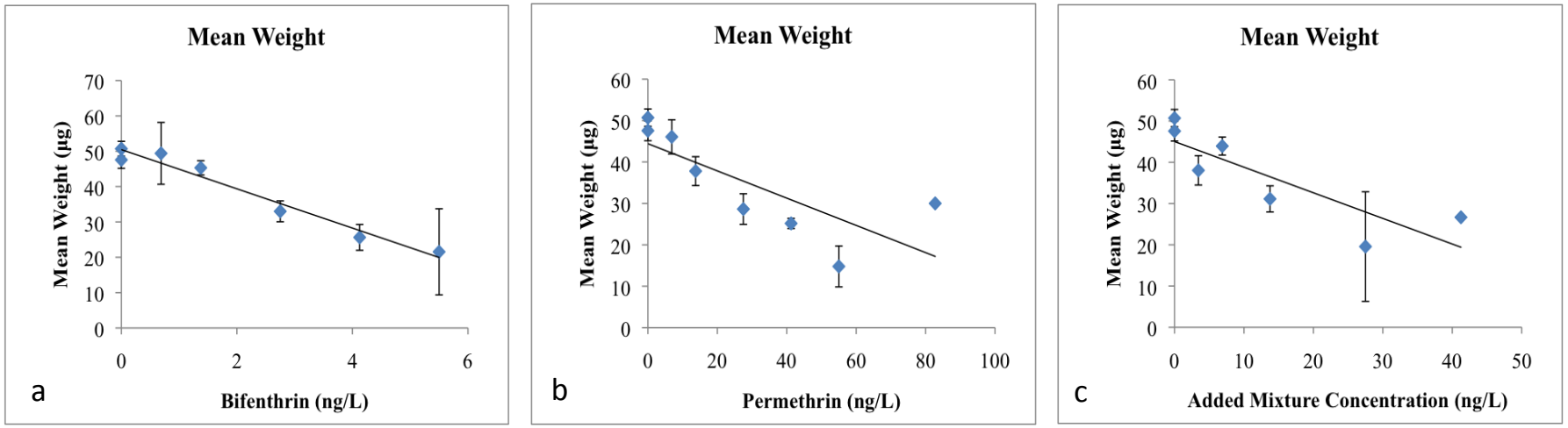
Figures 11a – 11c. Growth response in bifenthrin x chlorpyrifos toxicity experiment showing standard error bars and a linear fitted trend line.



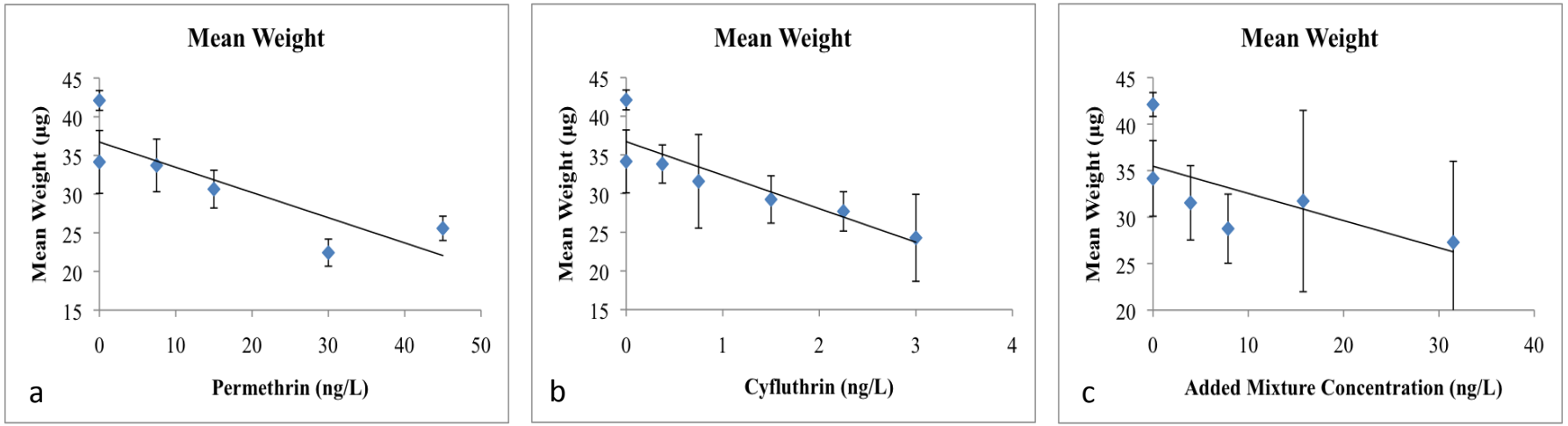
Figures 12a – 12c. Growth response in bifenthrin x ammonia toxicity experiment showing standard error bars and a linear fitted trend line.



Figures 13a – 13c. Growth response in bifenthrin x copper toxicity experiment showing standard error bars and a linear fitted trend line.



Figures 14a – 14c. Growth response in bifenthrin x permethrin toxicity experiment showing standard error bars and a linear fitted trend line.



Figures 15a – 15c. Growth response in permethrin x cyfluthrin toxicity experiment showing standard error bars and a linear fitted trend line.

Table 8. Generalized Linear Model statistics for chemical effects and interactions on growth after chronic, 10-day exposures. Low p-values indicate significant interactions, where negative parameter estimates = antagonistic interactions (i.e. less than additive by CA) and positive parameter estimates = synergistic.

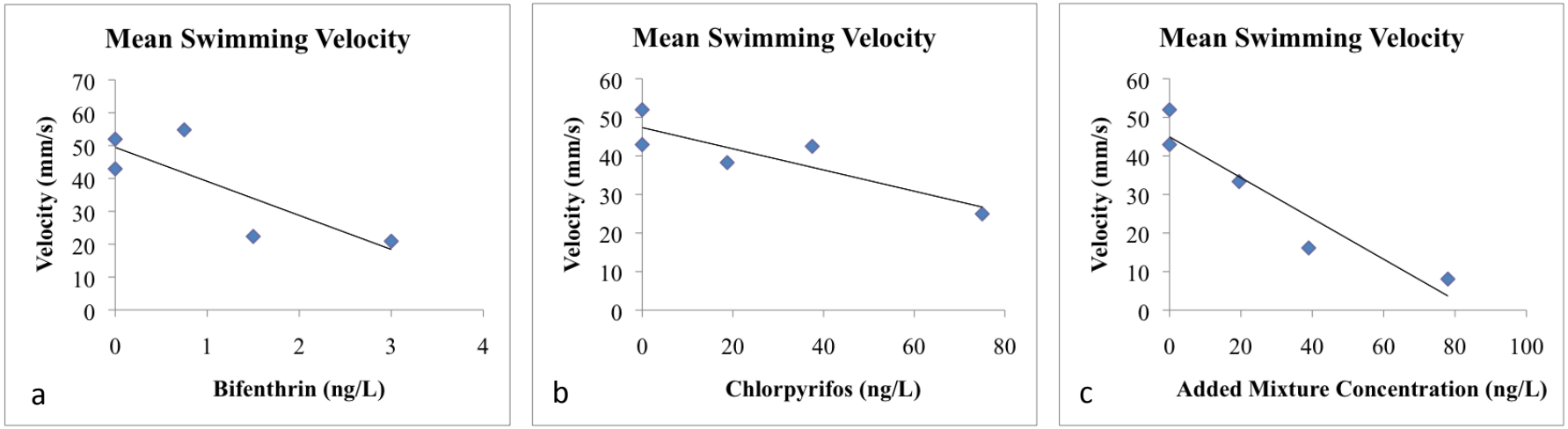
Effect		P-value	Parameter Estimate	Standard Error
Bifenthrin x Chlorpyrifos				
	Bifenthrin	<0.001**	-0.502538	0.099531
	Chlorpyrifos	0.408	-0.001585	0.001894
	Interaction	0.105[a]	-0.01752	0.010562
Bifenthrin x Ammonia				
	Bifenthrin	0.0209*	-0.570481	0.240578
	Ammonia	0.9257	-0.002192	0.023425
	Interaction	Not significant	NA	NA
Bifenthrin x Copper				
	Bifenthrin	0.0029**	-0.0975636	0.0311469
	Copper	<0.001**	-0.0039426	0.0009824
	Interaction	Not significant	NA	NA
Bifenthrin x Permethrin				
	Bifenthrin	<0.001**	-0.13649	0.02564
	Permethrin	<0.001**	-0.0186	0.0028
	Interaction	Not significant	NA	NA
Permethrin x Cyfluthrin				
	Permethrin	<0.001**	-0.013269	0.003461
	Cyfluthrin	0.003736**	-0.131339	0.043733
	Interaction	Not significant	NA	NA

*significant (p = 0.001 - 0.05), **highly significant (p < 0.001)

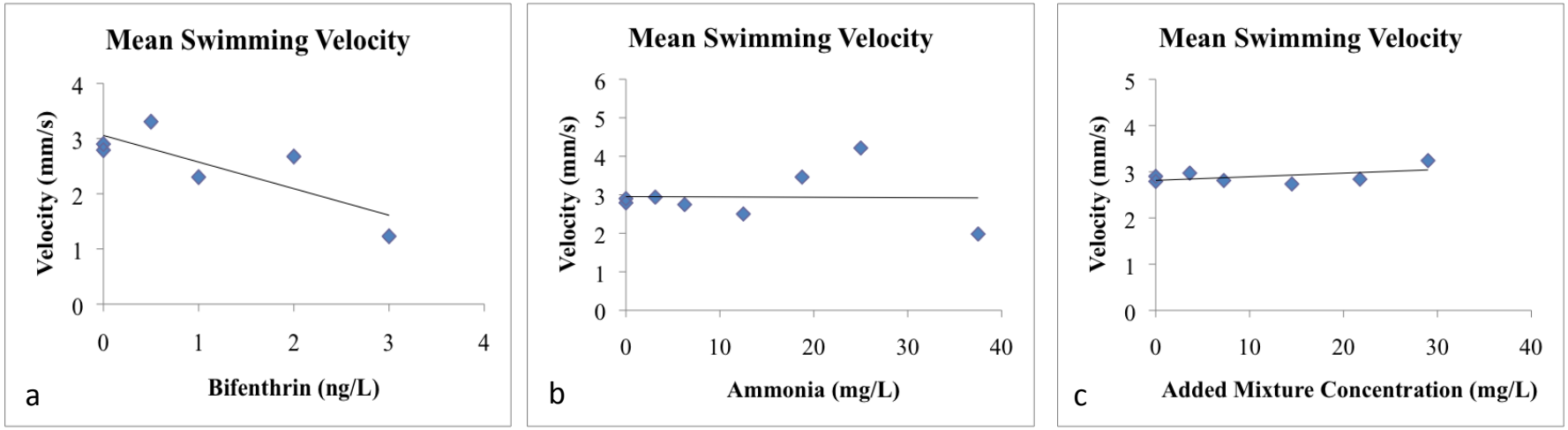
[a]Not significant, however model fit (based on a t-test of Akaike's Information Criteria) was significantly better with an interaction term included, therefore this value is reported. All other interactions were omitted from the models and thus are not shown.

Swimming

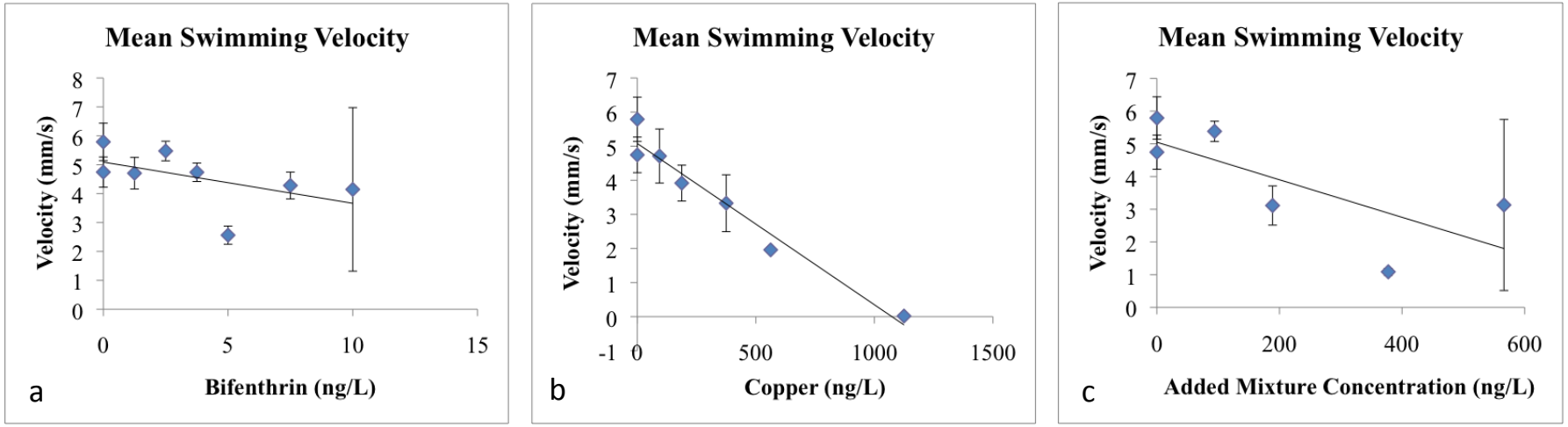
Figures 16 – 20 show concentration-response curves for all swimming results. Effects on swimming velocity were apparent in all chemicals except ammonia (Table 9). There were no significant interactions among any of the mixtures, therefore indicating a concentration additive response on swimming velocity. The mixture experiment containing ammonia and bifenthrin did not produce significant effects on swimming from either chemical, though bifenthrin resulted in significant effects across three other experiments. Similar results are apparent in the concentration-response curves shown in Figures 16 – 20 below, showing negative correlations of swimming velocity to exposures of all toxicants except ammonia. Mixtures within each experiment are also concentration-responsive as shown in the figures below.



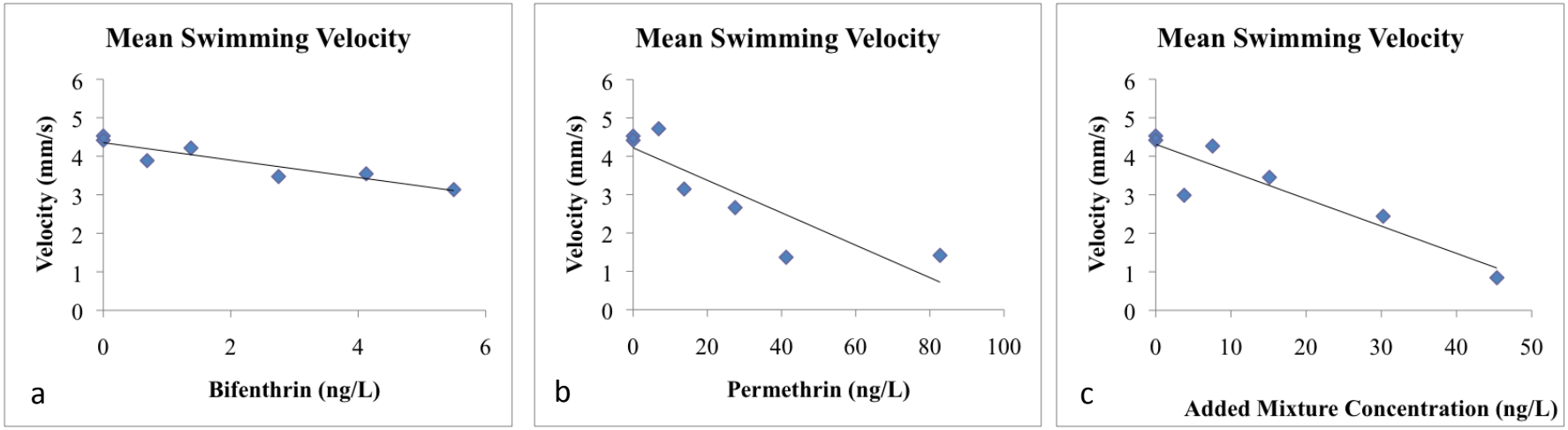
Figures 16a – 16c. Swimming response in bifenthrin x chlorpyrifos toxicity experiment showing standard error bars and a linear fitted trend line.



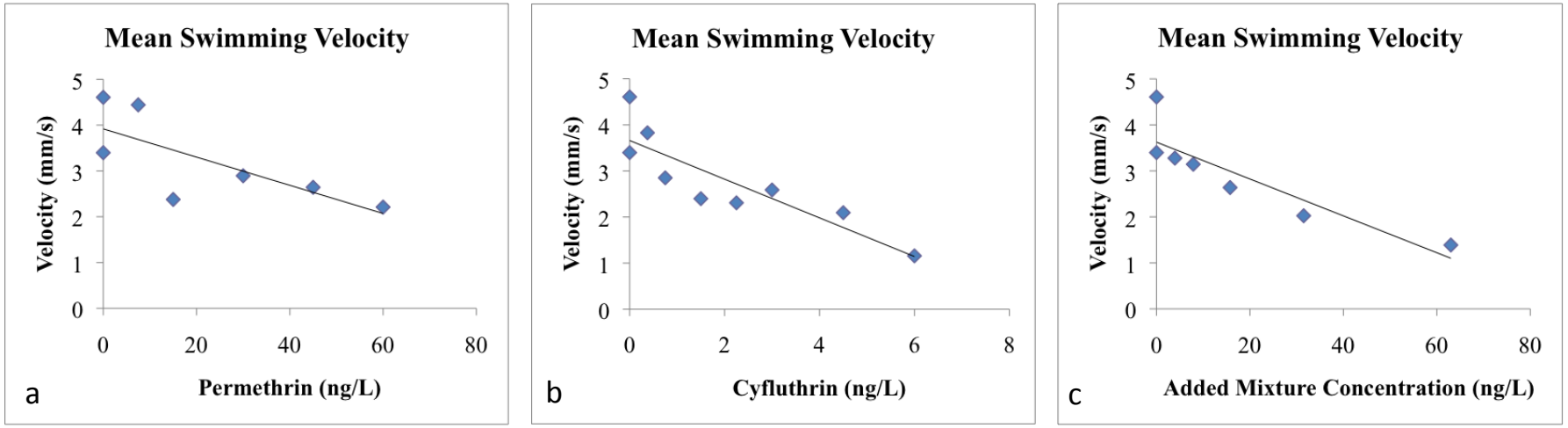
Figures 17a – 17c. Swimming response in bifenthrin x ammonia toxicity experiment showing standard error bars and a linear fitted trend line.



Figures 18a – 18c. Swimming response in bifenthrin x copper toxicity experiment showing standard error bars and a linear fitted trend line.



Figures 19a – 19c. Swimming response in bifenthrin x permethrin toxicity experiment showing standard error bars and a linear fitted trend line.



Figures 20a – 20c. Swimming response in permethrin x cyfluthrin toxicity experiment showing standard error bars and a linear fitted trend line.

Table 9. Generalized Linear Model statistics for chemical effects and interactions on swimming velocity after chronic, 10-day exposures. Low p-values indicate significant interactions, where negative parameter estimates = antagonistic interactions (i.e. less than additive by CA) and positive parameter estimates = synergistic.

Effect		P-value	Parameter Estimate	Standard Error
Bifenthrin x Chlorpyrifos				
	Bifenthrin	<0.001**	-0.33922	0.06392
	Chlorpyrifos	<0.001**	-0.0109	0.00221
	Interaction	Not significant	NA	NA
Bifenthrin x Ammonia				
	Bifenthrin	0.191	-0.14344	0.1087
	Ammonia	0.219	0.01546	0.01245
	Interaction	Not significant	NA	NA
Bifenthrin x Copper				
	Bifenthrin	0.0106*	-0.1890074	0.0722282
	Copper	<0.001**	-0.0045854	0.0007562
	Interaction	Not significant	NA	NA
Bifenthrin x Permethrin				
	Bifenthrin	0.00198*	-0.071454	0.02216
	Permethrin	<0.001**	-0.017029	0.002911
	Interaction	Not significant	NA	NA
Permethrin x Cyfluthrin				
	Permethrin	<0.001**	-0.01247	0.00214
	Cyfluthrin	<0.001**	-0.19873	0.03416
	Interaction	Not significant	NA	NA

*significant (p = 0.001 - 0.05), **highly significant (p < 0.001)

Note: Interactions are omitted from the generalized linear models where they are not significant and thus are not shown.

5. DISCUSSION

5.1 Mixture responses

5.1.1 Analyses of mixture effects and interactions

Generalized linear model analyses revealed significant antagonisms between three of the pesticide mixtures at the 4-day mortality endpoint, but not at the 10-day endpoint. Back calculations on the generalized linear models (described below) indicate that the antagonistic effects in these mixtures would reduce their combined toxicity by between 0 and 20%, compared to the toxicity they would incur if the mixture was additive by IA. Qualitative comparisons of equipotent mixture data to the additive models of CA and IA, on the other hand, point to additivity by the model of CA, indicating that there are no significant interactions within these three mixtures. All other mixture results agreed across GLM statistics and model comparisons, showing that bifenthrin x ammonia and bifenthrin x copper were significantly antagonistic and were less than concentration additive, while bifenthrin x permethrin, bifenthrin x lambda-cyhalothrin, permethrin x lambda-cyhalothrin and cyfluthrin x lambda-cyhalothrin did not interact significantly and most closely followed the model of CA.

In a GLM analysis, the sign and magnitude of an interaction between effects is indicated by the interaction parameter estimate (β_{mix}), representing the slope of the interaction on the odds ratio in the logit model (Eq. 3). Other parameters within the GLM include a y-intercept (β_o) and parameters for each chemical's individual effects (β_1 and β_2). The toxicity of a given mixture can thus be calculated by plugging these parameters as well as hypothetical concentrations for each chemical (X_1 and X_2) into the model equation (Eq. 3). The maximum difference between the toxicity of the three pesticide mixtures with and without their interaction term was between 10 and 20%. For the mixture with the most antagonistic interaction, bifenthrin x cyfluthrin (parameter estimate of -0.39), the interaction of the chemicals resulted in approximately 0 – 20% less toxicity than expected if the chemicals were perfectly additive, depending on the concentrations of the chemicals within the mixture. A mixture of roughly 1 TU of either chemical exhibited the greatest interaction effect, whereas non-equipotent mixtures or mixtures of greater or less potency were less affected by the interaction. The GLM model is shown below, where y (odds) represents the odds ratio of mortality.

$$y\ (\text{odds}) = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_{mix} X_1 X_2 \qquad \text{(Eq. 3)}$$

The antagonistic effects on the 4-day toxicity of bifenthrin x chlorpyrifos, bifenthrin x cyfluthrin and permethrin x cyfluthrin indicate that these mixtures are less than concentration additive. Data were further analyzed by comparison to the models of CA and IA. The most closely fitting model for all three mixtures was the model of CA, suggesting no mixture interactions (contrary

to the GLM result). The contradiction between GLM statistics and model comparisons is likely due the fact that GLM statistics were able to incorporate all mixture treatments within their analyses, while model comparisons only encompassed equipotent mixture treatments, excluding the six non-equipotent mixture treatments tested within each experiment. GLM results are the most reliable analytical tool, not only for their analytical coverage of data, but also because the analysis is statistically grounded. Model comparisons are used in this study only for qualitative and graphical purposes, and to more specifically characterize mixture effects across chemicals on the basis of their mechanism of action. Model comparisons are limited and do not provide real statistical evidence for their best-fit results. However, GLM results and model comparisons for most mixtures tested in this study concurred. Bifenthrin x ammonia and bifenthrin x copper resulted in significant antagonistic interactions and the mixtures most closely followed the model of IA or were less than additive by either model. Bifenthrin x permethrin, bifenthrin x lambda-cyhalothrin, permethrin x lambda-cyhalothrin and cyfluthrin x lambda-cyhalothrin did not interact significantly at either the four or 10-day mortality endpoint and most closely followed the model of CA.

Previous studies have applied one of the two analytical methods used in the current study, but not both, and results have been variable. Brander et al. (2009) tested a mixture of permethrin with cyfluthrin at an environmentally relevant ratio, and demonstrated antagonistic (less than additive) toxicity to *H. azteca* using a multiple logistic regression analysis (similar to GLM), but did not compare results to the additive mixture models of CA and IA. Trimble et al. (2009) tested equipotent mixtures of four pyrethroids, including mixtures of type I and type II forms, and found them to be predominantly additive by the model of CA, but data were not statistically analyzed for interactions. Interestingly, though the results of these studies appear to conflict, both agree with the conclusions reported in the current study. Belden and Lydy (2006) compared the toxicity of equipotent mixtures of chlorpyrifos (an organophosphate pesticide) and esfenvalerate (a pyrethroid pesticide) on midge larvae and fathead minnows to the models of CA and IA and found them to be either concentration additive (CA) or synergistic. No statistical analyses of mixture interactions were conducted. These results do vary from those found in the current experiment, which may be evidence for cross-species differences in mixture responses. Variable results across species are not surprising due to the diversity of mechanisms of action across chemicals and the probability of multiple target sites that could differ between species.

5.1.2 Acute versus chronic responses

Interactions were significant at 4-day but not 10-day exposures for three of the seven pesticide mixtures. This disparity could be a factor of the metabolic or biological effects of the chemicals over time. Different modes of metabolism may be able to deal with chemicals in mixtures over acute periods, while chronic stress loads overwhelm the metabolic response in the long term. Acute and chronic biological responses may also differ. Acute responses are fairly well characterized for organophosphate and pyrethroid pesticides, including neurotoxicity that results in paralysis and death (Vais et al. 2001, Weston et al. 2010, Reiss et al. [in press]). The different

modes of neurotoxic action between bifenthrin and chlorpyrifos, bifenthrin and cyfluthrin, and permethrin and cyfluthrin appear to be less than concentration additive on the nervous system of *H. azteca*. Long-term effects, on the other hand, may involve chronic oxidative stress, necrosis, apoptosis or reductions in crucial biological functions such as immune response, metabolism and respiration (Galloway and Handy 2002, Pena-Llopis et al. 2003, Valanadidis et al. 2006,), which could account for the more additive nature of response. It has been noted by the investigator that the physical appearance of dead *H. azteca* at the four and 10-day endpoints also tend to differ. Four-day moribund amphipods are generally distinguishable from living individuals only by their lack of movement or response, whereas moribund amphipods after ~5-6 days of exposure are generally found in a curled position and with a red-brown pigment. The red-brown color, like that seen in multiple crustaceans, could be a sign of irreversible hemocyanin oxidation due to the degeneration of cells and tissues. Further investigation on the different modes of toxic action at the acute and chronic exposure levels is necessary to understand the difference between these mixture responses. Additional mixture analyses, testing neurotoxic pesticides from different classes across four and 10-day exposures would be important to substantiate this trend.

5.1.3 Nominal and measured concentrations

Due to the limited number of treatment concentrations measured in this study, nominal concentrations were used for all analyses. Specific concentrations per treatment are not necessary for the analyses of mixture interactions because the comparisons/statistics are based on the toxicity of individual chemicals tested within a given experiment. On the other hand, determining effect concentrations as applicable to environmental exposures requires an extrapolation of measured concentrations. A mini analysis was conducted to compare nominal and measured concentrations attained from tests that occurred over the last three years within the Aquatic Health Program (AHP), UC Davis. Spiking accuracies (defined here as the percent measured concentration over nominal concentration) were similar in the current study to those found in previous studies conducted at the AHP lab. Historical analytical results also indicate that spiking accuracy, as a percentage of nominal values, is consistent across concentrations (data not shown). Table 10 compares the average measured to nominal concentration percentage in the current study to those attained from previous experiments. The ammonia percentage is shown as an approximation from historical results. There were no historical records for lambda-cyhalothrin in tests conducted at the AHP lab, therefore only the average spiking accuracy from the current study is provided. A copper percentage is only shown for historical results because copper concentrations were not tested in the current study. Table 10 summarizes the average percent accuracy per toxicant. From these percentages, approximate measured concentrations can be deduced for all chemicals in this study.

Table 10. Percent measured over nominal concentration per test chemical.

Test Chemical	Recovery - Current Study	SE	Recovery - Historical	SE
Bifenthrin	42%	4%	31%	3%
Permethrin	61%	9%	61%	9%
Chlorpyrifos	72%	2%	49%	5%
Cyfluthrin	58%	8%	64%	9%
Ammonia	114%	1%	100%	nd
Lambda-Cyhalothrin	31%	5%	nd	nd
Copper	nd	nd	95%	2%

nd = no data

5.2 Recommendations for Future Studies

Results from this study indicate that binary mixtures of pyrethroid pesticides, chlorpyrifos and copper and are more toxic then their component chemicals individually, while ammonia and bifenthrin are no more toxic as a mixture than each chemical alone. Additive responses varied across chemicals and exposure durations (acute or chronic), however all mixtures were close to additive by either the model of Concentration Addition or Independent Action. Sublethal responses, including reduced growth and swimming velocity, were concentration-additive across all mixtures, including those containing chemicals with different physiological mechanisms of action. Other toxicants occurring in the environment are likely additive in this manner. In addition, previous studies indicate that complex mixtures of many chemicals at trace (no effect) concentrations can add up to cause toxicity (Faust et al. 2003, Altenburger, et al. 2000, Backhaus, et al. 2000). Future research should incorporate additional aquatic contaminants that are commonly found in the environment, as well as more complex mixtures containing multiple contaminants at NOEC or LOEC concentrations to better assess the environmental risk of these water quality impairments.

Other considerations for future work to better understand the ecological risk of contaminant stress include further analysis of the acute versus chronic effects of toxicants, the analysis of additional factors present in field matrix water and cross-species comparisons of contaminant and mixture effects. Results from this study indicate that acute and chronic mixture effects may vary, particularly among combinations of chemicals from different classes with similar mechanisms of action. Additional analysis of the biochemical effects of contaminants on *H. azteca* after acute and chronic exposures would help to elucidate the mechanisms underlying the differences in mixture effects resulting from the current study. In addition, the complex matrix of nutrients and dissolved matter in ambient water needs to be accounted for in the analysis of contaminant toxicity. The experiments in this study, and most toxicity analyses in the literature, were conducted using laboratory control water that is not entirely representative of environmental conditions. Further testing with additional species will also be important in

understanding the environmental risk of chemical mixtures across trophic levels. Cross-species comparisons will be helpful in understanding mixture interactions, as it is possible that responses and interactions are variable among species. Results would be important for the understanding (and modeling) of indirect and direct effects of stressors under environmental conditions on a community and population scale.

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Appendix I

Table A1. Summary of acute and chronic *H. azteca* water column toxicity tests initiated on November 12, 2010 examining the toxicity of bifenthrin x chlorpyrifos mixtures.

Treatment	4-day Survival (%)		10-day Survival (%)	
	Mean	SE	Mean	SE
DIEPAMHR	100	0	100	0
DIEPAMHR + Methanol	98	4	94	9
Bifenthrin 0.75 ng/L (1/8 TU) + Methanol	100	0	100	0
Bifenthrin 1.5 ng/L (1/4 TU) + Methanol	100	0	76	11
Bifenthrin 3 ng/L (1/2 TU) + Methanol	96	5	61	13
Bifenthrin 6 ng/L (1 TU) + Methanol	42	26	4	9
Bifenthrin 9 ng/L (1.5 TU) + Methanol	12	11	0	0
Bifenthrin 12 ng/L (2 TU) + Methanol	15	33	0	0
Bifenthrin 18 ng/L (3 TU) + Methanol	2	4	0	0
Chlorpyrifos 18.75 ng/L (1/8 TU) + Methanol	100	0	100	0
Chlorpyrifos 37.5 ng/L (1/4 TU) + Methanol	100	0	100	0
Chlorpyrifos 75 ng/L (1/2 TU) + Methanol	92	8	58	18
Chlorpyrifos 150 ng/L (1 TU) + Methanol	22	8	4	5
Chlorpyrifos 225 ng/L (1.5 TU) + Methanol	6	9	0	0
Chlorpyrifos 300 ng/L (2 TU) + Methanol	4	5	0	0
Chlorpyrifos 450 ng/L (3 TU) + Methanol	0	0	0	0
Chlorpyrifos 75 ng/L (1/2 TU) + Bifenthrin 0.75 ng/L (1/8 TU) + Methanol	16	11	0	0
Chlorpyrifos 75 ng/L (1/2 TU) + Bifenthrin 1.5 ng/L (1/4 TU) + Methanol	46	28	2	4
Chlorpyrifos 75 ng/L (1/2 TU) + Bifenthrin 3 ng/L (1/2 TU) + Methanol	60	21	4	9
Chlorpyrifos 75 ng/L (1/2 TU) + Bifenthrin 6 ng/L (1 TU) + Methanol	42	35	0	0
Chlorpyrifos 75 ng/L (1/2 TU) + Bifenthrin 9 ng/L (1.5 TU) + Methanol	12	11	0	0
Chlorpyrifos 18.75 ng/L (1/8 TU) + Bifenthrin 0.75 ng/L (1/8 TU) + Methanol	98	4	88	4
Chlorpyrifos 37.5 ng/L (1/4 TU) + Bifenthrin 1.5 ng/L (1/4 TU) + Methanol	74	23	37	29
Chlorpyrifos 150 ng/L (1 TU) + Bifenthrin 6 ng/L (1 TU) + Methanol	4	10	0	0
Chlorpyrifos 225 ng/L (1.5 TU) + Bifenthrin 9 ng/L (1.5 TU) + Methanol	0	0	0	0
Chlorpyrifos 37.5 ng/L (1/4 TU) + Bifenthrin 3 ng/L (1/2 TU) + Methanol	72	42	17	17
Chlorpyrifos 37.5 ng/L (1/4 TU) + Bifenthrin 6 ng/L (1 TU) + Methanol	70	41	12	16
Chlorpyrifos 150 ng/L (1 TU) + Bifenthrin 1.5 ng/L (1/4 TU) + Methanol	14	11	0	0
Chlorpyrifos 150 ng/L (1 TU) + Bifenthrin 3 ng/L (1/2 TU) + Methanol	16	11	0	0

Table A2. Summary of water chemistry measured in a *H. azteca* toxicity test initiated on November 12, 2010 examining the toxicity of bifenthrin x chlorpyrifos mixtures.

Treatment	Laboratory Chemistry						
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH
DIEPAMHR	342	20.3	23.1	7.1	9.0	7.49	7.99
DIEPAMHR + Methanol	343	20.3	23.1	7.1	9.0	7.49	7.99
Bifenthrin 0.75 ng/L (1/8 TU) + Methanol	343	20.3	23.1	7.1	9.0	7.49	7.99
Bifenthrin 1.5 ng/L (1/4 TU) + Methanol	342	20.3	23.1	7.1	9.0	7.49	7.99
Bifenthrin 3 ng/L (1/2 TU) + Methanol	341	20.3	23.0	5.8	9.0	7.49	7.99
Bifenthrin 6 ng/L (1 TU) + Methanol	341	20.3	23.0	5.8	8.9	7.49	7.99
Bifenthrin 9 ng/L (1.5 TU) + Methanol	339	20.3	23.0	5.8	8.9	7.43	7.99
Bifenthrin 12 ng/L (2 TU) + Methanol	339	20.3	23.0	5.8	8.9	7.43	7.99
Bifenthrin 18 ng/L (3 TU) + Methanol	338	20.3	23.0	5.8	8.9	7.43	7.99
Chlorpyrifos 18.75 ng/L (1/8 TU) + Methanol	338	20.7	23.0	5.8	8.9	7.43	8.09
Chlorpyrifos 37.5 ng/L (1/4 TU) + Methanol	337	20.7	23.0	5.8	8.9	7.43	8.09
Chlorpyrifos 75 ng/L (1/2 TU) + Methanol	336	20.7	23.0	5.8	8.9	7.43	8.09
Chlorpyrifos 150 ng/L (1 TU) + Methanol	336	20.7	23.0	5.8	8.9	7.43	8.09
Chlorpyrifos 225 ng/L (1.5 TU) + Methanol	337	20.7	23.0	5.8	8.9	7.43	8.09
Chlorpyrifos 300 ng/L (2 TU) + Methanol	338	20.7	23.0	4.6	8.9	7.43	8.09
Chlorpyrifos 450 ng/L (3 TU) + Methanol	339	20.7	23.0	4.6	8.9	7.43	8.09
Chlorpyrifos 75 ng/L (1/2 TU) + Bifenthrin 0.75 ng/L (1/8 TU) +	340	20.7	23.0	4.6	8.9	7.46	8.09
Chlorpyrifos 75 ng/L (1/2 TU) + Bifenthrin 1.5 ng/L (1/4 TU) +	340	20.7	23.0	4.6	8.9	7.46	8.09
Chlorpyrifos 75 ng/L (1/2 TU) + Bifenthrin 3 ng/L (1/2 TU) + Methanol	339	20.7	23.0	4.6	8.9	7.46	8.09
Chlorpyrifos 75 ng/L (1/2 TU) + Bifenthrin 6 ng/L (1 TU) + Methanol	339	20.3	23.0	4.6	8.9	7.46	8.13
Chlorpyrifos 75 ng/L (1/2 TU) + Bifenthrin 9 ng/L (1.5 TU) + Methanol	340	20.3	23.0	4.6	8.9	7.37	8.13
Chlorpyrifos 18.75 ng/L (1/8 TU) + Bifenthrin 0.75 ng/L (1/8 TU) +	341	20.3	23.0	4.6	8.9	7.37	8.13
Chlorpyrifos 37.5 ng/L (1/4 TU) + Bifenthrin 1.5 ng/L (1/4 TU) +	341	20.3	23.1	4.6	8.9	7.37	8.13
Chlorpyrifos 150 ng/L (1 TU) + Bifenthrin 6 ng/L (1 TU) + Methanol	341	20.3	23.1	4.6	8.9	7.37	8.13
Chlorpyrifos 225 ng/L (1.5 TU) + Bifenthrin 9 ng/L (1.5 TU) + Methanol	340	20.3	23.1	5.6	8.9	7.37	8.13
Chlorpyrifos 37.5 ng/L (1/4 TU) + Bifenthrin 3 ng/L (1/2 TU) +	339	20.3	23.1	5.6	8.9	7.37	8.13
Chlorpyrifos 37.5 ng/L (1/4 TU) + Bifenthrin 6 ng/L (1 TU) + Methanol	339	20.3	23.1	5.6	8.9	7.37	8.13
Chlorpyrifos 150 ng/L (1 TU) + Bifenthrin 1.5 ng/L (1/4 TU) + Methanol	339	20.3	23.1	5.6	8.9	7.37	8.13
Chlorpyrifos 150 ng/L (1 TU) + Bifenthrin 3 ng/L (1/2 TU) + Methanol	339	20.3	23.1	5.6	8.9	7.37	8.13

Table A3. Summary of acute and chronic *H. azteca* water column toxicity tests initiated on December 13, 2010 examining the toxicity of bifenthrin x ammonia mixtures.

Treatment	4-day Survival (%)		10-day Survival (%)	
	Mean	SE	Mean	SE
DIEPAMHR	100	0	100	0
DIEPAMHR + Methanol	100	0	100	0
DIEPAMHR + Bifenthrin 0.5 ng/L (1/8 TU) + Methanol	98	4	96	9
DIEPAMHR + Bifenthrin 1 ng/L (1/4 TU) + Methanol	94	5	88	8
DIEPAMHR + Bifenthrin 2 ng/L (1/2 TU) + Methanol	49	25	39	16
DIEPAMHR + Bifenthrin 3 ng/L (3/4 TU) + Methanol	33	23	8	12
DIEPAMHR + Bifenthrin 4 ng/L (1 TU) + Methanol	4	5	0	0
DIEPAMHR + Bifenthrin 6 ng/L (1.5 TU) + Methanol	0	0	0	0
DIEPAMHR + Bifenthrin 8 ng/L (2 TU) + Methanol	0	0	0	0
DIEPAMHR + NH3 3.125 mg/L (1/8 TU) + Methanol	89	15	59	31
DIEPAMHR + NH3 6.25 mg/L (1/4 TU) + Methanol	82	17	78	14
DIEPAMHR + NH3 12.5 mg/L (1/2 TU) + Methanol	88	11	68	24
DIEPAMHR + NH3 18.75 mg/L (3/4 TU) + Methanol	54	15	36	4
DIEPAMHR + NH3 25 mg/L (1 TU) + Methanol	48	23	10	12
DIEPAMHR + NH3 37.5 mg/L (1.5 TU) + Methanol	18	11	6	9
DIEPAMHR + NH3 50 mg/L (2 TU) + Methanol	10	7	0	0
DIEPAMHR + Bifenthrin 1 ng/L (1/4 TU) + NH3 6.25 mg/L (1/4 TU) + Methanol	84	15	78	16
DIEPAMHR + Bifenthrin 1 ng/L (1/4 TU) + NH3 12.5 mg/L (1/2 TU) + Methanol	83	16	79	15
DIEPAMHR + Bifenthrin 1 ng/L (1/4 TU) + NH3 18.75 mg/L (3/4 TU) + Methanol	61	24	57	26
DIEPAMHR + Bifenthrin 2 ng/L (1/2 TU) + NH3 6.25 mg/L (1/4 TU) + Methanol	68	18	53	16
DIEPAMHR + Bifenthrin 2 ng/L (1/2 TU) + NH3 12.5 mg/L (1/2 TU) + Methanol	63	6	51	19
DIEPAMHR + Bifenthrin 2 ng/L (1/2 TU) + NH3 18.75 mg/L (3/4 TU) + Methanol	56	22	40	21
DIEPAMHR + Bifenthrin 3 ng/L (3/4 TU) + NH3 6.25 mg/L (1/4 TU) + Methanol	23	17	4	5
DIEPAMHR + Bifenthrin 3 ng/L (3/4 TU) + NH3 12.5 mg/L (1/2 TU) + Methanol	31	16	17	20
DIEPAMHR + Bifenthrin 3 ng/L (3/4 TU) + NH3 18.75 mg/L (3/4 TU) + Methanol	28	18	12	8
DIEPAMHR + Bifenthrin 0.5 ng/L (1/8 TU) + NH3 3.125 mg/L (1/8 TU) + Methanol	96	5	86	5
DIEPAMHR + Bifenthrin 4 ng/L (1 TU) + NH3 25 mg/L (1 TU) + Methanol	21	18	10	14

Table A4. Summary of water chemistry measured in a *H. azteca* toxicity test initiated on December 13, 2010 examining the toxicity of bifenthrin x ammonia mixtures.

Treatment	Laboratory Chemistry							
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH	Total Ammonia (mg/L)
DIEPAMHR	329	20.2	22.2	7.1	8.7	7.47	7.95	0.13
DIEPAMHR + Methanol	325	20.4	21.8	3.5	8.7	7.23	7.98	0.07
DIEPAMHR + Bifenthrin 0.5 ng/L (1/8 TU) + Methanol	328	20.3	21.5	4.0	8.7	7.24	7.95	0.13
DIEPAMHR + Bifenthrin 1 ng/L (1/4 TU) + Methanol	326	20.4	22.0	4.1	8.8	7.19	7.95	0.09
DIEPAMHR + Bifenthrin 2 ng/L (1/2 TU) + Methanol	327	20.4	22.4	4.4	8.8	7.14	7.97	0.09
DIEPAMHR + Bifenthrin 3 ng/L (3/4 TU) + Methanol	329	20.6	22.2	4.6	8.7	7.19	7.97	0.12
DIEPAMHR + Bifenthrin 4 ng/L (1 TU) + Methanol	321	20.6	21.7	5.8	8.8	7.30	7.94	0.08
DIEPAMHR + Bifenthrin 6 ng/L (1.5 TU) + Methanol	330	21.1	21.8	6.9	8.6	7.54	7.95	0.11
DIEPAMHR + Bifenthrin 8 ng/L (2 TU) + Methanol	330	21.1	21.7	6.7	8.6	7.53	7.93	0.00
DIEPAMHR + NH3 3.125 mg/L (1/8 TU) + Methanol	350	20.7	21.7	4.2	8.7	7.26	7.88	3.14
DIEPAMHR + NH3 6.25 mg/L (1/4 TU) + Methanol	381	20.6	22.3	4.0	8.8	7.16	7.84	6.95
DIEPAMHR + NH3 12.5 mg/L (1/2 TU) + Methanol	435	20.8	21.6	4.4	8.7	7.24	7.76	14.12
DIEPAMHR + NH3 18.75 mg/L (3/4 TU) + Methanol	486	20.6	21.5	3.8	8.7	7.16	7.72	20.96
DIEPAMHR + NH3 25 mg/L (1 TU) + Methanol	542	20.1	21.4	3.8	8.7	7.20	7.66	30.27
DIEPAMHR + NH3 37.5 mg/L (1.5 TU) + Methanol	653	20.6	22.2	4.6	9.0	7.24	7.60	42.82
DIEPAMHR + NH3 50 mg/L (2 TU) + Methanol	736	20.6	21.7	6.1	8.7	7.18	7.54	58.07
DIEPAMHR + Bifenthrin 1 ng/L (1/4 TU) + NH3 6.25 mg/L (1/4 TU) + Methanol	381	20.6	22.1	4.4	8.8	7.13	7.83	7.51
DIEPAMHR + Bifenthrin 1 ng/L (1/4 TU) + NH3 12.5 mg/L (1/2 TU) + Methanol	436	20.7	22.4	4.3	8.9	7.19	7.77	14.57
DIEPAMHR + Bifenthrin 1 ng/L (1/4 TU) + NH3 18.75 mg/L (3/4 TU) + Methanol	488	20.8	21.9	4.1	8.9	7.10	7.75	20.96
DIEPAMHR + Bifenthrin 2 ng/L (1/2 TU) + NH3 6.25 mg/L (1/4 TU) + Methanol	383	20.5	22.2	4.4	8.7	7.14	7.84	7.06
DIEPAMHR + Bifenthrin 2 ng/L (1/2 TU) + NH3 12.5 mg/L (1/2 TU) + Methanol	433	20.5	22.2	4.5	8.8	7.17	7.78	15.47
DIEPAMHR + Bifenthrin 2 ng/L (1/2 TU) + NH3 18.75 mg/L (3/4 TU) + Methanol	488	20.5	21.9	4.2	8.7	7.15	7.73	21.86
DIEPAMHR + Bifenthrin 3 ng/L (3/4 TU) + NH3 6.25 mg/L (1/4 TU) + Methanol	379	20.2	21.9	4.3	8.8	7.16	7.85	6.73
DIEPAMHR + Bifenthrin 3 ng/L (3/4 TU) + NH3 12.5 mg/L (1/2 TU) + Methanol	438	20.8	22.1	4.3	8.9	7.17	7.77	14.35
DIEPAMHR + Bifenthrin 3 ng/L (3/4 TU) + NH3 18.75 mg/L (3/4 TU) + Methanol	485	20.7	22.2	4.3	8.6	7.12	7.74	20.51
DIEPAMHR + Bifenthrin 0.5 ng/L (1/8 TU) + NH3 3.125 mg/L (1/8 TU) + Methanol	354	19.9	21.9	3.7	8.7	7.07	7.92	3.59
DIEPAMHR + Bifenthrin 4 ng/L (1 TU) + NH3 25 mg/L (1 TU) + Methanol	542	20.7	21.7	4.2	8.8	7.16	7.67	28.92

Table A5. Summary of acute and chronic *H. azteca* water column toxicity tests initiated on May 10, 2010 examining the toxicity of bifenthrin x copper mixtures.

Treatment	4-day Survival (%)		10-day Survival (%)	
	Mean	SE	Mean	SE
DIEPAMHR	98	4	87	10
DIEPAMHR + Methanol	100	0	98	5
DIEPAMHR + Bifenthrin 0.625 ng/L (1/8 TU) + Methanol	100	0	98	4
DIEPAMHR + Bifenthrin 1.25 ng/L (1/4 TU) + Methanol	96	5	96	5
DIEPAMHR + Bifenthrin 2.5 ng/L (1/2 TU) + Methanol	100	0	100	0
DIEPAMHR + Bifenthrin 3.75 ng/L (3/4 TU) + Methanol	94	9	86	11
DIEPAMHR + Bifenthrin 5 ng/L (1 TU) + Methanol	90	11	73	16
DIEPAMHR + Bifenthrin 7.5 ng/L (1.5 TU) + Methanol	48	20	32	22
DIEPAMHR + Bifenthrin 10 ng/L (2 TU) + Methanol	24	17	4	5
DIEPAMHR + Copper 93.75 ug/L (1/8 TU) + Methanol	92	8	92	8
DIEPAMHR + Copper 187.5 ug/L (1/4 TU) + Methanol	94	10	94	10
DIEPAMHR + Copper 375 ug/L (1/2 TU) + Methanol	82	8	49	18
DIEPAMHR + Copper 562.5 ug/L (3/4 TU) + Methanol	48	26	4	6
DIEPAMHR + Copper 750 ug/L (1 TU) + Methanol	33	19	0	0
DIEPAMHR + Copper 1125 ug/L (1.5 TU) + Methanol	17	13	2	4
DIEPAMHR + Copper 1500 ug/L (2 TU) + Methanol	4	5	0	0
DIEPAMHR + Bifenthrin 0.625 ng/L (1/8 TU) + Copper 93.75 ug/L (1/8 TU) + Meth.	98	5	98	5
DIEPAMHR + Bifenthrin 1.25 ng/L (1/4 TU) + Copper 187.5 ug/L (1/4 TU) + Methanol	94	6	87	9
DIEPAMHR + Bifenthrin 2.5 ng/L (1/2 TU) + Copper 375 ug/L (1/2 TU) + Methanol	90	12	36	16
DIEPAMHR + Bifenthrin 2.5 ng/L (1/2 TU) + Copper 562.5 ug/L (3/4 TU) + Methanol	47	7	4	5
DIEPAMHR + Bifenthrin 2.5 ng/L (1/2 TU) + Copper 750 ug/L (1 TU) + Methanol	32	9	0	0
DIEPAMHR + Bifenthrin 3.75 ng/L (3/4 TU) + Copper 375 ug/L (1/2 TU) + Methanol	88	13	22	12
DIEPAMHR + Bifenthrin 3.75 ng/L (3/4 TU) + Copper 562.5 ug/L (3/4 TU) + Methanol	47	16	4	6
DIEPAMHR + Bifenthrin 3.75 ng/L (3/4 TU) + Copper 750 ug/L (1 TU) + Methanol	42	13	4	5
DIEPAMHR + Bifenthrin 5 ng/L (1 TU) + Copper 375 ug/L (1/2 TU) + Methanol	74	12	12	15
DIEPAMHR + Bifenthrin 5 ng/L (1 TU) + Copper 562.5 ug/L (3/4 TU) + Methanol	62	17	2	5
DIEPAMHR + Bifenthrin 5 ng/L (1 TU) + Copper 750 ug/L (1 TU) + Methanol	27	15	0	0
DIEPAMHR + Bifenthrin 7.5 ng/L (2 TU) + Copper 1125 ug/L (2 TU) + Methanol	2	4	0	0

Table A6. Summary of water chemistry measured in a *H. azteca* toxicity test initiated on May 10, 2011 examining the toxicity of bifenthrin x copper mixtures.

Treatment	Laboratory Chemistry						
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH
DIEPAMHR	328	18.3	22.4	7.5	9.5	7.77	8.24
DIEPAMHR + Methanol	326	17.8	22.2	4.7	9.5	7.42	8.27
DIEPAMHR + Bifenthrin 0.625 ng/L (1/8 TU) + Methanol	325	17.6	22.4	4.8	9.7	7.45	8.24
DIEPAMHR + Bifenthrin 1.25 ng/L (1/4 TU) + Methanol	326	17.7	22.4	5.2	9.7	6.65	8.20
DIEPAMHR + Bifenthrin 2.5 ng/L (1/2 TU) + Methanol	327	17.8	24.5	5.9	9.5	7.48	8.23
DIEPAMHR + Bifenthrin 3.75 ng/L (3/4 TU) + Methanol	327	17.5	21.5	5.4	9.8	7.23	8.22
DIEPAMHR + Bifenthrin 5 ng/L (1 TU) + Methanol	325	17.5	22.8	4.4	9.6	7.45	8.22
DIEPAMHR + Bifenthrin 7.5 ng/L (1.5 TU) + Methanol	326	17.9	21.6	5.6	9.4	7.45	8.22
DIEPAMHR + Bifenthrin 10 ng/L (2 TU) + Methanol	328	17.7	22.4	4.6	9.6	7.50	8.22
DIEPAMHR + Copper 93.75 ug/L (1/8 TU) + Methanol	326	17.8	22.2	6.2	9.5	7.57	8.20
DIEPAMHR + Copper 187.5 ug/L (1/4 TU) + Methanol	328	17.6	23.3	6.0	9.7	7.65	8.19
DIEPAMHR + Copper 375 ug/L (1/2 TU) + Methanol	327	17.6	21.7	6.8	9.8	7.66	8.22
DIEPAMHR + Copper 562.5 ug/L (3/4 TU) + Methanol	328	17.7	22.1	6.7	9.8	7.79	8.15
DIEPAMHR + Copper 750 ug/L (1 TU) + Methanol	325	17.9	22.1	7.8	9.7	7.84	8.13
DIEPAMHR + Copper 1125 ug/L (1.5 TU) + Methanol	329	18.1	23.4	7.2	9.4	7.87	8.13
DIEPAMHR + Copper 1500 ug/L (2 TU) + Methanol	328	18.1	22.1	8.1	9.4	7.87	8.08
DIEPAMHR + Bifenthrin 0.625 ng/L (1/8 TU) + Copper 93.75 ug/L (1/8 TU) +	328	17.9	22.3	5.9	9.5	7.44	8.17
DIEPAMHR + Bifenthrin 1.25 ng/L (1/4 TU) + Copper 187.5 ug/L (1/4 TU) +	328	17.5	22.2	5.6	9.6	7.54	8.17
DIEPAMHR + Bifenthrin 2.5 ng/L (1/2 TU) + Copper 375 ug/L (1/2 TU) + Methanol	325	17.4	21.9	6.0	9.8	7.61	8.15
DIEPAMHR + Bifenthrin 2.5 ng/L (1/2 TU) + Copper 562.5 ug/L (3/4 TU) + Methanol	326	17.5	22.2	6.5	9.6	7.75	8.13
DIEPAMHR + Bifenthrin 2.5 ng/L (1/2 TU) + Copper 750 ug/L (1 TU) + Methanol	324	17.9	22.0	7.9	9.4	7.84	8.12
DIEPAMHR + Bifenthrin 3.75 ng/L (3/4 TU) + Copper 375 ug/L (1/2 TU) + Methanol	324	17.5	22.0	5.8	9.6	7.70	8.19
DIEPAMHR + Bifenthrin 3.75 ng/L (3/4 TU) + Copper 562.5 ug/L (3/4 TU) +	325	17.2	22.8	6.6	9.7	7.70	8.17
DIEPAMHR + Bifenthrin 3.75 ng/L (3/4 TU) + Copper 750 ug/L (1 TU) + Methanol	322	17.1	22.4	7.6	9.8	7.70	8.18
DIEPAMHR + Bifenthrin 5 ng/L (1 TU) + Copper 375 ug/L (1/2 TU) + Methanol	315	17.5	22.2	6.2	9.6	7.58	8.15
DIEPAMHR + Bifenthrin 5 ng/L (1 TU) + Copper 562.5 ug/L (3/4 TU) + Methanol	326	17.5	22.6	6.9	9.7	7.77	8.19
DIEPAMHR + Bifenthrin 5 ng/L (1 TU) + Copper 750 ug/L (1 TU) + Methanol	324	17.4	22.2	7.8	9.8	7.71	8.15
DIEPAMHR + Bifenthrin 7.5 ng/L (2 TU) + Copper 1125 ug/L (2 TU) + Methanol	332	17.9	20.4	7.8	9.2	7.71	8.12

Table A7. Summary of an acute *H. azteca* water column toxicity test initiated on June 21 2011 examining the toxicity of bifenthrin x cyfluthrin mixtures.

Treatment	4-day Survival (%)	
	Mean	SE
DIEPAMHR	100	0
DIEPAMHR + Methanol	100	0
DIEPAMHR + Bifenthrin 0.6875 ng/L (1/8 TU) + Methanol	98	4
DIEPAMHR + Bifenthrin 1.375 ng/L (1/4 TU) + Methanol	100	0
DIEPAMHR + Bifenthrin 2.75 ng/L (1/2 TU) + Methanol	88	16
DIEPAMHR + Bifenthrin 4.125 ng/L (3/4 TU) + Methanol	98	4
DIEPAMHR + Bifenthrin 5.5 ng/L (1 TU) + Methanol	76	21
DIEPAMHR + Bifenthrin 8.25 ng/L (1.5 TU) + Methanol	20	7
DIEPAMHR + Bifenthrin 11 ng/L (2 TU) + Methanol	16	5
DIEPAMHR + Cyfluthrin 0.25 ng/L (1/8 TU) + Methanol	94	9
DIEPAMHR + Cyfluthrin 0.5 ng/L (1/4 TU) + Methanol	46	23
DIEPAMHR + Cyfluthrin 1 ng/L (1/2 TU) + Methanol	27	20
DIEPAMHR + Cyfluthrin 1.5.25 ng/L (3/4 TU) + Methanol	2	4
DIEPAMHR + Cyfluthrin 2 ng/L (1 TU) + Methanol	2	4
DIEPAMHR + Cyfluthrin 3 ng/L (1.5 TU) + Methanol	0	0
DIEPAMHR + Cyfluthrin 4 ng/L (2 TU) + Methanol	0	0
DIEPAMHR + Bifenthrin 0.34375 ng/L (1/16 TU) + Cyfluthrin 0.125 ng/L (1/16 TU) + Methanol	96	6
DIEPAMHR + Bifenthrin 0.6875 ng/L (1/8 TU) + Cyfluthrin .25 ng/L (1/8 TU) + Methanol	89	14
DIEPAMHR + Bifenthrin 1.375 ng/L (1/4 TU) + Cyfluthrin 0.5 ng/L ng/L (1/4 TU) + Methanol	57	12
DIEPAMHR + Bifenthrin 1.375 ng/L (1/4 TU) + Cyfluthrin 1 ng/L (1/2 TU) + Methanol	4	9
DIEPAMHR + Bifenthrin 1.375 ng/L (1/4 TU) + Cyfluthrin 1.5 ng/L (3/4 TU) + Methanol	14	13
DIEPAMHR + Bifenthrin 2.75 ng/L (1/2 TU) + Cyfluthrin 0.5 ng/L ng/L (1/4 TU) + Methanol	25	6
DIEPAMHR + Bifenthrin 2.75 ng/L (1/2 TU) + Cyfluthrin 1 ng/L (1/2 TU) + Methanol	25	26
DIEPAMHR + Bifenthrin 2.75 ng/L (1/2 TU) + Cyfluthrin 1.5 ng/L (3/4 TU) + Methanol	9	13
DIEPAMHR + Bifenthrin 4.125 ng/L (3/4 TU) + Cyfluthrin 0.5 ng/L ng/L (1/4 TU) + Methanol	42	16
DIEPAMHR + Bifenthrin 4.125 ng/L (3/4 TU) + Cyfluthrin 1 ng/L (1/2 TU) + Methanol	18	4
DIEPAMHR + Bifenthrin 4.125 ng/L (3/4 TU) + Cyfluthrin 1.5 ng/L (3/4 TU) + Methanol	12	8
DIEPAMHR + Bifenthrin 5.5 ng/L (1 TU) + Cyfluthrin 2 ng/L (1 TU) + Methanol	0	0
DIEPAMHR + Bifenthrin 8.25 ng/L (1.5 TU) + Cyfluthrin 3 ng/L (1.5 TU) + Methanol	0	0

Table A8. Summary of water chemistry measured in a *H. azteca* toxicity test initiated on June 21, 2011 examining the toxicity of bifenthrin x cyfluthrin mixtures.

Treatment	Laboratory Chemistry						
	EC (uS/cm)	Min temp (°C)	Max temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH
DIEPAMHR	317	21.2	23.5	6.6	8.3	7.69	8.18
DIEPAMHR + Methanol	315	21.6	23.6	4.7	8.4	7.40	8.15
DIEPAMHR + Bifenthrin 0.6875 ng/L (1/8 TU) + Methanol	315	21.8	23.7	4.1	8.2	7.39	8.12
DIEPAMHR + Bifenthrin 1.375 ng/L (1/4 TU) + Methanol	317	21.8	23.5	4.4	8.5	7.43	8.16
DIEPAMHR + Bifenthrin 2.75 ng/L (1/2 TU) + Methanol	317	21.9	23.6	4.2	8.3	7.43	8.15
DIEPAMHR + Bifenthrin 4.125 ng/L (3/4 TU) + Methanol	318	22.0	23.5	4.8	8.2	7.48	8.15
DIEPAMHR + Bifenthrin 5.5 ng/L (1 TU) + Methanol	316	22.1	23.5	4.0	8.4	7.41	8.15
DIEPAMHR + Bifenthrin 8.25 ng/L (1.5 TU) + Methanol	321	21.3	23.5	4.4	8.4	7.53	8.15
DIEPAMHR + Bifenthrin 11 ng/L (2 TU) + Methanol	316	21.2	23.5	4.3	8.4	7.41	8.16
DIEPAMHR + Cyfluthrin 0.25 ng/L (1/8 TU) + Methanol	318	21.5	23.6	4.2	8.3	7.40	8.14
DIEPAMHR + Cyfluthrin 0.5 ng/L (1/4 TU) + Methanol	319	21.6	23.5	4.3	8.4	7.42	8.17
DIEPAMHR + Cyfluthrin 1 ng/L (1/2 TU) + Methanol	316	21.7	23.3	4.1	8.3	7.39	8.14
DIEPAMHR + Cyfluthrin 1.5.25 ng/L (3/4 TU) + Methanol	317	21.9	23.4	4.8	8.4	7.46	8.14
DIEPAMHR + Cyfluthrin 2 ng/L (1 TU) + Methanol	319	21.9	23.4	4.8	8.3	7.48	8.13
DIEPAMHR + Cyfluthrin 3 ng/L (1.5 TU) + Methanol	319	22.0	23.5	4.5	8.5	7.44	8.13
DIEPAMHR + Cyfluthrin 4 ng/L (2 TU) + Methanol	318	21.3	23.5	4.2	8.4	7.40	8.14
DIEPAMHR + Bifenthrin 0.34375 ng/L (1/16 TU) + Cyfluthrin 0.125 ng/L (1/16 TU) + Methanol	318	21.4	23.4	4.4	8.5	7.42	8.09
DIEPAMHR + Bifenthrin 0.6875 ng/L (1/8 TU) + Cyfluthrin .25 ng/L (1/8 TU) + Methanol	317	21.6	23.4	4.0	8.4	7.38	8.16
DIEPAMHR + Bifenthrin 1.375 ng/L (1/4 TU) + Cyfluthrin 0.5 ng/L ng/L (1/4 TU) + Methanol	317	21.8	23.3	4.6	8.4	7.44	8.13
DIEPAMHR + Bifenthrin 1.375 ng/L (1/4 TU) + Cyfluthrin 1 ng/L (1/2 TU) + Methanol	316	21.9	23.3	5.2	8.3	7.50	8.14
DIEPAMHR + Bifenthrin 1.375 ng/L (1/4 TU) + Cyfluthrin 1.5 ng/L (3/4 TU) + Methanol	319	21.9	23.1	4.5	8.4	7.33	8.16
DIEPAMHR + Bifenthrin 2.75 ng/L (1/2 TU) + Cyfluthrin 0.5 ng/L ng/L (1/4 TU) + Methanol	318	22.0	23.2	4.6	8.5	7.41	8.14
DIEPAMHR + Bifenthrin 2.75 ng/L (1/2 TU) + Cyfluthrin 1 ng/L (1/2 TU) + Methanol	319	22.1	23.2	4.4	8.4	7.40	8.15
DIEPAMHR + Bifenthrin 2.75 ng/L (1/2 TU) + Cyfluthrin 1.5 ng/L (3/4 TU) + Methanol	316	21.5	23.2	4.0	8.5	7.40	8.15
DIEPAMHR + Bifenthrin 4.125 ng/L (3/4 TU) + Cyfluthrin 0.5 ng/L ng/L (1/4 TU) + Methanol	318	21.6	23.2	4.1	8.5	7.38	8.14
DIEPAMHR + Bifenthrin 4.125 ng/L (3/4 TU) + Cyfluthrin 1 ng/L (1/2 TU) + Methanol	318	21.9	23.1	4.0	8.4	7.38	8.16
DIEPAMHR + Bifenthrin 4.125 ng/L (3/4 TU) + Cyfluthrin 1.5 ng/L (3/4 TU) + Methanol	318	21.9	23.2	4.9	8.4	7.46	8.16
DIEPAMHR + Bifenthrin 5.5 ng/L (1 TU) + Cyfluthrin 2 ng/L (1 TU) + Methanol	317	22.0	23.2	4.2	8.4	7.41	8.13
DIEPAMHR + Bifenthrin 8.25 ng/L (1.5 TU) + Cyfluthrin 3 ng/L (1.5 TU) + Methanol	319	21.2	22.9	4.8	8.6	7.26	8.07

Table A9. Summary of acute and chronic *H. azteca* water column toxicity tests initiated on May 27, 2011 examining the toxicity of bifenthrin x permethrin mixtures.

Treatment Description	4-day Survival (%)		10-day Survival (%)	
	Mean	SE	Mean	SE
DIEPAMHR	98	4	96	5
DIEPAMHR + Methanol	100	0	100	0
DIEPAMHR + Bifenthrin 0.6875 ng/L (1/8 TU) + Methanol	100	0	96	8
DIEPAMHR + Bifenthrin 1.375 ng/L (1/4 TU) + Methanol	100	0	100	0
DIEPAMHR + Bifenthrin 2.75 ng/L (1/2 TU) + Methanol	94	9	87	13
DIEPAMHR + Bifenthrin 4.125 ng/L (3/4 TU) + Methanol	96	5	67	20
DIEPAMHR + Bifenthrin 5.5 ng/L (1 TU) + Methanol	51	21	33	28
DIEPAMHR + Bifenthrin 8.25 ng/L (1.5 TU) + Methanol	20	16	0	0
DIEPAMHR + Bifenthrin 11 ng/L (2 TU) + Methanol	15	16	0	0
DIEPAMHR + Permethrin 6.875 ng/L (1/8 TU) + Methanol	100	0	100	0
DIEPAMHR + Permethrin 13.75 ng/L (1/4 TU) + Methanol	100	0	100	0
DIEPAMHR + Permethrin 27.5 ng/L (1/2 TU) + Methanol	96	9	90	10
DIEPAMHR + Permethrin 41.25 ng/L (3/4 TU) + Methanol	78	13	66	15
DIEPAMHR + Permethrin 55 ng/L (1 TU) + Methanol	50	19	32	24
DIEPAMHR + Permethrin 82.5 ng/L (1.5 TU) + Methanol	47	17	10	10
DIEPAMHR + Permethrin 110 ng/L (2 TU) + Methanol	12	11	0	0
DIEPAMHR + Bifenthrin 0.34375 ng/L (1/16 TU) + Permethrin 3.4375 ng/L (1/16 TU) + Meth.	90	12	90	12
DIEPAMHR + Bifenthrin 0.6875 ng/L (1/8 TU) + Permethrin 6.875 ng/L (1/8 TU) + Methanol	98	4	98	4
DIEPAMHR + Bifenthrin 1.375 ng/L (1/4 TU) + Permethrin 13.75 ng/L ng/L (1/4 TU) + Meth.	96	5	96	6
DIEPAMHR + Bifenthrin 1.375 ng/L (1/4 TU) + Permethrin 27.5 ng/L (1/2 TU) + Methanol	77	7	57	21
DIEPAMHR + Bifenthrin 1.375 ng/L (1/4 TU) + Permethrin 41.25 ng/L (3/4 TU) + Methanol	75	10	47	27
DIEPAMHR + Bifenthrin 2.75 ng/L (1/2 TU) + Permethrin 13.75 ng/L ng/L (1/4 TU) + Methanol	66	15	37	20
DIEPAMHR + Bifenthrin 2.75 ng/L (1/2 TU) + Permethrin 27.5 ng/L (1/2 TU) + Methanol	74	23	34	15
DIEPAMHR + Bifenthrin 2.75 ng/L (1/2 TU) + Permethrin 41.25 ng/L (3/4 TU) + Methanol	54	14	9	9
DIEPAMHR + Bifenthrin 4.125 ng/L (3/4 TU) + Permethrin 13.75 ng/L ng/L (1/4 TU) + Meth.	80	14	44	20
DIEPAMHR + Bifenthrin 4.125 ng/L (3/4 TU) + Permethrin 27.5 ng/L (1/2 TU) + Methanol	66	13	21	17
DIEPAMHR + Bifenthrin 4.125 ng/L (3/4 TU) + Permethrin 41.25 ng/L (3/4 TU) + Methanol	39	21	14	15
DIEPAMHR + Bifenthrin 5.5 ng/L (1 TU) + Permethrin 55 ng/L (1 TU) + Methanol	18	13	0	0
DIEPAMHR + Bifenthrin 8.25 ng/L (1.5 TU) + Permethrin 82.5 ng/L (1.5 TU) + Methanol	0	0	0	0

Table A10. Summary of water chemistry in a *H. azteca* toxicity test initiated on May 27, 2011 examining the toxicity of bifenthrin x permethrin mixtures.

Treatment	Laboratory Chemistry						
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH
DIEPAMHR	321	18.5	23.6	7.7	9.4	7.75	8.21
DIEPAMHR + Methanol	316	18.7	23.6	5.1	9.4	7.42	8.27
DIEPAMHR + Bifenthrin 0.6875 ng/L (1/8 TU) + Methanol	317	18.7	23.7	5.6	9.3	7.43	8.19
DIEPAMHR + Bifenthrin 1.375 ng/L (1/4 TU) + Methanol	320	18.7	23.7	5.9	9.3	7.51	8.22
DIEPAMHR + Bifenthrin 2.75 ng/L (1/2 TU) + Methanol	314	14.4	23.2	5.6	9.2	7.42	8.18
DIEPAMHR + Bifenthrin 4.125 ng/L (3/4 TU) + Methanol	321	18.6	23.3	5.4	9.5	7.45	8.20
DIEPAMHR + Bifenthrin 5.5 ng/L (1 TU) + Methanol	313	18.7	23.1	5.9	9.2	7.45	8.18
DIEPAMHR + Bifenthrin 8.25 ng/L (1.5 TU) + Methanol	311	18.7	23.0	6.5	9.3	7.64	8.19
DIEPAMHR + Bifenthrin 11 ng/L (2 TU) + Methanol	313	18.6	22.7	7.0	9.3	7.68	8.27
DIEPAMHR + Permethrin 6.875 ng/L (1/8 TU) + Methanol	315	18.6	22.8	5.6	9.4	7.45	8.16
DIEPAMHR + Permethrin 13.75 ng/L (1/4 TU) + Methanol	315	18.5	22.9	5.8	9.4	7.44	8.21
DIEPAMHR + Permethrin 27.5 ng/L (1/2 TU) + Methanol	318	18.7	23.0	5.8	9.3	7.48	8.20
DIEPAMHR + Permethrin 41.25 ng/L (3/4 TU) + Methanol	320	18.6	22.8	5.9	9.4	7.43	8.25
DIEPAMHR + Permethrin 55 ng/L (1 TU) + Methanol	317	18.8	22.8	5.9	9.1	7.33	8.19
DIEPAMHR + Permethrin 82.5 ng/L (1.5 TU) + Methanol	317	18.6	22.7	5.8	9.3	7.42	8.16
DIEPAMHR + Permethrin 110 ng/L (2 TU) + Methanol	313	18.6	22.7	6.5	9.3	7.56	8.20
DIEPAMHR + Bifenthrin 0.34375 ng/L (1/16 TU) + Permethrin 3.4375 ng/L (1/16 TU) + Methanol	287	18.6	22.3	5.4	9.1	7.37	8.17
DIEPAMHR + Bifenthrin 0.6875 ng/L (1/8 TU) + Permethrin 6.875 ng/L (1/8 TU) + Methanol	318	18.9	22.3	5.9	9.0	7.46	8.18
DIEPAMHR + Bifenthrin 1.375 ng/L (1/4 TU) + Permethrin 13.75 ng/L ng/L (1/4 TU) + Methanol	320	18.5	23.6	5.6	9.2	7.45	8.18
DIEPAMHR + Bifenthrin 1.375 ng/L (1/4 TU) + Permethrin 27.5 ng/L (1/2 TU) + Methanol	313	18.6	22.4	6.3	9.4	7.49	8.20
DIEPAMHR + Bifenthrin 1.375 ng/L (1/4 TU) + Permethrin 41.25 ng/L (3/4 TU) + Methanol	316	18.8	22.3	6.0	9.1	7.42	8.18
DIEPAMHR + Bifenthrin 2.75 ng/L (1/2 TU) + Permethrin 13.75 ng/L ng/L (1/4 TU) + Methanol	319	18.7	23.6	5.7	9.2	7.40	8.22
DIEPAMHR + Bifenthrin 2.75 ng/L (1/2 TU) + Permethrin 27.5 ng/L (1/2 TU) + Methanol	317	18.6	22.1	6.3	9.2	7.46	8.17
DIEPAMHR + Bifenthrin 2.75 ng/L (1/2 TU) + Permethrin 41.25 ng/L (3/4 TU) + Methanol	314	18.6	21.9	6.0	9.2	7.51	8.16
DIEPAMHR + Bifenthrin 4.125 ng/L (3/4 TU) + Permethrin 13.75 ng/L ng/L (1/4 TU) + Methanol	316	18.6	21.6	5.8	9.4	7.45	8.20
DIEPAMHR + Bifenthrin 4.125 ng/L (3/4 TU) + Permethrin 27.5 ng/L (1/2 TU) + Methanol	316	18.5	21.5	5.9	9.4	7.50	8.19
DIEPAMHR + Bifenthrin 4.125 ng/L (3/4 TU) + Permethrin 41.25 ng/L (3/4 TU) + Methanol	316	18.5	22.5	6.0	9.3	7.50	8.21
DIEPAMHR + Bifenthrin 5.5 ng/L (1 TU) + Permethrin 55 ng/L (1 TU) + Methanol	298	18.6	21.8	6.8	9.2	7.56	8.27
DIEPAMHR + Bifenthrin 8.25 ng/L (1.5 TU) + Permethrin 82.5 ng/L (1.5 TU) + Methanol	318	18.9	21.8	7.8	8.9	7.87	8.13

Table A11. Summary of acute and chronic *H. azteca* water column toxicity tests initiated on August 8, 2011 examining the toxicity of permethrin x cyfluthrin mixtures.

Treatment	4-day Survival (%)		10-day Survival (%)	
	Mean	SE	Mean	SE
DIEPAMHR	94	4	92	4
DIEPAMHR + Methanol	94	4	92	4
DIEPAMHR + Permethrin 7.5 ng/L (1/8 TU) + Methanol	100	0	100	0
DIEPAMHR + Permethrin 15 ng/L (1/4 TU) + Methanol	88	4	84	5
DIEPAMHR + Permethrin 30 ng/L (1/2 TU) + Methanol	58	7	58	7
DIEPAMHR + Permethrin 45 ng/L (3/4 TU) + Methanol	51	12	42	10
DIEPAMHR + Permethrin 60 ng/L (1 TU) + Methanol	38	10	20	4
DIEPAMHR + Permethrin 90 ng/L (1.5 TU) + Methanol	6	6	0	0
DIEPAMHR + Permethrin 120 ng/L (2 TU) + Methanol	0	0	0	0
DIEPAMHR + Cyfluthrin 0.375 ng/L (1/8 TU) + Methanol	96	2	96	2
DIEPAMHR + Cyfluthrin 0.75 ng/L (1/4 TU) + Methanol	84	7	84	7
DIEPAMHR + Cyfluthrin 1.5 ng/L (1/2 TU) + Methanol	94	2	90	5
DIEPAMHR + Cyfluthrin 2.25 ng/L (3/4 TU) + Methanol	92	4	90	3
DIEPAMHR + Cyfluthrin 3 ng/L (1 TU) + Methanol	72	8	57	13
DIEPAMHR + Cyfluthrin 4.5 ng/L (1.5 TU) + Methanol	36	11	14	7
DIEPAMHR + Cyfluthrin 6 ng/L (2 TU) + Methanol	35	14	14	10
DIEPAMHR + Permethrin 3.75 ng/L (1/16 TU) + Cyfluthrin 0.1875 ng/L (1/16 TU) + Methanol	92	2	92	4
DIEPAMHR + Permethrin 7.5 ng/L (1/8 TU) + Cyfluthrin 0.375 ng/L (1/8 TU) + Methanol	86	4	84	4
DIEPAMHR + Permethrin 15 ng/L ng/L (1/4 TU) + Cyfluthrin 0.75 ng/L (1/4 TU) + Methanol	76	4	58	8
DIEPAMHR + Permethrin 15 ng/L ng/L (1/4 TU) + Cyfluthrin 1.5 ng/L (1/2 TU) + Methanol	70	9	56	13
DIEPAMHR + Permethrin 15 ng/L ng/L (1/4 TU) + Cyfluthrin 2.25 ng/L (3/4 TU) + Methanol	66	5	24	4
DIEPAMHR + Permethrin 30 ng/L (1/2 TU) + Cyfluthrin 0.75 ng/L (1/4 TU) + Methanol	59	11	50	14
DIEPAMHR + Permethrin 30 ng/L (1/2 TU) + Cyfluthrin 1.5 ng/L (1/2 TU) + Methanol	50	7	44	8
DIEPAMHR + Permethrin 30 ng/L (1/2 TU) + Cyfluthrin 2.25 ng/L (3/4 TU) + Methanol	41	5	24	5
DIEPAMHR + Permethrin 45 ng/L (3/4 TU) + Cyfluthrin 0.75 ng/L (1/4 TU) + Methanol	33	7	12	7
DIEPAMHR + Permethrin 45 ng/L (3/4 TU) + Cyfluthrin 1.5 ng/L (1/2 TU) + Methanol	39	9	29	9
DIEPAMHR + Permethrin 45 ng/L (3/4 TU) + Cyfluthrin 2.25 ng/L (3/4 TU) + Methanol	56	6	23	7
DIEPAMHR + Permethrin 60 ng/L (1 TU) + Cyfluthrin 3 ng/L (1 TU) + Methanol	8	6	2	2
DIEPAMHR + Permethrin 90 ng/L (1.5 TU) + 4.5 ng/L (1.5 TU) + Methanol	4	2	0	0

Table A12. Summary of water chemistry measured in a *H. azteca* toxicity test initiated on August 8, 2011 examining the toxicity of permethrin x cyfluthrin mixtures.

Treatment	Laboratory Chemistry						
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH
DIEPAMHR	336	22.6	24.8	6.9	8.6	7.62	8.06
DIEPAMHR + Methanol	336	22.5	24.9	4.4	8.5	7.28	8.07
DIEPAMHR + Permethrin 7.5 ng/L (1/8 TU) + Methanol	332	22.5	24.4	5.7	8.6	7.33	8.10
DIEPAMHR + Permethrin 15 ng/L (1/4 TU) + Methanol	332	22.6	24.6	5.9	8.6	7.30	8.12
DIEPAMHR + Permethrin 30 ng/L (1/2 TU) + Methanol	331	21.6	24.4	6.2	8.8	7.45	8.13
DIEPAMHR + Permethrin 45 ng/L (3/4 TU) + Methanol	334	22.6	24.6	5.9	8.6	7.35	8.12
DIEPAMHR + Permethrin 60 ng/L (1 TU) + Methanol	332	22.4	24.6	6.2	8.6	7.42	8.14
DIEPAMHR + Permethrin 90 ng/L (1.5 TU) + Methanol	325	22.0	23.6	7.0	8.6	7.58	8.12
DIEPAMHR + Permethrin 120 ng/L (2 TU) + Methanol	328	22.7	23.5	6.5	8.6	7.59	8.14
DIEPAMHR + Cyfluthrin 0.375 ng/L (1/8 TU) + Methanol	330	21.8	24.8	5.9	8.8	7.40	8.15
DIEPAMHR + Cyfluthrin 0.75 ng/L (1/4 TU) + Methanol	335	22.9	24.4	6.0	8.6	7.30	8.15
DIEPAMHR + Cyfluthrin 1.5 ng/L (1/2 TU) + Methanol	337	23.1	24.5	6.0	8.7	7.42	8.15
DIEPAMHR + Cyfluthrin 2.25 ng/L (3/4 TU) + Methanol	333	22.3	24.4	6.1	8.6	7.39	8.16
DIEPAMHR + Cyfluthrin 3 ng/L (1 TU) + Methanol	333	22.3	24.1	6.4	8.5	7.44	8.19
DIEPAMHR + Cyfluthrin 4.5 ng/L (1.5 TU) + Methanol	331	22.4	24.4	6.0	8.6	7.38	8.08
DIEPAMHR + Cyfluthrin 6 ng/L (2 TU) + Methanol	332	22.4	24.3	5.9	8.5	7.40	8.07
DIEPAMHR + Permethrin 3.75 ng/L (1/16 TU) + Cyfluthrin 0.1875 ng/L (1/16 TU) +	332	21.9	24.5	6.1	8.6	7.45	8.14
DIEPAMHR + Permethrin 7.5 ng/L (1/8 TU) + Cyfluthrin 0.375 ng/L (1/8 TU) + Methanol	335	22.7	24.6	6.1	8.5	7.41	8.12
DIEPAMHR + Permethrin 15 ng/L ng/L (1/4 TU) + Cyfluthrin 0.75 ng/L (1/4 TU) +	329	21.9	24.0	6.2	8.7	7.42	8.11
DIEPAMHR + Permethrin 15 ng/L ng/L (1/4 TU) + Cyfluthrin 1.5 ng/L (1/2 TU) +	331	21.3	24.8	6.3	8.7	7.43	8.09
DIEPAMHR + Permethrin 15 ng/L ng/L (1/4 TU) + Cyfluthrin 2.25 ng/L (3/4 TU) +	330	21.7	24.1	6.2	8.7	7.44	8.05
DIEPAMHR + Permethrin 30 ng/L (1/2 TU) + Cyfluthrin 0.75 ng/L (1/4 TU) + Methanol	334	22.8	24.8	6.1	8.5	7.37	8.07
DIEPAMHR + Permethrin 30 ng/L (1/2 TU) + Cyfluthrin 1.5 ng/L (1/2 TU) + Methanol	334	22.3	24.6	6.1	8.6	7.48	8.12
DIEPAMHR + Permethrin 30 ng/L (1/2 TU) + Cyfluthrin 2.25 ng/L (3/4 TU) + Methanol	337	23.0	24.9	6.1	8.5	7.39	8.08
DIEPAMHR + Permethrin 45 ng/L (3/4 TU) + Cyfluthrin 0.75 ng/L (1/4 TU) + Methanol	332	22.4	24.3	6.1	8.6	7.34	8.07
DIEPAMHR + Permethrin 45 ng/L (3/4 TU) + Cyfluthrin 1.5 ng/L (1/2 TU) + Methanol	331	22.2	24.6	6.0	8.9	7.34	8.10
DIEPAMHR + Permethrin 45 ng/L (3/4 TU) + Cyfluthrin 2.25 ng/L (3/4 TU) + Methanol	332	22.4	24.2	6.1	8.6	7.38	8.06
DIEPAMHR + Permethrin 60 ng/L (1 TU) + Cyfluthrin 3 ng/L (1 TU) + Methanol	338	22.7	25.1	5.9	8.3	7.40	8.10
DIEPAMHR + Permethrin 90 ng/L (1.5 TU) + 4.5 ng/L (1.5 TU) + Methanol	331	22.5	24.3	6.6	8.6	7.57	8.07

Table A13. Summary of an acute *H. azteca* water column toxicity test initiated on September 12, 2011 examining the toxicity of lambda-cyhalothrin x cyfluthrin mixtures.

Treatment	4-day Survival ( )	
	Mean	SE
DIEPAMHR	100	0
DIEPAMHR + Methanol	100	0
DIEPAMHR + Lambda-Cyhalothrin 1.375 ng/L (1/4 TU) + Methanol	98	4
DIEPAMHR + Lambda-Cyhalothrin 2.75 ng/L (1/2 TU) + Methanol	92	4
DIEPAMHR + Lambda-Cyhalothrin 4.125 ng/L (3/4 TU) + Methanol	80	12
DIEPAMHR + Lambda-Cyhalothrin 5.5 ng/L (1 TU) + Methanol	64	9
DIEPAMHR + Lambda-Cyhalothrin 8.25 ng/L (1.5 TU) + Methanol	43	19
DIEPAMHR + Lambda-Cyhalothrin 11 ng/L (2 TU) + Methanol	12	22
DIEPAMHR + Lambda-Cyhalothrin 16.5 ng/L (3 TU) + Methanol	0	0
DIEPAMHR + Cyfluthrin 1 ng/L (1/4 TU) + Methanol	98	4
DIEPAMHR + Cyfluthrin 2 ng/L (1/2 TU) + Methanol	100	0
DIEPAMHR + Cyfluthrin 3 ng/L (3/4 TU) + Methanol	96	5
DIEPAMHR + Cyfluthrin 4 ng/L (1 TU) + Methanol	88	16
DIEPAMHR + Cyfluthrin 6 ng/L (1.5 TU) + Methanol	52	11
DIEPAMHR + Cyfluthrin 8 ng/L (2 TU) + Methanol	32	31
DIEPAMHR + Cyfluthrin 12 ng/L (3 TU) + Methanol	14	15
DIEPAMHR + Lambda-Cyhalothrin 0.6875 ng/L (1/8 TU) + Cyfluthrin 0.5 ng/L (1/8 TU) + Methanol	100	0
DIEPAMHR + Lambda-Cyhalothrin 1.375 ng/L ng/L (1/4 TU) + Cyfluthrin 1 ng/L (1/4 TU) + Methanol	88	16
DIEPAMHR + Lambda-Cyhalothrin 1.375 ng/L ng/L (1/4 TU) + Cyfluthrin 2 ng/L (1/2 TU) + Methanol	58	36
DIEPAMHR + Lambda-Cyhalothrin 1.375 ng/L ng/L (1/4 TU) + Cyfluthrin 3 ng/L (3/4 TU) + Methanol	89	8
DIEPAMHR + Lambda-Cyhalothrin 2.75 ng/L (1/2 TU) + Cyfluthrin 1 ng/L (1/4 TU) + Methanol	74	20
DIEPAMHR + Lambda-Cyhalothrin 2.75 ng/L (1/2 TU) + Cyfluthrin 2 ng/L (1/2 TU) + Methanol	65	26
DIEPAMHR + Lambda-Cyhalothrin 2.75 ng/L (1/2 TU) + Cyfluthrin 3 ng/L (3/4 TU) + Methanol	56	23
DIEPAMHR + Lambda-Cyhalothrin 4.125 ng/L (3/4 TU) + Cyfluthrin 1 ng/L (1/4 TU) + Methanol	73	12
DIEPAMHR + Lambda-Cyhalothrin 4.125 ng/L (3/4 TU) + Cyfluthrin 2 ng/L (1/2 TU) + Methanol	43	22
DIEPAMHR + Lambda-Cyhalothrin 4.125 ng/L (3/4 TU) + Cyfluthrin 3ng/L (3/4 TU) + Methanol	40	16
DIEPAMHR + Lambda-Cyhalothrin 5.5 ng/L (1 TU) + Cyfluthrin 4 ng/L (1 TU) + Methanol	20	16
DIEPAMHR + Lambda-Cyhalothrin 8.25 ng/L (1.5 TU) + Cyfluthrin 6 ng/L (1.5 TU) + Methanol	2	4

Table A14. Summary of water chemistry measured in a *H. azteca* toxicity test initiated on September 12, 2011 examining the toxicity of lambda-cyhalothrin x cyfluthrin mixtures.

Treatment	Laboratory Chemistry						
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH
DIEPAMHR	347	22.3	23.5	6.0	8.2	7.57	8.14
DIEPAMHR + Methanol	348	20.8	23.6	5.8	8.2	7.60	8.12
DIEPAMHR + Lambda-Cyhalothrin 1.375 ng/L (1/4 TU) + Methanol	341	21.0	23.5	5.8	8.3	7.50	8.11
DIEPAMHR + Lambda-Cyhalothrin 2.75 ng/L (1/2 TU) + Methanol	350	21.0	23.5	5.7	8.2	7.57	8.13
DIEPAMHR + Lambda-Cyhalothrin 4.125 ng/L (3/4 TU) + Methanol	346	21.7	23.2	5.7	8.2	7.56	8.13
DIEPAMHR + Lambda-Cyhalothrin 5.5 ng/L (1 TU) + Methanol	347	20.0	23.1	5.8	8.2	7.57	8.16
DIEPAMHR + Lambda-Cyhalothrin 8.25 ng/L (1.5 TU) + Methanol	346	21.0	23.3	6.0	8.3	7.55	8.06
DIEPAMHR + Lambda-Cyhalothrin 11 ng/L (2 TU) + Methanol	344	20.1	23.3	5.8	8.2	7.58	8.14
DIEPAMHR + Lambda-Cyhalothrin 16.5 ng/L (3 TU) + Methanol	349	22.3	23.5	5.0	8.3	7.46	8.11
DIEPAMHR + Cyfluthrin 1 ng/L (1/4 TU) + Methanol	350	22.3	23.5	5.4	8.3	7.48	8.12
DIEPAMHR + Cyfluthrin 2 ng/L (1/2 TU) + Methanol	347	22.2	23.1	5.6	8.3	7.49	8.08
DIEPAMHR + Cyfluthrin 3 ng/L (3/4 TU) + Methanol	345	22.1	23.3	5.8	8.3	7.51	8.08
DIEPAMHR + Cyfluthrin 4 ng/L (1 TU) + Methanol	350	21.8	23.6	5.6	8.3	7.53	8.11
DIEPAMHR + Cyfluthrin 6 ng/L (1.5 TU) + Methanol	345	20.1	23.5	6.0	8.3	7.55	8.12
DIEPAMHR + Cyfluthrin 8 ng/L (2 TU) + Methanol	352	22.2	23.7	5.8	8.5	7.56	8.07
DIEPAMHR + Cyfluthrin 12 ng/L (3 TU) + Methanol	349	22.3	23.4	5.9	8.3	7.57	8.13
DIEPAMHR + Lambda-Cyhalothrin 0.6875 ng/L (1/8 TU) + Cyfluthrin 0.5 ng/L (1/8 TU) + Methanol	350	20.7	23.2	5.8	8.2	7.52	8.17
DIEPAMHR + Lambda-Cyhalothrin 1.375 ng/L ng/L (1/4 TU) + Cyfluthrin 1 ng/L (1/4 TU) + Methanol	340	20.3	23.3	5.8	8.2	7.54	8.16
DIEPAMHR + Lambda-Cyhalothrin 1.375 ng/L ng/L (1/4 TU) + Cyfluthrin 2 ng/L (1/2 TU) + Methanol	340	20.1	23.7	5.5	8.6	7.52	8.13
DIEPAMHR + Lambda-Cyhalothrin 1.375 ng/L ng/L (1/4 TU) + Cyfluthrin 3 ng/L (3/4 TU) + Methanol	343	20.1	23.7	5.9	8.2	7.53	8.11
DIEPAMHR + Lambda-Cyhalothrin 2.75 ng/L (1/2 TU) + Cyfluthrin 1 ng/L (1/4 TU) + Methanol	344	20.0	23.5	5.6	8.3	7.45	8.13
DIEPAMHR + Lambda-Cyhalothrin 2.75 ng/L (1/2 TU) + Cyfluthrin 2 ng/L (1/2 TU) + Methanol	347	22.5	23.6	5.7	8.3	7.53	8.13
DIEPAMHR + Lambda-Cyhalothrin 2.75 ng/L (1/2 TU) + Cyfluthrin 3 ng/L (3/4 TU) + Methanol	343	19.9	23.2	5.9	8.2	7.54	8.16
DIEPAMHR + Lambda-Cyhalothrin 4.125 ng/L (3/4 TU) + Cyfluthrin 1 ng/L (1/4 TU) + Methanol	350	22.2	23.6	5.5	8.3	7.50	8.08
DIEPAMHR + Lambda-Cyhalothrin 4.125 ng/L (3/4 TU) + Cyfluthrin 2 ng/L (1/2 TU) + Methanol	344	20.5	23.0	5.8	8.2	7.56	8.05
DIEPAMHR + Lambda-Cyhalothrin 4.125 ng/L (3/4 TU) + Cyfluthrin 3ng/L (3/4 TU) + Methanol	342	20.1	23.6	5.7	8.2	7.55	8.12
DIEPAMHR + Lambda-Cyhalothrin 5.5 ng/L (1 TU) + Cyfluthrin 4 ng/L (1 TU) + Methanol	343	20.9	23.4	5.4	8.2	7.47	8.14
DIEPAMHR + Lambda-Cyhalothrin 8.25 ng/L (1.5 TU) + Cyfluthrin 6 ng/L (1.5 TU) + Methanol	338	19.8	23.5	5.5	8.3	7.54	8.12

Table A15. Summary of an acute *H. azteca* water column toxicity test initiated on September 16, 2011 examining the toxicity of lambda-cyhalothrin x permethrin mixtures.

Treatment	4-day Survival ( )	
	Mean	SE
DIEPAMHR	100	0
DIEPAMHR + Methanol	100	0
DIEPAMHR + Lambda-Cyhalothrin 1.75 ng/L + Methanol	98	4
DIEPAMHR + Lambda-Cyhalothrin 3.5 ng/L + Methanol	90	10
DIEPAMHR + Lambda-Cyhalothrin 5.25 ng/L + Methanol	69	18
DIEPAMHR + Lambda-Cyhalothrin 7 ng/L + Methanol	42	20
DIEPAMHR + Lambda-Cyhalothrin 10.5 ng/L + Methanol	6	5
DIEPAMHR + Lambda-Cyhalothrin 14 ng/L + Methanol	4	10
DIEPAMHR + Lambda-Cyhalothrin 21 ng/L + Methanol	0	0
DIEPAMHR + Permethrin 12.5 ng/L + Methanol	98	4
DIEPAMHR + Permethrin 25 ng/L + Methanol	92	8
DIEPAMHR + Permethrin 37.5 ng/L + Methanol	90	11
DIEPAMHR + Permethrin 50 ng/L + Methanol	73	29
DIEPAMHR + Permethrin 75 ng/L + Methanol	44	13
DIEPAMHR + Permethrin 100 ng/L + Methanol	13	12
DIEPAMHR + Permethrin 150 ng/L + Methanol	2	4
DIEPAMHR + Lambda-Cyhalothrin .875 ng/L + Permethrin 6.25 ng/L + Methanol	100	0
DIEPAMHR + Lambda-Cyhalothrin 1.75 ng/L + Permethrin 12.5 ng/L + Methanol	96	9
DIEPAMHR + Lambda-Cyhalothrin 1.75 ng/L + Permethrin 25 ng/L + Methanol	86	10
DIEPAMHR + Lambda-Cyhalothrin 1.75 ng/L + Permethrin 50 ng/L + Methanol	41	19
DIEPAMHR + Lambda-Cyhalothrin 3.5 ng/L + Permethrin 12.5 ng/L + Methanol	80	16
DIEPAMHR + Lambda-Cyhalothrin 3.5 ng/L + Permethrin 25 ng/L + Methanol	62	33
DIEPAMHR + Lambda-Cyhalothrin 3.5 ng/L + Permethrin 50 ng/L + Methanol	44	22
DIEPAMHR + Lambda-Cyhalothrin 7 ng/L + Permethrin 12.5 ng/L + Methanol	40	14
DIEPAMHR + Lambda-Cyhalothrin 7 ng/L + Permethrin 25 ng/L + Methanol	21	13
DIEPAMHR + Lambda-Cyhalothrin 7 ng/L + Permethrin 50 ng/L + Methanol	2	4
DIEPAMHR + Lambda-Cyhalothrin 5.25 ng/L + Permethrin 37.5 ng/L + Methanol	30	21
DIEPAMHR + Lambda-Cyhalothrin 10.5 ng/L + Permethrin 75 ng/L + Methanol	2	4

Table A16. Summary of water chemistry measured in a *H. azteca* toxicity test initiated on September 16, 2011 examining the toxicity of lambda-cyhalothrin x permethrin mixtures.

Treatment	Laboratory Chemistry						
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH
DIEPAMHR	343	21.7	22.3	7.1	8.4	7.61	7.96
DIEPAMHR + Methanol	339	21.8	23.2	6.5	8.5	7.54	7.97
DIEPAMHR + Lambda-Cyhalothrin 1.75 ng/L + Methanol	345	22.0	22.8	7.0	8.2	7.64	8.06
DIEPAMHR + Lambda-Cyhalothrin 3.5 ng/L + Methanol	344	21.9	23.7	6.7	8.5	7.58	7.99
DIEPAMHR + Lambda-Cyhalothrin 5.25 ng/L + Methanol	341	22.0	22.9	7.0	8.4	7.60	8.03
DIEPAMHR + Lambda-Cyhalothrin 7 ng/L + Methanol	342	21.5	22.3	6.9	8.5	7.60	8.08
DIEPAMHR + Lambda-Cyhalothrin 10.5 ng/L + Methanol	335	22.3	22.9	5.4	8.4	7.46	8.06
DIEPAMHR + Lambda-Cyhalothrin 14 ng/L + Methanol	340	21.9	22.5	6.2	8.5	7.56	8.04
DIEPAMHR + Lambda-Cyhalothrin 21 ng/L + Methanol	344	22.1	23.3	5.8	8.3	7.50	7.98
DIEPAMHR + Permethrin 12.5 ng/L + Methanol	341	21.7	23.4	5.8	8.4	7.50	8.01
DIEPAMHR + Permethrin 25 ng/L + Methanol	339	22.0	22.5	6.8	8.5	7.62	8.04
DIEPAMHR + Permethrin 37.5 ng/L + Methanol	344	22.4	23.5	6.5	8.4	7.57	8.00
DIEPAMHR + Permethrin 50 ng/L + Methanol	342	21.7	22.7	6.3	8.5	7.55	8.01
DIEPAMHR + Permethrin 75 ng/L + Methanol	344	22.1	23.3	6.8	8.4	7.60	8.04
DIEPAMHR + Permethrin 100 ng/L + Methanol	342	22.2	22.5	6.6	8.5	7.61	8.02
DIEPAMHR + Permethrin 150 ng/L + Methanol	346	22.1	22.4	7.0	8.4	7.10	8.03
DIEPAMHR + Lambda-Cyhalothrin .875 ng/L + Permethrin 6.25 ng/L +	343	22.0	22.7	6.3	8.5	7.58	8.04
DIEPAMHR + Lambda-Cyhalothrin 1.75 ng/L + Permethrin 12.5 ng/L +	343	22.1	23.4	6.4	8.4	7.51	8.00
DIEPAMHR + Lambda-Cyhalothrin 1.75 ng/L + Permethrin 25 ng/L + Methanol	344	22.3	23.0	6.4	8.4	7.55	8.12
DIEPAMHR + Lambda-Cyhalothrin 1.75 ng/L + Permethrin 50 ng/L + Methanol	344	22.4	23.3	5.6	8.4	7.52	8.01
DIEPAMHR + Lambda-Cyhalothrin 3.5 ng/L + Permethrin 12.5 ng/L + Methanol	340	22.1	22.6	6.3	8.5	7.54	8.07
DIEPAMHR + Lambda-Cyhalothrin 3.5 ng/L + Permethrin 25 ng/L + Methanol	346	21.9	22.7	6.9	8.4	7.48	8.11
DIEPAMHR + Lambda-Cyhalothrin 3.5 ng/L + Permethrin 50 ng/L + Methanol	340	22.1	23.2	6.4	8.3	7.55	8.07
DIEPAMHR + Lambda-Cyhalothrin 7 ng/L + Permethrin 12.5 ng/L + Methanol	342	21.9	22.9	7.0	8.3	7.66	8.04
DIEPAMHR + Lambda-Cyhalothrin 7 ng/L + Permethrin 25 ng/L + Methanol	343	22.4	23.1	5.6	8.4	7.50	8.03
DIEPAMHR + Lambda-Cyhalothrin 7 ng/L + Permethrin 50 ng/L + Methanol	344	22.4	23.4	6.8	8.4	7.60	8.03
DIEPAMHR + Lambda-Cyhalothrin 5.25 ng/L + Permethrin 37.5 ng/L +	339	21.7	22.7	5.8	8.6	7.53	8.04
DIEPAMHR + Lambda-Cyhalothrin 10.5 ng/L + Permethrin 75 ng/L + Methanol	346	22.0	22.8	6.7	8.5	7.59	8.03

Table A17. Summary of an acute *H. azteca* water column toxicity test initiated on October 18, 2011 examining the toxicity of bifenthrin x lambda-cyhalothrin mixtures.

Treatment	4-day Survival ( )	
	Mean	SE
DIEPAMHR	100	0
DIEPAMHR + Methanol	100	0
DIEPAMHR + Lambda-Cyhalothrin 1.625 ng/L (1/4 TU) + Methanol	94	9
DIEPAMHR + Lambda-Cyhalothrin 3.25 ng/L (1/2 TU) + Methanol	56	29
DIEPAMHR + Lambda-Cyhalothrin 4.875 ng/L (3/4 TU) + Methanol	58	22
DIEPAMHR + Lambda-Cyhalothrin 6.5 ng/L (1 TU) + Methanol	20	10
DIEPAMHR + Lambda-Cyhalothrin 9.75 ng/L (1.5 TU) + Methanol	0	0
DIEPAMHR + Lambda-Cyhalothrin 13 ng/L (2 TU) + Methanol	2	4
DIEPAMHR + Lambda-Cyhalothrin 19.5 ng/L (3 TU) + Methanol	0	0
DIEPAMHR + Bifenthrin 1.5 ng/L (1/4 TU) + Methanol	100	0
DIEPAMHR + Bifenthrin 3 ng/L (1/2 TU) + Methanol	94	5
DIEPAMHR + Bifenthrin 4.5 ng/L (36/4 TU) + Methanol	84	11
DIEPAMHR + Bifenthrin 6 ng/L (1 TU) + Methanol	56	21
DIEPAMHR + Bifenthrin 9 ng/L (1.5 TU) + Methanol	24	9
DIEPAMHR + Bifenthrin 12 ng/L (2 TU) + Methanol	2	4
DIEPAMHR + Bifenthrin 18 ng/L (3 TU) + Methanol	0	0
DIEPAMHR + Lambda-Cyhalothrin 0.8125 ng/L (1/8 TU) + Bifenthrin 0.75 ng/L (1/8 TU) + Methanol	98	4
DIEPAMHR + Lambda-Cyhalothrin 1.625 ng/L (1/4 TU) + Bifenthrin 1.5 ng/L (1/4 TU) + Methanol	86	11
DIEPAMHR + Lambda-Cyhalothrin 1.625 ng/L (1/4 TU) + Bifenthrin 3 ng/L (1/2 TU) + Methanol	78	19
DIEPAMHR + Lambda-Cyhalothrin 1.625 ng/L (1/4 TU) + Bifenthrin 6 ng/L (1 TU) + Methanol	52	22
DIEPAMHR + Lambda-Cyhalothrin 3.25 ng/L (1/2 TU) + Bifenthrin 1.5 ng/L (1/4 TU) + Methanol	54	17
DIEPAMHR + Lambda-Cyhalothrin 3.25 ng/L (1/2 TU) + Bifenthrin 3 ng/L (1/2 TU) + Methanol	44	11
DIEPAMHR + Lambda-Cyhalothrin 3.25 ng/L (1/2 TU) + Bifenthrin 6 ng/L (1 TU) + Methanol	12	16
DIEPAMHR + Lambda-Cyhalothrin 6.5 ng/L (1 TU) + Bifenthrin 1.5 ng/L (1/4 TU) + Methanol	8	8
DIEPAMHR + Lambda-Cyhalothrin 6.5 ng/L (1 TU) + Bifenthrin 3 ng/L (1/2 TU) + Methanol	4	5
DIEPAMHR + Lambda-Cyhalothrin 4.875 ng/L (3/4 TU) + Bifenthrin 4.5 ng/L (3/4 TU) + Methanol	12	11
DIEPAMHR + Lambda-Cyhalothrin 6.5 ng/L (1 TU) + Bifenthrin 6 ng/L (1 TU) + Methanol	0	0
DIEPAMHR + Lambda-Cyhalothrin 9.75 ng/L (1.5 TU) + Bifenthrin 9 ng/L (1.5 TU) + Methanol	0	0

Table A18. Summary of water chemistry measured in a *H. azteca* toxicity test initiated on October 18, 2011 examining the toxicity of bifenthrin x lambda-cyhalothrin mixtures.

Treatment	Laboratory Chemistry						
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH
DIEPAMHR	317	21.2	23.5	6.6	8.3	7.69	8.18
DIEPAMHR + Methanol	315	21.6	23.6	4.7	8.4	7.40	8.15
DIEPAMHR + Lambda-Cyhalothrin 1.625 ng/L (1/4 TU) + Methanol	315	21.8	23.7	4.1	8.2	7.39	8.12
DIEPAMHR + Lambda-Cyhalothrin 3.25 ng/L (1/2 TU) + Methanol	317	21.8	23.5	4.4	8.5	7.43	8.16
DIEPAMHR + Lambda-Cyhalothrin 4.875 ng/L (3/4 TU) + Methanol	317	21.9	23.6	4.2	8.3	7.43	8.15
DIEPAMHR + Lambda-Cyhalothrin 6.5 ng/L (1 TU) + Methanol	318	22.0	23.5	4.8	8.2	7.48	8.15
DIEPAMHR + Lambda-Cyhalothrin 9.75 ng/L (1.5 TU) + Methanol	316	22.1	23.5	4.0	8.4	7.41	8.15
DIEPAMHR + Lambda-Cyhalothrin 13 ng/L (2 TU) + Methanol	321	21.3	23.5	4.4	8.4	7.53	8.15
DIEPAMHR + Lambda-Cyhalothrin 19.5 ng/L (3 TU) + Methanol	316	21.2	23.5	4.3	8.4	7.41	8.16
DIEPAMHR + Bifenthrin 1.5 ng/L (1/4 TU) + Methanol	318	21.5	23.6	4.2	8.3	7.40	8.14
DIEPAMHR + Bifenthrin 3 ng/L (1/2 TU) + Methanol	319	21.6	23.5	4.3	8.4	7.42	8.17
DIEPAMHR + Bifenthrin 4.5 ng/L (36/4 TU) + Methanol	316	21.7	23.3	4.1	8.3	7.39	8.14
DIEPAMHR + Bifenthrin 6 ng/L (1 TU) + Methanol	317	21.9	23.4	4.8	8.4	7.46	8.14
DIEPAMHR + Bifenthrin 9 ng/L (1.5 TU) + Methanol	319	21.9	23.4	4.8	8.3	7.48	8.13
DIEPAMHR + Bifenthrin 12 ng/L (2 TU) + Methanol	319	22.0	23.5	4.5	8.5	7.44	8.13
DIEPAMHR + Bifenthrin 18 ng/L (3 TU) + Methanol	318	21.3	23.5	4.2	8.4	7.40	8.14
DIEPAMHR + Lambda-Cyhalothrin 0.8125 ng/L (1/8 TU) + Bifenthrin 0.75 ng/L (1/8 TU) + Methanol	318	21.4	23.4	4.4	8.5	7.42	8.09
DIEPAMHR + Lambda-Cyhalothrin 1.625 ng/L (1/4 TU) + Bifenthrin 1.5 ng/L (1/4 TU) + Methanol	317	21.6	23.4	4.0	8.4	7.38	8.16
DIEPAMHR + Lambda-Cyhalothrin 1.625 ng/L (1/4 TU) + Bifenthrin 3 ng/L (1/2 TU) + Methanol	317	21.8	23.3	4.6	8.4	7.44	8.13
DIEPAMHR + Lambda-Cyhalothrin 1.625 ng/L (1/4 TU) + Bifenthrin 6 ng/L (1 TU) + Methanol	316	21.9	23.3	5.2	8.3	7.50	8.14
DIEPAMHR + Lambda-Cyhalothrin 3.25 ng/L (1/2 TU) + Bifenthrin 1.5 ng/L (1/4 TU) + Methanol	319	21.9	23.1	4.5	8.4	7.33	8.16
DIEPAMHR + Lambda-Cyhalothrin 3.25 ng/L (1/2 TU) + Bifenthrin 3 ng/L (1/2 TU) + Methanol	318	22.0	23.2	4.6	8.5	7.41	8.14
DIEPAMHR + Lambda-Cyhalothrin 3.25 ng/L (1/2 TU) + Bifenthrin 6 ng/L (1 TU) + Methanol	319	22.1	23.2	4.4	8.4	7.40	8.15
DIEPAMHR + Lambda-Cyhalothrin 6.5 ng/L (1 TU) + Bifenthrin 1.5 ng/L (1/4 TU) + Methanol	316	21.5	23.2	4.0	8.5	7.40	8.15
DIEPAMHR + Lambda-Cyhalothrin 6.5 ng/L (1 TU) + Bifenthrin 3 ng/L (1/2 TU) + Methanol	318	21.6	23.2	4.1	8.5	7.38	8.12
DIEPAMHR + Lambda-Cyhalothrin 4.875 ng/L (3/4 TU) + Bifenthrin 4.5 ng/L (3/4 TU) + Methanol	318	21.9	23.1	4.0	8.4	7.38	8.16
DIEPAMHR + Lambda-Cyhalothrin 6.5 ng/L (1 TU) + Bifenthrin 6 ng/L (1 TU) + Methanol	318	21.9	23.2	4.9	8.4	7.46	8.16
DIEPAMHR + Lambda-Cyhalothrin 9.75 ng/L (1.5 TU) + Bifenthrin 9 ng/L (1.5 TU) + Methanol	317	22.0	23.2	4.2	8.4	7.41	8.13

Appendix II

RESPONSE TO COMMENTS ON DRAFT FINAL REPORT SUBMITTED 02-29-2012

I. Response to Comments by Tessa Fojut, Central Valley Regional Water Quality Control Board

1. p. 5 (third full sentence on page) "The model of CA calculates the concentration required to elicit a give response based on the proportional contribution from each chemical (*pi*) in the mixture and their individual effects (*ECxi*).

   "give" should be "given"

   This comment is addressed on page 7; "give" changed to "given" as recommended.

2. p.7 fig. 1. The shading does not show up clearly on my document as "striped" or "checkered," they just look like light gray and dark gray, not sure if it's a resolution problem, or if you just want to pick different fills, or just say light and dark gray.

   This comment is addressed on page 9. Figure shading simplified to light, dark and none.

3. p. 7. Section 3.2.1. The acronyms UCD, AHP, and SOP should be defined on the first use in the report.

   This comment is addressed on page 9. Acronyms were defined or removed as needed.

4. p. 8. "Beakers were haphazardly relocated…"
   Does this mean that locations were randomly assigned? Were they randomly assigned by a random number generator, or just by the scientist?

   This comment is addressed on page 10. To more clearly illustrate the type of randomization, the wording was changed to "Beakers were relocated by the investigator daily (haphazardly), to avoid spatial effects on treatments."

5. Also in this sentence: It says that the organisms were fed after renewal of water, feeding after renewal increases the chance of an ingestion exposure route – this possibility should be mentioned somewhere in the report. In other studies I have seen organisms are fed shortly (~2 hr) before renewal to decrease the possibility of exposure via ingestion. Or perhaps this was done intentionally because ingestion is a possible exposure route in the environment - it would be helpful to explain the exposure routes in the experiment and what might happen in the field.

The protocol used in this study was developed as such because H. azteca proved to be highly sensitive to starvation. Initial tests were conducted with feeding occurring just 2-3 hours prior to renewal (as recommended by the reviewer), however organisms displayed hypersensitivity to chemical exposures and were exhibiting cannibalism, therefore the protocol was modified so that food was always available in the treatment beakers (i.e. fed after renewal). The methods applied will likely affect the route of exposure, as mentioned by the reviewer, such that oral ingestion rather than dermal absorption would dominate in a water column containing food, however it is the author's belief that the procedures used are representative of what occurs in the environment, given that any contaminant input will occur in a mixture of food, nutrients, and water, and therefore results are not compromised.

6. p. 13. Tables 3 and 4. It's surprising that bifenthrin alone LC50s for 4-day and 10-day tests cover the same range of concentrations – I would expect 10-day LC50s to be lower. Do you have any insights into why the values are so similar?

   This comment is addressed on page 15, Tables 4 and 5. The 10-day LC50 for bifenthrin under the bifenthrin x copper mixture study was a typographical error and has been amended to the correct LC50 value.

7. Were separate single chemical toxicity tests conducted simultaneously with each binary toxicity test – could you describe this in more detail in the Experimental Design section (3.1)? Is that why single chemical LC50s vary across a given chemical? (e.g., I expected all of the 4-day bifenthrin alone LC50s to be the same, but it seems like you ran 6 separate 4-day bifenthrin alone toxicity tests according to Table 3 – is that correct?)

   To address this comment, the authors have more clearly explained the experimental design in the text on page 9. The experimental design used (as illustrated in Figure 2) includes 7 or more single chemical exposures within each mixture experiment. It is important that these occur within the same test to avoid any confounding affects because of the variability between experiments. Best-fit curves are fit to the concentration responses for each individual chemical and are used to generate the predictive models of Concentration Addition and Independent Action, therefore the accuracy of the single chemical dose-response curves are essential to correctly compare results to the two additive models of CA and IA.

8. p. 14. Section 4.2.2. Replace "contrastingly" with "in contrast"

   This comment is addressed on page 16, under section 4.2.2; "Contrastingly" was replaced with "In contrast."

9. "The level of interaction can be further investigated by comparing mixture date to the two additive models, CA and IA (Figures 2 – 15) as shown in the following section."

   Should "date" be "data"?

This comment is addressed on page 16, under section 4.2.2; "date" replaced with "data."

10. p. 13 and 14. Tables 5 and 6. Please state the p-value ranges that are designated as significant or highly significant.

    This comment is addressed on page 15 and 16, Tables 6 and 7 (and all other tables containing p-values). P-values are defined for significant and highly significant designations.

11. p. 14. The label Figure 2-3 is confusing, maybe change it to say Figures 2 and 3 or just call the whole thing Figure 2 and label each part as "a" and "b". This goes for all figures. Also, figure resolution didn't show up very well for me, you could try increasing resolution when you import them.

    This comment is addressed across figures 3-18. Figures displayed across the page are labeled as parts of one figure designated with letters a, b or c. The authors will make an effort to enhance the figure resolution, however this may be problematic because of the limit of size in the electronic version transmitted by email. The submitted hard copy and electronic copy will use the best resolution possible.

12. p. 19. Last sentence. "It must be concluded…" The authors chose to make this conclusion, and other researchers might not, so it's not that it "must be concluded," but that "the authors concluded…".

    This comment is addressed in the last sentence on page 21. "It must be concluded" was changed to "The authors concluded."

13. p. 23. Table 7. There is a footnote labeled "a" below the table, but there is nothing in the table with this footnote.

    This comment is addressed in Table 8. The label "a" was added to the data where appropriate.

14. p. 27. Table 7. This table is also labeled as Table 7 – there is another one on p. 23. It seems the number and title just need to be corrected because it shows different data.

    This comment is addressed such that the table of concern was renamed as "Table 9" on page 28 (and the subsequent table nomenclature also adjusted).

15. p. 28.
    "Excluding appropriate assumptions in an analysis can bias results such that the interpretation can be significantly altered. These factors are likely to be the major point of contingence between results from the two levels of analysis applied in this study."

These sentences are not clear to me - it may just be that this is not my area of expertise, but clarification would be helpful for people like me. When you say "excluding appropriate assumptions" are you saying the CA and IA models have fewer assumptions than GLM, and GLM gives less biased results because it includes these "appropriate assumptions"? If so, what are these specific assumptions and why do they make such a difference?
In the second sentence, what are "these factors" referring to – the biased results or the excluded assumptions or something else? With saying that they are "the major point of contingence between results," are you saying this is why there is overlap or similarity between the results?

It would be interesting to me if you included some discussion of what other researchers' results have been with mixtures of similar pesticides (e.g., Brander, Barata et al., Trimble et al.) and which models they have fit to their data.

The Discussion section of this report (pages 31-34) has been revised to more clearly describe the discrepancies in conclusions from either analytical method and compare results to previous mixture studies of similar chemical combinations.

II. Response to Comments by Dr. Afiqur Khan, Western Plant Health Association

16. On behalf of the Western Plant Health Association (WPHA) I am writing to provide comments on the recently released report "Toxicity and Interactions among Common Aquatic Contaminants in Binary Mixtures Contracts 09-093-150 and 10-067-150." WPHA appreciates the opportunity to provide comments on the subject mentioned in the draft final report. WPHA represents the interest of crop protection, and Fertilizer Manufactures, distributors, agricultural biotechnology providers, and agricultural retailers in California, Arizona and Hawaii.

    WPHA appreciates the State Water Resources Control Board's attempt to provide more transparent and scientific approaches to characterize and quantify the toxicity of binary combinations of aquatic contaminants. However, we believe current pesticide risk assessments are sufficiently conservative so that the potential for mixture toxicity is adequately considered. The conclusion of the European Centre for Eco-toxicology and Toxicology of Chemicals in a recent analysis of the risks of environmental mixtures was that *"There is no evidence to support an overhaul of current risk assessment schemes or any additional conservatism in the assessment factors in prospective risk assessment schemes"*. For the vast majority of chemical mixtures, including mixtures containing chemicals with a similar mode of action, an assumption of additivity of toxicity provides a conservative estimate of potential response in exposed organisms. Several recent reviews provide excellent summaries of the relevant literature.

Results from the reported study agree with the statement that an assumption of additive toxicity provides a conservative estimate of potential response in H. azteca for the mixtures tested.

17. Synergism of chemical activity, leading to an observed response greatly exceeding what would be expected from dose addition, is a very rare event. The potential for interaction depends on the mechanism of action and pharmacokinetics; the absorption, metabolism, disposition, and elimination of the compounds, as well as their likelihood of co-occurrence at levels high enough for any observed interaction to occur (Moore and Teed, 2012; Teed and Moore, 2012). The potential interactions range from none, to additive, to synergistic, to potentiate, to antagonism (Rodney et al., 2012). Laboratory experiments with mixtures suggest that, in the wide majority of instances, dose addition conservatively estimates potential toxicity. Dose addition typically overestimates the toxicity of mixtures of chemicals with dissimilar modes of action. An additive response also depends on the relative potency of the constituents of the mixtures, similarities and differences in pharmacokinetics and the selected dose relative to the effective dose. A few pesticides have been shown to exhibit synergistic or potentiating toxic effects. These effects, however, occur at levels that are not routinely seen in the environment.

    There were no synergistic effects found in any contaminant mixtures tested in this study, therefore the authors cannot speak to the comments regarding these effects. The authors agree that dose addition typically overestimates the toxicity of mixtures of chemicals with dissimilar modes of actions, however the reported study indicates that the neurotoxic pesticides bifenthrin and chlorpyrifos, though they target different biological sites, are concentration additive on a chronic and sublethal level.

18. Co-occurrence of pesticide residues in the field is often observed at levels below toxicity thresholds. For example, according to USGS National Water Quality Assessment (NWQA) program data "more than 6,000 unique 5-compound mixtures were found in samples from agricultural streams"; this number decreases to "less than 100 when only concentrations greater than 0.1 ppb are considered". The NWQA program is an on-going, far reaching monitoring program across the U.S. that has collected tens of thousands of samples. The program has found that, "pesticide concentrations are below 0.1 parts per billion (ppb) in 80% of the surface water samples, and below 1 ppb in 95% of the samples".

    Pyrethroid and organophosphate pesticides are highly toxic to aquatic invertebrates at concentrations in the low parts per trillion (ppt or ng/L) range. Pyrethroids, even in the absence of potentiating agents, can be lethal at concentrations as low as to 3 ppt, individually, and 1 ppt or less in a binary mixture (refer to Tables 4 and 5 of this report for LC50 values of individual chemicals and their mixture), therefore 20% of environmental sample water containing pesticide concentrations at or above 100 ppt is cause for concern. This study incorporated concentrations of contaminants that are environmentally relevant. For multiple pesticides tested, average detected concentrations were actually above the average concentration tested in the lab (CEDEN database 1989 – 2010).

19. The concentrations of pesticides in mixture toxicity tests are nearly always orders of magnitude above those observed in the environment. If chemicals in a mixture (i) do not have the same modes of action, or (ii) do not include a potentiating agent and a corresponding parent chemical that degrades to a more toxic metabolite, then the toxic effects of the mixture can be predicted based upon the toxicity and exposure level or dose of the individual chemicals.

    These comments are addressed above.

20. "The Toxicity and Interactions among Common Aquatic Contaminants in Binary Mixtures" report concludes that toxicity of all the mixtures studied was additive or close to additive. WPHA believes that this conclusion duplicates the conclusion of numerous literature references.

    Previous studies have investigated pesticide and contaminant interactions, however this study is novel in its robust experimental design and statistical approach. Please refer to the Discussion section starting on page 31 for details on how the reported study relates to and builds upon the current literature.

21. WPHA appreciates the efforts of the research team in conducting this study; however, we find that there are some basic problems with the research or report that makes its use problematic. In reviewing the report we found the background was not well described on specific biologically active compounds. Details of experimental procedure such as design, methodology, chemical analytical procedure was not transparent. For example, the dosing procedure is not described clearly, and the experimental conditions show variations that indicate the test system was not well controlled. We are concerned that due to the lack of transparency as well as figure captions and numbers were not well presented, that the report does not contain sufficient detail to support the conclusions of the researchers.

    The authors have made a substantial effort to report all aspects of this study including the design, methodology and chemical analytical procedures. Please refer to section 4.1, page 12 for details on the materials, methods and quality control procedures used in this study. Dosing conditions are described under the third paragraph of section 3.2.1. It is the authors' opinion that the water quality parameters of all treatments in this study were within the physiological tolerances of H. azteca. Figure captions and numbers have been amended as needed to be as clear as possible.

References:

ECETOC. 2011. Development of guidance for assessing the impact of chemical mixtures in the environment. European Centre for Eco-toxicology and Toxicology of Chemicals Technical Report No. 111.

Moore, D.R.J. and R.S. Teed. 2012. Risks of carbamate and organophosphate pesticide mixtures to salmon in the Pacific Northwest. IEAM (in review).

Rodney, S.I., Teed, R.S. and D.R.J. Moore. 2012. Toxicity of pesticide mixtures: A Review. HERA (in preparation).

Teed, R.S. and D.R.J. Moore. 2012. Occurrence and toxicity of pesticide mixtures in surface waters of the Central Valley of California. HERA (in review).