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TOXICITY OF A DISSOLVED PYRETHROID MIXTURE TO *HYALELLA AZTECA* AT ENVIRONMENTALLY RELEVANT CONCENTRATIONS

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Abstract—Use of pyrethroid pesticides, which are highly toxic to aquatic organisms, has increased substantially over the past decade. In 2006, the pyrethroid pesticides cyfluthrin and permethrin were measured in Sacramento–San Joaquin (SSJ) Delta (CA, USA) water at 5 and 24 ng/L (pptr), respectively. To elucidate any interactions between the two pyrethroids, a 10-d laboratory exposure was performed with 7- to 14-d-old amphipods (*Hyalella azteca*). Cyfluthrin and permethrin were tested singly and in combination at detected levels and also at half and twice the detected levels, both with and without the addition of 25 ppb of piperonyl butoxide (PBO). Mortality in all treatments was significantly higher than in controls, with the median lethal concentration (LC50) for permethrin with PBO (13.9 ng/L) and the LC50s with and without PBO for cyfluthrin (5.7 and 2.9 ng/L, respectively) at or below levels measured in SSJ Delta water samples. The LC50 for permethrin alone was estimated to be 48.9 ng/L. To evaluate combined toxicity, logistic regression models containing terms for concentrations of cyfluthrin, permethrin, and PBO, as well as models containing all possible combinations of these terms and interactions, were run and compared using Akaike's information criterion. The most parsimonious set of models indicated slight antagonism between cyfluthrin and permethrin. Results indicate that a dissolved mixture of cyfluthrin and permethrin is toxic at environmentally relevant concentrations in the water column.

Keywords—Hyalella Pyrethroids Mixture toxicity Akaike's information criterion Antagonism

INTRODUCTION

Pyrethroid pesticide use in the United States has increased substantially over the past decade as organophosphate pesticides are being gradually phased out in a number of states, including California [1–4]. Pyrethroids, which are not acutely toxic to mammals at concentrations applied or found in the environment, are highly toxic to fish and aquatic invertebrates at these levels because of a combination of factors, including the similar physiology of aquatic invertebrates and insects and the potential for osmoregulatory disruption in fish [2,4,5]. These pesticides disrupt the nervous system by binding to and prolonging the opening of voltage-dependent ion channels, the consequences of which are convulsions, paralysis, and death [2,4,6]. A toxic response to pyrethroids has been observed at the parts-per-trillion level in crustaceans, fish, and amphibians [2,4,7]. This is a special cause for concern in the Central Valley of northern California (USA), where aquatic ecosystems are surrounded by an area of intense agriculture.

Pyrethroids are highly lipophilic and tend to bind to sediments, and it has been argued that this decreases substantially their toxicity in aquatic environments [8]. These compounds may remain in the water column for days to weeks [9] after introduction, however, and may be soluble enough to render biological harm to vulnerable organisms [2,4]. Because of these chemical properties, pyrethroids may be harmful to both pelagic and benthic species.

The objective of the present study was to use a local, sensitive species to evaluate the toxicity of environmentally relevant concentrations as well as mixtures of two pyrethroid pesticides (cyfluthrin and permethrin) detected in the water column of the Sacramento–San Joaquin (SSJ) Delta (USA). *Hyalella azteca*, an epibenthic amphipod prevalent in northern California's SSJ Delta (a diffuse body of estuaries and sloughs that receives agricultural and urban runoff from the Central Valley of northern California) is exposed to these pesticides via both contaminated water and sediments. It has been confirmed that *H. azteca* is highly sensitive to sediment-bound pyrethroids [10,11].

Sediment-bound permethrin and cyfluthrin are toxic to *H. azteca* at the parts-per-billion range (57 and 12.5 ng/g organic carbon, respectively), which is well within the levels measured in this region [12,13]. Of the top-five pyrethroids in use in the region, permethrin (a type I pyrethroid) is the most frequently used but also the least toxic. Cyfluthrin (a type II pyrethroid) is the fifth most used but ranks second in toxicity [2,4]. Cyfluthrin (Fig. 1), like other type II pyrethroids, is chemically modified via the addition of various functional groups (e.g., cyano or halogen groups), and as such, it hydrolyzes more slowly than type I pyrethroids, resulting in a toxic potency up to 20-fold greater than that of permethrin (Fig. 2) [8]. A number of studies have used *H. azteca* to examine the toxicity of pyrethroids bound to sediments [11,13–15], including a recent study that evaluated a mixture of cyfluthrin and lambda-cyhalothrin in a constructed wetland using *H. azteca* [16]. To our knowledge, however, no study has yet evaluated the combined toxicity and interaction of cyfluthrin and permethrin at levels measured in the water column. Also, although numerous studies regarding the toxicity of individual pyrethroids with

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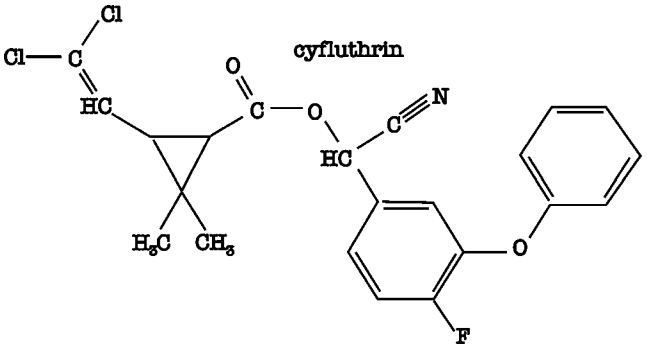

Fig. 1. Cyfluthrin, a type II pyrethroid pesticide. The cyano group stabilizes the ester linkage, making the molecule more resistant to metabolic enzymes.

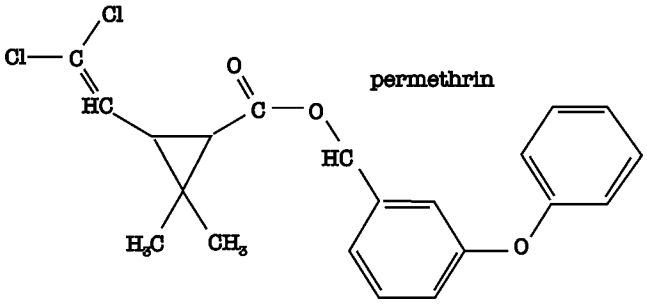

Fig. 2. Permethrin, a type I pyrethroid pesticide. Type I pyrethroids are more susceptible to breakage at the ester linkage than type II pyrethroids are.

and without the pesticide synergist piperonyl butoxide (PBO) have been conducted [2,4,12,17,18], little is known about the combined toxicity of specific type I and type II pyrethroids with or without the addition of PBO.

In the present study, we report the first investigation, to our knowledge, of the possibility of additivity, synergism, antagonism, or competitive agonism [19] between cyfluthrin and permethrin with and without PBO at concentrations relevant to water-column exposures in the SSJ Delta (5 and 24 ppt, respectively). Additionally, whereas the interactions of binary mixtures have been evaluated and modeled in previously published studies [19–21] (http//www.ecologyandsociety.org/vol9/iss6/art1]), the present study may be the first time that Akaike's information criteria, a widely utilized model selection method [22], has been used to select the most parsimonious set of models for mixture interaction.

MATERIALS AND METHODS

Materials

A water sample collected on August 22, 2006, at site 902 in the SSJ Delta (38°01′09.1″N, 121°34′55.9″W) caused a 52% reduction of *H. azteca* growth after PBO addition (I. Werner, unpublished data). Chemical analysis of the whole-water sample for the pyrethroids bifenthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, fenvalerate, lambda-cyhalothrin, and permethrin revealed the presence of 0.005 μg/L (ppb) of cyfluthrin and 0.024 μg/L (ppb) of permethrin. To verify that these compounds could be responsible for the observed negative effects on *H. azteca* growth, a laboratory experiment was performed in 2007 and repeated in 2008.

Cyfluthrin (Baythroid™, 98% mix of isomers) (Fig. 1) and permethrin (31.8% *cis*, 67.4% *trans*) (Fig. 2) were purchased from Chem Service in 2007. Stock solutions were made in methanol and spiked into laboratory control water consisting of deionized water amended to U.S. Environmental Protection Agency moderately hard standards [23]. Following an experimental design as described by Ferreira et al. [21] and Cassee et al. [24], treatments were spiked to yield nominal concentrations ranging from 0.5- to 2-fold the levels detected at site 902 (Table 1). Tests were conducted with and without PBO addition. A 5-ppm stock solution of PBO was prepared and added to 400 ml of water for a final concentration of 25 ppb. This concentration does not affect survival or growth of *H. azteca* in a 10-d toxicity test (I. Werner, unpublished data).

Chemical mixtures were confirmed by analyses performed at the Department of Fish and Game, Fish and Wildlife Water Pollution Control Laboratory (Rancho Cordova, CA, USA). In 2007, measured concentrations nearly matched nominal concentrations. In 2008, however, because of unexpected degradation of stocks (pesticides were marked with an expiration date of 2009), measured concentrations were substantially lower than nominal. Additionally, whereas the measured concentrations of permethrin were well above the analytical method detection limit (0.003 μg/L) in both 2007 and 2008, the method detection limit for cyfluthrin doubled from 0.001 μg/L in 2007 to 0.002 μg/L in 2008, and the percentage surrogate recovery decreased from greater than 95% in 2007 to less than 80% in 2008 because of slight variations in analytical technique. This raised the 2008 limit of quantitation to a concentration above the nominal concentration in two of the three treatments, rendering the measured values questionable. To compensate for this difficulty, the exponential decay equation was used to estimate the actual concentration of the cyfluthrin stock solution in 2008 from the measured concentration.

Assuming a constant rate of degradation from the purchase date of the chemical stock in January 2007, which had an initial concentration of $C_0 = 40$ ppb, the concentration at any time t is given by $C_t = C_0 e^{-\lambda t}$. We estimated the degradation rate λ as 0.019/month based on the laboratory-measured concentration of 30.6 ppb in April 2008, which predicted an actual stock concentration of 31.5 ppb when the test was performed in January 2008. This degradation correction to predict the 2008 stock concentration yielded parameter estimates for the 2008 data similar to those of the 2007 data (Table 1).

Table 1. Nominal and measured concentrations of cyfluthrin and permethrin to which *Hyallela azteca* were exposed in 2007 and 2008 experiments[a]

Treatment	Nominal concentration (ppb)	Measured concentration (ppb)	
		2007	2008[b]
Cyfluthrin (0.5× DL)	0.0025	0.0029	*0.002*
Cyfluthrin (1× DL)	0.0050	0.0051	*0.004*
Cyfluthrin (2× DL)	0.0100	0.0104	*0.008*
Permethrin (0.5× DL)	0.0120	0.0119	0.004
Permethrin (1× DL)	0.0240	0.0254	0.008
Permethrin (2× DL)	0.0480	0.0573	0.016

[a] Each treatment was conducted with and without 25 ppb of piperonyl butoxide. Also indicated is the relationship between the nominal concentration and the detected level (DL) of that pesticide in the Sacramento–San Joaquin Delta (CA, USA).

[b] Concentrations in italics are estimated based on concentration of the stock solution and percentage recovery (see text for details).

Experimental design

Biological testing conducted in the Aquatic Toxicology Laboratory at the University of California, Davis, was based on U.S. Environmental Protection Agency protocol for a 10-d chronic exposure using *H. azteca* [23]. Amphipods were purchased from Aquatic Research Organisms. Each treatment consisted of six replicate, 250-ml glass beakers, each containing 100 ml of sample, a one-square-inch piece of nitex screen (a substrate for *H. azteca* to cling to), and 10 organisms. We initiated tests with 7- to 14-d-old organisms. Animals in each replicate were fed YCT (a mixture of yeast, organic alfalfa, and trout chow) on test initiation and days 2, 4, 6, 8, as well as on day 5, when 75% of the test water was renewed. Each series of tests included a standard laboratory control, a solvent (0.025% MeOH) control, and a solvent-plus-PBO control (25 ppb). Tests were conducted in a 23 ± 2°C water bath with a 16:8-h light:dark photoperiod. We recorded mortality daily. On day 10, half of the surviving *H. azteca* were dried and weighed to determine dry tissue weight per individual and relative growth. Because of limited sensitivity of the laboratory scale, however, relative growth could not be determined. The remaining animals were flash-frozen in liquid nitrogen and stored for biochemical analysis.

After the 2007 testing only, a Bradford protein analysis [25] was performed on the animals that were still alive at test termination. To determine average total protein content per amphipod, animals from each replicate were pooled, and resulting protein content was divided by the number of animals per treatment. Bovine serum albumin was used for calibration.

Statistical analysis

We calculated LC50s for each of the individual pesticides in their respective solitary treatments (i.e., cyfluthrin only or permethrin only). Because concentration-based LC50s cannot be calculated for a mixture, we used toxicity units (TU) to estimate the combined concentrations of cyfluthrin and permethrin that would be required to cause a specific proportion of mortality [19,26].

We analyzed survival data using logistic regression, via an approach similar to the concentration-addition models employed in recent mixture toxicity analyses [19,21,26,27]. Regression models were as follows:

$$\text{Mortality} = \frac{1}{1 + (1/e^{\mathbf{Xb}})} \quad (1)$$

where b is an $m \times 1$ vector containing the regression parameters (where m is the number of regression parameters), X is an $n \times m$ matrix of predictor variables (where n is the number of observations), and mortality is binomially distributed. We considered univariate models containing terms for cyfluthrin concentration, permethrin concentration, and presence of PBO to determine the independent effects of each compound. For example, in the univariate model for cyfluthrin, the term Xb in Equation 1 expands to

$$\text{Xb} = b_0 + b_1[\text{cyfluthrin}] \quad (2)$$

where b_0 and b_1 are regression parameters and [cyfluthrin] is the concentration of cyfluthrin, which varied among treatments (and varied slightly among years for each treatment). To test for synergism/antagonism between cyfluthrin and permethrin, we considered models with an additional term for the cyfluthrin × permethrin interaction:

$$b_i[\text{cyfluthrin}][\text{permethrin}] \quad (3)$$

Hereafter, b_i and b_j denote individual regression parameters; the actual values of the indices i and j would depend on the order in which that term appears in the model ($1 \leq i, j \leq m$). To test for a dose-level effect, we considered models with a term of the form:

$$b_i(1 - b_j[\text{cyfluthrin}])[\text{cyfluthrin}][\text{permethrin}] \quad (4)$$

(or equivalent terms with b_j[permethrin]). When $b_j = 0$ (no dose-level effect), this term collapses to the original synergism/antagonism term:

$$b_i[\text{cyfluthrin}][\text{permethrin}] \quad (5)$$

When $b_j \neq 0$, the cyfluthrin–permethrin interaction switches between synergism and antagonism (or vice versa, depending on the sign of b_i) when [cyfluthrin] = $1/b_j$ (or [permethrin] = $1/b_j$, depending on the form of Eqn. 4). Henceforth, we refer to the value $1/b_j$ as the *switching threshold* [19]. In addition to the univariate and multivariate models already described, we also considered versions of these models with additional terms for interactions with PBO.

We used a conventional model selection procedure based on a version of Akaike's information criterion corrected for small sample sizes (AIC_c) [22] to select the most parsimonious model from among the 69 considered. Including additional parameters in a statistical model nearly always increases the model likelihood, so likelihood-based comparisons tend to select unnecessarily complex models [22]. Using AIC_c overcomes this tendency by penalizing the likelihood by the number of parameters in the model. The AIC_c method also is more flexible than other model-selection methods, such as likelihood-ratio tests, because it can compare non-nested models [22]. Additionally, it frequently is used in the analysis of ecological data [28]. All regressions were performed in Matlab® 7.6 (Mathworks). Analyses of the 2007 and 2008 data sets found that the same set of models was identified as being most parsimonious in each year, and these models described similar effects of pesticide action in both years (i.e., the signs of each parameter value were the same in both years). Therefore, for the results reported here, the data for both years were pooled to improve statistical power for estimating parameter values and testing their significance.

RESULTS

Analysis of permethrin and cyfluthrin alone confirmed that cyfluthrin is more toxic than permethrin. The LC50 for permethrin alone was calculated to be 0.0489 ppb (Fig. 3), and the LC50 for cyfluthrin alone was estimated to be 0.0057 ppb (Fig. 4). The addition of 25 ppb of PBO resulted in significantly lower LC50s for both cyfluthrin and permethrin, at 0.0029 ppb and 0.0138 ppb, respectively (Table 2). Piperonyl butoxide doubled the toxicity of cyfluthrin and more than tripled the toxicity of permethrin (Table 2).

For the analysis of mortality using the entire data set, including treatments with both pesticides, model selection using AIC_c did not identify a single most parsimonious regression model, but the best four models represented more than 99% of Akaike weight, providing strong evidence that the best model lies within that set [22]. All of these models contained a term representing a cyfluthrin dose–dependent antagonism/synergism. In each case, however, the maximum-likelihood estimate for the switching threshold exceeded the maximum cyfluthrin concentration used in the experiment; that is, the

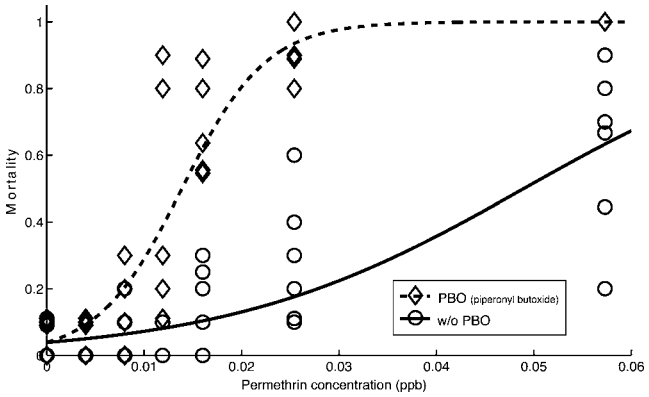

Fig. 3. Proportion of mortality caused by permethrin in 2007 and 2008. The dashed line indicates the proportion of mortality without piperonyl butoxide (PBO); the solid line represents the response with 25 ppb of PBO added to each treatment. The non-PBO and PBO treatments were significantly different ($p < 0.00001$).

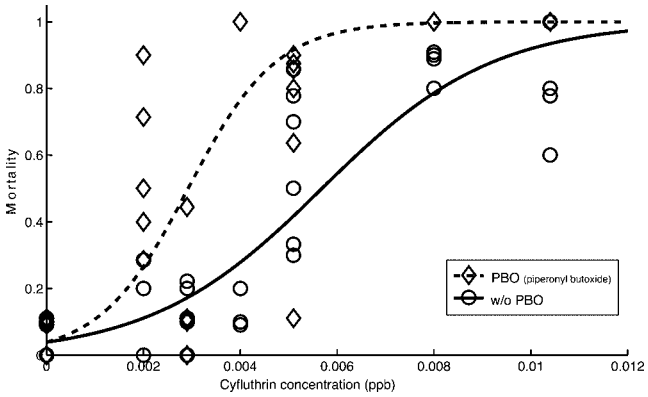

Fig. 4. Proportion of mortality caused by cyfluthrin in 2007 and 2008. The dashed line indicates proportion mortality without piperonyl butoxide (PBO); the solid line represents the response with 25 ppb of PBO added to each treatment. The non-PBO and PBO treatments were significantly different ($p < 0.00001$).

putative dose-level switch did not occur within the range of our data, calling into question the reliability of this result. As such, the models containing dose-level effects were discarded, and the model selection statistics were recalculated for a reduced set of 21 models.

The second round of model selection on the entire data set identified two models with a combined Akaike weight of greater than 99.3% (Table 3). One of these was the full model (containing terms for cyfluthrin, permethrin, PBO, pairwise interactions between PBO and each pesticide, and synergism–antagonism interactions), which contained all of the terms appearing in the other top model. Therefore, we focused on the full model in our interpretations of the data. As expected, this model contained positive terms for both cyfluthrin and permethrin, indicating that mortality increased with the concentrations of both pesticides, and positive terms for the interactions of each pesticide with PBO, indicating a synergistic effect of PBO. Additionally, this model described a negative interaction between cyfluthrin and permethrin (negative cyfluthrin × permethrin term for the full model in Table 2), indicating that mortality decreased when the two pesticides were both present (i.e., the two pesticides exhibited a slight antagonism).

To visualize the antagonism, we plotted observed mortality versus TU of permethrin plus cyfluthrin (Fig. 5). The expected (additive-only) dose–response curves assume an additive-interaction or concentration-addition modeling approach [19,21,27], based on a summation of the toxicities of cyfluthrin alone and permethrin alone. We took concentration addition to be the null model, because cyfluthrin and permethrin have the same mechanism of toxicity. The difference between the expected (additive-only) dose–response curve without PBO and the actual curve fitted to the data revealed the negative interaction between cyfluthrin and permethrin in the absence of PBO. At approximately 1.7 TU, 50% mortality was observed.

When PBO was present, 50% mortality was observed at a lower concentration of approximately 0.6 TU. This is equivalent to approximate concentrations of 0.0013 ppb of cyfluthrin and 0.0060 ppb of permethrin. Because no difference was found between the actual and expected dose–response curves for treatments with PBO (cyfluthrin × permethrin × PBO interaction term, $p > 0.05$) (Table 2), no evidence was observed for an interaction effect between cyfluthrin and permethrin when PBO was present at 25 ppb.

In addition to the acute toxicity results presented above, the Bradford protein analysis conducted in 2007 showed that even surviving amphipods were affected by exposure to pyrethroids. *Hyalella azteca* exposed to pyrethroid pesticides or to pyrethroids spiked with 25 ppb of PBO had significantly less protein than controls ($p < 0.05$) (Fig. 6).

DISCUSSION

Permethrin and cyfluthrin operate via the same mechanism of toxicity; therefore, in combination, one would expect them

Table 2. Regression coefficients (Coeff.), standard errors (SE), and *p* values for each term in the most parsimonious logistic regression models describing mortality as a function of pyrethroid pesticide concentration[a]

	Cyfluthrin			Permethrin			Cyfluthrin + Permethrin		
Parameter	Coeff.	SE	*p*	Coeff.	SE	*p*	Coeff.	SE	*p*
Intercept	−3.21	0.19	<0.00001	−3.30	0.18	<0.00001	−3.21	0.14	<0.00001
Cyf	560.22	39.95	<0.00001	—	—	—	563.61	32.38	<0.00001
Cyf × PBO	502.49	55.89	<0.00001	—	—	—	532.53	53.36	<0.00001
Per	—	—	—	70.99	5.99	<0.00001	65.47	5.29	<0.00001
Per × PBO	—	—	—	166.09	14.20	<0.00001	165.59	13.05	<0.00001
Cyf × Per	—	—	—	—	—	—	−5,252.57	1,302.65	0.00005
Cyf × Per × PBO	—	—	—	—	—	—	5,940.97	13,379.38	0.65701

[a] Results are presented for analyses performed on cyfluthrin (Cyf) exposure data alone, permethrin (Per) exposure data alone, and the full data set, including exposures to mixtures of cyfluthrin plus permethrin. For the cyfluthrin-alone and permethrin-alone analyses, the most parsimonious regression models are shown. For the full data set, results are shown for the full model with all parameters, which fell within the set of most parsimonious models. PBO = piperonyl butoxide.

Table 3. Models ranked from most to least parsimonious by Akaike's information criterion corrected for small sample sizes (AIC_c)[a]

Model:	Model no.	ΔAIC_c	AIC_c weight	Cum. AIC_c weight
int,c,p,c*p,c*pbo,p*pbo	*17*	*0*	*0.716354073*	*0.716354073*
int,c,p,c*p,c*pbo,p*pbo,c*p*pbo	*21*	*1.9*	*0.277043507*	*0.99339758*
int,c,p,c*pbo,p*pbo	14	10	0.004826756	0.998224336
int,c,p,c*pbo,p*pbo,c*p*pbo	20	12	0.001775664	1
int,c,p,c*p,p*pbo,c*p*pbo	19	128	1.15E−28	1
int,c,p,c*p,p*pbo	12	153	4.28E−34	1
int,c,p,p*pbo,c*p*pbo	16	159.4	1.75E−35	1
int,c,p,p*pbo	9	182.1	2.05E−40	1
int,c,p,c*p,c*pbo,c*p*pbo	18	232.6	2.22E−51	1
int,c,p,c*p,c*pbo	11	252.8	9.13E−56	1
int,c,p,c*pbo,c*p*pbo	15	255.6	2.25E−56	1
int,c,p,c*pbo	8	271.4	8.34E−60	1
int,c,p,c*p,c*p*pbo	13	351.9	2.76E−77	1
int,c,p,c*p*pbo	10	400.4	8.12E−88	1
int,c,p,c*p	7	498.2	4.70E−109	1
int,c,p	4	518.6	1.75E−113	1
int,c,c*pbo	5	700.5	5.54E−153	1
int,c	2	889.7	4.56E−194	1
int,p,p*pbo	6	1,191.4	1.40E−259	1
int,p	3	1,394.8	9.51E−304	1
int	1	1,692.6	0	1

[a] The ΔAIC_c indicates the difference in AIC_c scores between each model and the most parsimonious model. The AIC_c weights indicate the probability that the model falls within the set of most parsimonious models. The cumulative (Cum.) AIC_c weight, summed in ascending order, indicates the probability that a particular set of models is the most parsimonious. Model 21, which includes all parameters, was used to describe the response (Table 2 and Fig. 5). Model terms are given as follows: int = intercept; c = cyfluthrin; p = permethrin; pbo = piperonyl butoxide. Interactions are represented by an asterisk. Numbers in italics indicate concentrations estimated via exponential decay.

to have additive effects. Indeed, a mixture of the two at concentrations detected in the SSJ Delta did produce higher toxicity compared with toxicity from either of the pesticides alone at their respective detected concentrations. A slight but clear antagonism, however, was evident between the two pesticides, indicating that their effects are not completely additive (Fig. 5). Structural differences between type I and type II pyrethroids may provide a mechanistic explanation for our results. First, the observed antagonism could be a result of binding-site saturation. Cyfluthrin also may be out-competing permethrin for the same binding sites, particularly sodium channel–binding sites, for which both type I and type II pyrethroids have high affinity [8,29], a phenomenon known as competitive agonism [24]. The differing rates of metabolism between cyfluthrin and permethrin also may contribute to the observed antagonism. Cyfluthrin is metabolized more slowly by carboxylesterase and/or cytochrome P450, so it is more stable than, and can bind longer than, permethrin [30]. By the time cyfluthrin degrades and permethrin can access the binding site, permethrin may have already been metabolized and rendered inactive. This could contribute to our observation of a slightly antagonistic mixture effect. Interestingly, PBO seems to remove any antagonism between cyfluthrin and permethrin. Because both pyrethroids are more resistant to metabolic enzymes in the presence of PBO, perhaps this overrides any slight antagonism introduced by competition for the same binding sites, potentially because both cyfluthrin and permethrin are able to bind with similar affinity in the presence of PBO.

One of the more novel findings of the present study is that the data revealed a significant difference in the synergism of permethrin (type I) and cyfluthrin (type II) with PBO. Although a previous study found no difference in the synergism of toxicity with PBO between type I and type II pyrethroids [30], the results of the present study indicate that permethrin toxicity

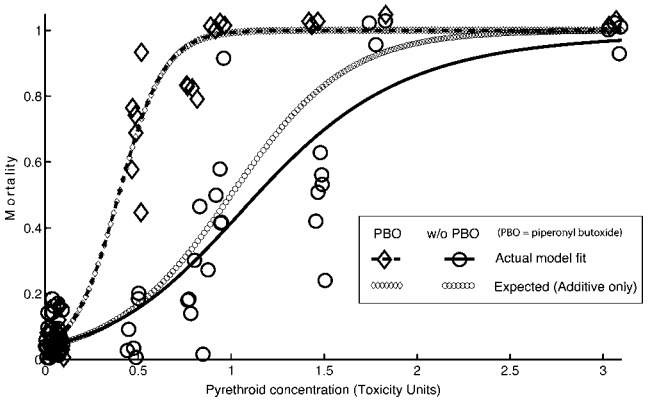

Fig. 5. Proportion of mortality caused by permethrin and cyfluthrin mixtures in 2007 and 2008. Slight antagonism ($p = 0.00005$) was observed between cyfluthrin and permethrin, but this antagonism was eliminated in the presence of 25 ppb of piperonyl butoxide (PBO).

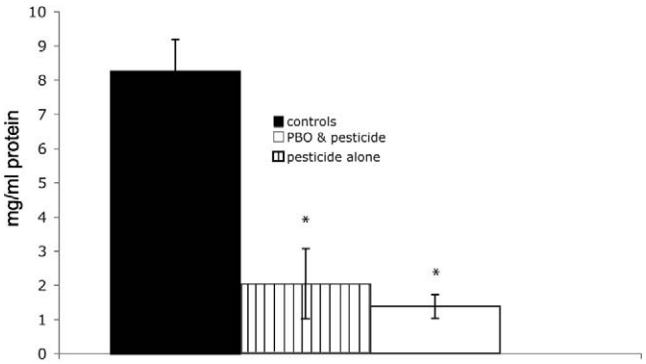

Fig. 6. Protein levels detected in *Hyalella azteca* that survived a 10-d exposure to cyfluthrin and permethrin in 2007. Protein levels in pyrethroid-exposed animals were too low to conduct a Western blot analysis for heat shock protein expression. An asterisk indicates that the result was significantly different than the control ($p < 0.05$).

was increased 3.5-fold by PBO, whereas the toxicity of cyfluthrin was only doubled. This effect is present in the data from 2007 and 2008. Because type II pyrethroids are designed to be more resistant to enzymatic breakdown, inhibition of pyrethroid-metabolizing enzymes by PBO might affect the toxicity of type II pyrethroids to a lesser degree. More simply, type II pyrethroids circumvent the problem of breakage at the ester linkage via carboxylesterase [8] without the addition of PBO because of the additional of chemical functional groups that strengthen this ester bond.

Total protein content of individual amphipods proved to be an excellent indicator of sublethal pyrethroid toxicity. The 2007 data indicated pyrethroid-treated animals that survived for 10 d had significantly lower protein compared with controls, indicating the extent of the sublethal impact by cyfluthrin and permethrin (Fig. 6). Total protein content can be used as a measure of organism growth at early life stages [31]. The potential ecological consequences of such sublethal growth effects are far-reaching, in that pyrethroid-exposed aquatic organisms may experience reduced fecundity and ecological fitness in the wild. Organisms exposed to sublethal concentrations of pyrethroids also exhibit altered swimming behavior, are ineffective at competing for food, and are more vulnerable to predation [32–35].

Although our analysis is similar to that used in several previously conducted mixture toxicity studies [19,21,26], the use of AIC_c improves on the likelihood-ratio model selection approach used by others, because it is possible to select the most parsimonious model from among a greater number of non-nested models [22]. For example, the AIC_c approach allowed a comparison between a model with terms for permethrin and PBO and a model with terms for permethrin and cyfluthrin; such non-nested comparisons are not possible using likelihood ratios. In practice, of course, the present analysis selected the model with the greatest number of parameters as the most parsimonious option. Additionally, studies examining the interactions between three or more pyrethroid pesticide mixtures should be conducted, because these types of treatments would more closely mimic conditions in the wild. Although a large toxicity assessment of pesticide mixtures present in the SSJ Delta was recently completed by Lydy and Austin [26], pyrethroid pesticides were not included. A similar study evaluating a suite of pyrethroid pesticides and their interactions at environmentally relevant concentrations would be timely.

The experimental design of the present study lacked a treatment including either sediment or organic matter, which other studies have shown can reduce or even eliminate acute pyrethroid toxicity [9,36]. What such tests do not take into account is the occurrence of high concentrations of pesticides in runoff that may be periodically present in the water column. Over an extended period of time, organic matter and sediment may mitigate pyrethroid toxicity, but acute toxicity may still occur, especially considering that the dissolved fraction can be as high as 16.7% and take more than 30 d to become completely bound to sediments [9]. The mitigating ability of organic matter in the water column, however, should not be understated. *Hyalella azteca* exposed to natural water samples that contained cyfluthrin and permethrin concentrations tested in the present study experienced only sublethal toxicity, and this occurred only after the addition of PBO.

Regardless of whether antagonism may be occurring, the toxicity of permethrin and cyfluthrin, both alone and combined, at parts-per-trillion concentrations is cause for concern, because the levels used in the present study have been detected in the SSJ Delta and other Central Valley surface waters (data not shown) [2]. Considering the number of other pyrethroids used in the Central Valley of northern California and other heavily farmed areas in the United States, as well as the possibility of additive and/or synergistic interactions with other pesticides [37] and/or residual PBO in the water column [12], obvious potential exists for toxicity, both lethal or sublethal, to aquatic organisms in the water column. Additionally, use of the SSJ Delta by endangered salmonids as a migratory route and recent findings of pesticide interference with important migratory behaviors [38] heightens the importance of minimizing the impact of heavy pesticide use in this region and others like it.

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