USE AND TOXICITY OF PYRETHROID PESTICIDES IN THE CENTRAL VALLEY, CALIFORNIA, USA

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Abstract- The use of pyrethroid insecticides is increasing in agriculture, commercial pest control, and in residential consumer use. In addition, there is a trend towards the use of newer and more potent compounds. Little is known about the toxicity of sediment-associated pyrethroid residues to aquatic organisms, yet recent work has shown they are commonly found in aquatic sediments in the heavily agricultural Central Valley of California, USA. Minimal data exist on the sensitivity of standard sediment toxicity testing species to pyrethroids, despite two or more decades of agricultural use of these compounds. Sediment concentrations causing acute toxicity and growth impairment to the amphipod *Hyalella azteca* (*H. azteca*) were determined for six pyrethroids in three sediments, ranging from 1.1 to 6.5% organic carbon (OC). In order of decreasing toxicity of sediment-associated residues, the compounds tested were lambda-cyhalothrin (average 10-d median lethal concentration (LC50) =0.45 µg/g OC), bifenthrin (0.52 µg/g OC), deltamethrin (0.79 µg/g OC), cyfluthrin (1.08 µg/g OC), esfenvalerate (1.54 µg/g OC), and permethrin (10.83 µg/g OC). In a sediment containing about 1% OC, most pyrethroids, except permethrin, would be acutely toxic to *H. azteca* at concentrations of 4 to 10 ng/g dry weight, a concentration only slightly above current analytical detection limits. Growth was typically inhibited at concentrations below the LC50; animal biomass was on average 38% below controls when exposed to pyrethroid concentrations roughly one-third to one-half the LC50. Survival data are consistent with current theory that exposure occurs primarily via the interstitial water rather than the particulate phase. A reanalysis of previously reported field data using these toxicity data confirms that the compounds are exceeding concentrations acutely toxic to sensitive species in many agriculture-dominated water bodies.

Keywords-   Pyrethroids   Sediment toxicity   *Hyalella azteca*

Introduction

In recent years, organophosphate use in California, USA agriculture has been declining due to concerns over the human toxicity of these compounds, as well as frequent periods of surface water toxicity detected by ambient monitoring [1,2]. Current agricultural use of diazinon and chlorpyrifos, the dominant organophosphate pesticides, is half of their peak use attained in the mid-1990s (www.cdpr.ca.gov). Concerns surrounding the aquatic toxicity of organophosphates have resulted in implementation of several water quality monitoring programs in the agricultural areas of California, yielding considerable data on organophosphates in surface waters. In contrast, use of pyrethroids has increased, though there has been little monitoring of sediments in these agricultural water bodies, and data on more hydrophobic pesticides such as the organochlorines and pyrethroids are minimal.

Peak statewide agricultural use of pyrethroids since 1993 occurred in that year at 151,900 kg (**Fig. 1a**; www.cdpr.ca.gov). Of this total, nearly 80% was permethrin. Since then (1994-2002), total pyrethroid use has averaged 125,000 kg/year. There is an indication of gradually declining use throughout the 1990s, with a minimum value in 1999, and a 25% increase in the past few years. However, this trend belies the fact that there has been a shift towards more recently developed compounds, which are much more toxic than permethrin. In 1993 there were five pyrethroids used in California agriculture, and permethrin comprised 60% of the total use. In 2002, there were ten compounds used, and permethrin was 45% of the total. Many of the newer compounds have toxicities to aquatic life nearly 20 times greater than permethrin. In order to account for these toxicity differences, use of all pyrethroids can be expressed in

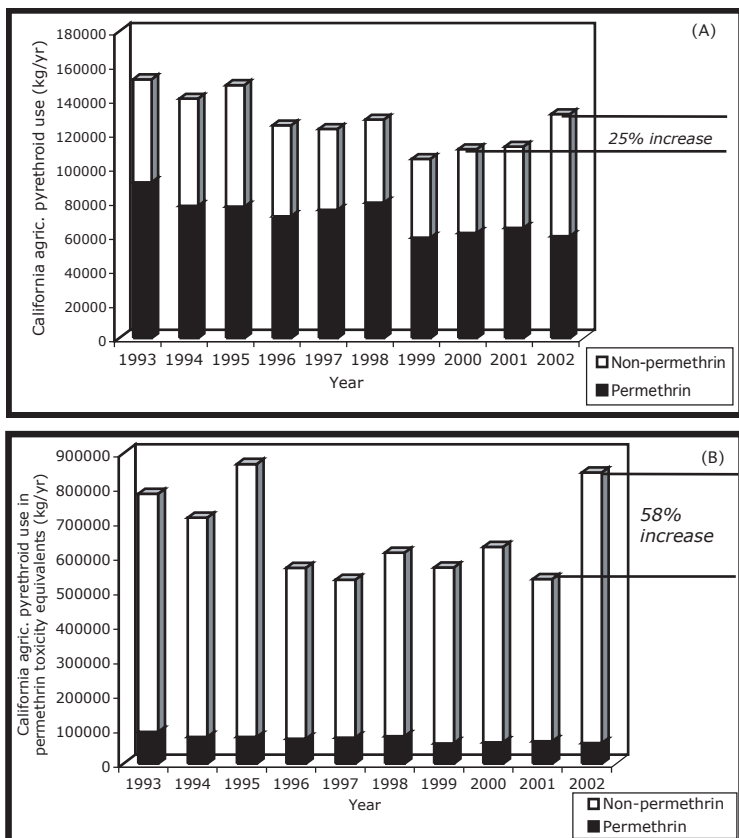*Correspondence: erinamweg@berkeley.edu*

Fig. 1. Pyrethroid use in California, USA agriculture from 1993 to 2002 (data from www.cdpr.ca.gov). (A) Total statewide agricultural use in kg/active ingredient per year; and (B) Statewide agricultural use of pyrethroids expressed as permethrin equivalents after adjusting for the relative aquatic toxicity of the compounds.

terms of permethrin equivalents, based on their relative toxicities. For example, the 10th percentile median lethal concentration (LC50) for all aquatic organisms tested with permethrin is 180 ng/L, whereas the 10th percentile LC50 for cypermethrin is 10 ng/L [3]. Application of 1 kg of cypermethrin can therefore be considered equivalent, in terms of aquatic toxicity, to application of 18 kg permethrin. When temporal patterns of use are expressed in permethrin equivalents to adjust for toxicity differences among the pyrethroids, there has been a large and very recent increase use in agriculture of permethrin toxicity equivalents (**Fig. 1b**). Between 2001 and 2002, there was a 58% increase in application of permethrin equivalents. Only a very small fraction of this toxicity-adjusted use is in fact permethrin; most of the potential aquatic toxicity is associated with compounds that are used in lesser amounts but are far more toxic.

Nonagricultural use of pyrethroids, though not shown in these figures, has also had large increases. In 2002, commercial nonagricultural use in California (231,000 kg) was five times greater than in the early 1990s, with structural pest control as the principal appli-

cation. Also, the State's database does not track consumer home and garden use, and in this area pyrethroids have entirely replaced organophosphates such as diazinon and chlorpyrifos, which have recently been withdrawn from the consumer market by their manufacturers. The trends in California reflect a nationwide shift that indicates an emerging need to better understand the environmental fate and aquatic toxicity of compounds in this class.

Pyrethroid pesticides have high log K_{ow} values of 5 to 7 and therefore partition into the organic carbon (OC) fraction of sediments [4]. In spiked stream water allowed to come to equilibrium, more than 97% of detected pyrethroids were adsorbed to suspended solids [5], indicating that transport on suspended solids will be the main vector for off-site movement of pyrethroids and that sediments are likely to be the principal reservoir for environmental residues. Even though half-lives in the environment are typically on the order of days to weeks in the aqueous phase [4], pyrethroids exhibit prolonged persistence in aquatic sediments. Lee et al. [6] report a bifenthrin half-life of 163 days in a creek sediment slurry, and deltamethrin was detectable in sediments of a pond mesocosm at concentrations of 3 to 5 ng/g after nearly one year [7]. Although pyrethroid-degrading bacteria exist in the environment and contribute to rapid pyrethroid disappearance in the dissolved phase, adsorption of the pesticide to sediments appears to render it unavailable for bacterial transformation [6].

In areas of agricultural pyrethroid use, residues of the compounds are often found in aquatic sediments, and are occasionally present at concentrations acutely lethal to sensitive species. Analysis of about 80 sediment samples from rivers, creeks, and irrigation canals throughout a 10-county area of California found detectable pyrethroids in 75% of the samples [8]. Twenty-eight percent of the samples caused mortality in 10-d tests with *H. azteca*, and in most of these cases, sediment pyrethroid concentrations were high enough to explain the observed toxicity. Toxicity to *Chironomus tentans*, a species that is less sensitive than *H. azteca* to pyrethroids, was seen in 13% of the samples, and about half of these instances of toxicity were attributed to pyrethroids by Weston et al. [8].

The indication of pyrethroid-related acute toxicity in aquatic sediments suggests more attention

needs to be given to toxicity of sediment-associated residues. Much of the risk assessment work to date has focused on dissolved phase pyrethroids [3,9] with only infrequent consideration of sediment toxicity [8,10]. Although most of the currently used pyrethroids have been available for over 20 years, there are few data on sediment median effective/lethal concentration values (EC50/LC50) for common sediment toxicity testing species like *H. azteca, Chironomus tentans*, or *Chironomus riparius*. Ten pyrethroids are in use in California agriculture, yet sediment LC50 data for *H. azteca* are available for only one (cypermethrin; [10]). Sediment EC50/LC50 data for *C. riparius* are available only for lambda-cyhalothrin [11] and permethrin [12]; sediment LC50 data for *C. tentans* are available only for cypermethrin [10]. In this study, sediment LC50 values and growth lowest-observable-effect concentrations (LOECs) for six commonly used agricultural pyrethroid pesticides were determined for *H. azteca*. These values were used to reanalyze the field data of Weston et al [8]. The previously published analysis had relied on estimates of pyrethroid LC50 values, but the results produced by the current study allow use of measured LC50 data and better understanding of the role pyrethroids may have played in the observed toxicity.

Materials and Methods

Sediment collection

Test sediments were collected in October 2003 from three locations in California's Central Valley, an area that encompasses two-thirds of the State's total cropland. Sampling locations were selected on the periphery of the Valley in order to obtain sediments upstream of agricultural inputs. Sediment from the South Fork of the American River was collected about 2 km west of the confluence with Weber Creek, in Placer County near Folsom Lake. Pacheco Creek was sampled from a site south of Pacheco Lake in Santa Clara County. Del Puerto Creek sediment was collected from Del Puerto Canyon in the hills of western Stanislaus County. Samples were collected from these water bodies by skimming the top 2 to 3 cm of sediment using a steel shovel. Sediments were stored at 4 °C for less than one week, until sieving on a 1 mm screen. Material passing through the screen was homogenized and frozen until use. Sediments were considered acceptable for test purposes after 10-d sediment toxicity tests with *H. azteca* showed survival rates greater

than 80%.

Chemical analyses

The sediments were analyzed for pesticides following the methods of You et al. [13] on an Agilent 6890 series gas chromatograph equipped with an Agilent 7683 autosampler and an electron capture detector (Agilent Technologies, Palo Alto, CA, USA). Two columns from Agilent, a HP-5MS (30m x 0.25mm; 0.25µm film thickness) and a DB-608 (30m x 0.25mm; 0.25µm film thickness) were used. Analytes included four pyrethroids (bifenthrin, esfenvalerate, lambda-cyhalothrin, and permethrin) as well as chlorpyrifos and 20 organochlorine pesticides or their degradation products. The same analytical procedures were used both to initially confirm low pesticide levels in the test sediments, and again after spiking with pyrethroids to confirm nominal concentrations. Cyfluthrin and deltamethrin were added as analytes later in the study to confirm spiking concentrations. Test sediments were wet sieved to determine grain size distribution and analyzed for organic carbon content on a CE-440 elemental analyzer from Exeter Analytical (Chelmsford, MA, USA), following acid vapor treatment to remove inorganic carbon.

Toxicity testing

The six compounds tested were purchased from Chem Service (West Chester, PA, USA), including bifenthrin, cyfluthrin (Baythroid™), deltamethrin, esfenvalerate (Asana™), lambda-cyhalothrin, and permethrin (20% cis, 78% trans). All of the pesticides were tested in at least two of three test sediments. Pesticides were dissolved in an acetone carrier and spiked into sediments using <200 µL acetone/kg wet sediment. The sediments were then mixed with a steel paint mixing attachment in an electric drill, and aged at 4 °C for 11 to 12 d. After aging, aliquots were removed for chemical verification of actual concentrations. These aliquots were placed into clean glass jars and frozen at –30 °C until analysis.

Ten-day toxicity tests with the freshwater amphipod *H. azteca* were performed using standard U.S. Environmental Protection Agency protocols [14]. All toxicity tests were conducted at 23 °C with a 16:8-h light:dark cycle in 400 ml beakers (3 replicates per concentration) containing 50 to 75 ml of spiked sediment and 300 ml of moderately hard water reconstituted from Milli-Q purified water (Millipore, Billerica, MA,

USA). Amphipods were sieved from cultures to collect animals passing through a 500 µm screen but retained on a 355 µm screen. These animals are typically 3 to 9 days old [14] and they were allowed to age 3 d prior to use in the tests. A yeast, cerophyll, and trout chow mixture was fed daily throughout this period and in the 10-d tests. During the tests, an 80% water change was performed every other day, and water samples were taken prior to water renewal on days two and ten for analysis of temperature, dissolved oxygen, pH, conductivity, alkalinity, hardness, and ammonia. All beakers were aerated gently and continuously. Tests were terminated by sieving contents of the beakers over a 425 µm screen. Surviving amphipods were dried overnight at 70 °C and weighed to determine relative biomass difference among the treatments.

Equilibrium partitioning

It has been suggested that partitioning of the pyrethroid cypermethrin between sediment and water is consistent with equilibrium partitioning predictions, and that the dissolved phase of the pesticide in pore water could account for apparent sediment toxicity [10]. This hypothesis was addressed using sediment LC50 data from the current study and published K_{oc} values [4] to estimate the pyrethroid concentration likely to be found in pore water when the sediments were at their LC50 concentration. Pore water concentrations were calculated by dividing the OC normalized sediment LC50 by the average K_{oc} value [4], according to the formula:

$$[LC50_{water}] = [LC50_{sediment\ oc}] / K_{oc}$$

Statistics

Toxicity data were analyzed using ToxCalc 5.0 software (Tidepool Scientific Software, McKinleyville, CA, USA). Survival data was arc-sin transformed prior to analysis. A one-tailed Bonferroni's *t*-test was used to identify no-observable-effect concentration (NOEC) and lowest-observable-effect concentration (LOEC) values for growth. The trimmed Spearman-Karber method was used to determine LC50s for each compound. Abbott's correction was applied in a few cases when the data was nonmonotonic.

Results and Discussion

Test sediment characteristics

Test sediments were analyzed prior to use for 25 pesticides, including four pyrethroids: bifenthrin,

esfenvalerate, lambda-cyhalothrin, and permethrin. None of the test sediments contained any analytes above the 1 ng/g detection limit. Organic carbon contents for the three test sediments were 1.1% for Del Puerto Creek, 1.4% for American River, and 6.5% for Pacheco Creek. Percentage of silts and clays for the same sediments were 31.7, 43.1, and 21.3%, respectively.

Pyrethroid recovery

Measured concentrations by gas chromatography were close to nominal concentrations. Recoveries averaged 70% for bifenthrin (range 58-77%), 89% for esfenvalerate (range 74-105%), and 67% for permethrin (range 56-78%). Cyfluthrin, deltamethrin, and lambda-cyhalothrin recoveries were 114, 80, and 106%, respectively. The analytical technique has previously been shown to provide approximately 100% recovery [13] though with no aging period between spiking and extraction.

Though the recoveries among sediments were relatively similar for any given compound, Pacheco Creek sediment consistently had the lowest value of the three sediments. This sediment was atypical in that it contained a large proportion of cow manure from surrounding rangeland, raising the possibility that matrix effects may have reduced extraction efficiency.

Toxicity and growth

Control survival in the tests averaged 94%, with no appreciable difference between control and solvent control survival (averaging 95%). On a dry weight basis, lambda-cyhalothrin and deltamethrin were the most toxic of all compounds tested with average 10-d LC50s for *H. azteca* of 5.6 and 9.9 ng/g, respectively (**Table 1**). Bifenthrin (12.9 ng/g) and cyfluthrin (13.7 ng/g) were slightly less toxic. Esfenvalerate's LC50 averaged 41.7 ng/g (range 18-83). Permethrin was the least toxic compound by about a factor of five (10-d LC50 averaging 201 ng/g, range 127-249). Among the various sediments, LC50s varied by factors of two to four. On a dry weight basis, Del Puerto Creek sediment yielded the lowest LC50s for five of the six compounds tested. This may be due to the low OC content of the sediment, making pyrethroids more biologically available to organisms [10].

Due to the hydrophobicity of pyrethroid pesticides, these compounds will partition in to the sediment OC fraction, and LC50 values could be less variable

	LC50		Growth LOEC	
	ng/g dry sediment (95% CI)[a]	µg/g OC (95% CI)[a]	µg/g OC	% Biomass reduction from control
Bifenthrin				
American River	8.6 (7.7-9.7)	0.63 (0.57-0.69)	0.23	22
Del Puerto Creek	6.6 (5.7-7.7)	0.57 (0.51-0.69)	0.60	49
Pacheco Creek	23.5 (18.9-28.0)	0.37 (0.29-0.43)	0.23	33
Cyfluthrin				
American River	14.9 (13.3-16.6)	1.07 (0.96-1.2)	0.46	32
Del Puerto Creek	12.5 (10.9-14.2)	1.09 (0.96-1.25)	0.77	40
Deltamethrin				
American River	9.8 (8.3-11.5)	0.71 (0.6-0.83)	0.20	37
Del Puerto Creek	10.0 (8.5-11.7)	0.87 (0.75-1.02)	>1.57	
Esfenvalerate				
American River	24.3 (21.3-27.9)	1.76 (1.5-2.1)	0.50	28
Del Puerto Creek	17.9 (15.3-21.2)	1.59 (1.3-1.9)	0.84	42
Pacheco Creek	83.1 (68.3-102)	1.28 (1.05-1.57)	0.50	48
Lambda-cyhalothrin				
American River	6.0 (5.3-6.8)	0.43 (0.38-0.49)	0.14	35
Del Puerto Creek	5.2 (4.5-6.0)	0.46 (0.40-0.53)	0.23	36
Permethrin				
American River	249 (206-278)	17.9 (14.9-20.0)	11.8	37
Del Puerto Creek	127 (111-142)	11.1 (9.8-12.4)	11.8	52
Pacheco Creek	226 (189-271)	3.51 (2.9-4.2)	1.51	39

[a] Confidence interval

Table 1. Pyrethroid sediment median lethal concentrations (LC50) and growth lowest- observable- effect concentrations (LOEC) for bifenthrin, cyfluthrin, deltamethrin, esfenvalerate, lambda-cyhalothrin, and permethrin using standard 10-d sediment toxicity tests with *Hyalella azteca*. The three test sediments were collected from sites in California, USA and ranged from 1.1 to 6.5% organic carbon.

and more applicable to other sediments if expressed on an OC-normalized basis. Sediments had been deliberately selected with a wide range in OC content (1.1-6.5%), though most Central Valley sediments typically contain 0.5 to 2% OC [8]. Mean OC-normalized LC50s for lambda-cyhalothrin, bifenthrin, and deltamethrin were all less than 1 µg/g OC (0.45, 0.52, and 0.79 µg/g OC, respectively). Cyfluthrin and esfenvalerate LC50s (1.08 and 1.54 µg/g OC, respectively) were slightly over 1 µg/g OC, and permethrin averaged 10.8 µg/g OC, the least toxic of the six pyrethroids analyzed by about a factor of seven.

The only pyrethroid for which there are published sediment LC50 values for *H. azteca* is cypermethrin. Reported cypermethrin 10-d LC50s range from 0.18 to 0.6 µg/g OC [10], a range that overlaps with our reported values for two other pyrethroids.

Except in the case of permethrin, normalizing for OC made LC50 concentrations more consistent among the three sediments. Normalized values were within a factor of 2 for any given pyrethroid. The only exception was permethrin for which normalization considerably increased the variability. The reason for this variability in permethrin LC50s is unknown.

Although data are limited, there is some evidence that *H. azteca* is one of the more sensitive of the very few species that have been tested with sediment-associated pyrethroids. The measured LC50 value for lambda-cyhalothrin (0.43-0.46 µg/g OC) is well below the *C. riparius* EC50 for emergence of 6.8 µg/g OC [11], given an OC content of the *C. riparius* test sediment of 3.7% (provided by J. Warinton, Syngenta, Berkshire, UK, personal communication). The *H. azteca* LC50 for permethrin (3.5-18 µg/g OC) is also slightly below the LC50 for *C. riparius* of 21.9 µg/g OC [12]. It has previously been shown that *H. azteca* is about three times more sensitive to sediment-associated cypermethrin than *C. tentans* [10]. The esfenvalerate 10-d LC50 for *H. azteca* (average 1.54 µg/g OC) is below the 96-h LC50 of fenvalerate for nematodes
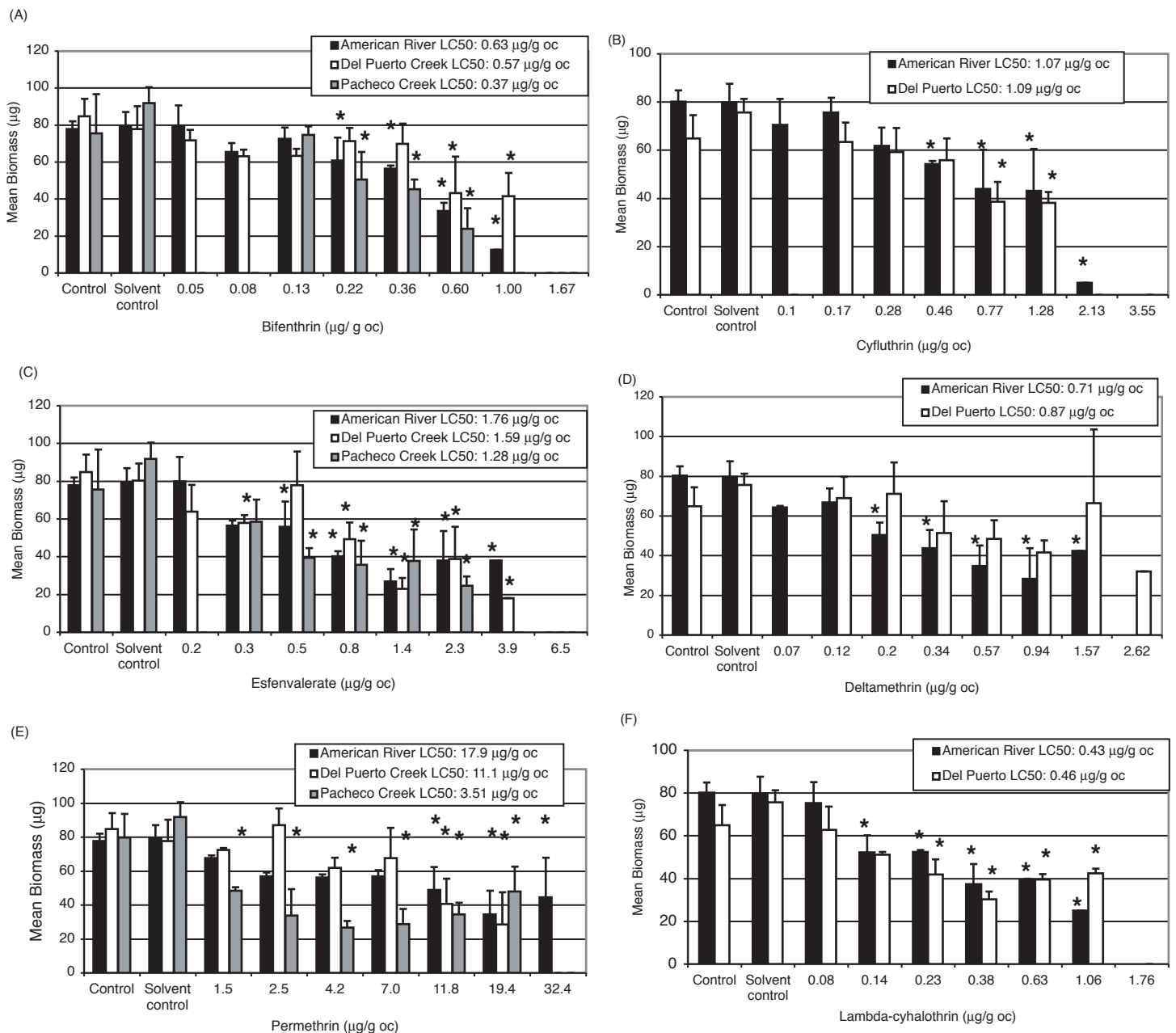
Fig. 2. Biomass of the amphipod *Hyalella azteca* after exposure to pyrethroid-spiked sediment in standard 10-d toxicity tests. Mean biomass in the treatment is shown with bars representing standard deviation. Treatments significantly lower than control ($p<0.05$) using a one-tailed Bonferroni *t*-test are denoted with an asterisk. The 10 day median lethal concentration (LC50) is shown for each sediment. All sediments were collected from sites in California, USA.

(33.2 µg/g OC) and two copepod species (74-84 µg/g OC) [15], although the different exposure durations and differences in isomer composition confound this comparison.

Growth in the 10-d tests was inhibited at concentrations typically one-third to one-half the LC50 in 12 out of 15 tests (**Figure 2a-f**, Table 1). At the LOEC, amphipod biomass was reduced 38% on average compared to control animals. In three tests (bifenthrin, deltamethrin, and permethrin in Del Puerto Creek sediment) there was no inhibition in growth until pyrethroid concentrations exceeded the reported LC50.

After normalizing for sediment OC content, lambda-cyhalothrin was still the most toxic pyrethroid

tested with a LOEC averaging 0.19 µg/g OC respectively. On a dry weight basis, this corresponds to sediment concentrations ranging from 2.0 to 2.6 ng/g for lambda-cyhalothrin. Average amphipod biomass was reduced 36% compared to control animals at these concentrations. Growth lowest-observable-effect concentrations for the least toxic compound, permethrin were 1.5 to 11.8 µg/g OC (or 98-162 ng/g) and occurred when mean amphipod body mass was reduced by about 43% compared to control animals. The individual pyrethroids showed similar relative toxicities for both the growth impairment and mortality endpoints.

A roughly 40% biomass reduction compared to control amphipods was required to detect an effect in

Pyrethroid	Average sediment 10-d LC50 (μg/g OC)	Average K_{oc} [a]	Estimated pore water concentration (ng/L)	Reported 5th percentile LC50 in water (ng/L) [b]	Reported 10th percentile LC50 in water (ng/L) [b]
Bifenthrin	0.52	237,000	2.2	0.38	15
Cyfluthrin	1.08	124,000	8.7	<4	12
Deltamethrin	0.79	704,000	1.1	<3	9
Esfenvalerate	1.54	No data	---	17	37
Lambda-cyhalothrin	0.45	326,000	1.4	<4	10
Permethrin	10.83	277,000	39.1	35 [c]	76 [c]

[a] K_{oc} data from Laskowski [4].

[b] Lethal concentration (LC) data from water-only exposures from Solomon et al. [3].

[c] Permethrin LC50 data only for arthropods; data for other compounds from all taxa tested.

Table 2. The estimated pore water concentration of pyrethroids when the bulk sediment is at median lethal concentration (LC50), in comparison to the levels found to be toxic to sensitive species in water-only exposures.

these experiments. In the environment, it seems plausible that a relative biomass loss of this magnitude would have a substantial impact on factors affecting population structure. These could include reduced fecundity and recruitment, as well as potentially reduced resistance to other environmental stressors as homeostatic energy demands are increased to deal with contaminant stress. Pyrethroid exposure has been shown to increase metabolic demands, resulting in utilization of energy stores and reduced growth in the estuarine shrimp *Palaemonetes pugio,* especially after metamorphosis from planktonic to benthic life stages increases sediment exposure [16]. Both lipid depletion and sublethal reproductive impairment were caused by fenvalerate exposure in *Daphnia magna* as well [17].

The concentrations at which effects were seen (often LC50s of 4-10 ng/g and growth impairment at about half these concentrations) are only slightly above analytical detection limits for pyrethroids in sediments. Various investigators have reported detection or quantification limits for pyrethroids in dry sediments of 0.5 to 1 ng/g [13], 0.75-1.5 ng/g [18], 1 ng/g [19], 5 ng/g [20], and 25 ng/g [16]. Toxicity to sensitive species may exist at field sites where pyrethroid concentrations are barely detectable, or not detected at all by some of the analytical methods that have been used.

These results indicate that in order to protect benthic species with pyrethroid sensitivities comparable to *H. azteca*, it is necessary to attain a detection limit of at least 1 ng/g, and, if possible, to improve analytical methods to achieve even lower detection limits.

Equilibrium partitioning

Pyrethroid pore water concentrations predicted at equilibrium when sediments were at the LC50 concentration ranged from 1.1 to 39 ng/L (**Table 2**). If the dissolved phase of the pesticide in pore water alone is the sole source of exposure, then these predicted concentrations should match *H. azteca* LC50 values in water exposures. Such data, however, are generally lacking. Therefore, the 5th and 10th percentile LC50s for all aquatic organisms tested were used as surrogates for the concentrations likely to be acutely toxic to a sensitive species [3]. The only data available specifically for *H. azteca* to test this assumption is the reported 96 h LC50 of lambda-cyhalothrin of 4 ng/l [11], a value close to lambda-cyhalothrin's 5th percentile estimate for all aquatic species. This analysis indicates that when the bulk sediments have a pyrethroid concentration equal to the *H. azteca* sediment LC50, the pore water pyrethroid concentration roughly approximates the 5th percentile of reported water LC50s for all aquatic species. Bifenthrin, cyfluthrin, and permethrin estimated pore water concentrations were between the 5th and 10th percentile range; estimated concentrations for deltamethrin and lambda-cyhalothrin were slightly lower than the 5th percentile, consistent with the reported values of <3 and <4 ng/L, respectively. If *H. azteca* were among the more sensitive species to the pyrethroids considered (though this possibility can not presently be confirmed), pore water concentrations alone could account for the observed mortality. This suggestion becomes even more plausible considering that the 5th and 10th percentile values are based on 24- to 96- h exposures, whereas the estimated pore water concentrations would apply to the 10-d exposure used in the sediment tests. While this study was not specifically intended to address the relative uptake of pyrethroids by *H. azteca* from the dissolved and particulate phases, the results are at least consistent with uptake from the dissolved phase alone.

Reanalysis of field data

The LC50s derived in this study are useful to improve interpretation of previously observed sediment toxicity in field samples from throughout California's Central Valley [8]. In 2002 and 2003, about 80 sediment samples were taken from primarily creeks and irrigation canals in areas of intensive agriculture area within the Central Valley. About half the samples were taken from areas of historically high pyrethroid use or reported sediment toxicity; the other half of the
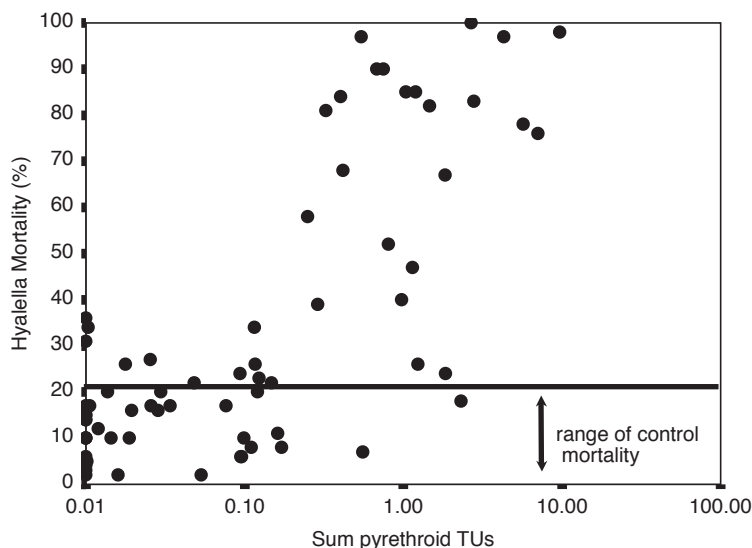
Fig. 3. Mortality of *Hyalella azteca* exposed to sediments collected from Central Valley, California, USA in comparison to the total pyrethroid toxicity units (TUs) present in the sediment at each site. Total pyrethroid toxicity units were calculated from previously reported pyrethroid concentrations at the sites [8] and the average 10-day median lethal concentration (LC50) data from the current study. Sediments having undetectable pyrethroid concentrations were assigned a TU value of 0.01.

samples were from water bodies receiving substantial amounts of irrigation return flow, but without regard to pesticide use practices or expectations of toxicity. Toxicity to *H. azteca* following a 10-d exposure was observed in 23 of the samples. The investigators applied a toxicity unit (TU) analysis to identify which compounds were likely responsible for the toxicity. Toxicity units were calculated as the measured sediment concentration of each compound divided by the 10-d sediment LC50 for *H. azteca*, with both values expressed on an OC normalized basis. It was concluded that in 12 of the 23 toxic samples a single pyrethroid was present in sufficient concentration to account for much or all of the toxicity, and in another four samples the additive effects of multiple pyrethroids could have been responsible. In total, pyrethroids were reported to approach or exceed acutely toxic concentration in 70% of the samples exhibiting *H. azteca* toxicity, or about 20% of the samples overall.

However, the analysis done by Weston et al. [8] was based on estimates of pyrethroid sediment LC50s since actual values were available only for cypermethrin. Sediment LC50s for the other pyrethroids were estimated by comparing the toxicity of each compound to cypermethrin in water-only exposures, and then using a relative toxicity factor to adjust the reported cypermethrin sediment LC50. The estimated LC50s previously reported were very close to actual LC50 val-ues measured in this study. LC50 values estimated by Weston et al. [8] for bifenthrin, esfenvalerate, lambda-cyhalothrin, and permethrin ranged from 63-110% of values measured in this study.

The TU analysis was recalculated with actual LC50 data from this study. The total TUs of pyrethroids at each site were summed assuming additivity of the individual pyrethroid TUs, and plotted against the mortality to *H. azteca* in 10-d tests as reported by Weston et al. [8] (**Fig. 3**). It is clear that when total pyrethroids reach about 0.4 TUs, mortality rises dramatically. The vast majority of samples with >0.4 TU show greater than 40% *H. azteca* mortality. The few stations with >0.4 TU but no increased mortality suggest the influence of sediment factors affecting bioavailability other than total organic carbon content, which has already been factored into the analysis. Below 0.4 TU of pyrethroids, mortality was equal or only slightly above the levels typically seen in control samples. Above about 3 TUs of pyrethroids, there was near total mortality in all samples.

The fact that elevated mortality was seen in these field samples at pyrethroid concentrations the present laboratory studies indicated should cause substantial mortality supports the accuracy of the reported LC50s. In addition, if other chemical agents or environmental conditions were frequently responsible for the observed *H. azteca* mortality in the field samples, such a clear relationship between total pyrethroids and mortality would be unlikely. It was reported that other pesticide analytes in these samples rarely approached concentrations at which acute *H. azteca* mortality would be expected [8]. It is likely that factors other than pyrethroids are responsible for the slightly elevated mortality seen in a few samples below 0.1 TU pyrethroids, but in any sample exhibiting more than about 40% mortality, pyrethroids alone were present in sufficient concentration to explain it. These sites include three creeks (Del Puerto, Ingram, and Little John Creeks), five irrigation canals, and two ponds receiving excess irrigation water. Only a few samples were taken from the major rivers in the region, and most of these showed low pyrethroid concentrations and little *H. azteca* toxicity. The one river site with elevated mortality (34-52%; San Joaquin River at the town of Vernalis, CA, USA; July, 2002) contained 0.12 to 0.81 TUs of pyrethroids in the sediment, suggesting these compounds were contributors to the toxicity.

Thus, reanalysis of the data using the measured LC50s supports the original conclusion that there are many Central Valley locations in which agricultural pyrethroids are present in the sediments at concentrations acutely toxic to sensitive species such as *H. azteca*. *Hyalella azteca* is a resident species within Central Valley water bodies, though it is typically reported only in areas upstream of substantial agricultural inputs (R. Holmes, Central Valley Regional Water Quality Control Board, Sacramento, CA, USA, personal communication).

Conclusions

In a sediment with about 1% OC, most pyrethroids, excluding permethrin, will cause 50% mortality to *H. azteca* in a 10-d exposure at concentrations in the range of 4 to 10 ng/g. Substantial growth impairment will occur at half these concentrations. While permethrin is the most widely used of the pyrethroids in agriculture, and the most often detected in aquatic sediments [8], its toxicity in sediments to *H. azteca* is lower than that of the other pyrethroids. These results indicate, as was suggested by the pesticide use analysis presented earlier based on water-only toxicity tests, that the other pyrethroids that are used less frequently and generally found at lower concentrations in sediments may be as or more responsible for toxicity to aquatic life than permethrin.

Over 360 metric tons of pyrethroids are used annually in California alone (agricultural and nonagricultural use, but excluding residential consumer use for which data are lacking), and residues of the compounds appear to be widespread in agriculture-affected water bodies. While exposure may occur through the dissolved phase in pore waters, sediments will serve as the primary ecological repository of these compounds. Thus, past emphasis on dissolved phase toxicity with testing on organisms that inhabit the water column appears insufficient, as exposure would be much reduced compared to sediment dwelling organisms. These data indicate the need to better understand the fate and toxicity of sediment-associated pyrethroids to properly assess ecological risk.

Bibliography
1. Kuivila KM, Foe CG. 1995. Concentration, transport and biological effects of dormant spray pesticides in the San Francisco Estuary, California. *Environ Toxicol Chem* 14:1141-1150.
2. Werner I, Deanovic LA, Connor V, de Vlaming VV, Bailey HC, Hinton DE. 2000. Insecticide-caused toxicity to *Ceriodaphnia dubia* (Cladocera) in the Sacramento-San Joaquin River Delta, California, USA. *Environ Toxicol Chem* 19:215-227.
3. Solomon KR, Giddings JM, Maund SJ. 2001. Probabilistic risk assessment of cotton pyrethroids: I. Distributional analyses of laboratory aquatic toxicity data. *Environ Toxicol Chem* 20:652-659.
4. Laskowski DA. 2002. Physical and chemical properties of pyrethroids. In Ware GW, ed, *Reviews of Environmental Contamination and Toxicology*, Vol 174. Springer-Verlag, New York, NY, USA, pp 49-170.
5. Liu W, Gan JJ, Lee S, Kabashima JN. 2004. Phase distribution of synthetic pyrethroids in runoff and stream water. *Environ Toxicol Chem* 23:7-11.
6. Lee S, Gan J, Kim J-S, Kabashima JN, Crowley DE. 2004. Microbial transformation of pyrethroid insecticides in aqueous and sediment phases. *Environ Toxicol Chem* 23:1-6.
7. Muir DCG, Rawn GP, Grift NP. 1985. Fate of the pyrethroid insecticide deltamethrin in small ponds: A mass balance study. *J Agric Food Chem* 33:603-609.
8. Weston DP, You J, Lydy MJ. 2004. Distribution and toxicity of sediment-associated pesticides in the agriculture-dominated water bodies of California's Central Valley. *Environ Sci Technol* 38:2752-2759.
9. Giddings JM, Solomon KR, Maund SJ. 2001. Probabilistic risk assessment of cotton pyrethroids: II. Aquatic mesocosm and field studies. *Environ Toxicol Chem* 20:660-668.
10. Maund SJ, Hamer MJ, Lane MCG, Farrelly E, Rapley JH, Goggin UM, Gentle WE. 2002. Partitioning, bioavailability, and toxicity of the pyrethroid insecticide cypermethrin in sediments. *Environ Toxicol Chem* 21:9-15.
11. Maund SJ, Hamer MJ, Warinton JS, Kedwards TJ. 1998. Aquatic ecotoxicology of the pyrethroid insecticide lambda-cyhalothrin: Considerations for higher-tier aquatic risk assessment. *Pestic Sci* 54:408-417.
12. Conrad A, Fleming R, Crane M. 1999. Laboratory and field response of *Chironomus riparius* to a pyrethroid insecticide. *Water Res* 33:1603-1610.
13. You J, Weston DP, Lydy MJ. 2004. A sonication extraction method for the analysis of pyrethroid, organophosphate, and organochlorine pesticides from sediment by gas chromatography with electron-capture detection. *Arch Environ Contam Toxicol* 47:141-147.
14. U.S. Environmental Protection Agency. 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates, second edition. EPA/600/R-99/064. Office of Research and Development, Washington DC.
15. Chandler G, Coull B, Davis J. 1994. Sediment-phase and aqueous-phase fenvalerate effects on meiobenthos - implications for sediment quality criteria development. *Mar Environ Res* 37:313-327.
16. McKenney C, Weber D, Celestial D, Macgregor M. 1998. Altered growth and metabolism of an estuarine shrimp

(*Palaemoaetes pugio*) during and after metamorphosis on to fenvalerate-laden sediment. *Archiv Environ Contam Toxicol* 35:464-471.

17. McKee MJ, Knowles CO. 1986. Effects of fenvalerate on biochemical parameters survival and reproduction of *Daphnia magna*. *Ecotoxicol Environ Saf* 12:70-84.

18. Sundaram KMS, Curry J. 1991. Partitioning and uptake of permethrin by stream invertebrates and periphyton. *J Environ Sci Health Part B Pestic Food Contam Agric Wastes* 26:219-240.

19. Helliwell S, Stevens M. 2000. Efficacy and environmental fate of alphacypermethrin applied to rice fields for the control of chironomid midge larvae (Diptera : Chironomidae). *Field Crops Res* 67:263-272.

20. Schulz R, Liess M. 2001. Toxicity of aqueous-phase and suspended particle-associated fenvalerate: Chronic effects after pulse-dosed exposure of *Limnephilus lunatus* (Trichoptera). *Environ Toxicol Chem* 20:185-190.