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Identifying the cause of sediment toxicity in agricultural sediments: The role of pyrethroids and nine seldom-measured hydrophobic pesticides

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HIGHLIGHTS

- Monitoring fails to test for many agricultural pesticides used in any given area.
- Nine seldom-analyzed pesticides (e.g., abamectin) were tested for in sediments.
- One-quarter of the sediment samples were toxic to the amphipod, *Hyalella azteca*.
- The seldom-analyzed pesticides may have contributed to toxicity in a few samples.
- Pyrethroid insecticides were responsible for the vast majority of toxicity.

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ABSTRACT

Few currently used agricultural pesticides are routinely monitored for in the environment. Even if concentrations are known, sediment LC_{50} values are often lacking for common sediment toxicity testing species. To help fill this data gap, sediments in California's Central Valley were tested for nine hydrophobic pesticides seldom analyzed: abamectin, diazinon, dicofol, fenpropathrin, indoxacarb, methyl parathion, oxyfluorfen, propargite, and pyraclostrobin. Most were detected, but rarely at concentrations acutely toxic to *Hyalella azteca* or *Chironomus dilutus*. Only abamectin, fenpropathrin, and methyl parathion were found at concentrations of potential concern, and only in one or two samples. One-quarter of over 100 samples from agriculture-affected waterways exhibited toxicity, and in three-fourths of the toxic samples, pyrethroids exceeded concentrations expected to cause toxicity. The pyrethroid Bi-fen-thrin in particular, as well as lambda-cyhalothrin, cypermethrin, esfenvalerate, permethrin, and the organophosphate chlorpyrifos, were primarily responsible for the observed toxicity, rather than the more novel analytes, despite the fact that much of the sampling targeted areas of greatest use of the novel pesticides.

1. Introduction

California's Central Valley has an extensive network of natural and manmade watercourses returning irrigation runoff to the rivers. Sampling in 2002–2006 found 27% of 200 sediment samples caused toxicity to the amphipod, *Hyalella azteca* (Weston et al., 2008). Based on pyrethroid concentrations, these insecticides were likely responsible for mortality in 61% of the toxic samples. The organophosphate, chlorpyrifos, was a secondary contributor. Organochlorine pesticides never attained concentrations of concern. After considering these three pesticide classes, toxicity in 33% of the instances were of undetermined cause. Finding toxicity of unknown cause is not surprising. Over 160 pesticides are applied in the Central Valley (Kuivila and Hladik, 2008) and few are routinely analyzed in environmental samples. Even if analyses are done, concentrations causing sediment toxicity are generally unknown, as are the potential interactions between pesticides.

This study was designed to determine if seldom-analyzed, hydrophobic insecticides, fungicides and herbicides were present in sediments and contributing to toxicity. We describe three approaches to determine if these compounds cause sediment toxicity. Sediments were collected from areas where these pesticides were most heavily used, and tested for their presence and toxicity. Archived sediments previously found to be toxic were also tested for these pesticides. Finally, toxic sediments were evaluated with toxicity identification evaluation (TIE) procedures to identify substances responsible.

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2. Materials and methods

2.1. Pesticide selection

We have typically analyzed sediment for chlorpyrifos and seven pyrethroids (bifenthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, lambda-cyhalothrin, and permethrin). Risk ranking of current-use Central Valley pesticides (Lu and Davis, 2009) was used to identify other pesticides of potential concern, focusing on those with high toxicity (based on water exposures since sediment toxicity data were lacking) and with a $K_{oc} > 1000$, since they would be most likely to be found in sediment. We added fenpropathrin, which was not in the risk-ranking document, but we had observed it in Central Valley sediments. We deleted compounds with major analytical difficulties, very low expected environmental persistence, or extremely low toxicity to *H. azteca* or the midge, *Chironomus dilutus* in preliminary testing. The final list included insecticides (abamectin, diazinon, fenpropathrin, indoxacarb, methyl parathion), acaricides (dicofol, propargite), a fungicide (pyraclostrobin), and a herbicide (oxyfluorofen). We refer to these pesticides as "novel", reflecting their absence in past sediment monitoring. Diazinon has been widely monitored in water, but rarely in sediment.

2.2. Sediment collection

Agricultural pesticide use in California is reported to the Department of Pesticide Regulation's Pesticide Use Reports (PUR) database. In the first phase, referred to as "targeted sampling", the PUR database was used to identify areas of greatest use for each pesticide of interest and to establish months of peak use. Waterways draining those areas were sampled at the end of the peak use period. Oxyfluorfen and diazinon were sampled in February, pyraclostrobin in June, and all others in July to September (all in 2007–2008). Five to 16 sites were identified for each pesticide, with 69 total samples collected. Collection sites were in creeks (56% of sites), agricultural drains (35%), and rivers (9%). The upper 1–2 cm of sediment were collected, and subsampled for toxicity testing, grain size, pesticides, and organic carbon (oc) analyses. Samples were analyzed for the pesticide(s) for which the site had been selected, and the traditional analytes of chlorpyrifos and pyrethroids. Targeting peak use locations and times would yield "worst-case" conditions if off-site transport occurs during or soon after application. For growing season applications, summer irrigation runoff would often be the primary transport mechanism, but in some locations and with some crops, there is little irrigation runoff, and winter rains provide the first opportunity for runoff. While winter rains could be important for some compounds, 4 months elapse between summer application and the first appreciable rain (usually December), providing opportunity for degradation of many pesticides in farm soils.

An additional 12 samples, referred to as "archived samples", were previously collected sediments toxic to *H. azteca*, but with insufficient pyrethroids or chlorpyrifos to explain the cause. They had been collected without regard to the intensity of use of any pesticide, and had been archived in a frozen state for 1–3 years. They were analyzed for all the novel pesticides.

In a third "TIE sampling" phase, sites found to be toxic in various monitoring programs were reported to us, and revisited to collect sediment as described above. Forty samples were tested, with 14 used for TIE procedures. These 14 were analyzed for pyrethroids and chlorpyrifos, with additional analysis for the novel pesticides should TIEs indicate other causes of toxicity. Most sites were in the Central Valley, but two were near Salinas, California.

2.3. Analytical chemistry

The traditional pyrethroids and chlorpyrifos were extracted by accelerated solvent extraction (ASE) followed by solid phase extraction (SPE) clean-up (You et al., 2008). Diazinon, dicofol, fenpropathrin, indoxacarb, methyl parathion, oxyfluorfen, and pyraclostrobin, were extracted by the ASE-SPE method of Wang et al. (2010). Analyses were performed using an Agilent 6890 series gas chromatography (GC) with a micro-electron capture detector (μECD) and a nitrogen phosphate detector (NPD) (Agilent Technologies, Palo Alto, CA, USA). An HP-5MS and a DB-608 column were used. Diazinon and methyl parathion were detected by NPD and all other pesticides by μECD. Two surrogates, 4,4′-dibromooctafluorobiphenyl (DBOFB) and decachlorobiphenyl (DCBP), were added prior to the ASE extraction, with recoveries of 80–113% and 88–121%, respectively. Propargite was extracted using a sonication extraction method modified from EPA method 3550B, and analyzed by GC/mass spectrometry (Ding et al., 2011). Abamectin was quantified using high-performance liquid chromatography with fluorescence detection after sonication extraction and derivatization (Ding et al., 2011). Matrix spikes, matrix spike duplicates and blanks (clean sand) were run every 20 samples. Some data are presented in toxic units (TUs), calculated as actual concentration divided by the *H. azteca* or *C. dilutus* 10-d sediment LC_{50}, with all values oc-normalized.

2.4. Toxicity testing

Sediments were tested with *H. azteca* following standard protocols (USEPA, 2000). Approximately 75 mL of sediment was placed in five replicate 400-mL beakers, and the beakers filled with 250 mL water made moderately hard by adding salts to Milli-Q purified water (Millipore, Billerica, MA, USA). Ten 7–14-d old *H. azteca* were added to each beaker, held at 23 °C with a 16 h:8 h light:dark cycle, and fed daily with 1 mL yeast/cerophyll/trout chow. An automated system delivered 500 mL water to each beaker daily. After 10 d, survivors were recovered on a 425 μm screen. All tests included a 2% organic carbon control sediment collected from a drinking water reservoir. This sediment was collected far from any agricultural influence, but was screened for pyrethroids that could also be of urban origin, and none were found.

Three TIE procedures developed for pyrethroids were used. First, addition of piperonyl butoxide (PBO) inhibits enzymatic detoxification of pyrethroids, increasing toxicity if they are responsible. The PBO was added to overlying water at 25 μg L^{-1} (Amweg and Weston, 2007). About 80% of water was removed daily and replaced with fresh PBO solution. Second, tests were done at 18 °C, roughly doubling pyrethroid toxicity compared to the standard 23 °C test (Weston et al., 2009). Third, samples were treated with engineered enzymes, developed to hydrolyze specific pesticides. Enzyme OpdA degrades chlorpyrifos and other organophosphates (Sutherland et al., 2004). Enzyme E3-013 degrades bifenthrin, permethrin, and possibly other pyrethroids (Weston and Jackson, 2009). Both enzymes reduce toxicity of contaminated sediments (Weston and Jackson, 2009), though it is not clear if they hydrolyze adsorbed pesticides or only those in interstitial and overlying water. The enzymes were obtained through research collaboration with Orica Ltd., Melbourne, Australia. Enzyme was added to the overlying water daily (10 mg L^{-1}) with the water change. To establish if toxicity reduction was due to enzymatic activity, or simply complexation of toxicant with dissolved organic matter (DOM) contributed by the enzyme, trials included a DOM control. Bovine serum albumin (BSA) at 10 mg L^{-1} was initially used, but later OpdA enzyme was used as a DOM control for E3-013, and vice versa.

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These TIE procedures were tested with control sediments spiked with abamectin and diazinon. Compounds were spiked into sediment in an acetone carrier (<0.7 mL kg^{-1}), mixed using a paint-mixing attachment in an electric drill, and held at 4 °C for 12 d before use. The 10-d sediment LC_{50} was determined for each TIE manipulation using at least five concentration steps (three replicates per step) with results reported based on initial actual concentrations.

The TIE tests also helped identify toxicants in field samples exhibiting high toxicity. Early TIEs (seven tests) were done with undiluted sediments, using five replicate beakers for each TIE manipulation. Later TIEs (seven tests) were done as dilution series, using test sediment diluted with control sediment (100%, 50%, 25%, 12%, and 6%). Three replicate beakers were used at each concentration, and the LC_{50} determined. TIE testing conditions followed those described for the general 10-d exposures, except water was automatically exchanged over 30 min, rather than throughout the day, so that PBO and enzyme concentrations could be restored quickly.

Toxicity data were analyzed using CETIS (Tidepool Scientific Software, McKinleyville, CA, USA). Mortality in field sediments was compared to controls using *t*-tests when parametric assumptions were met, or Wilcoxon Rank Sum when they were not. Dunnett's test was used to compare TIE treatments to controls when testing undiluted field sediments. For TIEs done as a dilution series, the Spearman–Karber LC_{50} method was used, and the significance of differences in LC_{50}s determined by non-overlapping 95% confidence intervals.

Twenty-five field samples were also tested with the midge, *C. dilutus*, following standard protocols (USEPA, 2000). Wet sediment (60 g) was distributed into five replicate beakers with 300 mL overlying water. Ten 3rd instar larvae were added and held at 23 °C with a 16 h:8 h light: dark photoperiod. Moderately hard overlying water was renewed three times daily (60 mL each renewal). Organisms were fed 1 mL of a 6 g L^{-1} Tetrafin® suspension daily. After 10 d, surviving organisms were recovered using a 500 μm sieve.

3. Results and discussion

3.1. Sediment chemistry

Two of the novel analytes were found in most samples. Dicofol, primarily used in California on cotton, beans, and oranges, was in 75% of samples up to 250 ng g^{-1} (Table 1). The herbicide oxyfluorfen was in 81% of samples up to 265 ng g^{-1}. Indoxacarb and methyl parathion had detection frequencies ranging from 25% to 50%. The remaining novel analytes were found in one sample (abamectin, diazinon, fenpropathrin, propargite) or not at all (pyraclostrobin).

Measured concentrations were compared to sediment 10-d LC_{50} values (Ding et al., 2011). Abamectin is extremely toxic to *C. dilutus* with an LC_{50} of 0.18 μg g^{-1} oc, or 1–4 ng g^{-1} in typical Central Valley sediments. It was in a single sample (Nile Road Drain), at 0.5 ng g^{-1}, just slightly below the *C. dilutus* LC_{50}. Fenpropathrin was in a single sample (Mosher Creek) at 20.6 ng g^{-1}, about half the *H. azteca* LC_{50}. Methyl parathion was in five samples, but reached concentrations of concern in only one archived sample (Elbow Creek) at 1360 ng g^{-1}, many times its LC_{50} to *H. azteca*. No other novel analyte reached concentrations causing acute toxicity to *H. azteca* or *C. dilutus*. Since samples were collected at times and in areas of peak use, our finding that only three compounds (abamectin, fenpropathrin, methyl parathion) reached concentrations of concern, and only in a single sample each, suggests none present widespread risk.

On the other hand, pyrethroids remain of toxicological concern. Bifenthrin was found in 30% of samples, and presented risk of acute toxicity to *H. azteca* in nearly half of them (Table 1). Its presence at toxic concentrations in one of every seven samples is noteworthy since locations were selected based on use of novel analytes, not bifenthrin use. Bifenthrin reached a maximum of 32 ng g^{-1} (Bear Creek), compared to an LC_{50} of 3–10 ng g^{-1} in typical Central Valley sediments. Lambda-cyhalothrin was of occasional concern, reaching toxic concentrations in 4% of samples. Cypermethrin, esfenvalerate, and permethrin approached levels of concern to *H. azteca* in one or two samples. No pyrethroids reached concentrations representing an acute threat to *C. dilutus*.

The organophosphate chlorpyrifos was in 49% of samples, a finding not surprising since its California agricultural use is twice that of all pyrethroids combined. Only one sample reached a concentration of concern (98.6 ng g^{-1} in the Tuolumne River).

3.2. Toxicity

Throughout the study, *H. azteca* survival in control sediments was 85–98% (mean = 93%). Of 69 samples collected during the targeted sampling phase for the novel pesticides 29% caused mortality statistically exceeding controls (Table 2). In the TIE phase, using sediments from historically toxic sites, 25% of 40 samples were toxic.

Using 0.5 TU as a rough approximation of when concentrations may be approaching a threshold of toxicity, and not considering interactive effects among pesticides, there was at least one pesticide >0.5 TU in 23 of 30 toxic samples. Bifenthrin was the dominant toxicant in half the toxic samples and a secondary toxicant in two more (57% overall). Lambda-cyhalothrin likely contributed to toxicity in four samples, cypermethrin in three, and permethrin in one. One or more pyrethroids exceeded 0.5 TU in 77% of toxic samples, but only 4% of non-toxic samples.

Among novel pesticides, only one was present above 0.5 TU in a toxic sample. Fenpropathrin occurred at 0.5 TU in Mosher Creek, a site with low but statistically significant toxicity.

C. dilutus control survival ranged from 81% to 100% (mean = 94%). Of 25 samples tested, three caused mortality (Cottonwood Creek; Tuolumne River; Unnamed drain at Monte Vista Ave.). Toxicity in Cottonwood Creek could not be explained by any measured pesticides, but Tuolumne River toxicity may have been due to chlorpyrifos, present at 0.6 TU for *C. dilutus*. Toxicity in the unnamed drain may have been due to high esfenvalerate concentration (203 ng g^{-1}), but the *C. dilutus* esfenvalerate LC_{50}, and therefore the TU, is unknown.

3.3. Toxicity identification evaluations

Addition of PBO, reducing temperature, and addition of enzymes are effective TIE tools for identifying pyrethroid-related toxicity (Phillips et al., 2006; Amweg and Weston, 2007; Weston and Jackson, 2009; Weston et al., 2009; Weston and Lydy, 2010). The TIE response profiles for pyrethroids are different than profiles for chlorpyrifos, DDT, fluoranthene, and cadmium. In this study, these TIE procedures were used with two novel pesticides: abamectin and diazinon.

Bifenthrin shows the typical pyrethroid profile for these TIE procedures (Fig. 1). Adding PBO more than doubles bifenthrin toxicity. Reducing temperature also increases toxicity. Enzyme E3-013 reduces bifenthrin toxicity, although it is not effective against all pyrethroids. Enzyme OpdA, which hydrolyzes organophosphates, has no effect on bifenthrin toxicity, nor does BSA used as a DOM control.

All treatments had little or no effect on abamectin toxicity. PBO slightly decreased toxicity. BSA and E3-013 slightly increased toxicity, and although similar in magnitude, only BSA effects were

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Table 1
Concentration of the pesticide analytes in Central Valley sediments, in comparison to published sediment 10-d LC_{50} concentrations for *H. azteca* and *C. dilutus*. Fifty percent of the LC_{50} is used as a rough approximation of when concentrations may be approaching the point of acute toxicity.

Pesticide	*H. azteca* 10-d LC_{50} ($\mu g\ g^{-1}$ oc)	*C. dilutus* 10-d LC_{50} ($\mu g\ g^{-1}$ oc)	Samples tested	Detection frequency (%)	Maximum concen. ($ng\ g^{-1}$ d.w.)	% Samples exceeding 50% *H. azteca* LC_{50}	% Samples exceeding 50% *C. dilutus* LC_{50}
Standard analytes							
Bifenthrin	0.52	6.2	79	30	32.2	13	0
Cyfluthrin	1.08	2.34	78	1	3.0	0	0
Cypermethrin	0.38	1.3	78	1	6.7	1	0
Deltamethrin	0.79	No data	78	0	<1	0	LC_{50} unknown
Esfenvalerate	1.54	No data	79	30	203	1	LC_{50} unknown
λ-Cyhalothrin	0.45	2.8	79	14	11.7	4	0
Permethrin	10.83	24.5	79	37	158	2	0
Chlorpyrifos	4.16	7.73	78	49	98.6	1	1
Novel analytes							
Abamectin	19.9	0.18	6	17	0.5	0	17
Diazinon	15.4	54.3	18	6	3.6	0	0
Dicofol	>573	915	28	75	250	0	0
Fenpropathrin	1.6	8.9	79	4	20.6	1	0
Indoxacarb	>1420	11.3	20	50	118	0	0
Methyl parathion	6.9	318	20	25	1360	5	0
Oxyfluorfen	>6140	630	21	81	265	0	0
Propargite	>467	964	19	5	41	0	0
Pyraclostrobin	>1920	346	8	0	<1	0	0

Most LC_{50} values from Amweg et al. (2005), Maul et al. (2008), or Ding et al. (2011), using means when multiple values provided. A few LC_{50} values from Weston et al. (2009), Maund et al. (2002), Ankley et al. (1994), and Xu et al. (2007).

Table 2
Sites demonstrating significantly greater mortality to *H. azteca* than the corresponding control. See Supplementary Information for site coordinates.

Site	Sample date	Percent survival	Pesticides >0.5 TU
Targeted phase sampling			
August drain	August 2007	16	Bifenthrin (1.3 TU)
Bear Creek	September 2007	35	Bifenthrin (1.3 TU)
Cottonwood Creek	February 2008	23–49	Permethrin (0.6 TU)
Cottonwood Creek	June 2008	63	None
Del Puerto Creek	July 2008	1	Bifenthrin (2.9 TU) Lambda-cyhalothrin (0.7 TU)
Helm Canal	August 2007	41	None
Holland Drain	September 2007	0	Cypermethrin (1.2 TU)
Hospital Creek	July 2007	3	Bifenthrin (4.5 TU)
Ingram Creek	July 2007	1	Lambda-cyhalothrin (2.9 TU)
Ingram Creek	June 2008	23	Bifenthrin (1.7 TU) Lambda-cyhalothrin (0.7 TU)
Middle Paddy Creek	September 2007	91	None
Mokelumne River	February 2008	54	None
Mosher Creek	September 2007	90	Fenpropathrin (0.5 TU)
Orestimba Creek (Morris Rd.)	July 2007	84–88	Bifenthrin (0.6 TU)
Orestimba Creek (River Rd.)	August 2008	71	None
Poso Slough	September 2007	46	Bifenthrin (0.6 TU)
Prairie Flower Drain	August 2007	60	Bifenthrin (0.7 TU)
Unnamed Drain (Jack Tone Rd.)	August 2007	61	None
Unnamed Drain (Monte Vista Ave.)	February 2008	0	Esfenvalerate (4.5 TU) Bifenthrin (0.6 TU)
Unnamed Drain (Monte Vista Ave.)	June 2008	16	Esfenvalerate (1.5 TU) Bifenthrin (1.6 TU)
TIE phase sampling			
Blewett Drain	October 2008	76	Bifenthrin (2.0 TU)
Chualar Creek	August 2010	3	Chlorpyrifos (5.4 TU)
Cottonwood Creek	June 2010	10	Bifenthrin (3.9 TU) Esfenvalerate (1.1 TU)
Hatch Drain	October 2008	0	Bifenthrin (9.3 TU)
Stadiler Drain	October 2008	76	Bifenthrin (1.1 TU)
Holland Drain	September 2010	8	Bifenthrin (2.8 TU) Cypermethrin (1.2 TU)
Marsh Creek	November 2009	62	Bifenthrin (1.1 TU)
Poso Slough	June 2010	22	Bifenthrin (1.6 TU)
Quail Creek	August 2010	12	Lambda-cyhalothrin (1.6 TU)
Unnamed Drain (Lone Tree Creek)	July 2010	12	None

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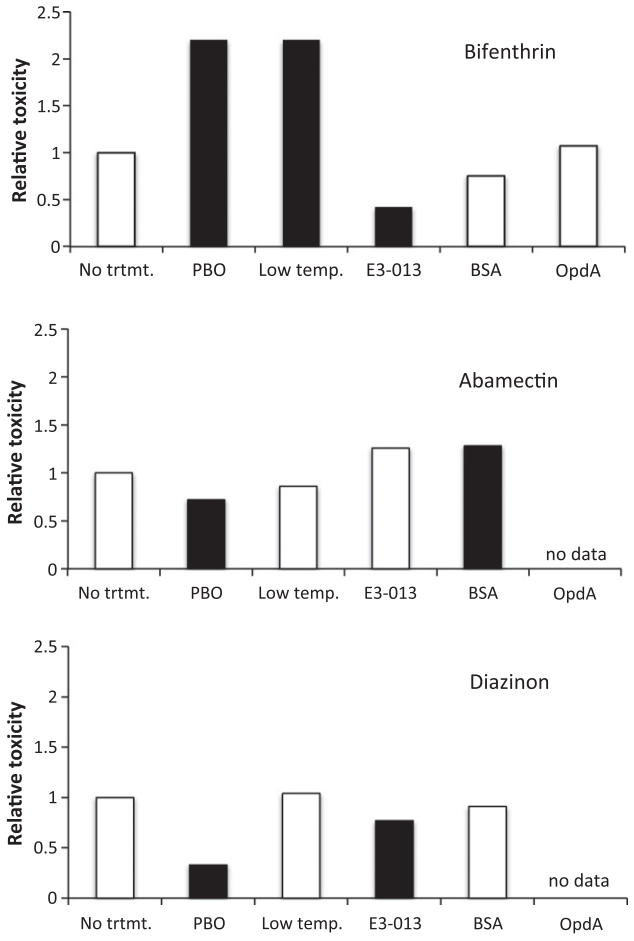

Fig. 1. The effect of various TIE treatments on toxicity of bifenthrin, abamectin and diazinon. Effects are shown as the ratio of the 10-d sediment LC_{50} without any treatment divided by the LC_{50} with the given treatment (i.e., a value of two indicates the treatment doubled toxicity; a value of 0.5 indicates toxicity was halved). Filled bars are significantly different from the "no treatment" LC_{50} as indicated by non-overlapping 95% confidence intervals. The "no treatment" condition is set at one, but is equivalent to 8.1 $\mu g\, g^{-1}$ oc for abamectin, and 19.3 $\mu g\, g^{-1}$ oc for diazinon. Bifenthrin data compiled from Amweg and Weston (2007), Weston and Jackson (2009), and Weston et al. (2009), with the "no treatment" LC_{50} varying from 0.26 to 0.99 $\mu g\, g^{-1}$oc among these studies.

statistically different from the control because of a narrower 95% confidence interval.

The PBO dramatically reduced diazinon toxicity (Fig. 1) as expected given it inhibits transformation of organophosphates to the toxic oxon form. Low temperature had no effect on diazinon toxicity. There was a slight toxicity reduction due to E3-013, indicating its activity may not be entirely specific to pyrethroids, although it is far more effective against them. The OpdA enzyme was not tested, but is known to reduce diazinon concentration and toxicity (Scott et al., 2011).

When field samples with high toxicity were found, early TIEs were done using undiluted field sediments (Table 3), and later as dilution series to derive an LC_{50} (Table 4). All tests included controls in which the TIE procedure was applied to water overlying control sediment, and there was no effect on *H. azteca* survival. The PBO increased toxicity in every field sample tested, usually by at least threefold. This result is consistent with finding pyrethroids in every sample with the exception of Chualar Creek; it contained no reported pyrethroids, but did contain 258 $ng\, g^{-1}$ chlorpyrifos (3 TU). The PBO effect was slight, and may have been due to lambda-cyhalothrin at approximately 0.3 $ng\, g^{-1}$, below the nominal 1 $ng\, g^{-1}$ reporting limit. PBO is far more effective at enhancing pyrethroid toxicity than it is in mitigating organophosphate toxicity (Amweg and Weston, 2007).

The low temperature TIE treatment was erratic, with increased toxicity at low temperature in four instances, but no significant effect in nine instances. In no case was there a decrease in toxicity at low temperature, as observed with some toxicants like cadmium (Weston et al., 2009). We regard the low temperature treatment as the least reliable of the procedures employed. It sometimes provides the expected increase in pyrethroid toxicity, but occasionally does not even when a pyrethroid is the known cause of toxicity (e.g., cypermethrin; (Weston and Lydy, 2010)).

The E3-013 enzyme resulted in decreased toxicity in seven of 13 samples. In these cases, the presence of bifenthrin, or occasionally permethrin and esfenvalerate, was analytically confirmed. Of six samples on which E3-013 had no effect on toxicity, no effect was expected in Chualar Creek because the primary toxicant was an organophosphate. In two samples, the toxicant was lambda-cyhalothrin, against which E3-013 has minimal effect (Weston and Jackson, 2009). In the three remaining samples, all containing bifenthrin, the enzyme decreased toxicity but results were not statistically significant (survival was 14% greater in Stadiler Drain, 15% in unnamed drain to Lone Tree Creek, and 26% in Marsh Creek).

When BSA was used as the DOM control, it had no effect in three of four instances. In the single sample having an organophosphate as the primary toxicant (chlorpyrifos in Chualar Creek), addition of OpdA enzyme nearly removed all toxicity, increasing survival in the undiluted sediment from 3% to 78%, and raising the LC_{50} from 35% to >100%.

The BSA has traditionally been used as a DOM control when using enzymes (Wheelock et al., 2006; Weston and Lydy, 2010), but simultaneous use of E3-013 and OpdA is a better alternative. The assumption that BSA and enzymes would be equally effective in complexation of pyrethroids is unvalidated, so suitability of a BSA control remains questionable. However, enzymes are provided as a crude preparation of lysed bacteria cells, very little of which is the enzyme of interest. Thus, most DOM added with E3-013 and OpdA would be essentially identical. The OpdA enzyme acts as a DOM control for E3-013 when pyrethroids are present, and E3-013 acts as a control for OpdA when organophosphates are present. The interpretive complications when both organophosphates and pyrethroids are present could be avoided by using as a control some other enzyme active against pesticides not found in study area sediments. Enzymes have been produced against triazine herbicides and carbamates (Scott et al., 2011). Unfortunately, only OpdA is commercially available. It is marketed as Landguard™ in Australia to treat organophosphate-contaminated wastewaters and could be a powerful tool to identify organophosphate toxicity.

4. Conclusions

Based on testing with *H. azteca* and *C. dilutus*, the novel pesticides do not appear to be frequently present in Central Valley sediments at concentrations posing significant risk. Abamectin, fenpropathrin, and methyl parathion were each found in a single sample at concentrations approaching or exceeding levels of concern. Fenpropathrin has been reported in another Central Valley sediment at 52 $ng\, g^{-1}$ (Weston et al., 2008), more than twice the highest concentration of this study. Abamectin is highly toxic to *C. dilutus*, with a 10-d LC_{50} in very low parts per billion. However, California agriculture used only 8770 kg of abamectin in 2010, compared to, for example, 134,000 kg of bifenthrin (http://cdpr.ca.gov/docs/pur/purmain.htm). Fenpropathrin and methyl parathion were the most toxic of the novel compounds to *H. azteca*, but fenpropathrin use in California is approximately

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Table 3
Results of Toxicity Identification Evaluation manipulations conducted at 100% field-collected sediment without dilution. The effect of the TIE manipulation on toxicity is characterized as an increase, decrease, or no statistically significant effect. Values shown are the mean percent survival of *H. azteca* and its standard deviation.

Sample	% Survival unamended	% Survival with PBO	% Survival at 18 °C	% Survival with E3-013	% Survival with BSA	Pesticides detected
Marsh Creek November 2009	62 ± 44	Increase 12 ± 18	No effect 78 ± 13	No effect 88 ± 16	No effect 88 ± 11	Bifenthrin (1.1 TU) Chlorpyrifos (<0.1 TU)
Cottonwood Creek February 2008	12 ± 8	Increase 0 ± 0	No effect 16 ± 5	Decrease 70 ± 14		Permethrin (0.6 TU)
Stadiler Drain October 2008	76 ± 11	Increase 0 ± 0	Increase 26 ± 11	No effect 90 ± 7	Decrease 94 ± 9	Bifenthrin (1.1 TU)
Blewett Drain[a] October 2008	76 ± 15	Increase 6 ± 9	No effect 54 ± 29	Decrease 98 ± 4	No effect 90 ± 17	Bifenthrin (1.0 TU) Esfenvalerate (0.1 TU)
Mokelumne River February 2008	72 ± 11	Increase 36 ± 21	No effect 44 ± 34	No effect 76 ± 9		Lambda-cyhalothrin (0.3 TU)
Unnamed Drain, Monte Vista Ave.[b] February 2008	30 ± 16	Increase 0 ± 0	No effect 32 ± 22	Decrease 78 ± 18		Esfenvalerate (0.6 TU) Bifenthrin (0.1 TU) Permethrin (<0.1 TU) Chlorpyrifos (<0.1 TU)
Bear Creek September 2007	40 ± 32	Increase 0 ± 0	No effect 18 ± 18			Bifenthrin (1.3 TU) Permethrin (<0.1 TU)

[a] Tested as a 76% dilution, with 24% control sediment.
[b] Tested as a 10% dilution, with 90% control sediment.

Table 4
Results of toxicity identification evaluation manipulations conducted as dilution series. The effect of the TIE manipulation on toxicity is characterized as an increase, decrease, or no statistically significant effect. Values shown are 10-d LC_{50}, as percent original sediment diluted with control material, and the 95% confidence interval of the LC_{50}.

	10-d LC_{50} unamended	10-d LC_{50} with PBO	10-d LC_{50} at 18 °C	10-d LC_{50} with E3-013	10-d LC_{50} with OpdA	Pesticides detected
Chualar Creek August 2010	34.8 (32.8–37.0)	Increase 26.4 (23.7–30.5)	Increase 22.6 (19.9–25.7)	No effect 29.0 (25.7–32.8)	Decrease >100	Chlorpyrifos (5.4 TU)
Cottonwood Crk. June 2010	29.4 (23.9–36.2)	Increase 8.3 (7.3–9.5)	Increase 17.3 (12.9–23.2)	Decrease 53.1 (39.3–71.6)	No effect 33.6 (27.4–41.3)	Bifenthrin (3.9 TU) Esfenvalerate (1.1 TU) Lambda-cyhalothrin (0.1 TU) Permethrin (<0.1 TU) Chlorpyrifos (<0.1 TU)
Hatch Drain October 2008	14.8 (9.8–21.2)			Decrease 30.4 (23.0–38.8)	No effect[a] 22.5 (18.4–27.5)	Bifenthrin (9.3 TU)
Holland Drain September 2010	50.4 (43.0–59.2)	Increase 18.2 (15.0–22.1)	No effect 50.9 (38.8–66.8)	Decrease 98.1 (75.3–>100)	No effect 68.0 (56.2–82.3)	Bifenthrin (2.8 TU) Cypermethrin (1.2 TU) Cyfluthrin (0.3 TU) Chlorpyrifos (<0.1 TU)
Poso Slough June 2010	75.6 (69.6–82.1)	Increase 49.9 (43.5–57.4)	No effect 73.3 (69.8–76.9)	Decrease >100	No effect 95.4 (80.5–>100)	Bifenthrin (1.6 TU) Chlorpyrifos (<0.1 TU)
Quail Creek August 2010	24.1 (19.2–30.3)	Increase 8.8 (7.4–10.5)	No effect 19.8 (16.5–23.9)	No effect 27.6 (19.8–35.9)	No effect 23.7 (19.9–28.1)	Lambda-cyhalothrin (1.6 TU) Cypermethrin (0.3 TU) Chlorpyrifos (0.2 TU) Esfenvalerate (<0.1 TU)
Unnamed Drain to Lone Tree Crk. July 2010	59.3 (52.3–67.2)	Increase 18.4 (15.9–21.4)	Increase 40.9 (35.2–47.6)	No effect 73.6 (60.3–89.8)	No effect 49.8 (41.2–60.3)	Bifenthrin (0.2 TU) Chlorpyrifos (<0.1 TU)

[a] This trial done with BSA, not OpdA.

13,400 kg yr^{-1}, one fourth of which is on strawberries predominantly grown outside the Central Valley. Methyl parathion use is 9700 kg yr^{-1}, nearly all on walnuts. Our results suggest there are only infrequent, isolated instances of toxicity related to abamectin, fenpropathrin, and methyl parathion in the Central Valley, though they could be of concern elsewhere where use is greater.

The remaining novel pesticides (diazinon, dicofol, indoxacarb, oxyfluorfen, propargite, and pyraclostrobin) were not found at concentrations likely to contribute to *H. azteca* or *C. dilutus* toxicity. Water column toxicity due to diazinon is well documented (Kuivila and Foe, 1995), but it has occasionally been reported as a sediment-associated toxicant (Szeto et al., 1990).

Our study reaffirms the contribution of pyrethroids to sediment toxicity. Even though sampling was not specifically focused on areas of high pyrethroid use, 28% of samples exhibited toxicity and in over three-fourths of these, pyrethroids exceeded concentrations expected to cause toxicity. The TIE data support a role of pyrethroids in most instances. Bifenthrin was usually responsible, with occasional contributions from lambda-cyhalothrin, cypermethrin, esfenvalerate and permethrin.

There remained a small number of samples for which cause of toxicity could not be established. It is inevitable that this will occasionally be the case. There are over 66 herbicides, 49 insecticides, 32 fungicides, and 14 pesticides in other categories applied to

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Central Valley agricultural lands (Kuivila and Hladik, 2008). Many have never been analyzed in environmental samples. While our study attempted to close the gap between the large number of pesticides used and the small number measured, the gap remains quite large, and is likely to be a continuing source of unexplained toxicity.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.06.039.

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