Toxicity of Sediment-Associated Pesticides to *Chironomus dilutus* and *Hyalella azteca*

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Abstract Two hundred sediment samples were collected and their toxicity evaluated to aquatic species in a previous study in the agriculturally dominated Central Valley of California, United States. Pyrethroid insecticides were the main contributors to the observed toxicity. However, mortality in approximately one third of the toxic samples could not be explained solely by the presence of pyrethroids in the matrices. Hundreds of pesticides are currently used in the Central Valley of California, but only a few dozen are analyzed in standard environmental monitoring. A significant amount of unexplained sediment toxicity may be due to pesticides that are in widespread use that but have not been routinely monitored in the environment, and even if some of them were, the concentrations harmful to aquatic organisms are unknown. In this study, toxicity thresholds for nine sediment-associated pesticides including abamectin, diazinon, dicofol, fenpropathrin, indoxacarb, methyl parathion, oxyfluorfen, propargite, and pyraclostrobin were established for two aquatic species, the midge *Chironomus dilutus* and the amphipod *Hyalella azteca*. For midges, the median lethal concentration (LC_{50}) of the pesticides ranged from 0.18 to 964 µg/g organic carbon (OC), with abamectin being the most toxic and propargite being the least toxic pesticide. A sublethal growth endpoint using average individual ash-free dry mass was also measured for the midges. The no–observable effect concentration values for growth ranged from 0.10 to 633 µg/g OC for the nine pesticides. For the amphipods, fenpropathrin was the most toxic, with an LC_{50} of 1–2 µg/g OC. Abamectin, diazinon, and methyl parathion were all moderately toxic (LC_{50}s 2.8–26 µg/g OC). Dicofol, indoxacarb, oxyfluorfen, propargite, and pyraclostrobin were all relatively nontoxic, with LC_{50}s greater than the highest concentrations tested. The toxicity information collected in the present study will be helpful in decreasing the frequency of unexplained sediment toxicity in agricultural waterways.

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Pesticide residues in runoff from farmland can be a threat to benthic biota especially for creeks that receive return flow from irrigated fields (Schulz and Liess 1999). Toxicity to *Chironomus dilutus* larvae was seen in 13% of 39 sediment samples collected from a 10-county area in the agriculturally dominated Central Valley of California from 2002 to 2003 (Weston et al. 2004). Two hundred sediment samples were collected from 2002 to 2005 in the same area, and 27% of those sediments were toxic to the epibenthic amphipod *Hyalella azteca* (Weston et al. 2008). Comparison of measured pesticide concentrations in the toxic sediments with those concentrations known to cause mortality indicated that pyrethroid insecticides (mainly bifenthrin, esfenvalerate, *lambda*-cyhalothrin, and permethrin), and occasionally the organophosphate (OP) insecticide chlorpyrifos, were likely responsible for the toxicity in the majority of sediments. After accounting for 20 organochlorine (OC) pesticides, one OP pesticide, and seven pyrethroid pesticides (assuming toxicity is additive based on the assumption of a concentration addition model)

in California agricultural sediments, the cause of toxicity remained unexplained in one third of the toxic samples (Weston et al. 2008).

Currently, hundreds of pesticides are used in California, but only a few dozen are analyzed in standard environmental monitoring. A significant amount of unexplained sediment toxicity may be due to pesticides that are in widespread use but have not been routinely monitored in the environment, and even if some of them were, the concentrations harmful to aquatic organisms are unknown. To decrease the frequency of unexplained toxicity in sediments affected by irrigated agriculture, chemical analysis and toxicity endpoints for more pesticides are needed. The present study focused on pesticides that possessed the following characteristics: relatively high use in the southern portion of California's Central Valley, ranked as high or very high for toxicity based on water exposure data reported in the United States Environmental Protection Agency (USEPA) Ecotox database, and soil absorption coefficients ≥1000, suggesting that residues may be found in bed sediments. Based on a compilation of these data for agricultural pesticides in California (Lu and Davis 2009), 17 candidate pesticides were identified. This list was further decreased by eliminating those compounds that presented analytical difficulties, that had impending regulatory restrictions that were expected to substantially decrease use, that had expected low environmental persistence, or had been found to have very low *C. dilutus* or *H. azteca* toxicity in preliminary testing. The final list of compounds selected for the present study included two OPs (diazinon and methyl parathion), a pyrethroid insecticide (fenpropathrin), an OC acaricide (dicofol), an avermectin insecticide (abamectin), an oxidiazine insecticide (indoxacarb), a phenyl ether herbicide (oxyfluorfen), a sulfite ester acaricide (propargite), and a strobilurin fungicide (pyraclostrobin). The polarity of these pesticides varied, and the log values of their octanol–water partition coefficient (log K_{ow}) ranged from 3.3 to 6.0 (Table 1). Although most of the target pesticides have been applied for several decades, there are few or no data on sediment toxicity for common testing species, such as *C. dilutus* and *H. azteca*. Research on the toxicity of these compounds to aquatic algae, invertebrates, or fish has primarily focused on the water column (Burkepile et al. 2000; Tisler and Erzen 2006; Bringolf et al. 2007), although fewer studies have addressed sediment-associated toxicity. In the present study, sediment LC_{50} values of the nine pesticides were determined using *C. dilutus* and *H. azteca* according to USEPA standard protocols (USEPA 2000). Both lethal and sublethal effects (growth no-observable-effect concentrations [NOECs] using individual ash-free dry mass [AFDM]) of the nine target pesticides to *C. dilutus* was tested using one sediment, whereas three different sediments were used to determine the LC_{50} for *H. azteca*.

Materials and Methods

Chemicals

Propargite and the internal standard phenanthrene-d_{10} were purchased from AccuStandard (New Haven, CT), and all

Table 1 Pesticide type, chemical abstracts service numbers (CAS no.), annual use, and physicochemical parameters of the nine target pesticides

Test pesticides	Type of pesticide	CAS no.	Annual agricultural use in California (kg)[a]	Log K_{ow}[b]	Log K_{oc}[c]
Abamectin	Avermectin insecticide	71751-41-2	5,590	4.0	4.0
Diazinon	OP insecticide	33-41-5	116,000	3.8	2.3
Dicofol	OC acaricide	115-32-2	11,600	5.0	3.8–3.9
Fenpropathrin	Pyrethroid insecticide	39515-41-8	14,800	6.0	5.6–5.7
Indoxacarb	Oxadiazine insecticide	173584-44-6	24,200	4.5	3.3–3.9
Methyl parathion	OP insecticide	298-00-0	16,300	3.3	3.7
Oxyfluorfen	Phenyl ether herbicide	42874-03-3	275,000	4.5	6.2
Propargite	Sulfite ester acaricide	2312-35-8	174,000	3.7	3.6–3.9
Pyraclostrobin	Strobilurin fungicide	175013-18-0	51,400	4.0	3.8–4.2

[a] 2008: http://www.cdpr.approximatelygov/docs/pur/purmain.htm

[b] Barceló et al. (1994), Hazardous Substances Data Bank 2008, Saito et al. (1993), (http://chemicalland21.com/lifescience/agro/FENPROPATHRIN.htm), Environmental Monitoring Branch, Department of Pesticide Regulation (2003). DPR Pesticide, Fisher et al. (1993), U.S. Department of Agriculture, Agriculture Research Service, Pesticide Properties database (http://www.arsusda.gov/services/docs.htm?docid=14199), Xu (2001), European commission, health and consumer protection directorate-general (2001)

[c] Wislocki et al. (1989), http://entweb.clemson.edu/pesticid/document/leeorg1/leeorg3.htm, Tillman (1992), Xu et al. (2007), Brugger and Kannuck (1997), Appelo and Postma (1996), Pesticide Properties database (http://www.arsusda.gov/services/docs.htm?docid=14199), Xu (2001), European commission, health and consumer protection directorate-general (2001)

other standards were from ChemService (West Chester, PA). The purities of the pesticides were >95% as certified by the manufacturers. The surrogates 4,4′-dibromoocta-fluorobiphenyl (DBOFB) and decachlorobiphenyl (DCBP), Florisil solid-phase extraction (SPE) cartridges, and dual-layer SPE cartridges packed with 300 mg granular carbon black (GCB) and 600 mg polymerically bonded ethylene-diamine-N-propyl (PSA) were obtained from Supelco (Bellefonte, PA). Anhydrous sodium sulfate (Na_2SO_4), copper powder, silica gel, and all pesticide grade solvents were purchased from Fisher Scientific (Pittsburgh, PA). Diatomaceous earth was obtained from Dionex Corporation (Sunnyvale, CA). Whatman no. 41 filter paper was from the Whatman Company (Maidstone, UK).

Sediment Collection and Preparation

Test sediments were collected from drinking water reservoirs and a lake in the vicinity of Berkeley, California. Sediment BRSP was a blend of sediments from Briones Reservoir and San Pablo Dam Reservoir. Sediment BR was from Briones Reservoir (different location in the reservoir than BRSP). Sediment LS was a blend of sediments from Lake Anza and San Pablo Dam Reservoir. The sediment was sieved through a 1-mm screen, homogenized, and then frozen until use. These sediments were selected to span a range of OC contents, and contained 0.5% (BR), 2.0% (BRSP), and 2.8% (LS) OC, respectively. Sediment toxicity data were all normalized to OC content. The test sediments were analyzed for the target pesticides before pesticide spiking and the toxicity tests, and no target pesticides exceeded the reporting limit of 1 ng/g dry weight (dw).

Sediments were spiked with each of the target pesticides using acetone as a carrier (<700 μl acetone/kg wet sediment). A solvent control was prepared by adding the same quantity of acetone alone into the sediment. Sediments were thoroughly mixed for 0.5 h using a stainless steel paddle driven by an overhead motor. Then sediments were covered with aluminum foil and aged at 4°C in the dark for 12 days before use in the toxicity tests.

Toxicity Tests

Ten-day sediment toxicity tests with *C. dilutus* (third-instar larvae) and *H. azteca* (juveniles) were performed based on standard USEPA protocols (USEPA 2000). The midges were obtained from stock cultures maintained at the Fisheries and Illinois Aquaculture Center, Southern Illinois University at Carbondale, from cultures originally obtained from the USEPA Environmental Research Laboratory, Duluth, MN. Bioassays with *C. dilutus* were conducted with sediment spiked with each pesticide at four to six concentrations. Five replicates at each concentration were

used for the experiments, and negative and solvent controls were included. After the 12-day aging period, 60 g wet sediment was distributed into each 350-ml beaker (approximately 300 ml overlying water), and the sediment settled overnight. Ten organisms were randomly placed into each beaker and held at 23°C with a 16:8-h light-to-dark photoperiod. Overlying water was renewed by addition of moderately hard water three times per day by an automatic water delivery system (total additions were approximately 180 ml/d), with the excess water overflowing of the beaker through a screened hole. Organisms were fed 1.0 ml 6 g/l Tetrafin (TetraFin® (Tetra Holding, Blacksburg, VA, USA)) suspension daily. After the 10-day exposure, organisms were removed from the sediment by sieving through a 500-μm sieve. Surviving midge larvae were counted and preserved in 10% formalin for later biomass measurements. A subsample of sediment at a mid-range concentration for each pesticide was set aside at the beginning and end of each toxicity test and stored at −20°C for chemical analysis. Mortality was calculated as total midge larvae added minus the surviving midges and, in addition to mortality, growth measured by AFDM was used as a sublethal endpoint for midge toxicity. The midge larvae surviving after the 10-day bioassays were placed in preashed aluminum pans at 60°C for 48 h. The dried organisms were weighed to the nearest 0.001 mg using a Mettler analytical balance (Schwiez, Switzerland) to obtain total mass and mean weights per surviving organism per replicate. Finally, the larvae and pans were ashed at 550°C for 3 h and reweighed to determine the AFDM.

Hyalella azteca toxicity tests generally followed the same procedures as the midge tests with the following exceptions. Organisms were obtained from cultures maintained at the University of California, Berkeley. Juveniles, 7–14 days old, were used for testing, with five to seven concentrations per pesticide and three replicates per concentration. Test organisms were fed daily with a yeast, cerophyll, and trout food mixture, and overlying water (300 ml) was replaced by the addition of 500 ml water/d (each renewal was 100 ml, and five renewals were used daily). After the 10-day testing period, survivors were recovered by sieving on a 425-μm screen. Mortality was the sole endpoint evaluated for *H. azteca.*

In the *C. dilutus* tests, sediments were spiked with pesticides as high as necessary to obtain LC_{50} estimates. In the *H. azteca* tests, pesticides were not spiked >20,000 ng/g nominal (except 153,000 ng/g in one case) on the assumption that higher concentrations would be environmentally unrealistic. If the LC_{50} was not reached at the highest concentration, the LC_{50} value was simply reported as "greater than" the maximum concentration used.

To verify good water quality, pH, conductivity, ammonia, hardness, and alkalinity were measured at the

beginning and end of each test, and temperature and dissolved oxygen were measured daily.

Sediment Pesticide Analyses

Sediment samples were analyzed for the target pesticides after the 12-day aging period (at the beginning of toxicity test) and the end of the 10-day toxicity test. Diazinon, dicofol, fenpropathrin, indoxacarb, methyl parathion, oxyfluorfen, and pyraclostrobin were analyzed according to the method of Wang et al. (2010). In brief, 10 g wet sediment was freeze dried (Labconco Corporation, Kansas City, MO) at $-50°C$ for 24 h and then extracted using an accelerated solvent extractor (ASE 200; Dionex, Sunnyvale, CA) with 1:1 dichloromethane:acetone (v/v) at 100°C and 1500 pounds per square inch (psi) for two 5-min static cycles. The sample cleanup was performed using dual-layer GCB/PSA SPE cartridges. After conditioning the cartridge with 3 ml hexane, the extract was loaded onto the cartridge. Diazinon, dicofol, fenpropathrin, indoxacarb, methyl parathion, and oxyfluorfen were eluted with 7 ml 30% dichloromethane in hexane, whereas pyraclostrobin was eluted off the GCB/PSA cartridge with 7 ml toluene, concentrated to 1 ml, loaded onto a Florisil cartridge, and then eluted with 7 ml hexane. All the eluents were concentrated to 1 ml under nitrogen before instrumental analysis. Granular copper was added to the extracts to remove sulfur interference except for the OPs, and no copper was used for these extracts to avoid the potential loss of OPs by copper-induced hydrolysis and degradation (Tse et al. 2004; Wang et al. 2010; You et al. 2004).

Instrumental analyses for these seven pesticides were performed using an Agilent 6890 series gas chromatograph (GC) equipped with a micro-electron capture detector (μECD) and a nitrogen phosphorous detector (NPD) (Agilent, Palo Alto, CA). Dicofol, fenpropathrin, indoxacarb, oxyfluorfen, and pyraclostrobin were detected using μECD, and diazinon and methyl parathion were detected by NPD. The detector temperature for the ECD and NPD were set at 320°C and 290°C, respectively. Two μl of the sample were injected using an Agilent 7683 autosampler in pulsed splitless mode, and the quantification of analytes were confirmed using dual columns: an HP-5MS column (30 m × 0.25 mm × 0.25 μm film thickness; Agilent) and a DB-608 column (30 m × 0.32 mm × 0.25 μm film thickness; Agilent). The GC oven for the HP-5MS column was ramped from an initial temperature of 100–180°C at a rate of 10°C/min, then to 205°C at 3°C/min and held at 205°C for 4 min and then to 280°C at 20°C/min and held at 280°C for 10.92 min. The GC oven for the DB-608 column was ramped from 100 to 250°C at 10°C/min and then to 280°C at 3°C/min and held at 280°C for 20 min. The flow rates of carrier gas were 3.8 and 1.8 ml/min for the

HP-5MS and DB-608 columns, respectively. Calibrations were based on peak area using five external standards. The standard solutions were made by dissolving 5, 10, 50, 200, and 250 ng each pesticide and surrogate into 1 ml hexane. The calibration curve was linear within the concentration range with linear regression coefficient $r^2 > 0.995$. To verify the performance of the analytical process, two surrogates (DBOFB and DCBP) were added to the freeze-dried sediment before ASE extraction. Validation of the sediment extraction method yielded extraction efficiencies of 76.5–126.6% in three different types of sediments for the target pesticides (Wang et al. 2010).

Propargite was extracted from sediment using a sonication-extraction method modified from USEPA method 3550B and analyzed by GC/mass spectrometry (GC/MS). Propargite samples were extracted with 60 ml 50:50 (v/v) mixture of acetone-dichloromethane using three 15-min sonication periods and then filtered although a 0.45-μm filter paper containing a small amount of anhydrous Na_2SO_4. Finally, the extract was concentrated using a Turbovap Evaporator (Hopkinton, MA) at 30°C to a final volume of 1 ml. Quantification of propargite was performed on an Agilent 6890 GC-5975 MS (Agilent) operated in the selected ion monitoring mode. A J&W DB-5MS column (30 m × 0.25 mm × 0.25 μm film thickness; Agilent) was used. Helium was employed as the carrier gas at a flow rate of 1.5 ml/min. The oven temperature program was 70°C held for 2 min, increased at 25°C/min to 150°C, and at 3°C/min to 200°C, then at 8°C/min to 280°C and held for 10 min. The extract (5 μl) was injected into the GC's silico-steel coated injection port using a pressure-pulsed splitless injection at 75 psi for 0.5 min. The quantitation, target, and qualifier ions had masses of 135, 173, and 81, respectively, and phenanthrene-d_{10} was used as the internal standard for calibration.

Finally, abamectin sediment concentrations were quantified using a high-performance liquid chromatograph (HPLC) with fluorescence detection after sonication extraction and derivatization. Approximately 10 g wet sediment was dried by mixing with 8 g anhydrous $MgSO_4$ and 5 g anhydrous Na_2SO_4; a 50-ml aliquot of a 50:50 mixture of acetone and dichloromethane was added; and the sample was sonicated for 5 min in 3-s pulse mode using a high-intensity ultrasonic processor (Tekmar, Cincinnati, OH). The extract was decanted and filtered through Whatman no. 41 filter paper. The procedure was repeated twice, and the extracts were combined and evaporated to approximately 5 ml using a Turbovap. The extract was solvent-exchanged with hexane and the volume further reduced to 1 ml under nitrogen. The extract was cleaned using a dual-layer GCB/PSA SPE cartridge. After conditioning the cartridge with 3 ml hexane, the extract was loaded, and abamectin was eluted off the cartridge with 10 ml a mixture of acetone

and hexane (1:1, v/v). The effluent was blown down to dryness under a gentle flow of nitrogen and derivatized by adding 300 μl trifluoroacetic anhydride and acetonitrile (1:2, v/v) and 200 μl N-methylimidazole and acetonitrile (1:1, v/v) (Diserens and Henzelin 1999). The derivatization product was analyzed by Agilent 1100 HPLC equipped with a fluorescence detector, and a Stablebond C18 analytical column was used for analyte separation. The fluorescence detector settings were an excitation wavelength of 365 nm and an emission wavelength of 475 nm. The mobile phase (acetonitrile–water-methanol, 47.5:5:47.5, v/v/v) was at a flow rate of 1.1 ml/min. Calibrations were based on peak area using five external standards: 10, 50, 200, 250, and 500 ng abamectin in acetonitrile, and all standards were derivatized before injection. The method showed good accuracy and precision with recoveries of abamectin of 88.1% ± 6.6% ($n = 7$) and 76.1% ± 13.6% ($n = 3$) at spiked concentrations of 2 and 20 ng/g dw, respectively, and the method detection limit was 0.41 ng/g dw.

Statistical Analyses

LC_{50} values for the pesticides were estimated by using either probit or trimmed Spearman–Karber analyses, and Abbott's correction was applied in cases where the data were nonmonotonic (SAS version 8.02; SAS [Cary, NC] or CETIS, Tidepool Scientific [McKinleyville, CA]). Analysis of variance and Dunnett's tests (SAS version 8.02) were used to calculate NOECs by determining significant differences between control and treatment individual biomass ($p < 0.05$).

Results

Persistence of Target Pesticide Concentrations in Sediment During Bioassays

All analytical quality-control data including accuracy of surrogates (70–130%), clean blank samples, accuracy of matrix spikes (75–125%), and relative % difference between matrix spikes (<25%) fell within the satisfactory range. Fenpropathrin was persistent in all three types of sediment, with concentrations only decreasing 1–12% during the 10-day exposure (Table 2). Pyraclostrobin concentrations were also relatively stable, with concentrations decreasing 5–35% during the tests. Abamectin and propargite concentrations decreased only slightly in BRSP and LS sediments, but their concentrations decreased quickly in BR sediment, which had the lowest OC content. Dicofol was relatively persistent in LS sediment (28% loss), but its concentrations decreased quickly in BRSP and BR sediments (77–82% loss and 65% loss, respectively). Concentrations of indoxacarb and oxyfluorfen were decreased from 26 to 41% and from 3 to 51%, respectively. Concentrations of the two OP insecticides, diazinon and methyl parathion, quickly decreased in all tested sediments, with % losses from 50 to 77% and 74 to 100%, respectively.

Regardless of the amounts of loss of pesticides from the sediments, the measured sediment concentrations were considered a better measure of actual exposure concentrations than the nominal values. Therefore, the reported toxicity and growth data were adjusted to actual concentrations using the mean values of the measured concentrations at the beginning and end of the 10-day toxicity tests.

Table 2 Percent loss of pesticides from the test sediments during the 10-day toxicity tests, based on measured concentrations at the beginning and end of the tests[a]

Test pesticides	BRSP (*Chironomus* tests)	BRSP (*Hyalella* tests)	BR (*Hyalella* tests)	LS (*Hyalella* tests)
Abamectin	6	(22)	77	24
Diazinon	64	77	50	57
Dicofol	77	82	65	28
Fenpropathrin	11	1	12	2
Indoxacarb	38	41	26	26
Methyl parathion[b]	99	86–97	74–96	93–100
Oxyfluorfen[b]	20	4–47	3–51	(22)–13
Propargite	(14)	9	64	26
Pyraclostrobin	35	7	5	17

[a] Increases in concentrations are indicated within parentheses and are likely due to heterogeneity of the pesticides within the sediment or analytical errors rather than actual gains

[b] Some tests repeated twice; range of values is shown

Lethality and Growth Impairment of Midges Exposed
to Target Pesticides

Temperature, DO, pH, conductivity, ammonia, hardness,
and alkalinity of overlying water were monitored through-
out the bioassays, and all water parameters were within the
acceptable range required by the USEPA (USEPA 2000).
Survival of *C. dilutus* in both negative and solvent controls
were > 88% in all tests, and average AFDM values ranged
from 0.58 to 0.67 mg for control organisms after the 10-day
bioassays. There were no significant differences ($p > 0.05$)
in the measurements between the negative and solvent
controls. LC_{50} values of the nine pesticides (Table 3),
ranged from 0.18 to 964 µg/g OC. Abamectin was the most
toxic of all tested compounds, with a 10-day LC_{50} of
0.18 µg/g OC, and propargite was the least toxic pesticide,
with a 10-day LC_{50} of 964 µg/g OC. Pesticide toxicity to
midges, from the most to least toxic, in BRSP sediment
was in the order of abamectin, fenpropathrin, indoxacarb,
diazinon, methyl parathion, pyraclostrobin, oxyfluorfen,
dicofol, and propargite.

NOECs values for growth, based on average individual
AFDM, are also listed in Table 3. The growth NOECs were
approximately one half of the LC_{50} values in most cases,
ranging from 19 to 69% of the LC_{50}s.

Mortality of Amphipods Exposed to Target Pesticides

Three sediments with various OC contents were used
for the *H. azteca* bioassay tests, with no appreciable dif-
ference found between negative and solvent control sur-
vival among sediments (mean survival was 95%). All
water-quality parameters were within acceptable ranges.
Fenpropathrin was the most toxic of the nine pesticides

tested, with an average 10-day LC_{50} of 1.6 µg/g OC
(Table 4). The two OP insecticides were less toxic, with
LC_{50} values of diazinon ranging from 2.8 to 24.4 µg/g OC,
and LC_{50} values of methyl parathion ranging from 2.8 to
12.7 µg/g OC. Abamectin was comparably toxic, with an
LC_{50} averaging approximately 20 µg/g OC (range
11.3–26.2). LC_{50} concentrations for dicofol, indoxacarb,
oxyfluorfen, and pyraclostrobin were not determined
because *H. azteca* mortality did not reach 50% even at the
highest spiked concentration (LC_{50}s > 200 µg/g OC).
Propargite had a measurable LC_{50} value in BRSP sediment
(576 µg/g OC) but was also relatively nontoxic.

Discussion

Persistence of Pesticide Concentrations During
Bioassays

Fenpropathrin was the only pyrethroid insecticide tested in
the present study, and it was persistent in all sediments
during the 10-day toxicity tests (Table 2). Similar persis-
tence has been found for other pyrethroid insecticides in
sediments in *C. dilutus* and *H. azteca* testing with the %
losses <10% (Trimble et al. 2009) or <25% (Maul et al.
2008). Bondarenko et al. (2006) also reported that sedi-
ment-associated pyrethroids were stable at 4°C in the dark
and that loss due to sorption to the container was negli-
gible. Abamectin photodegrades in water to less bioactive
compounds by oxidative and photo-oxidative mechanisms
(Tisler and Erzen 2006); however, it is readily sorbed by
sediment particles, and the present study showed that
abamectin could be relatively stable in sediment, with the
exception of BR sediment, which had extremely low OC

Table 3 Ten-day sediment LC_{50} values, LC_{50} values normalized to sediment OC content, and NOEC values normalized to sediment OC content for *C. dilutus*[a]

Pesticide	LC_{50} (ng/g_{dw}) (95% CI)	LC_{50} (µg/g_{oc}) (95% CI)	Growth NOEC (µg/g_{oc})
Abamectin	3.6 (2.8–4.3)	0.18 (0.15–0.21)	0.10
Diazinon	1085 (954–1235)	54.3 (47.7–61.8)	15.9
Dicofol	18308 (14362–26383)	915 (718–1319)	630
Fenpropathrin	177 (107–353)	8.9 (5.4–17.7)	1.7
Indoxacarb	226 (191–255)	11.3 (9.6–12.8)	3.2
Methyl parathion	6362 (6099–6636)	318 (305–332)	115
Oxyfluorfen	12603 (8657–14323)	630 (433–716)	312
Propargite	19279 (17122–25252)	964 (856–y1263)	633
Pyraclostrobin	6913 (4958–8443)	346 (248–422)	160

CI 95% confidence interval

[a] All data are from BRSP sediment (2.0% OC)

Table 4 Ten-day sediment LC$_{50}$ values for *H. azteca* with the target pesticides, shown on both a dw basis and normalized to sediment OC content (0.5%, 2.0%, and 2.8% in BR, BRSP, and LS sediments, respectively)

Pesticide	Sediment BR LC$_{50}$ (ng/g$_{dw}$) (95% CI)	Sediment BRSP LC$_{50}$ (ng/g$_{dw}$) (95% CI)	Sediment LS LC$_{50}$ (ng/g$_{dw}$) (95% CI)	Sediment BR LC$_{50}$ (µg/g$_{oc}$) (95% CI)	Sediment BRSP LC$_{50}$ (µg/g$_{oc}$) (95% CI)	Sediment LS LC$_{50}$ (µg/g$_{oc}$) (95% CI)
Abamectin	131 (128–135)	446 (332–557)	316 (266–373)	26.2 (25.5–26.9)	22.3 (16.6–27.8)	11.3 (9.5–13.3)
Diazinon	95.0 (89.5–101.3)	489 (442–540)	78.2 (71.2–85.8)	19.0 (17.9–20.3)	24.4 (22.1–27.0)	2.8 (2.5–3.1)
Dicofol	>6160	>5480	>6030	>1230	>274	>215
Fenpropathrin	11.0 (8.3–14.6)	22.3 (18.0–25.9)	39.5 (33.0–46.3)	2.2 (1.7–2.9)	1.1 (0.9–1.3)	1.4 (1.2–1.7)
Indoxacarb	>13,100	>20,200	>17,200	>2630	>1010	>616
Methyl parathion	14.2 (14.0–14.3)	103 (95–113)	356 (324–391)	2.8 (2.8–2.9)	5.2 (4.7–5.6)	12.7 (11.6–13.9)
Oxyfluorfen	>61,400	>68,000	>76,100	>12,300	>3400	>2720
Propargite	2880 (1780–5770)	>8150	>11,650	576 (355–1150)	>408	>416
Pyraclostrobin	>21,600	>19,300	>13,200	>4330	>963	>470

CI 95% confidence interval

(0.5%). Methyl parathion showed rapid loss of 74% to 100% in all three sediments used in the present study. This result is in agreement with previously published field data, where high concentrations of methyl parathion in Mediterranean sediments decreased to approximately 1% within 2 months (Badawy et al. 1984). The other OP, diazinon, also disappeared in the sediments fairly rapidly and slightly more quickly than the sediment half-life of 14–31 days reported previously (Bondarenko and Gan 2004). In addition to possible degradation in sediment, apparent loss of the compound may have been accelerated by dissolution into the overlying water that was regularly replaced throughout the toxicity tests.

Most persistence studies focus on soil or water column persistence, and few or no sediment-persistence data were available in the literature for the other pesticides, including dicofol, indoxacarb, oxyfluorfen, and pyraclostrobin. However, in development of the analytical methods for pesticides used in the present study, persistence of these compounds in sediment at 4°C during a 14-day aging period were evaluated (Wang et al. 2010). In that study, indoxacarb was relatively stable in all three sediments (<20% loss); however, the stability of diazinon, oxyfluorfen, and pyraclostrobin was sediment-dependent. After 14 days, sediment concentrations of diazinon, oxyfluorfen, and pyraclostrobin decreased from 5 to 60%, from 20 to 60%, and from 5 to 40% in the three sediments, respectively.

The loss of the test pesticides during the 10-day testing period may be attributed to several reasons, including the following: (1) the loss of pesticides caused by degradation processes, such as photodegradation or biodegradation; (2) a decrease in extractability by sequestration to sediment particles (Peterson and Batley 1993); and, (3) low log K_{ow} values and relatively high water solubility of some compounds, which could lead to loss during overlying water exchanges. For example, methyl parathion has a log K_{ow} of 3.3 (Fisher et al. 1993) and a water solubility of 55–60 mg/l at 25°C (Verschueren 1996), and thus it could have been susceptible to loss from the water exchanges.

Comparison With Previous Toxicity Studies

To our knowledge, the present study is the first report of sediment toxicity thresholds for the nine target pesticides to midges or amphipods, with the exception of diazinon, fenpropathrin, and methyl parathion for which limited data were available. The threshold for diazinon toxicity in aqueous exposure is well established, but in a sediment matrix the only data available are reports of *H. azteca* mortality at ≥60 ng/g with a substrate composed of leaf litter (Moore et al. 2007a). Although that study did not

provide a precise LC_{50} or data to permit OC normalization, the reported value is nearly within the range of our LC_{50} values on a dw basis, ranging from 78 to 489 ng/g depending on the sediment.

Fenpropathrin's LC_{50} value for *C. dilutus*, determined in the present study to be 9 μg/g OC, was approximately four-fold higher than other recently published values. Xu et al. (2007) reported fenpropathrin LC_{50} values of 2.36, 2.5, and 2.23 μg/g OC for sediments containing 1.44%, 1.88%, and 5.03% OC, respectively. The difference in toxicity results between the present study and those of Xu et al. (2007) could have been due to the different instar larvae and/or the different sediment matrices used. Reported *C. dilutus* LC_{50} values for other pyrethroids included 24.5 μg/g OC for permethrin, 6.2 μg/g OC for bifenthrin, 2.8 μg/g OC for lambda-cyhalothrin (Maul et al. 2008), 13–67 μg/g OC for cypermethrin (Maund et al. 2002), and 2.21–2.43 μg/g OC for cyfluthrin (Xu et al. 2007). Compared with *C. dilutus*, *H. azteca* were more sensitive to pyrethroid insecticides, with sediment 10-day LC_{50} values of approximately 0.5–1 μg/g OC for many pyrethroids (bifenthrin, lambda-cyhalothrin, deltamethrin, and cyfluthrin), and nearly 11 μg/g OC for permethrin (Amweg et al. 2005). The fenpropathrin LC_{50} value of 1.1–2.2 μg/g OC observed in the present study indicates fenpropathrin toxicity to *H. azteca* that is comparable with that of many of the other pyrethroids.

Few sediment toxicity data were reported for the rest of the target compounds to the two test species. The 24-h EC_{50} of methyl parathion to the congeneric benthic midge *C. riparius*, determined with spiked sediment containing 3% OC, was 0.13 μg/g OC (Fisher et al. 1993), and this value was much lower than the NOEC value found in the present study (115 μg/g OC) using a 10-day test with *C. dilutus*. These results suggest that different species from the same genus may show very different sensitivity to the same contaminant.

No data were available in the literature for abamectin sediment toxicity thresholds for either midges or amphipods; however, some toxicity values have been published for soil-dwelling organisms. For example, Diao et al. (2007) evaluated the sublethal toxicity of abamectin to three groups of soil-dwelling invertebrates (springtails, earthworms, and enchytraeids). The EC_{10} values for reproduction were 50 ng/g for springtails, 60 ng/g for earthworms, and 12,800 ng/g for enchytraeids. Sun et al. (2005) tested the 14-day acute toxicity of abamectin for earthworms in an artificial soil and found an LC_{50} value of 17,100 ng/g. The midge seems to be a much more sensitive species than these soil-dwelling invertebrates, with an LC_{50} value of 3.6 ng/g dw or 0.18 μg/g OC, making abamectin the most acutely toxic of the pesticides tested in the present study.

Comparative Toxicity of Tested Pesticides Between Two Tested Species

Both *C. dilutus* and *H. azteca* were tested using BRSP sediment, so results from this matrix can be used to evaluate the relative sensitivity of the two species. *C. dilutus* was more sensitive to abamectin, indoxacarb, oxyfluorfen, and pyraclostrobin than *H. azteca*, whereas *H. azteca* was more sensitive to fenpropathrin, diazinon, and methyl parathion. The greater sensitivity of *H. azteca* to fenpropathrin is not surprising and is consistent with previous results of species sensitivity of other pyrethroids (Amweg et al. 2005; Maul et al. 2008). The relative sensitivity to dicofol and propargite were difficult to compare between the two species due to the lack of precise LC_{50} values for *H. azteca*.

The differences in species sensitivity among compounds illustrate the shortcomings of relying on a single species for toxicity testing in environmental monitoring. Of the seven pesticides for which our data allow comparison of sensitivity, *C. dilutus* is the more sensitive species to four pesticides, and *H. azteca* is more sensitive to three of them. Neither species is the obvious choice if sensitivity is the criterion for selection as a testing species. Although simultaneous testing with both species in environmental monitoring would take advantage of these differences in relative sensitivity, few programs do so, largely because of cost constraints. In California, where all of these pesticides are used, virtually all sediment testing is currently performed using *H. azteca* (Weston et al. 2004, Phillips et al. 2006, Holmes et al. 2008, Brown et al. 2010), but the data of the present study indicate that this is not the most protective choice with respect to all pesticides.

Organism Toxicity Endpoints and Reported Environmental Field Concentrations

To assess the potential ecological risk caused by these pesticides, it is necessary to compare toxicity endpoints with the reported sediment concentrations in the environment in which these compounds are applied. For at least fenpropathrin, methyl parathion, and diazinon, there are indications that environmental concentrations in sediment can exceed toxicity thresholds. Sediment from an agriculture-affected stream in northern California contained 8.5 μg/g OC fenpropathrin (Weston et al. 2009). In southern California, fenpropathrin was found in 68% of the dry season and 90% of the wet season sediment samples that were collected throughout a mixed land-use, and concentrations of fenpropathrin reached 19.4 ng/g (1.6 μg/g OC) at one site near a nursery outfall (Budd et al. 2007). Both of these sediment concentrations are higher than the *H. azteca* LC_{50} (1.1 μg/g OC) value, and within the range

of the *C. dilutus* NOEC and LC$_{50}$ (1.7 and 8.9 µg/g OC) values determined in the present study, suggesting that fenpropathrin in field sediments may negatively impact populations of amphipods and chironomids and, furthermore, may influence the aquatic community and disrupt ecosystem balance.

Methyl parathion and diazinon were also widely detected in the environment. Methyl parathion concentration in sediment collected from Spittelwasser, Germany, was 1600 ng/g dw (Brack et al. 1999). Methyl parathion concentrations in three Mississippi Delta oxbow lake sediments ranged from 24.9 to 36.5 ng/g dw (Moore et al. 2007b). Both of these instances indicate potential toxicity to *H. azteca* (LC$_{50}$ values of 14.2–356 ng/g dw), although they are lower than the *C. dilutus* LC$_{50}$ (6362 ng/g dw) value. Diazinon is only moderately hydrophobic (log K_{ow} = 3.8), and its water solubility (40 mg/l) suggests much of it may be in the water column, but high sediment concentrations have occasionally been reported. Diazinon accumulated in sediment at concentrations as high as 47,000 ng/g dw in sediments of an urban creek in Alameda County, CA (Katznelson and Wetzig 1996). Irrigation ditches within a cranberry bog treated with diazinon reached 21,200 ng/g dw (Szeto et al. 1990). These reported diazinon concentration exceeded the *C. dilutus* NOEC (318 ng/g) or LC$_{50}$ (1085 ng/g) and *H. azteca* LC$_{50}$ (78–489 ng/g dw) values determined in the present study. These high diazinon concentrations may negatively impact amphipod and chironomid populations in the field.

Although many OCs have been banned or restricted for use since the 1970s, dicofol is currently in use. A dicofol concentration of 0.06 ng/g dw was detected in sediment samples collected in a reservoir from Beijing, China (Xue et al. 2005), and the highest concentration of dicofol found in sediments of south Florida from 1991 to 1995 was 26 ng/g dw (Miles and Pfeuffer 1997). In addition, a dicofol concentration of 24 ng/g dw was reported in an agricultural stream in California (Pereira et al. 1996). However, these published environmental concentrations are all much lower than the *C. dilutus* NOEC (12,600 ng/g dw) and LC$_{50}$ (18,308 ng/g dw) or *H. azteca* LC$_{50}$ (>5480 ng/g dw) values.

Few or no bed sediment data were available for abamectin, indoxacarb, oxyfluorfen, propargite and pyraclostrobin, and these compounds are rarely among the analytes in environmental monitoring. The absence of data for abamectin was of particular concern given the high toxicity demonstrated to *C. dilutus* observed in the present study. Researchers in our group are collecting and analyzing sediments from the Central Valley of California where these target pesticides are in widespread use. Future field sediment data will be collected and compared with the lethal and sublethal thresholds of the two species

established in the present study to assess the potential contribution of these pesticides to the observed toxicity effects and help to identify the cause of sediment toxicity, which can not be explained by the more typically monitored pesticides.

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