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Changing Patterns in Water Toxicity Associated with Current Use Pesticides in Three California Agriculture Regions

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ABSTRACT

Regulation of agriculture irrigation water discharges in California, USA, is assessed and controlled by its 9 Regional Water Quality Control Boards under the jurisdiction of the California State Water Resources Control Board. Each Regional Water Board has developed programs to control pesticides in runoff as part of the waste discharge requirements implemented through each region's Irrigated Lands Regulatory Program. The present study assessed how pesticide use patterns differ in the Imperial (Imperial County) and the Salinas and Santa Maria (Monterey County) valleys, which host 3 of California's prime agriculture areas. Surface-water toxicity associated with current use pesticides was monitored at several sites in these areas in 2014 and 2015, and results were linked to changes in pesticide use patterns in these areas. Pesticide use patterns appeared to coincide with differences in the way agriculture programs were implemented by the 2 respective Regional Water Quality Control Boards, and these programs differed in the 2 Water Board Regions. Different pesticide use patterns affected the occurrence of pesticides in agriculture runoff, and this influenced toxicity test results. Greater detection frequency and higher concentrations of the organophosphate pesticide chlorpyrifos were detected in agriculture runoff in Imperial County compared to Monterey County, likely due to more rigorous monitoring requirements for growers using this pesticide in Monterey County. Monterey County agriculture runoff contained toxic concentrations of pyrethroid and neonicotinoid pesticides, which impacted amphipods (*Hyalella azteca*) and midge larvae (*Chironomus dilutus*) in toxicity tests. Study results illustrate how monitoring strategies need to evolve as regulatory actions affect change in pesticide use and demonstrate the importance of using toxicity test indicator species appropriate for the suite of contaminants in runoff in order to accurately assess environmental risk. *Integr Environ Assess Manag* 2018;14:270–281. © 2017 SETAC

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INTRODUCTION

Regulation of agriculture irrigation water discharges in California is assessed and controlled by its 9 Regional Water Quality Control Boards under the jurisdiction of the California State Water Resources Control Board. Each regional board has developed programs to control pesticides in runoff as part of the waste discharge requirements implemented through each region's Irrigated Lands Regulatory Program. The Central Coast Region (Region 3) has developed its own version of this program, which is referred to as the Central Coast Irrigated Lands Regulatory Program (CCILRP; https://www.waterboards.ca.gov/centralcoast/water_issues/programs/ag_waivers/). The CCILRP requires growers to monitor toxicity and pesticides in receiving water, and for some, in

agriculture irrigation water discharges. These regulations also have stricter requirements for growers using pesticides that have been demonstrated to be linked to receiving water toxicity and ecological impacts, specifically the organophosphate pesticides diazinon and chlorpyrifos. As currently implemented, receiving water toxicity monitoring for the CCILRP requires water testing with the cladoceran *Ceriodaphnia dubia*, the alga *Selenastrum capricornutum* (now called *Pseudokirchneriella subcapitata*), and the fish *Pimephales promelas* (also known as US Environmental Protection Agency "3-species tests") 4 times/y. In addition, sediment toxicity is monitored annually with the amphipod *Hyalella azteca*. Synoptic water and sediment samples are analyzed for both pesticides and toxicity during 1 y of the 5-y Agriculture Order (Central Coast Water Board 2012).

One consequence of the CCILRP discharge requirements has been a shift in pesticide use patterns by growers away from the more regulated organophosphate pesticides,

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especially chlorpyrifos, to an increased use of pyrethroid and neonicotinoid pesticides. There has been no corresponding shift in water toxicity testing protocols with different chemical sensitivities; monitoring continues with the US Environmental Protection Agency (USEPA) 3-species tests and sediment toxicity using *H. azteca*. The current project was designed to sample discharges monitored as part of the CCILRP and to test these with freshwater test protocols more sensitive to pyrethroids and neonicotinoids. The goal was to evaluate whether recent monitoring results that showed a decreasing trend in detections of toxicity by the CCILRP program were a consequence of using indicator species less sensitive to current use pesticides.

In addition, monitoring was conducted in the Imperial Valley, another important agricultural area in California, which is under the regulatory jurisdiction of the Colorado River Basin Regional Water Quality Control Board (Region 7). In place of an Irrigated Lands Regulatory Program, Region 7 has 4 conditional waivers for agriculture dischargers, which are implemented by its Total Maximum Daily Load (TMDL) program. This water board region does not yet require monitoring of agriculture discharges. Surface-water toxicity and chemistry monitoring is conducted in Surface Water Ambient Monitoring Program (SWAMP; https://www.waterboards.ca.gov/water_issues/programs/swamp/monitoring/regional_monitoring_programs/region_7.shtml). Region 3 imposes greater scrutiny on the use of the organophosphate pesticides diazinon and chlorpyrifos through more rigorous monitoring and reporting requirements, and data from this region were used for comparison to those from Region 7 where restrictions are less stringent. This comparison was used to illustrate how different levels of regulations affect pesticide use patterns and to show how adaptive management of toxicity monitoring may alter conclusions drawn from current monitoring conducted as part of California's Irrigated Lands Regulatory Programs.

METHODS

Sampling and study areas

The Salinas, Santa Maria, and Imperial valleys comprise 3 of the largest agricultural regions of California where intensive industrial-scale row crop agriculture produces much of the nation's lettuce, broccoli, cauliflower, strawberries, and asparagus. Sampling in Region 3 was conducted in agriculture-influenced watersheds of the Salinas and Santa Maria River valleys on California's central coast. Nine sites were sampled in September 2014, and these were either creeks or drains that receive direct agriculture runoff or larger rivers that receive urban, agriculture, and natural flows. Sampling in Region 7 was conducted in agriculture-influenced water bodies of the Imperial Valley in the southeastern part of California. Eight sites were sampled in this region in October 2014 and again in October of 2015 by personnel from the California Department of Pesticide Regulation (CDPR). Grab water samples were collected by hand at all sites in 2-L amber glass bottles, then stored on ice and

transported overnight to the testing laboratory where they were refrigerated prior to testing. All toxicity testing was conducted within 48 h except for samples collected in Imperial County. Tests of the Imperial County samples were conducted within 4 d of sample collection. This exceeded the 48-h holding time required for water toxicity testing by SWAMP, but was necessary to accommodate the sampling schedule required by CDPR. Synoptic samples were collected for pesticide analysis, as described in the *Methods* section.

Toxicity testing. Toxicity of water samples was assessed using the amphipod *H. azteca* and the midge *Chironomus dilutus*. *Hyalella azteca* was used because it is 1 of the species most sensitive to pyrethroid pesticides (Solomon et al. 2001; Amweg et al. 2005), and *C. dilutus* was used because chironomids are sensitive to neonicotinoid pesticides (Morrissey et al. 2015; Cavallaro et al. 2016). Toxicity tests with amphipods were conducted in 300-mL glass beakers with 200 mL of test solution and containing ten 9- to 15-d-old animals in each of 5 replicates. Tests were conducted for 10 d and 50% of the test solution was renewed every 48 h. Each beaker was fed every 48 h with 1.5 mL of yeast-cerophyll-trout chow (YCT) after the renewal (USEPA 2002a). Final survival and growth in water samples were compared to 10-d survival and growth in laboratory well water. Growth was measured as dry weight per amphipod at 10 d.

Tests with chironomids were conducted in 300-mL glass beakers with 200 mL of test solution and containing twelve 7-d-old animals in each of 4 replicates. Each test container was supplied with 5 mL of sand as substrate for tube building by the larvae. Tests were conducted for 10 d and 50% of the test solution was renewed every 48 h. Each beaker was fed daily with an increasing amount of Tetramin[®] slurry (4 g/L) as follows: days 0 to 3 = 0.5 mL/d, days 4 to 6 = 1.0 mL/d, and days 7 to 10 = 1.5 mL/d. Final survival and growth were compared to 10-d survival in laboratory well water. Growth of surviving animals was measured as ash-free dry weight.

For all toxicity tests, dissolved O, pH, and conductivity were measured with an Accumet meter and appropriate electrodes (Fisher Scientific). Un-ionized ammonia was measured using a Hach 2010 spectrophotometer (Hach). Hardness and alkalinity were measured at initiation and termination of tests (Hach). Water temperature was recorded with a continuous recording thermometer (Onset Computer Corporation). Additional daily temperatures were measured using a glass spirit thermometer.

Results of the toxicity tests conducted at sites in the Salinas and Santa Maria River valleys were compared to results of toxicity tests conducted at the same sites as part of routine monitoring for the Central Coast Water Quality – Cooperative Monitoring Program (CMP) (data provided by Sarah Lopez, Central Coast Water Quality Preservation Inc., Cooperative Monitoring Program). This is a monitoring program for participating growers, which at the time was required by the 2012 Agriculture Order (Schmidt and Lopez 2016). Water for these tests was collected from the same sites in August 2014, approximately 4 wk prior to tests conducted as part of the present study (Central Coast Water Board

2012). Toxicity of the CMP samples was assessed using USEPA standard water test methods for 3 species (USEPA 2002b): *C. dubia* 6- to 8-d survival and reproduction, *P. promelas* 7-d survival and growth, and *S. capricornutum* 96-h algal growth. One of the August 2014 CMP samples had conductivity above the tolerance limit for these 3 species and was tested with alternate species. This sample was tested with *H. azteca* (10-d survival) in place of *C. dubia*, *Cyprinodon variegatus* (7-d growth and survival) in place of *P. promelas*, and *Thalassiosira pseudonana* (96-h growth) in place of *S. capricornutum*. All CMP toxicity testing was conducted by a certified commercial testing laboratory using USEPA (2002b) test methods.

Chemistry. Pesticides were analyzed by the Center for Analytical Chemistry, California Department of Food and Agriculture (CDFA). Analyte groups for the present study emphasized organophosphates (malathion, diazinon, chlorpyrifos, dimethoate, methomyl), pyrethroids (bifenthrin, cyhalothrin, permethrin, cyfluthrin, cypermethrin, esfenvalerate), and 1 neonicotinoid pesticide, imidacloprid, based on prioritizations derived from the pesticide risk model developed by Luo et al. (2014). Hexane extracts of water were analyzed for pyrethroids using a Varian CP 3800 gas chromatograph and a

Varian 320 triple quadrupole mass spectrometer. Reporting limits range from 1 to 50 ng/L. Methylene chloride extracts of water were analyzed for organophosphate pesticides using gas chromatography/mass spectrometry. Methylene chloride extracts of water were also analyzed for imidacloprid by ultraperformance liquid chromatography coupled to a triple quadrupole mass spectrometer using electrospray ionization in positive ion mode. Laboratory quality assurance and quality control procedures followed CDFA guidelines and consisted of laboratory blanks and matrix spikes. Only pesticides detected at or above the method reporting limit for each analyte are shown in the results.

Data analysis

Toxicity data were analyzed using the Test of Significant Toxicity approach (Denton et al. 2011). This approach tests whether the response in the treatment (i.e., a sample) is greater than or equal to a defined proportion of the control response. Chemical concentrations were compared to established median effect concentrations and median lethal concentrations (EC50s and LC50s) for the various toxicity test species and endpoints to determine their potential to cause toxicity (Table 1). Chemical concentrations were converted to toxic units (TUs) by dividing the measured concentration by

Table 1. Sensitivity of *Hyalella azteca*, *Chironomus dilutus*, and *Ceriodaphnia dubia*: LC50s and EC50s used to calculate TUs for selected chemicals detected in agriculture drain water

Chemical	<i>Hyalella azteca</i>	Endpoint	<i>Chironomus dilutus</i>	Endpoint	<i>Ceriodaphnia dubia</i>	Endpoint
Bifenthrin (ng/L)	7.7 ^a	96-h LC50	23.0 ^g	10-d LC50	142 ⁱ	48-h LC50
Cypermethrin (ng/L)	2.3 ^a	96-h LC50	6.9 ^h	48-h LC50	683 ⁱ	48-h LC50
Esfenvalerate (ng/L)	11.3 ^b	96-h LC50	210 ^l	96-h EC50	267 ⁱ	48-h LC50
L-cyhalothrin (ng/L)	2.3 ^c	48-h EC50	37.9 ^m	96-h LC50	200 ⁱ	48-h LC50
Permethrin (ng/L)	21.1 ^d	96-h LC50	99.0 ^g	10-d LC50	250 ⁱ	48-h LC50
Imidacloprid (μg/L)	7.01 ^e	96-h EC50	5.75 ^e	96-h EC50	2.070 ^j	48-h LC50
Chlorpyrifos (μg/L)	0.086 ^f	96-h EC50	0.140 ^g	96-h EC50	0.053 ^k	96-h LC50
Diazinon (μg/L)	6.510 ⁿ	96-h LC50	—	—	0.320 ^k	96-h LC50
Malathion (μg/L)	0.190 ^o	96-h LC50	—	—	2.120 ^q	48-h LC50
Dimethoate (μg/L)	—	—	12.90 ^p	96-h LC50	—	—

TU = toxic unit.

^aWeston and Jackson 2009.

^bWestfall et al. 2007.

^cMaund et al. 1998.

^dAnderson, Phillips, Hunt, Connor et al. 2006.

^eStoughton et al. 2008.

^fPhipps et al. 1995.

^gDing et al. 2012.

^hMaund et al. 2002.

ⁱWheelock et al. 2004.

^jChen et al. 2010.

^kBailey et al. 1997.

^lBelden and Lydy 2006.

^mHarwood et al. 2009.

ⁿAnkley and Collyard 1995.

^oCothran et al. 2009.

^pLeBlanc et al. 2012.

^qAnkley et al. 1991.

the species-specific median concentration. It should be noted that there is a discrepancy between the test durations for *C. dilutus* and *H. azteca* in the present study (10 d) and the shorter term 48-h to 96-h EC50 and LC50s used to calculate TUs. No 10-d LC50s were found for the specific pesticides analyzed. Use of acute effect thresholds likely underestimated the toxicity potential for some of the pesticides, particularly imidacloprid, as described in the *Discussion*. Toxic units for pyrethroids were summed to account for joint toxicity based on evidence of pyrethroid additive toxicity (Trimble et al. 2009). A similar approach was used for diazinon and chlorpyrifos TUs (Bailey et al. 1997).

Use data for selected pesticides were obtained from the CDPH's Pesticide Use Report (PUR) database to evaluate changing trends in pesticide use in Water Board Regions 3 and 7 (<http://calpip.cdpr.ca.gov/main.cfm>). Eight currently used pesticides from both regions were targeted for the present comparative study: chlorpyrifos, diazinon, malathion, imidacloprid, and the pyrethroids bifenthrin, esfenvalerate, lambda-cyhalothrin, and permethrin. The organophosphate pesticides chlorpyrifos and diazinon are subjected to more rigorous monitoring and reporting requirements in Region 3 due to their previously documented role in causing surface-water toxicity. Malathion is often used as an alternative organophosphate. The pyrethroid pesticides lambda-cyhalothrin, bifenthrin, permethrin, and esfenvalerate were summed to represent the pyrethroid class of pesticides, which have been replacing organophosphates in California. Imidacloprid was used to represent neonicotinoid pesticides, a class whose use is increasing in California, particularly in agriculture (Starnes and Goh 2012).

Use of these pesticides was summarized to illustrate how regulatory policy may be influencing pesticide use in each region. Data from 2011 were analyzed to reflect pesticide use prior to adoption of more rigorous monitoring requirements for growers who use chlorpyrifos in Region 3 (implemented in 2012). Data for 2014 were summarized to coincide with the 2014 monitoring conducted in Regions 3 and 7 as part of the present study. The PUR data were screened to reflect use in a representative agricultural county in Regions 3 and 7. Pesticide use in Imperial County (Region 7) and Monterey County (Region 3) were analyzed by summing total pounds of active ingredients of each pesticide applied to all agriculture crops in each county by year. Total pounds of active ingredients of the 4 pyrethroids (lambda-cyhalothrin, bifenthrin, permethrin, and esfenvalerate) were summed and presented as total pyrethroids. Total acres treated by each pesticide for all agriculture crops for each of the 2 counties were also summed for 2011 and 2014.

RESULTS

Pesticide use

California Department of Pesticide Regulation PUR data for 4 pesticides used in Imperial and Monterey counties showed a changing pattern between 2011 and 2014 (Figures 1 and 2). Use of chlorpyrifos, malathion, total pyrethroids, and

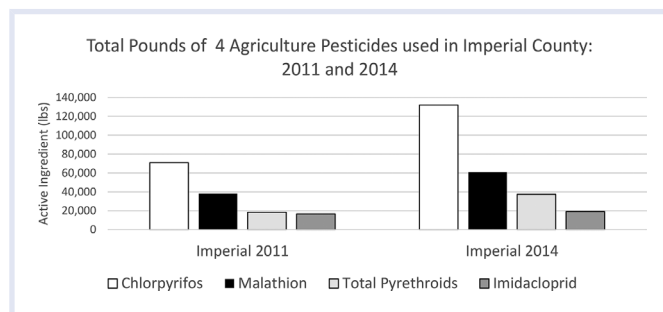


Figure 1. Pesticide use in 2011 and 2014 in Imperial County California. See *Methods* for details.

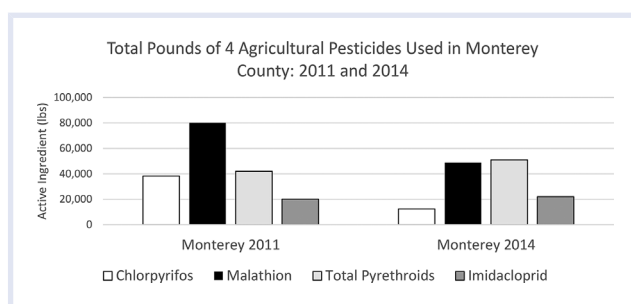


Figure 2. Pesticide use in 2011 and 2014 in Monterey County California. See *Methods* for details.

imidacloprid increased in Imperial County from 2011 to 2014. Total pounds of chlorpyrifos active ingredient increased from 70 972 to 132 008 pounds from 2011 to 2014 (46%), and total pounds of malathion increased from 38 062 to 60 923 pounds (38%). The only pesticide with decreasing use in Imperial County was the organophosphate diazinon. Total pounds of diazinon were the lowest of all pesticides analyzed for the present study and decreased by more than 99% between 2011 and 2014 in Imperial County. Chlorpyrifos use was much lower in Monterey County in 2011 and 2014 than in Imperial County. In addition, chlorpyrifos use decreased by 68% in Monterey County from 38 284 pounds in 2011 to 12 358 pounds in 2014, and malathion use decreased by 39% in Monterey County from 80 184 pounds in 2011 to 48 831 pounds in 2014. Diazinon use in Monterey County decreased by more than 80% between 2011 and 2014, more than any pesticide analyzed in the present study. Although chlorpyrifos, diazinon, and malathion use decreased, use of the 4 pyrethroids increased from 42 003 to 50 938 pounds (18%). The total pounds of pyrethroids used in Monterey County in 2011 and 2014 were dominated by permethrin (Table 2). Use of imidacloprid increased from 20 098 to 22 106 pounds (10%) in Monterey County during these reporting periods.

Trends in the total acres treated using these pesticides were similar to those observed for the total pounds applied, even though the total acreage treated using each pesticide was much greater in Monterey County (Table 3). There was an increase in total acreage treated with each of the pesticides analyzed in Imperial County between 2011 and 2014.

Table 2. Total pounds of pesticide active ingredient used on all agriculture crops in Imperial and Monterey counties, California, USA, in 2011 and 2014

County and year	Chlorpyrifos	Diazinon	Imidacloprid	Malathion	Lambda-cyhalothrin	Permethrin	Bifenthrin	Esfenvalerate	Total pyrethroids
Imperial 2011	70 972	2273	16 725	38 062	4793	8118	2417	3145	18 473
Imperial 2014	132 008	263	19 211	60 923	7919	23 553	2443	3517	37 432
Percent change	+86%	-99.96%	+15%	+60%	+65%	+190%	+1%	+12%	+102%
Monterey 2011	38 284	19 773	20 098	80 184	4358	31 137	4465	2043	42 003
Monterey 2014	12 358	4008	22 016	48 831	6107	37 390	5714	1727	50 938
Percent change	-68%	-80%	+10%	-39%	+40%	+20%	+28%	-15%	+18%

Table 3. Total acres of pesticide active ingredient used on all agriculture crops in Imperial and Monterey counties, California, USA, in 2011 and 2014

County and year	Chlorpyrifos	Diazinon	Imidacloprid	Malathion	Lambda-cyhalothrin	Permethrin	Bifenthrin	Esfenvalerate	Total pyrethroids
Imperial 2011	148 894	4435	53 732	31 757	171 048	47 088	27 221	74 584	319 941
Imperial 2014	203 551	201	61 834	50 519	274 564	129 609	28 805	80 511	513 489
Percent change	+37%	-95%	+15%	+59%	+61%	+175%	+6%	+8%	+61%
Monterey 2011	1 869 715	3863066	5 139 586	190 858	2 148 608	16 957 452	2 339 756	39 819	21 485 635
Monterey 2014	1 691 597	2970	6 781 419	177 462	1 924 479	25 776 555	4 272 453	37 828	32 011 315
Percent change	-10%	-99%	+32%	-7%	-10%	+52%	+83%	-5%	+49%

Chlorpyrifos-treated acreage increased 37% between 2011 and 2014, and malathion-treated acreage increased 59% in this period. The largest increases for acreage treated was observed for the pyrethroids permethrin (175%) and lambda-cyhalothrin (61%), and the total pyrethroids acreage treated in Imperial County increased by 61% between 2011 and 2014 (Table 3).

In all cases except for esfenvalerate, acreage treated with each pesticide was orders of magnitude greater in Monterey County compared to Imperial County. Unlike Imperial County, there were decreases in acreage treated in Monterey County between 2011 and 2014 for chlorpyrifos (–10%), diazinon (–99%), and malathion (–7%). These decreases mirrored decreases in pounds of active ingredient applied for these 3 organophosphates. There were also decreases in acreage treated in Monterey County using the pyrethroids lambda-cyhalothrin (–10%) and esfenvalerate (–5%) between 2011 and 2014 (Table 3). Large increases in acreage treated using total pyrethroids in Monterey County were driven by bifenthrin (+83%) and permethrin (+52%). Monterey County acreage treated using imidacloprid increased by 32% between 2011 and 2014.

Water toxicity and pesticide chemistry

Results of September 2014 monitoring of sites in the Salinas and Santa Maria River valleys (Region 3) showed widespread water toxicity to both amphipods and midges (Table 4). Samples from 8 of the 9 sites (89%) were toxic to *H. azteca* or *C. dilutus*. One month prior to the present project's tests, August 2014, water toxicity was monitored at the same Region 3 stations. These tests were conducted by a contract laboratory as part of regulatory monitoring for the Region's CMP. In contrast to widespread toxicity detected with *H. azteca* and *C. dilutus*, results of 3 species water tests conducted with *C. dubia*, *P. promelas*, and *S. capricornutum* showed little toxicity at the Region 3 sites (Table 5). The CMP criteria for designating a sample "significantly toxic" requires the response (e.g., survival, growth, or reproduction) to be statistically lower than the control response and to be less than 80% of the control response. Based on these criteria, 1 sample (Chualar Creek) reduced *C. dubia* survival and reproduction. Two additional samples inhibited *C. dubia* reproduction, but survival in these samples was 100%. No samples inhibited larval fish survival or growth, and 1 sample inhibited algal growth.

Chemical analyses of the September 2014 Region 3 samples showed mixtures of current use pesticides, including several pyrethroids, imidacloprid, and 3 organophosphates. Four pyrethroids were detected (bifenthrin, esfenvalerate, lambda-cyhalothrin, and permethrin), and all samples except Solomon Creek and the Main Street Ditch had at least 1 detection. All samples contained the neonicotinoid imidacloprid, and 6 of 7 of the samples contained malathion (86%). No samples contained detectable chlorpyrifos, but 3 contained diazinon, another organophosphate. A comparison of the pesticide concentrations with LC50 values for *H. azteca* and *C. dilutus* demonstrated much of the toxicity

could be accounted for by TUs of pyrethroids. Pyrethroid toxicity is additive (Trimble et al. 2009); therefore individual pyrethroid TUs were summed. Summed pyrethroid TUs exceeded 1 TU in 4 of the 7 samples toxic to *H. azteca*. One of these samples also contained greater than 1 TU of imidacloprid (Solomon Creek), and another contained greater than 1 TU of malathion (Oso Flaco Creek). Solomon Creek was the only sample toxic to *C. dilutus*, but not to *H. azteca*, and based on pesticides detected, this was likely due to its greater sensitivity to imidacloprid. It should also be noted that growth did not appear to be a more sensitive endpoint than survival in these tests. There were no cases in which growth of *H. azteca* or *C. dilutus* was inhibited in samples not significantly reducing survival. Although detected in 2 samples, diazinon concentrations were below toxicity thresholds for *H. azteca* (and *C. dubia*; Table 1). In addition to diazinon and malathion, dimethoate was also detected. The concentration of this organophosphate did not exceed the toxicity threshold for *C. dilutus* (Table 1).

As was observed in Region 3 monitoring, results of October 2014 monitoring of sites in the Imperial Valley (Region 7) showed widespread water toxicity to amphipods (Table 4). Six of the 8 samples were toxic to *H. azteca* (75%), and 2 of 8 were toxic to *C. dilutus*, containing sufficient chlorpyrifos TUs to account for toxicity (Table 4). In contrast to Region 3 samples, more toxicity in Region 7 was associated with the organophosphate chlorpyrifos. Chlorpyrifos was detected in all Imperial Valley samples, with 4 of the 6 toxic samples having sufficient chlorpyrifos TUs to account for toxicity to *H. azteca*. No malathion, dimethoate, or diazinon were detected in these samples. Pyrethroids bifenthrin, esfenvalerate, lambda-cyhalothrin, and permethrin were also detected, and there were sufficient summed pyrethroid TUs to account for amphipod mortality in the 2 other samples that were significantly toxic to *H. azteca*. Imidacloprid was detected in 88% of the Imperial Valley samples but was below 96-h acute toxicity thresholds for *H. azteca* and *C. dilutus*.

Results of October 2015 monitoring in the Imperial Valley (Region 7) again showed widespread water toxicity to amphipods (Table 6). Six of the 8 samples were toxic to *H. azteca* (75%), and 2 samples were toxic to *C. dilutus*. As was observed in the 2014 monitoring, toxicity to *H. azteca* was associated with chlorpyrifos and/or pyrethroids. Three of the 6 samples toxic to *H. azteca* contained sufficient pyrethroid TUs to account for toxicity, and 3 of these samples contained sufficient chlorpyrifos TUs to account for toxicity. The Verde Drain sample showed a high concentration of lambda-cyhalothrin at 447 ng/L, which represented 194 TUs to amphipods. Samples significantly toxic to *C. dilutus* contained sufficient pyrethroid and/or chlorpyrifos TUs to account for toxicity. No malathion, dimethoate, or diazinon was detected in these samples. Two samples toxic to *C. dilutus* were accounted for by elevated TUs of pyrethroids, and in 1 case, chlorpyrifos.

DISCUSSION

The present study assessed water toxicity associated with pesticides in agricultural irrigation runoff in 3 of the most

Table 4. Toxicity testing and pesticide chemistry results in Region 3 (Monterey County) and Region 7 (Imperial County) sites in 2014

Sites	HA survi- val mean (%)	HA growth mean (mg/ind)	CD survi- val mean (%)	CD growth mean (mg/ind)	Bifen- thrin (ng/ L)	(Es)			Perme- thrin total (ng/L)	Imida- cloprid (µg/L)	Chlor- pyri- fos (µg/L)	Dime- thoate (µg/L)	Mala- thion (µg/L)	Pyre- thro- ids	Imida- clo- prid	Organ- ophos- phates	Sum H. azteca TUs		Sum C. dilutus TUs														
						Fen- vale rate (ng/L)	Lambda- cyhalo- thrin (ng/L)	Fen- thrin (ng/ L)									Pyre- thro- ids	Imida- clo- prid	Organ- ophos- phates	Pyre- thro- ids	Imida- clo- prid	Organ- ophos- phates											
September 2014 Region 3																																	
Alisal Slough	38 ^a	0.067 ^a	0 ^a	NA	2.07	ND	ND	5.3	1.33	ND	0.022	0.551	0.024	0.52	0.19	0.13	0.14	0.23	—	—													
Chualar Creek	0 ^a	NA	73 ^a	0.301 ^a	11.4	ND	ND	17.1	1.74	ND	0.037	ND	Trace	2.29 ^a	0.25	0.01	0.67	0.30	—	—													
Main St. Ditch	94	0.111	92	2.095	ND	ND	ND	ND	1.39	ND	ND	ND	0.03	0.20	0.20	0.16	—	0.24	—	—													
Orcutt Creek	50 ^a	0.051 ^a	48 ^a	0.121 ^a	ND	8.8	ND	ND	1.54	ND	ND	ND	0.036	0.78	0.22	0.19	0.04	0.27	—	—													
Oso Flaco Creek	0 ^a	NA	42 ^a	0.223 ^a	ND	ND	9.45	ND	0.58	ND	ND	ND	0.227	4.11 ^a	0.08	1.19 ^a	0.25	0.10	—	—													
Quail Creek	0 ^a	NA	2 ^a	NA	5.74	ND	4.24	71.7	0.92	ND	ND	ND	0.145	5.99 ^a	0.13	0.76	1.09 ^a	0.16	—	—													
Rec Ditch III	30 ^a	0.058 ^a	4 ^a	NA	1.52	ND	5.25	ND	2.01	ND	ND	ND	ND	2.48 ^a	0.29	—	0.20	0.35	—	—													
Solomon Creek	98	0.078 ^a	0 ^a	NA	ND	ND	ND	ND	9.14	ND	ND	ND	0.123	—	1.30 ^a	0.65	—	1.59 ^a	—	—													
Tembladero Slough	59 ^a	0.065 ^a	83	1.860	3.22	ND	ND	ND	0.17	ND	ND	0.098	0.136	0.42	0.02	0.72	0.14	0.03	—	—													
Control	82	0.099	100	2.399	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—													
October 2014 Region 7																																	
Alamo River Garst	0 ^a	NA	69 ^a	2.729	ND	ND	ND	ND	0.35	0.22	ND	ND	ND	—	0.05	2.58 ^a	—	0.06	1.59 ^a	—													
Alamo River Ruth.	0 ^a	NA	79	2.008	ND	ND	ND	ND	0.41	0.12	ND	ND	ND	—	0.06	1.44 ^a	—	0.07	0.89	—													
Holtville Main	0 ^a	NA	82	1.143	1.68	ND	ND	2.33	0.18	0.09	ND	ND	ND	0.33	0.03	0.99	0.10	0.03	0.61	—													
Malva Drain	0 ^a	NA	0 ^a	NA	ND	ND	ND	ND	ND	1.22	ND	ND	ND	—	—	14.2 ^a	—	—	8.71 ^a	—													
New River	78	0.106	96	1.475	ND	ND	2.21	ND	0.07	0.04	ND	ND	ND	0.96	0.01	0.41	0.06	0.01	0.25	—													
Rice Drain III	40 ^a	0.138	88	1.404	ND	7.16	2.08	ND	0.21	0.05	ND	ND	ND	1.54 ^a	0.03	0.57	0.09	0.04	0.35	—													
Vail Drain	96	0.119	86	1.529	ND	ND	ND	2.39	0.90	0.03	ND	ND	ND	0.11	0.13	0.33	0.02	0.16	0.20	—													
Verde Drain	22 ^a	0.160	96	0.803	ND	5.43	3.39	3.57	0.10	ND	ND	ND	ND	2.12 ^a	0.01	0.15	0.02	0.02	—	—													
Control	96	0.096	96	0.923	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—													
Reporting limits					1	5	2	5	50	10	10	40	20	—	—	—	—	—	—	—													

CD = *Chironomus dilutus*; HA = *Hyalella azteca*; ind = individual; NA = not applicable; ND = not detected; TU = toxic unit.^aToxicity test results mean survival or growth was significantly less than the control value. Chemistry TUs exceeded 1.0.

Table 5. Results of 3-species toxicity tests with *Ceriodaphnia dubia*, *Pimephales promelas*, and *Selenastrum capricornutum* conducted using samples from August 2014 at Region 3 (Monterey County) agriculture sites^a

Sites August 2014	<i>Ceriodaphnia dubia</i> survival	<i>C. dubia</i> reproduction	<i>Pimephales promelas</i> survival	<i>P. promelas</i> growth	<i>Selenastrum capricornutum</i> cell growth
Alisal Slough	NS	NS	NS	NS	NS
Chualar Creek	0 ^b	1.1 ^b	94.7	114	169
Main St. Ditch	100	89.4	125	99.6	17.0 ^b
Orcutt Creek	100	78.9 ^b	100	113	146
Oso Flaco Creek	100	75.1 ^b	125	105	126
Quail Creek	100	80.9	105	100	188
Rec Ditch III	NS	NS	NS	NS	NS
Solomon Creek	86 ^c	NR	200 ^d	93.1 ^d	228 ^e
Tembladero Slough	100	116	94.7	111	225

NR = not reported; NS = not sampled.

^aResults from the Central Coast Water Quality Preservation, Inc. Cooperative Monitoring Program. All values are given as percent of the control response.

^bToxic = significantly less than control and less than 80% of control value.

^cTested with *Hyalella azteca* due to high conductivity = significantly different from control survival but greater than 80% of control value.

^dTested with *Cyprinodon variegatus* due to high conductivity.

^eTested with *Thalassiosira pseudonana* due to high conductivity.

productive row-crop agricultural regions in California. Cropping patterns are similar in Imperial and Monterey counties, dominated by row crops that include leafy greens such as lettuce and spinach, as well as celery, cauliflower, and broccoli. Two major cropping differences include larger scale cultivation of alfalfa and corn in Imperial County and relatively larger acreages in strawberry and wine grape production in Monterey County. Discussions with pest management advisors in Monterey County did not indicate changes in cropping patterns or pest management practices in Monterey County between 2011 and 2014. There was an increase in the use of chlorpyrifos for aphid control on alfalfa in the Imperial Valley between 2013 and 2015 (E Natwick, University of California Cooperative Extension, Imperial County, Imperial, CA, USA, personal communication). Different patterns of pesticides in runoff may reflect some regional differences in cropping patterns, but also likely reflect regional differences in use of pesticides based on Irrigated Lands Regulatory Program requirements in the 2 regions.

The Central Coast (Region 3) and Colorado River Basin (Region 7) Regional Water Quality Control Boards are at different phases in the implementation of their respective Irrigated Lands Regulatory Programs. Both programs follow California Water Code Section 13269 waiver requirements for agricultural discharges (http://leginfo.ca.gov/faces/codes_displaySection.xhtml?lawCode=WAT§ionNum=13269). The process begins with the issuance of Conditional Waivers of Waste Discharge Requirements (WDRs). These waivers regulate discharges of water that could directly or indirectly affect the quality of waters in California. Dischargers develop water quality management plans, implement management practices to protect water

quality, and participate in surface- and groundwater monitoring and reporting programs. Monitoring programs were implemented by a grower-formed nonprofit in 2005 in Monterey County and by grower coalitions in 2015 in Imperial County. As a consequence, the Region 3 CMP has compiled a decade of monitoring data that have largely corroborated previous studies showing surface-water toxicity associated with agriculture pesticides in the Salinas and Santa Maria River valleys. Because monitoring data in this region have previously linked the organophosphate pesticides diazinon and chlorpyrifos with toxicity and ecological impacts (Anderson et al. 2003; Hunt et al. 2003; Anderson, Phillips, Hunt, Richard et al. 2006; Phillips et al. 2006), growers who use these pesticides were required to comply with more rigorous (Tier 3) reporting and monitoring requirements in this region as part of a revised agriculture order beginning in 2012 (Central Coast Water Board 2012). Once the 2012 agriculture order was issued in Region 3, pest control contractors largely stopped using chlorpyrifos in Monterey County (e.g., Heather Healy, Office of the Monterey County Agricultural Commissioner, and Shimat Joseph, University of California Cooperative Extension, Monterey County, Salinas, CA, USA, personal communications). To avoid more rigorous monitoring requirements, growers have switched to alternative pesticides, including pyrethroids and neonicotinoids. This pattern is reflected in the California Department of Pesticide Regulation PURs for the year preceding implementation of the new agricultural order (2011) and in the monitoring year when the present study was conducted (2014). Monterey County agricultural applications of chlorpyrifos and diazinon declined in 2011, likely in anticipation of the revised agricultural order's

Table 6. Toxicity testing and pesticide chemistry results in Region 7 (Imperial County) sites sampled by CDPR in 2015

Sites	HA survival mean (%)	HA growth mean (mg/ind)	CD survival mean (%)	CD growth mean (mg/ind)	(Es)			Permethrin			Chlorpyrifos (μg/L)	Diazinon (μg/L)	Dimethoate (μg/L)	Sum HA TUs			Sum CD TUs			
					Bifenthrin (ng/L)	Fenvalerate (ng/L)	Lambda Cyhalothrin (ng/L)	Total (ng/L)	Imidacloprid (μg/L)	Pyrethroids (μg/L)				Imidacloprid	Pyrethroids	Organophosphates				
September 2015 Region 7																				
Alamo River Garst	0 ^a	NA ^a	90	0.878	ND	ND	ND	ND	0.460	0.121	ND	ND	ND	ND	—	0.07	1.41 ^a	—	0.08	0.86
Alamo River Ruth.	0 ^a	NA ^a	98	2.004	1.21	6.84	ND	ND	0.618	0.116	ND	ND	ND	ND	3.13 ^a	0.09	1.35 ^a	1.04 ^a	0.11	0.83
Holtville Main	54 ^a	0.091 ^a	98	0.972	3.03	ND	ND	ND	0.914	0.037	ND	ND	ND	ND	0.39	0.13	0.43	0.13	0.16	0.26
Malva Drain	0 ^a	NA ^a	98	2.641	ND	ND	ND	ND	ND	0.084	ND	ND	ND	ND	—	—	0.98	—	—	0.60
New River	85	0.105	100	1.180	ND	ND	ND	ND	0.234	0.019	ND	ND	ND	ND	—	0.03	0.22	—	0.04	0.14
Rice Drain III	0 ^a	NA ^a	13 ^a	0.175 ^a	ND	11.1	6.57	ND	3.48	0.202	ND	ND	ND	ND	7.68 ^a	0.50	2.35 ^a	1.78 ^a	0.61	1.44 ^a
Vail Drain	94	0.122	100	3.317	ND	ND	ND	ND	0.178	0.018	ND	ND	ND	ND	—	0.03	0.21	—	0.03	0.13
Verde Drain	0 ^a	NA ^a	0 ^a	NA ^a	1.43	39.7	447	ND	0.103	ND	ND	ND	ND	ND	212 ^a	0.01	—	17.6 ^a	0.02	—
Reporting limits																				
					1	5	2	5	50	10	10	40	20	—	—	—	—	—	—	—

CDPR = California Department of Pesticide Regulation; CD = Chironomus dilutus; HA = Hyalella azteca; ind = individual; NA = not applicable; ND = not detected.

^aSignificantly toxic sample to either *H. azteca* or *C. dilutus*. Toxic Units exceed 1 TU threshold for *H. azteca* or *C. dilutus*.

implementation in 2012. In addition, TMDLs were adopted for diazinon and chlorpyrifos in the lower Salinas River in 2011, and this likely also influenced use of these pesticides in Monterey County in subsequent years (http://www.swrcb.ca.gov/rwqcb3/water_issues/programs/tmdl/docs/salinas/pesticide/index.shtml). Use of chlorpyrifos in Monterey County continued to decline in 2014. Based on discussions with Monterey County pest control advisors, reductions in malathion use between 2011 and 2014 in Monterey County were likely due to preferences for newer, more effective classes of pesticides (Heather Healy, Office of the Monterey County Agricultural Commissioner, Salinas, CA, USA, personal communication).

Toxicity testing in Region 3 in 2014 by 2 separate monitoring studies showed different results depending on the suite of test species used. Although 89% of samples tested in Region 3 in September 2014 were toxic to either *H. azteca* or *C. dilutus* in the present study, CMP water tests conducted at the same sites in August 2014 showed less toxicity to *C. dubia* and little to no toxicity to *P. promelas* or *S. capricornutum*. This was more likely due to the comparatively low sensitivity of these species to the contaminants present at these sites, rather than to a change in contaminants in the month separating the sampling periods. Synoptic chemical analysis was conducted on a subset of the Salinas Valley samples as part of the CMP monitoring in August 2014. These samples were analyzed for selected carbamate and organophosphate pesticides (malathion, diazinon, chlorpyrifos) as well as nutrients, metals, and herbicides (Schmidt 2016; chemistry data not shown here). These samples were not analyzed for pyrethroids or neonicotinoids. These analyses detected 61 ng/L of chlorpyrifos in the August 2014 Chualar Creek sample, which accounted for 1.2 TUs and was sufficient to explain the toxicity to *C. dubia* in this sample (Table 5). No carbamates and few other organophosphate pesticides were detected in the CMP samples, and those that were present were below toxicity thresholds to *C. dubia*.

Data from the present study showed most toxicity was linked to concentrations of the pyrethroids bifenthrin, esfenvalerate, lambda-cyhalothrin, and permethrin. Both *H. azteca* and *C. dilutus* are more acutely sensitive to these pyrethroids than are *C. dubia* (Table 1). *Pimephales promelas* larvae and *S. capricornutum* are also less sensitive than amphipods and chironomids to the pyrethroid, organophosphate, and neonicotinoid pesticides present in these samples. Given the current mixtures of contaminants present in agriculture runoff, these results suggest continued use of routine USEPA 3-species testing underrepresents surface-water toxicity in Region 3 streams receiving agricultural runoff.

Similar findings were reported in 2 recent toxicity studies of agriculture and stormwater runoff in Monterey County. Phillips et al. (2016) monitored water toxicity and pesticides in Quail Creek, an agriculture drainage creek that previous studies showed was consistently toxic to *C. dubia* due to mixtures of the organophosphate pesticides chlorpyrifos and diazinon (Anderson et al. 2003; Hunt et al. 2003). These authors monitored water toxicity using *H. azteca* and *C. dubia* and

found 67% of samples were toxic to amphipods, whereas 17% were toxic to daphnids. Although the past studies showed toxicity was caused by organophosphates, the more recent results showed toxicity was due to toxic concentrations of pyrethroids. In a second study, contaminants associated with stormwater toxicity were monitored in the City of Salinas as part of an evaluation of the efficacy of parking lot bioswales to treat stormwater (Anderson et al. 2016). A comparison of toxicity tests showed no samples from 3 storms monitored at 3 sites were toxic to *C. dubia* or *P. promelas*, whereas most of the samples were toxic to *H. azteca* and *C. dilutus*. Toxic unit analysis suggested toxicity of these samples was associated with mixtures of pyrethroid pesticides, Zn, and Cu. Evidence from these studies illustrates that urban and agriculture pesticide use patterns also change, and this sometimes requires a revision of toxicity tests used for surface-water monitoring to accurately reflect risk to receiving systems.

Pesticides detected in Region 7 in 2014 and 2015 reflected the CDPR PUR agriculture use data for these pesticides (Figures 1 and 2). These differed from pesticides detected in Region 3 primarily because of the high detections of toxic concentrations of chlorpyrifos in Region 7 (88% of samples). The difference between the 2 regions likely reflects the greater regulatory restrictions on using chlorpyrifos in Region 3. Toxicity of Region 7 samples was associated with toxic mixtures of pyrethroid pesticides and chlorpyrifos. Water toxicity was detected in 2014 and 2015 in 75% of the samples using *H. azteca* and 25% of the samples using *C. dilutus*. It should be noted that because *C. dubia* is more acutely sensitive to chlorpyrifos (LC50 = 0.053 µg/L) than *H. azteca* (LC50 = 0.086 µg/L) and *C. dilutus* (LC50 = 0.140 µg/L), testing with *C. dubia* would have been a more sensitive indicator of chlorpyrifos toxicity in the Region 7 samples.

Imidacloprid was detected in 88% of the Region 7 samples in 2014 and 2015. None of the samples contained concentrations sufficient to cause acute toxicity (96-h LC50 for *C. dilutus* = 5.75 µg/L; Stoughton et al. 2008), and only 1 sample in 2014 and 2 samples in 2015 exceeded the reported 28-d LC50 of 0.91 µg/L (Stoughton et al. 2008). Recent research has shown that chronic exposures to imidacloprid and other neonicotinoids affect emergence and other chronic endpoints at lower concentrations than those affecting short- and long-term survival and growth. Morrissey et al. (2015) suggested a long-term chronic protective value based on a probabilistic risk assessment of 0.035 µg/L imidacloprid. Cavallaro et al. (2016) reported an imidacloprid EC50 of 0.39 µg/L for inhibition of emergence using a 40-d exposure to *C. dilutus*. All detected imidacloprid concentrations in Regions 3 and 7 exceeded this 40-d emergence EC50 for imidacloprid. Given that 40-d exposures are not practical for routine monitoring required for the Irrigated Lands Regulatory Programs, the present project used 10-d exposures of *C. dilutus* as a compromise. There has been no reported 10-d LC50 for imidacloprid toxicity to *C. dilutus*, but Cavallaro et al. (2016) reported a 14-d LC50 of 1.52 µg/L. This was exceeded in 1 sample from Region 7 in 2015 and in several samples from Region 3 in 2014 (Tables 4 and 6). It should be

noted that there are currently no standardized 10-d USEPA water-only toxicity test protocols for *C. dilutus* and *H. azteca* that can be used for monitoring. The USEPA describes acute toxicity test methods for *C. dubia* in its freshwater acute toxicity test manual (USEPA 2002a). This method allows a range of test durations from 24 to 96 h. In addition, the manual includes a supplemental list of test species, including *H. azteca* and *C. dilutus*. These test species are now included in waste discharge requirements by California regulators for certain applications, such as stormwater and agriculture discharge monitoring, and the newly revised monitoring requirements for the Region 3 agricultural receiving water monitoring program. The USEPA and US Geological Survey (USGS) describe 10- and 42-d sediment toxicity test protocols for *H. azteca* and *C. dilutus* (USEPA 2000). The 10-d sediment exposure procedure was adapted for use as a 10-d water-only static renewal exposure with both *H. azteca* and *C. dilutus* in the present project. Long-term tests can also be adapted for shorter durations, such as the 28-d exposure with *H. azteca* (measuring growth and survival), and *C. dilutus* (measuring growth, survival, and potentially, emergence). The USEPA and USGS are currently in the process of updating the USEPA (2000) sediment toxicity manual, which will include methods for testing both species in water and sediment using different exposure durations that range from 10 to 42 d for *H. azteca* and 10 to ~50 d for *C. dilutus*. This revision is currently undergoing internal review within these agencies (D Mount, USEPA, Duluth, MN, USA, personal communication).

Pesticide use patterns change in California agriculture due to a combination of factors, including regulatory actions. The present study provides evidence that different monitoring requirements associated with Irrigated Lands Regulatory Programs in 3 agriculture regions in California have influenced use patterns and surface-water contamination by pesticides. Results suggest water quality regulators responsible for structuring monitoring programs associated with irrigated land policies should consider how monitoring requirements may influence agricultural practices, including use of alternative pesticide products, and should design monitoring requirements to include test protocols that will adequately address potential environmental risk from the complex mix of pesticides likely to be in runoff. The Central Coast Regional Water Quality Control Board (Region 3) is in the process of revising its CCILRP, and more recent data showing toxicity to *H. azteca* and *C. dilutus* due to pyrethroid and neonicotinoid pesticides will be taken into consideration in that revision process. Sediment testing with *H. azteca* is also monitored annually in the current CCILRP and will likely continue in the new CCILRP. The Colorado Basin Regional Water Quality Control Board (Region 7) is now in the process of designing an irrigated lands monitoring program. To date, this region has relied on regional toxicity data generated from SWAMP, which includes a combination of USEPA 3-species testing with *C. dubia*, *P. promelas*, and *S. capricornutum*. Region 7 SWAMP was also one of the first to incorporate water column monitoring with the amphipod *H. azteca* to account

for potential pyrethroid toxicity in agricultural runoff (Phillips et al. 2007). Recent findings of increased detections of neonicotinoids in this region and elsewhere in California suggest that testing with *C. dilutus* should be considered as part of irrigated lands water monitoring, because this species is among the most sensitive to this class of pesticide. Due to their high solubility (imidacloprid log K_{ow} = 0.057), neonicotinoids do not partition to sediment, so toxicity monitoring for these pesticides requires water-only exposures. These data may be useful to other agricultural regions of the state, such as California's Central Valley (Region 5), where existing irrigated lands monitoring is being conducted with USEPA 3-species tests. As in other regions, Region 5 incorporates intermittent sediment testing with *H. azteca*, partly to address the potential for sediment toxicity due to pyrethroids.

Data from past and current monitoring illustrate there is a persistent cycle of pesticide-associated toxicity in California surface waters (Anderson et al. 2011). These data lead to impaired water body designations as part of Clean Water Act 303(d) listing cycles in California (California State Water Resources Control Board 2005). In the regions monitored for the present study, listings have been most often for toxicity associated with organophosphate pesticides, and more recently for toxicity associated with pyrethroids (http://www.swrcb.ca.gov/centralcoast/water_issues/programs/tmdl/303d_list.shtml#New_link). In many cases these same water bodies are now showing increased detections of neonicotinoids, such as imidacloprid. In recognition that this cycle needs to be addressed more proactively, California water quality regulators and environmental scientists are in the process of integrating monitoring and regulatory programs to more quickly identify and manage problem pesticides before they enter runoff and pollute receiving systems. This will require more cohesive cooperation between various state agencies and stakeholder groups involved in pest management, food production, and environmental protection. Adaptive management of surface-water monitoring programs plays a key role in this process, by providing current data using relevant pesticide analyte lists that reflect current pesticide use. Monitoring programs should also be revised periodically to include relevant toxicity test species to accurately reflect potential for environmental risk.

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Data Accessibility—Readers interested in the data and associated metadata and calculation tools for this research should contact the corresponding author Brian Anderson at anderson@ucdavis.edu.

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