EPA Public Access

Author manuscript

Environ Toxicol Chem. Author manuscript; available in PMC 2019 January 01.

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Published in final edited form as:

Environ Toxicol Chem. 2018 January ; 37(1): 99–106. doi:10.1002/etc.3938.

Enantiomer-specific measurements of current-use pesticides in aquatic systems

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Abstract

Some current-use pesticides are chiral and have nonsuperimposable mirror images called enantiomers that exhibit identical physical–chemical properties but can behave differently when in contact with other chiral molecules (e.g., regarding degradation and uptake). These differences can result in variations in enantiomer presence in the environment and potentially change the toxicity of pesticide residues. Several current-use chiral pesticides are applied in urban and agricultural areas, with increased potential to enter watersheds and adversely affect aquatic organisms. The present study describes a stereoselective analytical method for the current-use pesticides fipronil, *cis*-bifenthrin, *cis*-permethrin, cypermethrin, and cyfluthrin. We show use of the method by characterizing enantiomer fractions in environmental sample extracts (sediment and water), and laboratory-dosed fish and concrete extracts previously collected by California organizations. Enantiomer fractions for most environmental samples are the same as racemic standards (equal amounts of enantiomers, enantiomer fraction = 0.5) and therefore are not expected to differ in toxicity from racemic mixtures typically tested. In laboratory-derived samples, enantiomer fractions are more frequently nonracemic and favor the less toxic enantiomer; permethrin enantiomer fractions range from 0.094 to 0.391 in one type of concrete runoff and enantiomer fractions of bifenthrin in dosed fish range from 0.378 to 0.499. We use enantiomer fractions as a screening tool to understand environmental exposure and explore ways this uncommon measurement could be used to better understand toxicity and risk.

Supplemental Data

The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3938.

Data Availability

Data and associated metadata pertaining to this manuscript are available in the Supplemental Data.

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INTRODUCTION

Current-use insecticides such as pyrethroids and fipronil are used in agricultural and indoor and outdoor urban settings 1–8. In California during 2014, pyrethroids and fipronil were applied heavily, with a large percentage used for structural pest control as follows: 124 000 kg of permethrin with 44.7% for structural pest control; approximately 122 000 kg of bifenthrin with 32.5% for structural pest control; and approximately 36 000 kg each of fipronil, cypermethrin (including [S] cypermethrin), and cyfluthrin (including beta-cyfluthrin) with 97.6, 56.1, and 76.3%, respectively, for structural pest control 9,10. Research has shown that pesticides can be removed from application areas through particles (such as sediment, soil, and dust) and water (such as through irrigation and rain) 5, 11–18. These pesticides can migrate into local surface water and sediment 6, 16, 19–22 and habitats of sensitive aquatic species 23. For some of these pesticides, especially pyrethroids, low water solubility and a high octanol–water partition coefficient cause the compounds to partition into the organic compartment of sediment, which becomes a repository 20.

Increasingly, researchers are finding conditions in California water and sediment toxic to a variety of organisms such as *Hyalella Azteca* and *Ceriodaphnia dubia* 1, 7, 16, 21, 22, 24–30. To explain the toxicity, a wide array of water quality 3, 25 and chemical measurements have been made, including measurements for current-use pesticides such as the pyrethroids permethrin, bifenthrin, cypermethrin, cyfluthrin, and the phenylpyrazole fipronil 1, 16, 19, 20, 25, 28–31. Some of these measurements allow clear identification of a cause of toxicity, whereas others do not permit a full explanation of the toxic conditions, suggesting that additional information might be helpful.

Chiral pesticides often are formulated as a racemic mixture (equal amounts of each enantiomer) but, increasingly, some pesticides are being enriched with the more toxic (+) enantiomer for target pests 32. Research indicates that for various aquatic species, the difference in toxicity between the (+) enantiomer and the less toxic (–) enantiomer of 3 of the pesticides assessed in the present study—fipronil, *cis*-bifenthrin, and *cis*-permethrin—ranges from a 2-fold to a more than 35-fold difference 33–36. Once pesticides enter the environment, a variety of processes can alter the enantiomer signature (the direction and degree of enantioselectivity), especially degradation and metabolism 37. The environmental fate can shift the enantiomer signature away from that of the original formulation mixture (racemic or enriched), resulting in unpredictable and largely unstudied effects on toxicity and risk for nontarget organisms 38.

The primary goal of the present study was to develop a method to measure and characterize the enantiomer signatures of selected current-use pesticides and then to show use of the method on samples of environmental media. The present study investigated enantiomers of 5 current-use pesticides—fipronil, bifenthrin, permethrin, cypermethrin, and cyfluthrin—in California water and sediment samples collected by several California organizations. A central outcome of the present study is increasing the number of enantiomer-specific measurements for current-use pesticides in aquatic systems, and raising awareness for the need for and uses of enantioselective measurements in future exposure and risk assessments.

Because some of the environmental samples obtained for the present study had corresponding toxicity data, a lesser goal was to explore whether differences in enantiomer environmental fate and resulting exposure could refine the understanding of sediment and water toxicity in California. Greater understanding of both the exposure and toxicity of chiral chemicals can reduce risk assessment uncertainty and result in better protection of aquatic ecosystems.

MATERIALS AND METHODS

Enantiomer analysis

Enantioselective analysis was conducted in US Environmental Protection Agency (USEPA) laboratories using gas chromatography on a tert-butyl dimethyl silyl β -cyclodextrin (BGB-172) column (BGB Analytik) with electron impact mass spectrometry (MS; 6890 gas chromatograph with 5973 MS; Agilent Technologies). The injector temperature was 230 °C, and the carrier gas was helium at 25 psi under constant flow conditions. The gas chromatograph temperature program was 50 °C for 1 min, ramped at 5 °C per min to 160 °C, ramped at 1 °C per min to 230 °C, and held at 230 °C for 20 min, for a total run time of 113 min. The MS source temperature was maintained at 230 °C and the quadrupoles at 150 °C.

Enantiomer elution orders were determined by injecting single or enriched enantiomer standards. Fipronil eluted at approximately 60 min, with the (S, +) enantiomer preceding the (R, -) enantiomer, and was monitored using ions m/z 213, 367, and 379. Bifenthrin eluted at approximately 79 min, with the (R, +) enantiomer preceding the (S, -) enantiomer, and was monitored using ions m/z 165, 166, and 181. Permethrin (*cis*- and *trans*-) eluted at approximately 95 min, with an elution order of *cis*- (1R, +), *cis*- (1S, -), and *trans*- ($\pm$), and was monitored using ions m/z 163, 165, and 183. Cyfluthrin eluted at approximately 100 min, with 5 of 8 stereoisomer peaks separated, and was monitored using ions m/z 163, 165, and 206. Cypermethrin eluted at approximately 105 min, with 5 of 8 stereoisomer peaks separated, and was monitored using ions m/z 163, 165, and 181. Figure 1 presents the total ion chromatogram of this separation for a racemic standard.

Stereoisomer separation can be improved for cyfluthrin and cypermethrin with different temperature programs but these programs take a significantly longer time (more than 6 h) than the program used and are impractical for routine analysis. The method described is sufficient as a screen for cyfluthrin and cypermethrin. Because these chemicals were not detected in the samples, no further refinement of the method was pursued.

Calculation of enantiomer fractions

Enantiomer fractions were calculated using the following equation: enantiomer fraction = $\text{area (+)} \div [\text{area (+)} + \text{area (-)}]$ where: Area (+) = peak area of the (+) enantiomer and Area (-) = peak area of the (-) enantiomer. The theoretical value for a racemic (50:50) mixture is enantiomer fraction = 0.5. Raw time and abundance data were extracted using ChemStation software (Agilent Technologies) and PeakFit software (Ver 4.11; SYSTAT) by a previously described method 39. Eight racemic standards were used containing a mixture of fipronil, *cis*-bifenthrin, *cis*- and *trans*-permethrin, cypermethrin, and cyfluthrin ranging in

concentration from 10 picograms (pg)/ μ L to 100 ng/ μ L. The standards were run at the beginning and end of each analysis sequence, as well as interspersed with the environmental sample sequences for statistical comparison within each batch. This analysis was important to determine if analytical interferences caused the measured enantiomer fraction value to differ from the actual value in the sample.

Quality-control criteria similar to that described in Ulrich et al. 40 were also used for these analyses. Briefly, enantiomer fractions for ions were required to be within 5% of the average enantiomer fraction for that sample run. Ion ratios for both enantiomer peaks were required to have a relative percentage difference of less than 10%. In addition, ion retention times were also required to have a relative percentage difference of less than 10%.

In the present study, enantiomer fractions for racemic standards ranged from 0.35 to 0.54, indicating that the racemic environmental samples should have measured enantiomer fractions in the same range. For this reason, racemic versus nonracemic status was determined by comparing sample values with racemic standard values analyzed in the same batch; sample enantiomer fractions outside the range of racemic standard enantiomer fractions were considered nonracemic. Statistical differences among sets of samples were determined by using Microsoft Excel to run a single-factor analysis of variance and a 2-sample *t* test, assuming unequal variances ($\alpha = 0.05$).

Samples used in the present study

The method described in *Enantiomer analysis* and *Calculation of enantiomer fractions* was applied to 90 environmental samples from various aquatic matrices. Caltest Analytical Laboratory (Napa, CA, USA) contributed 45 water samples collected in May 2013 from the American River in Sacramento, CA ($n = 5$), southern California freshwater ($n = 3$), northern California publicly owned treatment works effluent ($n = 3$), California seawater ($n = 13$), southern and northern California storm water ($n = 11$), 2 samples of unknown origin, and matrix spikes from the different water matrices ($n = 8$). Figure 2A shows the enantiomer fraction results for these samples (light-colored symbols), and Supplemental Data, Table S1, summarizes these results. The Southern California Coastal Water Research Project also contributed 45 sediment samples (including 5 blanks) collected between October 2007 and December 2009 from 6 Ballona Creek sites, all located within the city of Los Angeles (CA, USA) 26, 41. Figure 2B shows the enantiomer fraction results for these samples (light-colored symbols), and Supplemental Data, Table S2, summarizes these results.

For comparison, the method also was applied to 51 laboratory-generated concrete runoff and dosed-fish samples. Although these samples were not collected from the natural environment, results from these samples provide insight into aquatic environment processes such as transport and nontarget organism uptake. The University of California at Riverside (USA) contributed 28 runoff samples collected 1 to 20 d after treatment of concrete with 3 formulations of bifenthrin and permethrin: ready-to-use liquid, ready-to-use solid, and professional concentrate. Figure 2C shows the enantiomer fraction results for these samples (light-colored symbols), and Supplemental Data, Table S3, sums up these results. Figure 3 gives enantiomer fractions measured in University of California at Riverside's concrete runoff water grouped by formulation. The US Geological Survey (USGS; Sacramento, CA,

USA) contributed 23 whole juvenile salmon samples collected 1 to 3 d after aqueous bifenthrin exposure. The USGS study included 2 exposure concentrations; the higher concentration of 0.2 µg/L was selected for the present study to ensure unambiguous and easy detection of bifenthrin enantiomers. Figure 2D displays the enantiomer fraction results for these samples (light-colored symbols), and Supplemental Data, Table S4, sums up these results. Figure 4 summarizes bifenthrin enantiomer fractions measured in USGS-dosed whole juvenile fish samples grouped by day after dosing.

In addition, the California Department of Pesticide Regulation (Sacramento, CA, USA) contributed 36 surface water samples and the California Department of Fish and Wildlife (Sacramento, CA, USA) contributed 15 surface water and urban sediment samples. Because no specific details about these samples were provided and their results were similar to results for other environmental samples, these samples are discussed in the Supplemental Data only.

All samples were previously extracted and analyzed by the organization that provided the samples to USEPA and were selected because they had detectable quantities of at least 1 of the analytes of interest (permethrin, bifenthrin, fipronil, cypermethrin, and cyfluthrin). Details of the previous research on these samples are outside the scope of the present study but in some cases have been published 14, 26, 38.

RESULTS

Five compounds were targeted for enantioselective analysis in the contributed samples: fipronil, *cis*-bifenthrin, *cis*- and *trans*-permethrin, cypermethrin, and cyfluthrin. Cyfluthrin and cypermethrin were not detected in any of the samples. The chromatogram in Figure 1 shows a very low response for cypermethrin and cyfluthrin compared with the other 3 compounds using the study method. Different results may have been obtained if a more sensitive technique had been used (such as electron-capture negative ionization or tandem mass spectrometry) or if the samples contained higher concentrations. Cypermethrin and cyfluthrin results are not discussed.

General enantiomer fraction results

Enantiomer fractions were determined for 44, 17, and 14 environmental samples for fipronil, *cis*-bifenthrin, and *cis*-permethrin, respectively (Figure 2A,B; details presented in Supplemental Data, Tables S1 and S2). Enantiomer fractions were calculated for 9, 36, and 22 laboratory-generated samples for fipronil, *cis*-bifenthrin, and *cis*-permethrin, respectively (Figure 2C,D; details given in Supplemental Data, Tables S3 and S4). The enantiomer fraction results are reasonable given that the samples were selected for enantioselective analysis because 1 or more of the 5 target analytes had been detected and quantitated using other methods. No fipronil or permethrin was detected in bifenthrin-dosed fish samples (Figure 2D), as expected. However, fipronil was detected and enantiomer fractions identified in 9 of 28 samples from concrete slabs that had been treated with bifenthrin and permethrin but not with fipronil (Figure 2C). The source of fipronil in these samples is unknown. Because the samples were selected as a result of detections of target analytes, there is no further statistical discussion of detections.

Sample enantiomer fraction values above or below the range of concurrently measured racemic reference standard enantiomer fractions were classified as nonracemic. As shown in Figure 2, enantiomer fraction results for nonracemic samples fall outside the range of enantiomer fractions for standards shown directly left of the sample results. Of the 44 environmental and 9 laboratory fipronil enantiomer fraction measurements, one (2.7%) publicly owned treatment works effluent sample was nonracemic. Of the 17 environmental and 36 laboratory bifenthrin enantiomer fraction measurements, 27 (51%) were nonracemic and heavily influenced by nearly all of the dosed-fish samples having nonracemic enantiomer fractions. Of the 14 environmental and 22 laboratory permethrin enantiomer fraction measurements, 17 (47%) were nonracemic, split almost evenly between the environmental and concrete runoff water samples. There is an obvious difference in the occurrence of nonracemic enantiomer fractions between fipronil and the 2 pyrethroid insecticides. For pyrethroids (compared with fipronil), this finding indicates either that enriched or single enantiomer formulations are being used or that enantioselective processes are occurring in the environment.

Caltest Analytical Laboratory water sample results

For the Caltest Analytical Laboratory water samples (Figure 2A and Supplemental Data, Table S1), fipronil enantiomer fractions were measured in freshwater ($n = 6$), publicly owned treatment works effluent ($n = 2$), seawater ($n = 3$), and storm water ($n = 7$); bifenthrin enantiomer fractions were measured in freshwater ($n = 2$) and seawater ($n = 10$); and permethrin enantiomer fractions were measured in seawater only ($n = 8$). Concentrations of enantiomers may be expected to differ in salty or brackish water compared with freshwater because of changes in solubility; however, salt should not affect enantiomer signatures because its presence should not be an enantioselective situation or cause enantioselective processes. The sample type did not contribute a statistically significant difference to fipronil or bifenthrin enantiomer fractions ($p > 0.45$). Fipronil enantiomer fractions showed no statistically significant difference among samples collected on different days ($p > 0.49$), and enantiomer fractions were not determined on enough different collection days for a comparison of bifenthrin and permethrin results.

As shown in Table 1, for all 3 compounds discussed in the present study, the (–) enantiomer is less toxic to several species of aquatic organisms than either the racemic mixture or the (+) enantiomer. Based on this finding, enantiomer fractions < 0.5 are expected to be less toxic and carry less risk, and enantiomer fractions > 0.5 are expected to be more toxic and carry more risk for the aquatic species studied and possibly for other species 38. The enantiomer fractions in these water samples were by and large racemic or had enantiomer fraction values measuring less than racemic standards. This finding suggests that aquatic organisms in the areas from which the samples were collected would not have been exposed to unusually toxic enantiomer mixtures of these compounds at the time of sample collection.

Southern California Coastal Water Research Project sediment sample results

The Southern California Coastal Water Research Project reported current-use pesticide concentrations as well as the percentage survival of *Eohaustorius estuarius* at the 6 different Ballona Creek sediment sampling sites during different sampling campaigns 41. For the

Ballona Creek sediment samples (Figure 2B and Supplemental Data, Table S2), neither sampling station ($p > 0.09$), sample collection date ($p > 0.70$), concentration ($R^2 = 0.0015$), nor toxicity measure ($R^2 = 0.0307$) contributed a statistically significant difference to the fipronil enantiomer fraction 41. Too few enantiomer fractions were measured for bifenthrin and *cis*-permethrin to generate meaningful statistics. It is worth noting that the 3 nonracemic bifenthrin samples all were collected from site 6, which was farthest from tidal influences and most likely to have longer water and sediment residence times than other sites 26. These results imply that residence time could be a factor in the degree of enantioselectivity, but additional measurements or observations are required to investigate this hypothesis. The sample collection sites have a range of salinity but, as previously mentioned, this condition is not expected to impact enantiomer signatures.

As indicated in Table 1 by the values for the lethal concentration required to kill 50% of the population (LC50), enantiomer fractions < 0.5 are expected to be less toxic and enantiomer fractions > 0.5 are expected to be more toxic to the species tested. Although not statistically significant, it is interesting to note that the general trend is for higher percentage survival of *E. estuarius* 41 with lower fipronil enantiomer fractions (Supplemental Data, Table S2). Nevertheless, not all individual enantiomer fraction and survival measurements support this trend; high enantiomer fractions of 0.562 and 0.532 correspond to *E. estuarius* survival at 18 and 49%, respectively, and low enantiomer fractions of 0.500 and 0.460 correspond to *E. estuarius* survival at 8 and 23%, respectively. These results indicate that something other than enantiomer signature is contributing to the toxicity, and further research is required to ascertain the cause. A variety of factors can impact the toxicity of a specific sample; thus the more known about the sample's properties and constitution, the clearer and more certain the understanding of toxicity.

University of California at Riverside concrete runoff sample results

The University of California at Riverside concrete runoff sample results provide insight into how current-use pesticides are removed from urban and residential application sites and transported to local aquatic systems. Because of the large amount of pesticides used for structural pest control, results from these samples are quite relevant to understanding pesticide fate and transport. Three different pesticide formulations of bifenthrin and permethrin were applied to the concrete to simulate real-world scenarios: ready-to-use liquid, professional concentrate, and ready-to-use solid (Figure 2C and Supplemental Data, Table S3). The concrete runoff samples showed no statistically significant difference ($p > 0.15$) among the enantiomer fractions measured on days 1, 7, and 20 after application of bifenthrin and permethrin, although the small number of samples in each group ($n = 4, 16$, and 8, respectively) may account for this lack of difference. A statistically significant difference was found among the enantiomer fractions measured for different permethrin formulations (t test, $p < 0.01$) but not for bifenthrin (Figure 3). Because fipronil was not intentionally applied to the concrete slabs 14, no enantiomer fraction comparisons were made.

The average permethrin enantiomer fractions for ready-to-use liquid, professional concentrate, and ready-to-use solid formulations were 0.197, 0.488, and 0.467, respectively

(Figure 3). These results suggest that nonracemic pesticide residues are removed from application sites and transported to local waterways. In this case, the nonracemic enantiomer pattern is expected to be less toxic than the racemic mixture or the more toxic (+) enantiomer for the aquatic species tested (Table 1).

Figure 3 shows 4 permethrin enantiomer fractions < 0.2 for the ready-to-use liquid formulation. All 4 of these samples were collected on day 7 after application. The ready-to-use liquid formulation sample yielding the enantiomer fraction value near 0.4 was collected on day 20. The day 7 samples are more nonracemic than the day 20 sample, but the exact mechanism for this pattern is unknown. Perhaps a nonracemic product was applied or a series of enantioselective processes quickly shifted the enantiomer fraction. However, it seems unlikely that permethrin would be formulated to be less toxic for target species, and concrete is not a matrix where enantioselective processes are expected. Unfortunately, no ready-to-use liquid formulation samples from day 1 or the applied product were available for analysis to further explore these hypotheses. Additional research into enantiomer absorption for a range of environmental surfaces is desirable to better understand if these mechanisms are enantioselective.

USGS sample results for salmon dosed with bifenthrin

In the present study, dosed whole juvenile salmon fish samples were analyzed for enantiomer signatures. This information was collected to provide insight into water uptake, metabolism, and excretion by higher trophic-level aquatic organisms. For the USGS dosed-fish samples (Figure 2D and Supplemental Data, Table S4), all but 2 sample enantiomer fractions were nonracemic, with only 1 enantiomer fraction greater than the racemic standards and the rest less than the racemic standards. This finding is not unexpected because absorption, distribution, metabolism, and excretion potentially are enantioselective processes in organisms such as fish 37, 42. Directly dosing fish removes many variables such as natural diet and habitat. Consequently, based on enantiomer fraction values and concentrations alone, it is not possible to distinguish during which phase of absorption, distribution, metabolism, and excretion enantioselectivity may have occurred. The samples studied were from whole fish; however, additional absorption, distribution, metabolism, and excretion information may be obtained by analyzing different compartments and tissues of the fish. For example, much absorption is likely to occur across the gills or in the digestive system, and metabolism is likely to be highest in the liver. Experiments designed to address different absorption, distribution, metabolism, and excretion processes could greatly increase the understanding of enantioselectivity.

Although the USGS investigators have not published their study's data, they did share their sample extract data for time since dose; fish status, length, and weight; and tissue concentration (Supplemental Data, Table S4). None of the control or low-dose ($0.02 \mu\text{g/L}$) fish died, but approximately one-half of the fish dosed at $0.2 \mu\text{g/L}$ died in the course of this experiment (only the high-dose fish underwent enantioselective analysis). As shown in Table 1, the less toxic bifenthrin enantiomer is (S, -) for 2 fish species (carp and tilapia), which is the more abundant enantiomer in these fish samples. However, no statistical difference was observed among enantiomer fractions in fish that were alive (mean = 0.429) or dead (mean =

0.438) at the time of harvest ($p > 0.50$). Given a racemic dosing and assuming similar toxicity for the salmon species in this experiment (compared with carp and tilapia), the fish were dosed close to or above the LC50 for the more toxic (R, +) enantiomer. Toxicity or death may have preempted enantioselective processes at a 3-d time scale. The reason for one-half of the fish dying possibly is related to concentration; nonetheless, because the enantiomer fractions were not > 0.5 , the deaths are not likely solely caused by enantiomer toxicity. As previously stated, the more that is known about sample history and constitution, the clearer the understanding of toxicity.

As shown in Figure 4, the most nonracemic mean (0.418) and sample enantiomer fractions (0.392, 0.384, and 0.378) were from day 3 samples, suggesting that enantioselective processes occur over time. Nevertheless, there was no statistically significant difference in bifenthrin enantiomer fractions measured on different days after dosing ($p > 0.070$). There also was no enantiomer fraction trend with bifenthrin concentration ($R^2 = 0.0057$), fish weight ($R^2 = 0.0025$), or fish length ($R^2 = 0.10$). Again, the fact that one-half of the fish sampled were dead when collected at day 2 and 80% were dead when collected at day 3 may have impacted these relationships.

DISCUSSION

Enantiomer fraction measurements were made for fipronil, bifenthrin, and *cis*-permethrin in a variety of environmental samples and samples collected by laboratories related to the aquatic environment. These samples included various types of water, sediment, and biological tissue. The present study shows that in these (and possibly similar) samples for the compounds studied, enantiomer exposure does not further explain the aquatic toxicity. This finding is based on the fact that enantiomer exposure generally is racemic and therefore not solely responsible for aquatic toxicity.

Sample descriptors (including water type, sample collection date, sample collection location, applied pesticide formulation, and mortality status; see Supplemental Data) and measurements (including pesticide concentration and percentage of *E. estuarius* survival; see Supplemental Data) were investigated for their impact on measured enantiomer fractions. The vast majority of the comparisons showed no predictable impact or correlation among the sample descriptors (listed above) and enantiomer fraction measurements. However, permethrin enantiomer fractions measured in concrete runoff samples that used the ready-to-use liquid formulation were statistically different from enantiomer fractions for the other 2 formulations studied (ready-to-use solid and professional concentrate). Unfortunately, this difference is difficult to explain because of product and sample availability limitations and because it seems unlikely that less efficacious nonracemic product would be produced or that enantioselective processes would occur on concrete. Regardless of the reason for the differences in permethrin enantiomer fractions, this nonracemic result for ready-to-use liquid formulation was unknown before the present study, leading to incorrect default assumptions of racemic residues and exposures.

In aquatic environmental samples, 98% of fipronil, 82% of bifenthrin, and 43% of *cis*-permethrin enantiomer fractions measured ($n = 75$) were racemic. These mostly racemic

enantiomer fractions indicate that the pesticides measured in the samples had not undergone enantioselective formulation or degradation. For *cis*-permethrin, 75% of the nonracemic samples ($n = 8$ total) had enantiomer fraction values < 0.5 . Therefore, the nonracemic *cis*-permethrin residues measured are expected to be less toxic to the aquatic organisms tested (Table 1) than a racemic mixture. The enantiomer fraction measurements also indicate that aquatic organisms were not being exposed to a more toxic enantiomer mixture in the environment. The screening measurements reported in the present study can be included to reduce uncertainty in future exposure and risk assessments for these chiral pesticides.

In the laboratory-generated samples, 0% of fipronil, 67% of bifenthrin, and 41% of the *cis*-permethrin enantiomer fractions measured ($n = 67$) were nonracemic. It was not surprising to find nonracemic bifenthrin enantiomer fractions in fish where enantioselective uptake, metabolism, and excretion were likely to occur. However, it was surprising to find that runoff water from concrete where a ready-to-use liquid formulation of permethrin was applied had the most nonracemic values measured in any of the contributed samples. Especially for pyrethroids, these nonracemic enantiomer fractions indicate that enantioselective processes can and do occur, although these measurements alone do not explain the exact mechanism of enantioselectivity.

For racemic enantiomer fractions, traditional environmental modeling, exposure, and risk assessments would produce accurate results. When enantioselective processes have occurred and nonracemic enantiomer fractions were measured, however, assumption of a racemic mixture for risk assessments usually would be more protective because the tendency is toward persistence of the less toxic (–) enantiomer. In all cases, environmental sample enantiomer fractions were between 0.4 and 0.6, indicating a small deviation from a racemic mixture. Although these small differences can be statistically significant, further investigation and research are required to determine if these small deviations in enantiomer exposure should be considered in risk assessments. Because of the varied and sometimes unexpected enantiomer fractions measured in the present study, consideration of the enantiomer disposition for chiral pesticides in the environment may reduce uncertainty in exposure and risk assessments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

The authors thank the following people for contributions of samples and sample information and for feedback on the interpretation and presentation of data: M. Hladik, K. Kuivila, and J. Teerlink (US Geological Survey); M. Ensminger, S. Peoples, and K. Goh (California Department of Pesticide Regulation); R. Reif López and J. Richards (University of California at Riverside); N. Dodder (Southern California Coastal Water Research Project); T. Albertson (Caltest Analytical Laboratory); and G. Cho (California Department of Fish and Wildlife).

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Figure 1.

Total ion chromatogram of a 100-ng/ μ L standard containing all analytes of interest. Elution order: (S, +) fipronil, (R, -) fipronil (60 min); (R, +) *cis*-bifenthrin, (S, -) *cis*-bifenthrin (79 min); (1R, +) *cis*-permethrin, (1S, -) *cis*-permethrin, ($\pm$) *trans*-permethrin (95 min); 5 stereoisomers of cyfluthrin (unknown stereoisomer assignment, 100 min); 5 stereoisomers of cypermethrin (unknown stereoisomer assignment, 105 min).

Figure 2.

Enantiomer fractions measured in racemic standards and environmental samples. **(A)** Data for river water, storm water, freshwater, seawater, and effluent water samples contributed by the Caltest Analytical Laboratory. **(B)** Urban estuary sediment samples provided by the Southern California Coastal Water Research Project. **(C)** Runoff samples from concrete treated with bifenthrin and permethrin supplied by the University of California at Riverside. **(D)** Whole juvenile salmon dosed with bifenthrin from the US Geological Survey. Details for these standards and samples are listed in the Supplemental Data, Tables S1–S4.

Figure 3. Enantiomer fractions measured in University of California Riverside's concrete runoff water grouped by formulation (ready-to-use liquid, professional concentrate, and ready-to-use solid).

Figure 4.
Bifenthrin enantiomer fractions measured in US Geological Survey dosed whole juvenile fish samples grouped by day after dosing (days 1, 2, and 3).

Table 1.

Median lethal concentration values for current-use pesticides on aquatic organisms

Compound [reference]	Organism	Racemic (µg/L)	(+) (µg/L)	(-) (µg/L)
Fipronil 33	<i>Ceriodaphnia dubia</i>	17.9	11.3	35.4
Fipronil 34	<i>Procambarus clarkii</i>	125	82	164
<i>cis</i> -bifenthrin 35	<i>Ceriodaphnia dubia</i>	0.144	0.076	1.34
<i>cis</i> -bifenthrin 35	<i>Daphnia magna</i>	0.175	0.081	1.80
<i>cis</i> -bifenthrin 36	<i>Daphnia pulex</i>		2.13 ^a	28.97 ^a
<i>cis</i> -bifenthrin 36	<i>Cyprinus carpio</i>		0.99 ^a	2.08 ^a
<i>cis</i> -bifenthrin 36	<i>Tilapia</i> spp.		0.19 ^a	0.80 ^a
<i>cis</i> -permethrin 35	<i>Ceriodaphnia dubia</i>	0.539	0.156	>6.0
<i>cis</i> -permethrin 35	<i>Daphnia magna</i>	0.788	0.388	>6.0

^a It appears that the enantiomer assignment for bifenthrin was incorrectly assigned in Liu et al. 36. Therefore, the enantiomer assignment was revised for this Table. See the Supplemental Data for details.