ENANTIOSELECTIVITY IN ENVIRONMENTAL CHEMISTRY AND ECOTOXICOLOGY OF SYNTHETIC PYRETHROIDS

Weiping Liu[1]*, Chao Xu[2], Jin Liu[1]

[1]College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China
2Research Center of Environmental Science, Zhejiang University of Technology, Hangzhou 310032, China

Introduction

Chiral pesticides currently constitute about 25% of all pesticides used, and this ratio may further increase as more complex and natural product-like compounds are introduced. Chirality occurs widely in synthetic pyrethroids (SPs), which are the mainstay of modern insecticides. Chiral pesticides consist of enantiomers that may have contrasting toxicological characteristics and may also degrade at significantly different rates in the environment. The enantioselectivity in these processes can result in enhanced or reduced ecotoxicological risks that cannot be predicted from our current level of understanding. In contrast to the increasing importance of chiral pesticides, very little has been done by the scientific community to understand their environmental implications, especially for currently used chiral insecticides. In this interdisciplinary study, we resolved enantiomers of a number of SPs on enantioselective columns and evaluated the occurrence of enantioselectivity in biodegradation and ecotoxicity.

Materials and Methods

***Synthetic pyrethroid insecticides*:** Analytical standards of racemic (*Z*)-*cis*-bifenthrin [BF, 96%, 2-methylbiphenyl-3-yl methyl (*Z*)-(*1RS*)-*cis*-3-(2-chloro-3, 3, 3-trifluoroprop-1-enyl)-2, 2-dimethylcyclopropane carboxylate], Racemic permethrin [PM, 20% *cis*- and 78% *trans*- pure, phenolxybenzyl-(*1SR*)-*cis*- *trans*-3-(2,2-di-chlorovinyl)-2,2-dimethylcyclopropane carboxylate], racemic cypermethrin [CP, 98%, (*RS*)-α-cyano-3-phenoxybenzyl (*1RS*)-*cis*-*trans*-3-(2,2-dichloro- vinyl)-1,1-dimethylcyclopropane carboxylate], isomer-enriched formulations β-CP (86%, enriched in *1R*-*cis*-α*S* + *1S*-*cis*-α*R* and *1S*-*trans*-α*R* + *1R*-*trans*-α*S*) and racemic cyfluthrin [CF, 98%, (*RS*)-α-cyano-4-fluro-3-phenoxybenzyl (*1RS*)-*cis*-*trans*-3-(2,2-di- chlorovinyl)-1,1-dimethyl cyclopropane carboxylate] were purchased from Chem Service (West Chester, PA, USA). *cis*-permethrin (*cis*-PM, 99.3%), *trans*-permethrin (*trans*-PM, 99.4%), and enantiopure *1R*-*cis*-bifenthrin (*R*-*cis*-BF, 97.2%) and the isomer-enriched formulations α-CP (99%, enriched in *1R*-*cis*-α*S* + *1S*-*cis*-α*R*) and θ-CP (99%, enriched in *1R*-*cis*-α*S*) were provided by FMC (Princeton, NJ, USA). Each of the selected insecticides (or isomers) contains one or more pairs of enantiomers.

***Chiral high performance liquid chromatography*:** Separation of individual enantiomers was carried out on an Agilent 1100 Series HPLC system (Wilmington, DE, USA) or a JASCO LC-2000 Series HPLC system (JASCO Co., Tokyo, Japan) using chiral columns. The polarity (i.e., rotation sign) of each resolved enantiomer was determined by an on-line laser polarimeter detector (PDR-Chiral, Lake Park, FL, USA). The light source for the chiral detector was a laser (675 nm), and the cell path was 50 mm. The polarity was indicated directly from the appearance of a positive (+) or negative (–) peak on the polarimeter concurrent to the response on the UV detector. The JASCO CD-2095 circular dichroism chiral detector was also used to measure the Cotton effect of each enantiopure.

***Chiral gas chromatography*:** An Agilent 6890N GC-electron capture detector (ECD) system (Wilmington, DE, USA) was used for quantitative analysis in experiments to evaluate enantioselectivity in degradation of SPs in sediments and laboratory conditions. An Agilent 5973 mass selective detector (MSD) was used for structural confirmation. The temperature of the ECD was 310°C, and the detector makeup gas was N_2 (60 mL min^{-1}). A suite of chiral capillary columns was tested, and optimal separation of enantiomers from BF, PM, *cis*-PM, CP and CF was achieved on a BGB-172 column (20% *tert*-butyldimethylsilyl- β-cyclodextrin dissolved in 15% diphenylpolysiloxane and 85% dimethylpolysiloxane, GBG Analytik, Adliswil, Switzerland). Preliminary experiments showed that no inter- conversion between BF and PM enantiomers occurred under these conditions. Concentrations were determined by using peak area, assuming the same response factor for enantiomers originating from the same compound.

***Aged sediment samples*:** Sediments containing residues of BF and *cis*-PM were collected at a site next to a nursery in Irvine, CA, USA and were used for evaluation of changes in *ER* as a result of natural attenuation. The dried sediment was accumulated from surface runoff over a 4-year period. Samples were taken by using a hand auger to depths of 0–15, 15–30, and 30–45 cm. The sediment samples were air dried, mixed, and passed through a 0.5-mm sieve. Aliquots (5.0 g) of sediments were extracted by mixing twice with acetone/hexane (1:1 v/v), and the extracts were concentrated to a small volume for analysis by GC. Preliminary experiments showed that the recovery of the above procedure was >90% for BF or *cis*-PM in soil or sediment. Three replicates were used for each sediment sample.

***Incubation experiments*:** Enantioselectivity in BF and *cis*-PM degradations was further evaluated through incubation experiments. Sediment samples were collected from a sedimentation pond and a runoff channel at a nursery site in Southern California. The pond sediment contained 0.65% organic carbon and 5% clay; the channel sediment contained 6.4% organic carbon and 19% clay. Compared with sediments used in the above experiment, sediments from the sedimentation pond and channel were newly deposited. The sediments were sampled from the surface layer (0–5 cm) and were used without air-drying to preserve the original microbial activity. Five grams (dry weight equivalent) of the sediment was placed in a 20-mL glass vial and mixed with 5 mL of deionized water. The sample vials were covered with aluminum foil and incubated at room temperature (21 ± 1°C). Replicate samples were removed at different times and stored in a freezer (–21°C) before analysis. For analysis, the sediment was quantitatively transferred to a Teflon centrifuge tube, mixed with hexane-acetone (1:1 v/v), and then centrifuged. After the supernatant was decanted, the sediment phase was extracted two more times with fresh solvents. The extracts were combined, dried with 50 g of anhydrous sodium sulfate, and then concentrated to a small volume to be used for analysis by GC.

***Acute water-phase toxicity assays*:** In these assays, LC50 values will be obtained for individual enantiomers of the test compounds using *Ceriodaphnia dubia* (*C. dubia*) and *Daphnia magna* (*D. magna*) as the test animals. Both *C. dubia* and *D. magna* are widely used freshwater indicators. EPA guidelines for effluent toxicity tests using these organisms will be followed (Weber, 1995). The general procedures are given below. Individual enantiomers will be prepared in a water miscible solvent (e.g., acetone) at known concentrations. For *C. dubia* test, spring water will be used to prepare the reconstituted, moderately hard water (RMHW). The prepared RMHW will be aerated by bubbling air through the water before use for more that 24 h. Test solutions containing enantiomers at 7 different concentrations will be prepared from stock solutions (≤20 μL) using RMHW. The prepared solution (15 mL) will be dispensed into 20 mL borosilicate glass vials, with 4 replicates for each concentration. A treatment with acetone only (≤20 μL) will be included as the control. Five *C. dubia* neonates less than 20 h old will be added into each vial. The test organisms will be fed with yeast cerophylla trout (YCT) chow and *Selenastrum* sp. (Aquatic Research Organisms, Hampton, NH, USA) 4 h prior to the exposure. All vials will be monitored at 24-h intervals for 96 h. The concentration that causes 50% mortality of the test population will be determined by probit analysis using ToxCalc (v5.0) (Tidepool Scientific Software, McKinleyville, CA) and defined as LC_{50} (μg L^{-1}).

A procedure similar to that for *C. dubia* will be used for the *D. magna* test, except that the assay will be conducted in 125-mL glass jars containing 100 mL solution. Ten organisms (adult) will be introduced into each container, and mortality of *D. magna* will be monitored at 24 h intervals for 96 h. The concentration that causes 50% mortality of the test population will be determined by probit analysis and defined as LC_{50} (μg L^{-1}). The obtained LC_{50} values will be subjected to statistical analysis to determine the significance in difference between enantiomers. From these assays, enantioselectivity in water-phase toxicity will be known and the active enantiomers will be identified.

Results and Discussion

This study focused on the enantioselectivity in biodegradation and ecotoxicology of SPs and OPs insecticides. In the final chapter of this thesis, I will try to discuss how the results contribute to a broader understanding of separation, isolation and analysis, and enantioselective degradation, ecotoxicology of SPs and OPs enantiomers.

Separation, isolation and analysis of pesticidal enantiomers. Isomers of four common- ly used SPs (BF, PM, CP and CF) were separated at the enantiomeric level by enantioselective high-performance liquid chromatography (HPLC). Enantiomers of BF and PM were completely resolved on a Sumichiral OA-2500-I or Chiralcel OJ-H column. The enantiomer of *trans*-PM was baseline separated on Chiralcel OJ-H column under

loose mobile phase. All eight isomers of CP and CF were completely separated on two chained Chirex 00G-3019-DO columns. Enantiomers of four organophosphates (fonofos, profenofos, trichloronate and crotoxyphos) were absolutely resolved on a Chiralcel OJ-H column. Otherwise single enantiomers can be "prepared" by collecting the eluent fractions after chiral HPLC separation. Chiral HPLC columns can be sufficient for preparing adequate amounts of single enantiomers to be used in the bioassay treatments and as enantiomeric standards in GC quantitative analysis.

A quantitative analysis of SP diastereomers or enantiomers was developed by capillary gas chromatographic (CGC). HP-5MS column to separate the diastereomers and a β-cyclodextrin-based enantioselective column (BGB-172) were used to separate the *cis*- enantiomers of BF, PM, CP and CF. Resolved peaks were identified by comparing chromatograms of BF, *1R-cis*-BF and isomer-enriched CP products. Diastereomers of both CP and CF were separated on the HP-5MS column. On the BGB-172 column, enantiomers of all *cis*- diastereomers were separated, while those of *trans* diastereomers were not separated. The elution order appears to be regulated by configuration, a finding that may allow peak identification in the absence of isomer standards. When coupled with electron capture detection, the developed methods had low detection limits and may be used for analysis of SP diastereomers and *cis*-enantiomers in environmental samples.

The chiral stability in SPs depends on the origin of chirality. For SPs with chirality deriving solely from the cyclopropyl ring, such as BF and PM, the chiral configurations were found to be relatively stable. Therefore, the isomer composition for these SPs will be preserved during sample preparation and GC analysis. However, for pyrethroids with chirality on the αC, such as CP and CF, isomer conversion may occur at αC position under heat or in water. Isomer conversion for CP and CF also occurred in water at a slow rate. As the conversion proceeded at the same rate for all stereoisomers from the same SP, isomer conversion would affect the analytical outcome only when stereoisomer-enriched samples are analyzed. However, using a lower inlet temperature, on-column injection, or both may effectively minimize isomer conversion during GC analysis.

Enantioselective biodegradation of synthetic pyrethroids. Enantioselectivity was eva- luated for SPs during their biodegradation in pesticide-degrading bacteria isolates and in whole sediments under field and laboratory conditions. Incubation with pesticide- degrading bacteria showed that the *trans*- diastereomer of PM was selectively degraded over the *cis*- diastereomer, whereas the *1S-cis* enantiomer in BF or *cis*-PM was preferentially degraded over the corresponding *1R-cis* enantiomer. The enantioselectivity was significantly greater for *cis*-PM than for BF and also varied among different strains of bacteria. Isomer selectivity in biodegradation of CP by microbial isolates and in sediment was shown that the *trans* diastereomers were preferentially degraded over the *cis* diastereomers. Of the two active enantiomers, *1R-cis-*α*S* was degraded slower, whereas 1R-trans-αS was degraded faster than the other stereoisomers. Similar isomer selectivity was observed during CP degradation in whole sediment.

Enantioselective ecotoxicology of synthetic pyrethroids. Great differences were found between enantiomers in the acute toxicity to aquatic invertebrates *C. dubia* or *D. magna*. In BF and cis-PM, the *1R-cis* enantiomer was 15-38 times more active than the *1S-cis* isomer, while in *trans*-PM, the *1R-trans* isomer was substantially more toxic than the *1S-trans* enantiomer. In cypermethrin or cyfluthrin, two of the eight isomers, *1R-cis-*α*S* and *1R-trans-*α*S*, contributed for almost all the toxicity in the racemate, while the other six enantiomers were inactive. For the selected four chiral OPs, it was observed that the (−)- enantiomer was consistently more active than the (+)-enantiomer. The activity of the racemate was attributable primarily to the (−)-enantiomer for both *C. dubia* (92-94%) and *D. magna* (87–94%).

SPs are a group of hydrophobic compounds with significant aquatic toxicity. Their strong affinity to suspended solids and humic materials suggests that SPs in natural surface water are distributed in solid-adsorbed, DOM–adsorbed, and freely dissolved phases. The freely dissolved phase is of particular importance because of its mobility and bioavailability. We used SPME to detect the freely dissolved phase, and evaluated the phase distribution of BF and PM in surface waters. In stream water, most SPs were associated with the suspended solids and, to a lesser extent, with DOM. The predominant partitioning into the adsorbed phases implies that the toxicity of SPs in surface water is reduced because of decreased bioavailability.

In finite ELISA for endocrine disrupting chemicals (EDCs), the significant enantio- selectivity of vitellogenin concentration was observed in the liver of male *J. medaka*. The enantiomer of *1R-cis*-BF was not found significant abduction of vitellogenin level in liver for fish. The result suggests that the environmental safety and high efficiency pesticide should come from single configuration (or some enantiomers) of racemic compounds.

The significant enantioselectivity was displayed in all of three rest animals (two water fleas and one fish).

The enantiomeric concentration of *1R-cis*-BF was higher than that of *1S-cis*-BF in animals. The concentration of both enantiomers in liver was higher than that in other parts of *J. medaka*. It implies that *1S-cis*-BF enantiomer should be less biouptake or easy to biodegradation by the enzymes in fish. Otherwise, the capacity of bioaccumulation of organism was significant different. The accumulative ability of liver of fish was much higher than that of other parts of fish.

Linking enantioselectivity in biodegradation and ecotoxicity. Occurrence of enantio- selectivity in either degradation or toxicity alone would have limited environmental significance. For instance, if two enantiomers of a chiral compound have the same aquatic toxicity (i.e., nonenantioselective), changes in *ER* alone will not result in different effects on the organism, because the combined toxicity will remain unchanged in relation to time. Conversely, if enantioselectivity occurs only in toxicity but not in degradation, *ER* will remain unchanged over time, and the ecotoxicological effects are predictable from the racemate. Although previous studies showed that legacy chiral insecticides and some chiral herbicides or fungicides could undergo enantioselective degradation in the environment, the ecotoxicological significance was not revealed, because enantioselectivity in ecotoxicity was not simultaneously determined for these compounds.

We demonstrated that enantioselectivity occurred concurrently in both aquatic toxicity and degradation for chiral compounds from the SP classes. This finding may have several important implications. First, as only specific enantiomers possess significant toxicity, the adverse ecotoxicological effects will closely depend on the environmental behavior of the toxicologically active enantiomer instead of on that of the racemate. Monitoring for the racemate concentration, as currently practiced, will give an inadequate or misleading basis for assessing the environmental risk of chiral contaminants. For instance, if the toxicologically active enantiomer is preferentially degraded, the use of racemate concentration will result in an overestimation of ecotoxicity. However, if the inactive enantiomer is selectively degraded, the use of racemate concentration will underestimate the ecotoxicological effect. Products enriched in biologically active enantiomers are increasingly used for achieving improved pest-control efficacy. For instance, enantiomer-enriched formulations of the SPs cyfluthrin, cypermethrin, and deltamethrin are currently in use. If the adverse ecological effects are mainly attributable to the enriched enantiomer, the environmental value of using enantiomer-enriched products must be questioned. However, if the adverse effects are caused only by or mostly by the excluded enantiomer, using enantiomer-enriched products may offer great environmental benefits. In addition, studies show that enantioselectivity may closely depend on environmental conditions and may vary significantly even for the same compound. Further studies are needed to characterize interactions of environmental factors, such as soil sediment properties, vegetation types, redox conditions, and microbial structures, with enantioselectivity in the behavior of chiral pesticides.

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