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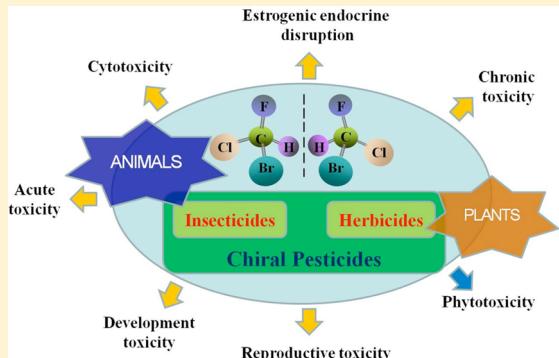
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ABSTRACT: The enantioselective environmental toxic effect of chiral pesticides is becoming more important. As the industry develops, increasing numbers of chiral insecticides and herbicides will be introduced into use, potentially posing toxic effects on nontarget living beings. Chiral pesticides, including herbicides such as acylanilides, phenoxypropanoic acids, and imidazolinones, and insecticides such as synthetic pyrethroids, organophosphates, and DDT often behave enantioselectively during agricultural use. These compounds also pose unpredictable enantioselective ecological threats to nontarget living beings and/or humans, affecting the food chain and entire ecosystems. Thus, to investigate the enantioselective toxic effects of chiral insecticides and herbicides is necessary during environmental protection. The environmental toxicology of chiral pesticides, especially the findings obtained from studies conducted in our laboratory during the past 10 years, is reviewed.



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1. INTRODUCTION

Rachel Carson's *Silent Spring* (1962) raised awareness of the threat posed to humans and the environment by pesticides. The concept of "environmental toxicology" was subsequently developed,¹ and research in this area has been remarkably rapid during the past 40 years. Since the latter half of the 19th century, because the industries of chemical manufacturing expanded rapidly, events of environmental pollution have been more frequent. Currently, new pollutants are under development. Chiral pesticides, including current chiral pesticides and persistent organic pollutants (POPs), are becoming important.

Approximately 25% of the insecticides and herbicides sold in 1996 were chiral.² Since more complex compounds are introduced, this proportion is expected to increase. In currently

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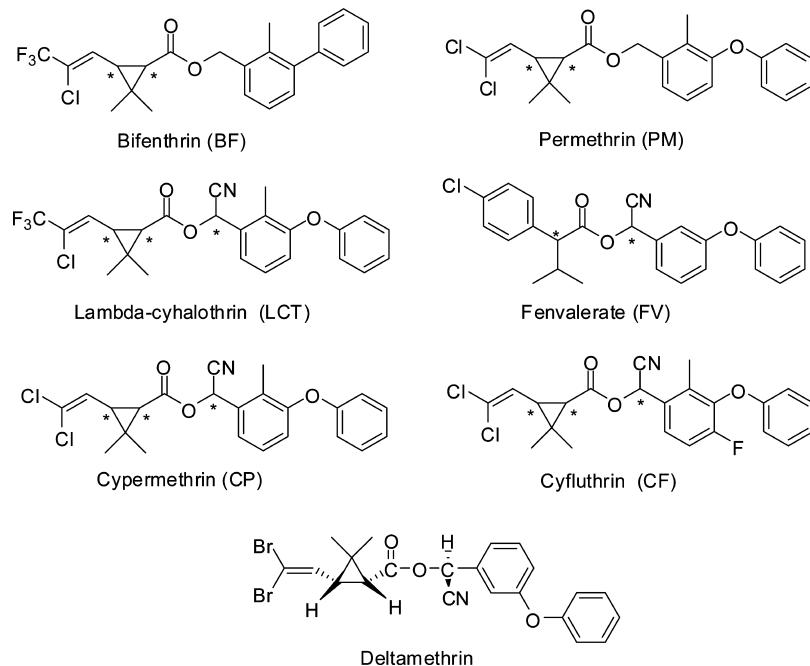


Figure 1. Chemical structures of several SPs. *Asymmetric atom.

registered pesticides, approximately 30% of the active ingredients contain asymmetric centers,³ and over 40% of current insecticides and herbicides in China are chiral.⁴ The enantioselective effects of chiral pesticides have received broad attention from the 1990s and are gaining increasing attention.^{5–10} Stereoisomers of chiral insecticides and herbicides usually pose different effects on weeds or insect control.¹¹ The registrations of chiral phenoxyalkanoic acids racemate have been revoked in Switzerland and The Netherlands based on the enantioselective bioactivities, and single-isomer registration was continuing to be approved. On the basis of the active ingredient weight, a tax on agrochemicals has been implemented in Sweden.

In ecology, chiral pesticides also undoubtedly behave enantioselectively. The active enantiomer may pose required effects on a target organism, but the inactive enantiomer would be harmful to nontarget organisms. Understanding the environmental toxic effects of chiral insecticides and herbicides is important for environmental risk assessment. If environmental toxic effects show differences, toxicity data for chiral pesticides are additionally needed. These data can be used in relevant environmental risk assessment and would guide the correct and appropriate use of pesticides. For more than 20 years, environmental scientists have been probing the enantiomer selectivity of chiral pesticides.

The purpose of this review is to summarize the enantioselective environmental toxic effects of some chiral insecticides including organophosphates (OPs), synthetic pyrethroids (SPs), and some organochlorines, and herbicides such as imidazolinones, acylanilides, and phenoxypropanoic acids; in particular, we will draw on studies conducted in our group during the past 10 years. Pure enantiomers that are used for toxicology tests are obtained mainly by chiral HPLC separation and analysis.^{12–14} In this review, two aspects of environmental toxicology are discussed: the enantioselective toxicology of chiral herbicides on nontarget plants and the enantioselective toxicology of chiral insecticides on nontarget animals.

2. ENANTIOSELECTIVE TOXICITY OF CHIRAL INSECTICIDES ON NONTARGET ORGANISMS

2.1. Synthetic Pyrethroids (SPs). Analogues of the natural pyrethrins, which are extracted from dried *Chrysanthemum cinerariaefolium* flowers, are synthetic pyrethroids (SPs) (Figure 1).¹⁵ These compounds have been used broadly to control agricultural and domestic insect species since the 1980s.¹⁶ A majority of SPs have 2 or 3 asymmetric elements, exhibiting 4 or 8 stereoisomers; thus, this family of insecticides is among those that exhibit the highest number of chiral centers. Owing to high acute aquatic toxicity to invertebrates and fish at low levels, SPs are of environmental concern.¹⁷

2.1.1. Acute toxicity. Bifenthrin (BF) possesses 2 asymmetric centers and presents two pairs of enantiomers. The commercial form of BF is *cis*-BF, which contains a 1*S*-*cis*-enantiomer and a 1*R*-*cis*-enantiomer. Acute toxicities of the racemate and the *cis*-BF enantiomers were measured using *Daphnia magna* (*D. magna*) and *Ceriodaphnia dubia* (*C. dubia*); the racemate was toxic, and a significant difference was observed in the LC₅₀ between 1*S*-*cis*-BF and 1*R*-*cis*-BF. 1*R*-*cis*-enantiomer is 17- and 22-fold more toxic than the 1*S*-*cis*-enantiomer to *C. dubia* and *D. magna*, respectively.^{17–19}

The acute oral toxicity of permethrin (PM) stereoisomers was studied in mice by Miyamoto in 1976.²⁰ The 1*R*-*cis*-isomer was the most toxic, the second is 1*R*-*trans*-PM, and the two 1*S*-enantiomers were least toxic. The toxicity of these compounds was studied in *D. magna* or *C. dubia* recently; using LC₅₀ as an end point, *S*-*cis*-isomer and *S*-*trans*-isomer were inactive in both species, whereas the *R* enantiomer in the racemate contributed 94–96% of the toxicity to *D. magna* and 95–97% to *C. dubia*.¹⁷ The same study group also indicated that 1*R*-*trans*-PM was more active than 1*S*-*trans*-PM toward *D. magna* and *C. dubia*; the LC₅₀ of the inactive enantiomer was at least 20–30 times higher than that of the active enantiomer.¹⁸

Enantiomers of *cis*-lambda cyhalothrin (LCT) pose enantioselective acute toxicity toward zebrafish and the zebrafish embryo. LCT enantiomers are distinguishable using a CD

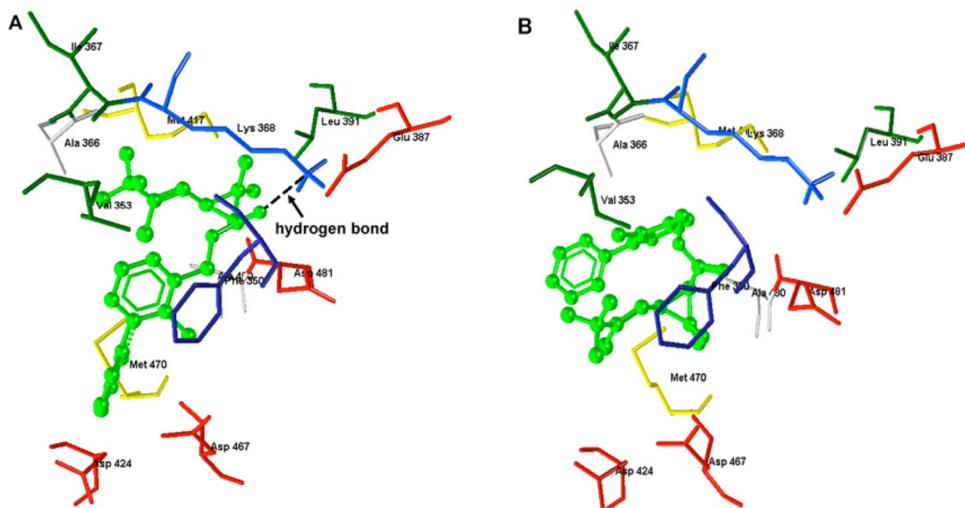


Figure 2. Molecular modeling evidence for the interaction between BF enantiomers and PKC. (A) The statistical evaluation of the ligand binding mode of 1S-cis-BF to PKC. (B) The statistical evaluation of the ligand binding mode of 1R-cis-BF to PKC. The BF isomers are represented as sticks and proteins as cartoons. The black dotted-line represents a hydrogen bond.

detector (236 nm), and (+) and (−) enantiomers are recognized. The results of a study showed that using LC₅₀ as an end point, the (−)-enantiomer was more than 162-fold more potent than the (+)-enantiomer to zebrafish, and the embryo assay indicated that LCT caused yolk sac edema, pericardial edema, and a crooked body. (−)-LCT yielded 7.2-fold higher 96-h mortality than (+)-LCT. At concentrations of 50 µg/L, (−)-LCT and the racemate caused malformations, while at concentrations above 100 µg/L, the (+)-enantiomer produced malformations.²¹

Fenvalerate (FV) has 2 asymmetric centers, and 2 pairs of enantiomers exist. Using zebrafish (*Danio rerio*), zebrafish embryos-larvae, and *D. magna* as test animals, enantioselectivity in toxicity assays for the racemate and each stereoisomer has been detected. In the toxicity assay to *D. magna*, 24-h EC₅₀ for αR-2R-FV was 51-fold higher than that for αS-2S-FV, suggesting that the latter was more toxic, and the results of a 48-h LC₅₀ assay demonstrated that αR-2R-FV was 99-fold less potent than αS-2S-FV. LC₅₀ values for αR-2R-FV were 17, 22, 39, and 56 times higher than those for αS-2S-FV on 1, 2, 3, and 4 d in the toxicity assay on *D. rerio*, respectively. Assays using 96-h-old zebrafish embryo larvae demonstrated that FV induced yolk sac edema, pericardial edema, and a crooked body enantioselectively and that αS-2S-FV exhibited 96-h mortality values that were 3.8 times greater than those of other stereoisomers.²²

For cypermethrin (CP), only 1R-cis-αS and 1R-trans-αS stereoisomers have significant activity toward *C. dubia*; the remaining 6 stereoisomers are less potent, with LC₅₀ values 10-fold higher than those of the two stereoisomers.²³ For cyfluthrin (CF), a nearly identical situation was found; the 1R-cis-αS and 1R-trans-αS stereoisomers were 50- to 100-fold more active than the other stereoisomers.^{23,18} Leicht et al.²⁴ tested the toxicity of CF in *D. magna* and found that the toxic effect consistently resulted from the 1R-cis-αS and 1R-trans-αS stereoisomers.

2.1.2. Chronic Toxicity. The chronic toxic effect of the *cis*-BF enantiomers toward *D. magna* showed a clear enantioselectivity in the same direction as the acute toxicity: 1S-cis-BF was evidently less potent than 1R-cis-BF. For survival and fecundity, on days 7 and 14, the lowest-observed-effective concentration

(LOEC) for 1S-cis-BF were nearly 40 and 80 times, respectively, higher than those for 1R-cis-BF. In addition, in *D. magna*, 1S-cis-BF accumulated to levels that were about 14- to 40-fold lower than those observed for 1R-cis-BF. The findings support the notion that in aquatic organisms the enantioselective chronic toxicity might be mainly attributed to an enantiomeric specific biological process.²⁵

FV showed enantioselective chronic toxicity in mammals as well. In nontarget organisms such as mice and rats, 2S-αS-FV induces changes of microgranulomatous in the liver, spleen, and adrenal glands and/or mesenteric lymph nodes. The enantioselective toxicity resulted from the stereospecific formation of a lipophilic metabolite.²⁶

2.1.3. Cytotoxicity. Genotoxicity and cytotoxicity in human amnion epithelial cell (FL) was induced by BF. The 1R-cis-enantiomer was less potent than the 1S-cis-enantiomer in cell proliferation and cytoflow analyses at concentrations greater than 7.5 mg/L. The cells showed a concentration-related accumulation of intracellular reactive oxygen species (ROS) exposed to 1S-cis-BF. In a comet assay, more cells experienced DNA damage after exposure to the 1S-cis-enantiomer than after 1R-cis-enantiomer exposure.²⁷ Liu et al.²⁸ used the Hep G2 cell (human hepatocellular liver carcinoma) to further evaluate enantioselectivity in terms of apoptosis and cytotoxicity mediated by the MAPK signaling pathway (mitogen-activated protein kinase). MAPKs are Ser/Thr protein kinases which transfer extracellular signals to the nucleus. The MAPK pathway is a key process which is related to reproduction, immunotoxicity, neurological symptoms, and endocrine disruption. Three MAPK subfamilies were identified: JNKs (Jun-N-terminal kinases), p38 kinases, and ERKs (extracellular regulated kinases). MAPKs regulate gene expression via phosphorylating downstream transcription factors. By detecting the phosphorylation status of JNK, ERK, and ROS production, this study²⁸ investigated MAPK signaling involvement. When treated with 1R-cis-BF, levels of phosphorylated JNK was not affected; whereas when treated with 1S-cis-BF, the phosphorylated JNK levels increased. Cytotoxicity and apoptosis induced by 1S-cis-BF was blocked by pretreatment with SP600125, the JNK inhibitor. Neither the 1R-cis-enantiomer nor the 1S-cis-enantiomer induced phosphoactivation of p38 and ERK1/2.

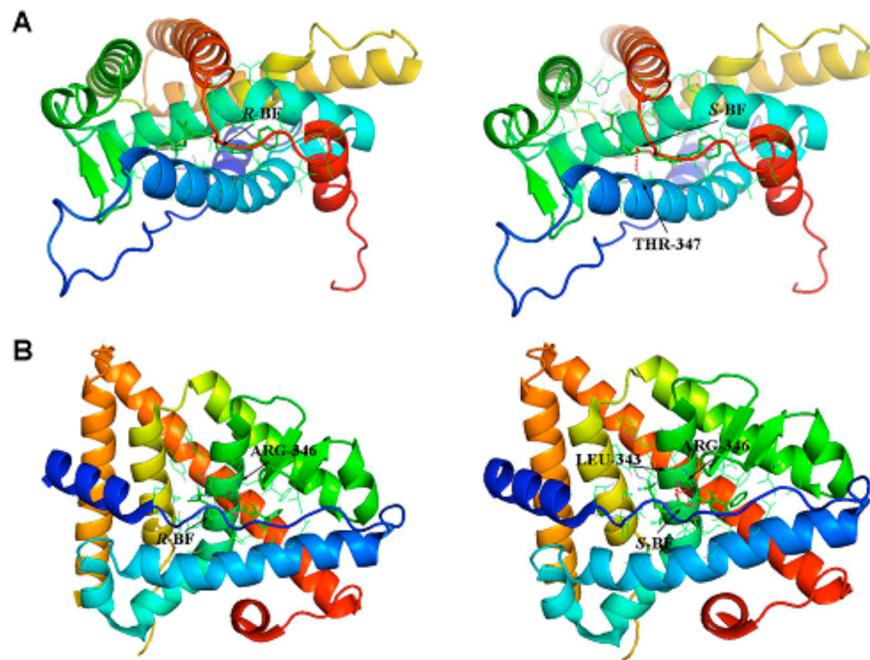


Figure 3. Binding mode of BF enantiomers to ER α LBD (A) and ER β LBD (B). The ligands are represented as sticks, and the proteins are presented as cartoons.

The results showed that *cis*-enantiomer-inducing apoptosis in Hep G2 cells might happen via activation of the JNK/MAPK signaling pathway; the exact molecular mechanisms still remain unknown.

2.1.4. Estrogenic Endocrine Disruption. The estrogenic potential of *cis*-BF is enantioselective. The proportions of relative proliferative effects of 1*R*-*cis*-BF and 1*S*-*cis*-BF were 20.9% and 74.2% using an human breast carcinoma MCF-7 cell proliferation test (*in vitro*). Cell proliferation caused by both the 1*R*-enantiomer and 1*S*-enantiomer might occur via the classical estrogen response pathway through the estrogen receptor (ER). Besides, vitellogenin (VTG) levels can be used as a molecular marker in male fish when exposed to estrogenic endocrine disruptive chemicals (EDCs). Experiments in Japanese medaka indicated that the VTG induction response to 1*R*-enantiomer was approximately 123-fold lower than that in response to the S-enantiomer at 10 ng/mL.²⁹ Later, Zhao et al.³⁰ also indicated that 1*S*-*cis*-BF exhibits greater estrogenic potential (in MCF-7 human breast carcinoma cell) and immunocytotoxicity (macrophage cells RAW264.7) than the *R*-enantiomer. Similarly, Wang et al.³¹ indicated that estrogenic potential of the *cis*-enantiomer is primarily due to the 1*S*-*cis*-enantiomer by applying the yeast two-hybrid model.

To probe the effects of BF on the biosynthesis of progesterone and prostaglandin E2 (PGE2), rat ovarian granulosa cells were used as an *in vitro* model by Liu et al.³² It was shown that in granulosa cells that the 1*S*-*cis*-enantiomer reduced the secretion of progesterone and PGE2 significantly. 1*S*-BF reduced the expression of the genes PBR, StAR, and P450sc, and COX-2, as well as DBI. 1*S*-*cis*-BF disrupted the transcriptional activation of StAR and the COX-2 promoter. Further, the 1*S*-enantiomer differentially inhibited the activity of protein kinase C (PKC). Molecular docking suggested that between the PKC protein and 1*S*-*cis*-BF a hydrogen bond was formed (Figure 2).

Furthermore, the effects of BF on hormone secretion, cell viability, steroidogenesis and the gene expression of trophoblast

cells were *in vitro* evaluated, and interactions of the estrogen receptor (ER) with BF enantiomers were predicted.³³ At noncytotoxic or low concentration, the secretion of both human chorionic gonadotropin and progesterone have been induced. Expression of the human leukocyte antigen G genes and progesterone receptor were enhanced. GnRH type-I and its receptor, the key regulators of the hormonal cascade, were both up-regulated. Furthermore, the *R*-enantiomer exhibited lower effects than the *S*-enantiomer in interference in hormone signaling. The enantioselective endocrine disruption of BF was supposed to be partially due to enantiospecific ER binding affinity in molecular docking studies (Figure 3). Therefore, BF may act enantioselectively via ER to disrupt the hormonal network. The findings indicate that current chiral insecticides should be significantly concerned regarding maternal–fetal health.

Two pairs of enantiomers of PM were separated and recognized according to the CD signals at 230 nm. An *in vivo* study observed that a 48-h exposure to racemic PM and the stereoisomers increased the transcription of 2 VTG genes in male adult zebrafish. The two enantiomers induced hepatic gene transcription at significantly different levels. The (−)-*trans*-enantiomer induced the levels of VTG 1 and VTG 2 mRNA in zebrafish by 2.6- and 1.8-fold higher than (+)-*trans*-enantiomer. Among these stereoisomers, (−)-*trans*-PM exhibited the highest estrogenic activity. These results definitely indicated that the estrogenic activity of PM is significantly enantioselective.³⁴

Similar to results obtained from adult zebrafish, a study³⁵ reported that in embryonic-larval zebrafish, after 7-day treatment with 250 ng/L racemic PM and the stereoisomers, the expression of VTG 1, ESR α , and CYP19b was stimulated. Estrogen-responsive gene expression was induced at levels that significantly differed between the two enantiomers. CYP19b, ESR α , and VTG1 response to the 1,000 ng/L (−)-*trans*-enantiomer were approximately 3.2, 1.8, and 1.5 times higher than that in fish exposed to the same concentration of

Table 1. Toxicity of Chiral Insecticides on Nontarget Organisms

insecticides	more toxic	less toxic	test animals	references
cis-BF (acute)	1R-cis	1S-cis	<i>C. dubia</i> <i>D. magna</i>	17–19
cis-BF (chronic)	1R-cis	1S-cis	<i>D. magna</i>	25
cis-BF (cytotoxicity)	1S-cis	1R-cis	FL cell lines; MCF-7 cell lines;	27
cis-BF (estrogenic endocrine disruption)	1S-cis	1R-cis	<i>J. medaka</i> trophoblast cells rat ovarian granulosa cells	29
cis-BF (estrogenic endocrine disruption)	1S-cis	1R-cis	rat ovarian granulosa cells	32
cis-BF (developmental toxicity)	1R-cis	1S-cis	zebrafish	36
PM (acute)	1R-cis	1S-trans	mice	20
	1R-trans	1S-cis		
	R-cis	S-cis	<i>C. dubia</i>	17
	R-trans	S-trans	<i>D. magna</i>	
PM (estrogenic endocrine disruption)	(−)-trans-PM	(+)-trans-PM	zebrafish; embryo-larval zebrafish	34
	(−)	(+)	zebrafish; zebrafish embryo	35
cis-LCT			zebrafish;	21
FV (acute)	αS-2S	αR-2R	<i>D. magna</i> ; <i>Danio rerio</i>	22
FV (chronic)	αS-2S	αR-2R	mice, rats, dogs	26
CP	1R-cis-αS	other six stereoisomers	<i>C. dubia</i>	23
	1R-trans-αS			
CF	1R-cis-αS	other six stereoisomers	<i>C. dubia</i>	18
	1R-trans-αS			
	1R-cis-αS	other six stereoisomers	<i>D. magna</i>	24
	1R-trans-αS			

(+)-trans-PM, respectively. Among these stereoisomers, (−)-trans-enantiomer was the most estrogenic active.

2.1.5. Developmental Toxicity. BF is harmful to the development and behavior of zebrafish. Jin et al.³⁶ found that the 1R-enantiomer caused more morphological damage than 1S-BF. At 96 h postfertilization under conditions of dark and light alternation, the administration of BF enantiomers at 20 µg/L posed different effects on locomotor activity. Zebrafish larvae exposed to 1R-BF were not sensitive to light to dark alteration, and the locomotor activity decreased to a level similar to that in light, which otherwise rose quickly and notably. 1S-BF did not affect the response pattern to the dark or light. These results showed that the different effects of BF enantiomers on development might cause enantioselective locomotor activity. The 1S-enantiomer inhibited the hatching process and movement, but 1R-BF showed an acceleration effect. The consistency of this enantioselective effect with insecticidal activity might suggest a common action mode.

2.1.6. Reproductive Toxicity. After maternal ablation, (+)-cis, (−)-cis, (+)-trans, and (−)-trans-PM were orally administered separately daily in 3-week-old male ICR mice for 3 weeks at doses of 0, 0.025, 0.05, and 0.1 g/kg/day, respectively. Results demonstrated that 0.1 g/kg of (+)-cis, (−)-cis, and (−)-trans-PM induced severe damage of testicular histopathology and reduction in serum testosterone (T) concentration and testis weight. Besides, PM enantiomers (especially (+)-cis-PM) also selectively influenced T synthesis and key gene transcription status. In the (+)-cis-PM group, peripheral benzodiazepine receptor (PBR) and 17 β-hydroxysteroid dehydrogenase (17β-HSD) mRNA levels decreased, whereas (+)-cis and (−)-trans-PM significantly down-regulated steroidogenic acute regulatory protein (StAR) levels. These results showed that (+)-cis-PM resulted in the greatest

disruption of endocrine activities; the effects of (−)-trans and (−)-cis-PM were moderate, and (+)-trans-PM was the weakest. The observations indicated that regarding the reproductive toxicity during pubertal exposure in mice, PM exhibits significant enantioselectivity.³⁷

The enantioselective toxicity of the chiral insecticides studied is summarized in Table 1.

2.1.7. Mode of Action of SPs. On the basis of these results, 1R-cis-BF exhibits higher aquatic toxicity (including acute and chronic) toward nontarget organisms *D. magna* and *C. dubia*, whereas 1S-cis-BF exhibits higher toxic effect in VTG induction in *J. medaka* and human cell lines (Table 1); however, not enough toxicity studies have been carried out on target organisms. A study²⁷ reported that 1S-cis-BF was 300-fold less potent than the 1R-cis-enantiomer toward *P. rapae* L. In general, the enantiomer of PM with the highest insecticidal effect is usually different from that with the highest estrogenic effect. For example, (−)-trans-PM exhibited higher estrogenic activity but lower insecticidal potency. Thus, with regard to the direction of enantioselectivity, a general rule might not exist between chronic and acute toxicities or among vertebrates, invertebrates, and mammals, regardless of whether the organisms are target or nontarget. Therefore, different experimental models need to be used to study the enantioselective toxic effects of SP stereoisomers. Various types of experiments may be helpful in probing the action modes of chiral molecules. Enantiomer-enriched pesticide products should be developed by selecting enantiomers with high potencies toward target species but exhibiting low adverse effects to nontarget organisms. Thus, “green pesticides”, which have high pesticidal efficiency and are environmentally friendly, should be developed.

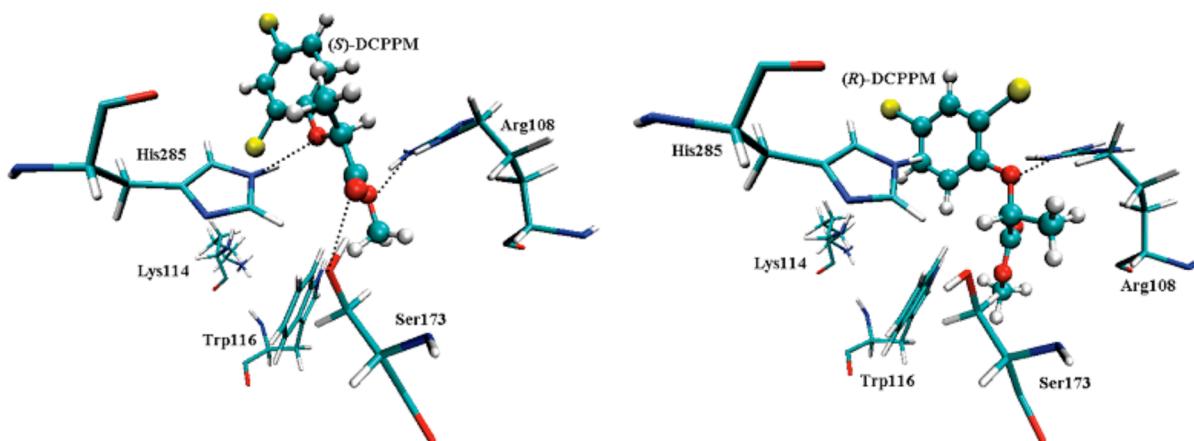


Figure 4. Binding mode of (*R*)- and (*S*)-DCPPM isomers to ANL. The dashed line indicates the hydrogen bond formed between DCPPM isomers and ANL. The DCPPM isomers are shown as sticks and the residues of ANL in licorice representation.

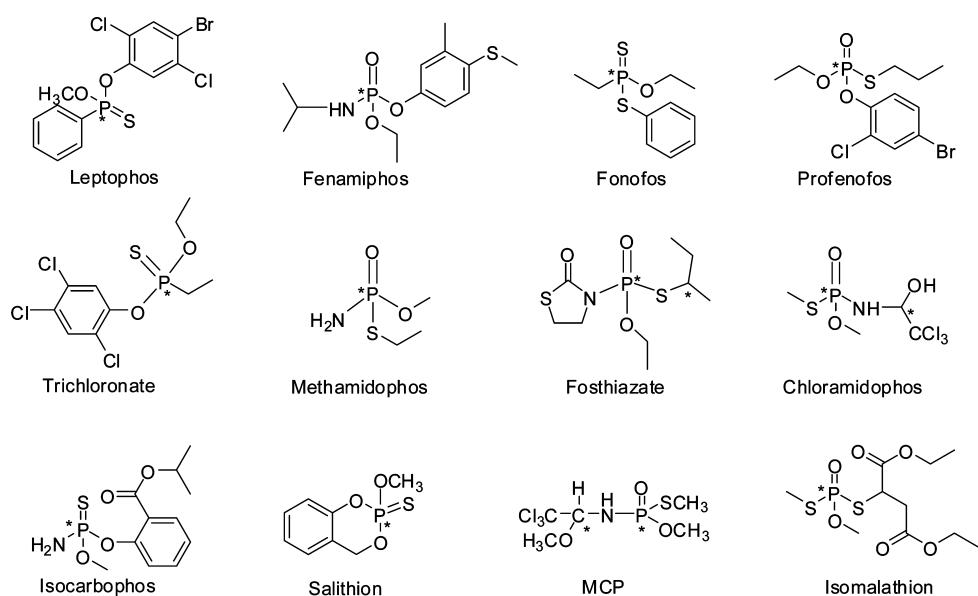


Figure 5. Chemical structures of several OPs. *Asymmetric atom.

The primary target sites of SPs are generally the voltage-gated sodium channels that are related with nerve cell membranes in insects and mammals.¹⁸ The specific bindings of SP stereoisomers to the sodium channel were studied, and stereoisomers exhibited differences in their ability to induce membrane depolarization. The nerve membrane potential response to SPs relies largely on the chirality of the SPs. *1R-cis*- and *1R-trans*-forms of phenothrin and tetramethrin were 100-fold more active on potentials than their *1S*-forms. αS -*2S*-FV was approximately 100-fold more potent than the αR -*2R*-isomer. The αS -isomers of deltamethrin was at least 300-fold more active than the αR -isomers.³⁸

To explore the interaction mechanisms of chiral pesticides with enzymes, *Aspergillus niger* lipase (ANL, EC3.1.1.3) was modified chemically by bromoacetic acid (BrAc), *N*-bromo-succinimide (NBS), methanol, and 2,3-butanedione (BD), and chiral GC was applied to investigate the enantioselectivity in the enzymatic hydrolysis of 2,4-dichloroprop-methyl (DCPPM).³⁹ The results indicated that histidine, tryptophan, and arginine were necessary for the activity of lipase and may be part of the catalytic sites of ANL. Besides, in determining the enantioselective hydrolysis of DCPPM, lysine and histidine play

important roles. Molecular modeling suggested that the enantioselectivity of DCPPM may be due to the forming of hydrogen bonds between the catalytic residues of ANL and DCPPM. Enantioselectivity may be lost due to alterations of the nature of ANL enzyme conformation and the binding pattern of DCPPM due to modification of the amino acids (Figure 4).

2.2. Organophosphates (OPs). OPs (Figure 5) are widely applied worldwide in controlling insects in agriculture. They also used for the treatment of infestations in humans, domestic animals, and buildings. In 2001, approximately 240 t of OPs was applied in a large agricultural region in California named Salinas Valley.⁴⁰ The pesticidal activity of OP is usually derived from one enantiomer, but at present, all chiral OPs are applied in agriculture as the racemates.

2.2.1. Acute Toxicity. Profenofos and fonofos each has two enantiomers. They had similar acute toxic effects to *D. magna* and *C. dubia*, and the (+)-enantiomers were less active than the (-)-enantiomers. The activities of the racemates mainly resulted from the (-)-enantiomers for both *D. magna* (87–94%) and *C. dubia* (92–94%).¹⁷

Trichloronate has a pair of enantiomers. The enantiomers ((+)) and ((-)) can be distinguished by GC-MS qualitative analysis and by detecting the optical rotation at 675 nm. The enantioselective toxic effects of trichloronate were measured using 4-d static tests with *D. magna* and *C. dubia*. The LC₅₀ value of the ((-)-form was 8–11 lower than that of the ((+)-enantiomer, and the ((-)-form contributed approximately 72% of the activity to *D. magna* and 68% of the activity to *C. dubia* in the racemate.⁴¹

In the 48 h static test to *D. magna*, S-methamidophos was 7.0-fold less toxic than R-methamidophos. The observation is consistent with that obtained by Miyazaki et al. using an *in vivo* assay,⁴² which demonstrated that ((+)-methamidophos was more toxic to nontarget houseflies.

Fenamiphos has a pair of enantiomers. The enantiomer was distinguished by GC-MS qualitative analysis and by testing the rotation (589 nm). However, absolute configurations of these two enantiomers have not yet been noted. The LC₅₀ values of the racemate, ((+)-fenamiphos, and ((-)-fenamiphos toward *Daphnia pulex* were 1.9, 1.6, and 6.1 µg/mL, respectively. No notable difference was observed between the racemate and ((+)-fenamiphos; however, ((-)-fenamiphos exhibited a lower toxic effect. The IC₅₀ values for the racemate, ((+)-fenamiphos, and ((-)-fenamiphos to cholinesterase are 0.46, 0.008, and 0.15 mg/mL, respectively.⁴³

Four fosthiazate stereoisomers were separated successfully. An optical rotation detector and a CD were used to distinguish the resolved isomers, identifying pk1 (the first) and pk3 (third) eluted peaks as one pair of enantiomers and pk2 (second) and pk4 (forth) as the other pair. The toxicity of fosthiazate stereoisomers was evaluated using *D. magna*. The toxic potencies at 1, 2, 3, and 4 d were ranked as follows: racemate < pk3 < pk2 < pk4 < pk1, pk3 ≈ racemate < pk4 < pk2 < pk1, pk3 < pk4 ≈ racemate < pk2 < pk1, and pk3 < pk4 ≈ racemate < pk2 ≈ pk1, respectively.⁴⁴

CD (UV = 230 nm) was used to identify the two enantiomers of isocarbophos; however, their absolute configurations are not known. The LC₅₀ values of the ((+)-enantiomer, ((-)-enantiomer, and the racemate of isocarbophos to *D. magna* were 7.08, 353, and 13.9 µg/L, respectively. A 50-fold difference between ((+)-isocarbophos and ((-)-isocarbophos was observed after a 48-h static test.⁴⁵

The 96-h LC₅₀ values of the racemate, R-salithion, and S-salithion to *D. magna* were 3.54, 1.10, and 0.36 µg/L, respectively, indicating that the R-enantiomer was approximately 3-fold less potent than S-salithion. The racemic salithion was less active than either enantiomer, indicating that antagonistic interactions may occur during the toxic action between the enantiomers.⁴⁶

2.2.2. Cholinesterase Inhibition. Methamidophos enantiomers behave differently in the *in vitro* inhibition of acetylcholinesterases (AChE) of bovine erythrocytes and *Electrophorus electricus*. The S-enantiomer inhibited BE-AChE (acetylcholinesterases of bovine erythrocytes) and EE-AChE (acetylcholinesterases of *E. electricus*) more strongly than R-enantiomer and the racemate. The IC₅₀ values indicated that the S-enantiomer inhibited both enzymes 8.0–12.4-fold more strongly than R-methamidophos. Enantioselective inhibition of BE-AChE and EE-AChE by methamidophos resulted mainly from the preferential binding of S-methamidophos.⁴⁷ The same research group separated four stereoisomers of fosthiazate denoted pk1, 2, 3, and 4 mentioned above. Stereoselective inhibitory effects of these four stereoisomers on AChE of *E.*

electricus have been observed. Among these four stereoisomers, pk2 inhibited EE-AChE most strongly, pk4 was the weakest inhibitor, and pk2 was about 1.4 times more toxic than pk4.⁴⁴

The two enantiomers of leptophos ((+) and ((-))) enantiomers were identified by GC-MS qualitative analysis and by detecting the specific rotation (589 nm). However, the absolute configurations of the enantiomers have not yet been identified. Toxicity tests were performed using *Daphnia pulex*. AChE from the housefly heads and butyrylcholinesterases (BChE) from horse serum and were inhibited by leptophos. On the basis of the inhibition of BChE, the IC₅₀ values of the racemate, ((+)-leptophos, and ((-)-leptophos were 1.05, 0.241, and 1.17 µg/mL, respectively; the IC₅₀ value of ((+)-leptophos was notably different from those of the other isomers. On the basis of AChE inhibition, the IC₅₀ values of the racemate, ((+)-leptophos, and ((-)-leptophos were 13.22, 14.01, and 24.32 g/mL, respectively, and the IC₅₀ value of ((-)-leptophos was notably different from those of the other isomers. On the basis of the results, leptophos, both the racemate and the enantiomers, appears to pose a higher neurotoxic effect on mammals than on the target insects. In a toxicity assay for *Daphnia*, the LC₅₀ values of the racemate, ((+)-leptophos, and ((-)-leptophos were 0.0409, 0.0387, and 0.802 g/L, respectively. Between ((+)-leptophos and ((±)-leptophos, there was no significant difference; however, the LC₅₀ of ((-)-leptophos was much higher than those of the other forms. On the basis of these results, ((+)-leptophos exhibited a higher toxic effect than the ((-)-form and the racemate toward *Daphnia*.⁴⁸

Both the racemate and the enantiomers of fenamiphos exhibited significant differences in cholinesterase inhibition; however, the ((+)-enantiomer was approximately 20-fold more active to *Daphnia* but was approximately 4-fold more inhibitory to BChE than ((-)-enantiomer.⁴³

Toxicological assays conducted by Wang et al.⁴⁹ also showed that R-((+)-fenamiphos was 3-fold more toxic to rat pheochromocytoma 12 (PC12) cells than S-((+)-fenamiphos and about 2.4 times more potent toward *D. magna*. In molecular docking studies, a dynamic simulation suggested that a key hydrogen bond and strong hydrophobic interactions can exist between R-((+)-fenamiphos and AChE, partly explaining the preferred binding of the R-((+)-enantiomer to AChE over the S-((+)-enantiomer.

Zhou et al.⁵⁰ separated four stereoisomers of chloramidophos which were denoted as pk1, 2, 3, and 4. The stereoisomers were distinguishable on their mass and CD spectra. The toxicity assay for *D. magna* (*in vivo*) and AChE inhibition (*in vitro*) of the four stereoisomers were tested, and the results showed that the inhibitory potency of the compounds to AChE decreased as follows: pk1 < pk2 < pk3 < pk4. The acute toxic effect toward *D. magna* was in the following order pk4 < pk1 < pk2 < pk3. Further, 1.1–1.8 times differences *in vitro* and 1.2–13 times differences *in vivo* among these stereoisomers were observed. Peak 4 was the least potent toward *D. magna* (*in vivo*) and was the most potent in inhibiting EE- and BE-AChE (*in vitro*). The reversal of activity was also found for methamidophos. Such a result is usually attributed to several factors and requires further investigation.

Unlike acute toxicity, the half BChE inhibition concentrations of the rac-salithion, R-enantiomer, and S-enantiomer are 33.09, 2.92, and 15.60 mg/L, respectively, indicating that the R-enantiomer was approximately 5.0-fold more potent than S-salithion.⁴⁶

Toxicity of the stereoisomers of *O,S*-dimethyl-*N*-(2,2,2-trichloro-1-methoxyethyl)phosphoramidothioate (MCP) were tested on *D. magna*, the inhibition of AChE, and the axon-like outgrowth of the SH-SY5Y cells. In SH-SY5Y cells, inhibitory effects of these stereoisomers on AChE were low and slightly stereoselective. However, a notable difference was observed in the delayed neurotoxicity of the stereoisomers. The 2-d acute toxicities of the stereoisomers toward *D. magna* was in the following order $\text{pk3} < \text{pk2} < \text{pair 2}$ (equimolar mixture of pk 2 and 4) $<$ racemate $\approx \text{pk 4} < \text{pair 1}$ (equimolar mixture of pk 1 and 3) $\approx \text{pk 1}$, and 1.0 to 6.3 times differences were found among the stereoisomers. Inhibition potency of MCP stereoisomers in SH-SY5Y cells to axon growth declined as $\text{pk 2} > \text{pair 2} > \text{pk 4} > \text{racemate} > \text{pk 3} > \text{pair 1} \approx \text{pk 1}$, and a 60 times difference between the weakest and strongest enantiomers was observed.⁵¹ The findings indicated that pk 1 exhibits the highest target selectivity and the best ecological profile. Approximately 2/3 of the usual usage of MCP could be reduced with a decrease in neuropathic risk if MCP were used only in the form of pk 1 instead of the racemate. Because feasible economical synthetic methods to manufacture enantiomer-enriched OPs are absent, based on the biological predominance and cost effectiveness, pair 1 exhibits potential for application in the future.

2.2.3. α -Naphthyl Acetate Esterase Inhibition. The inhibition kinetics and reversal of the spontaneous reactivation of α -naphthyl acetate esterase by enantiomers of malaoxon, isomalathion, and methamidophos were investigated.⁵² According to the bimolecular rate constants, the inhibition potency order was (*S*) $<$ (*R*) for malaoxon, (*1S,3S*) $<$ (*1S,3R*) $<$ (*1R,3S*) $<$ (*1R,3R*) for isomalathion, and (*R*) $<$ (*S*) for methamidophos; this order of potency is consistent with that found for the enantioselective inhibition of AChE by malaoxon, isomalathion, and methamidophos. These observations indicate that in response to chiral OPs, α -naphthyl acetate esterase and AChE exhibit similar selective inhibition kinetics and post-inhibitory reactions.

2.2.4. Cytotoxicity. To determine the cytotoxicity of the isocarbophos (ICP) enantiomers, Liu et al.⁵³ used Hep G2 cells as a model (*in vitro*). The results from cytoflow assays and cell viability suggested a clear enantioselectivity in hepatocyte toxicity for ICP: (–)-ICP was approximately twice as potent as (+)-ICP, and (–)-ICP up-regulated the expression of Bax protein and down-regulated the expression of Bcl-2 levels, increasing the Bax/Bcl-2 ratio in coordination with apoptosis. This indicates that (–)-ICP-induced hepatocyte toxicity was caused mainly by sustained activation of the JNK pathway and only partially through the ERK cascade. Further, (–)-ICP caused ROS production, whereas (+)-ICP did not affect ROS generation. The results provided further insight into the toxicity pathways of the enantiomers; thus, these pathways can be used in distinguishing activities among stereoisomers at the molecular level.

2.3. Other Chiral Insecticides. **2.3.1. Acetofenate (AF).** Acetofenate enantiomers (Figure 6) exhibited no differences in

acute toxicity, but using zebrafish embryo larva assays, enantioselectivity was detected in their developmental toxicities, like pericardial edema and yolk sac edema.⁵⁴ The ER α expression in zebrafish embryos using qRT-PCR demonstrated that there was an approximately 3.2 times difference in ER α mRNA induction between fish treated with *R*-AF or *S*-AF.

Enantioselective cytotoxicity of AF on the mouse macrophage cell line RAW264.7 was observed,²⁵ suggesting that the immunotoxicity is enantioselective. The immune system consists of several action modes which protect against disease via identifying and killing tumor cells and pathogens. Balance between the modes could be disrupted chemically, possibly enhancing the immune response and causing allergy or autoimmunity, or, in the case of immunosuppression, increasing cancer susceptibility and infection risk. The immune response can be divided into two categories: adaptive and innate immunities. The major components of the innate immunity are macrophages. Macrophages functions can be used as a biomarker for immunotoxic compounds. By testing the impacts of environmental compounds on cell growth and apoptosis of target cells, the cytotoxicity assay can be used as an *in vitro* assay to assay the toxic effects of compounds. ROS is regarded as a second messenger in multiple signaling pathways that cause apoptosis. ROS levels are also important biomarkers to assess genotoxicity and cytotoxicity. The p53 gene is a vital regulator of apoptosis, and p53 mRNA expressions levels reflect cell apoptosis. *S*-AF poses more potent toxicity than *R*-AF or *rac*-AF to macrophages. *S*-AF caused the highest values of intracellular ROS induction, damage in DNA, and the up-regulation expression of p53 gene.

2.3.2. *o,p'*-DDT (DDD). In several developing countries, the continued use of dichlordiphenyltrichloroethane (DDT) in controlling indoor vectors has raised debates recently on the ban of the persistent legacy compound. Studies have indicated that exposure to DDT is a contributor to breast cancer.⁵⁵

The enantiomers of *o,p'*-DDT were baseline separated using HPLC on a Chiracel OJ chiral column, and the absolute configuration was identified. Notable differences were found between the two enantiomers in estrogenic potential in the MCF-7 cell proliferation test and qRT-PCR. The enantioselective estrogenicity of *o,p'*-DDT can possibly occur via the estrogen receptor α (ER α) and estrogen receptor β (ER β) signaling pathways.⁵⁶

Zhao et al.⁵⁷ studied the enantioselective cytotoxicity of *o,p'*-DDT and found that the *R*-(–)-enantiomer caused more neuronal cell death than the *S*-(+)-enantiomer. The *R*-(–)-enantiomer also caused greater cellular apoptosis than the *S*-(+)-enantiomer at levels comparable to exposure levels in areas in which malaria is endemic (parts per million). Enantioselective apoptosis may involve 3 signaling pathways, involving caspase 3, NF κ B, and tumor p53. On the basis of the stereochemistry of DDT and results obtained from other chiral pesticides, it is indicated that the directional enantioselectivity of DDT to mammalian cells was the same as that described above.

By testing the activation of cellular apoptosis and oxidative stress systems and microarray analysis, the cytotoxic effects of *o,p'*-DDD on rat cells (PC12) were assayed,⁵⁸ and the *R*-(+)-enantiomer increased apoptosis. The *R*-enantiomer is more detrimental for both *o,p'*-DDT and *o,p'*-DDD. The stereostructural effects are consistent with the structure-activity relationships formulated at other structural levels.

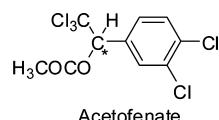


Figure 6. Chemical structures of acetofenate. *Asymmetric atom.

3. ENANTIOSELECTIVE TOXICITY OF CHIRAL HERBICIDES ON NONTARGET ORGANISMS

3.1. Acylanilides Herbicides. Metolachlor and metalaxyl are representatives of acylanilides (Figure 7), which are an

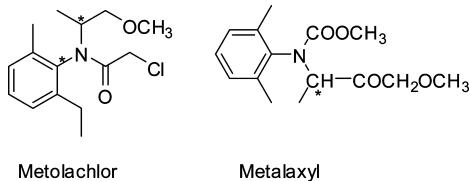


Figure 7. Chemical structures of metolachlor and metalaxyl. *Asymmetric atom. Metolachlor contains two chiral elements: an asymmetrically substituted carbon and a chiral axis.

important class of herbicides. Metolachlor is used in controlling a wide range of broad-leaf weeds on corn and other crops. The herbicidal activity of metolachlor is caused mainly by 1'S-isomers (αSS and αRS), which are now used widely because they exhibit more herbicidal activity than their racemate.¹⁴ Metolachlor was enriched by its manufacturer (Syngenta) to contain 86% of the active S-enantiomers. The enrichment enables the same herbicidal effect to be achieved while using 40% less herbicide. Metalaxyl is applied in controlling plant diseases resulting from pathogens of the *Oomycota* division.

3.1.1. Acute Toxicity. The 24-h LC₅₀ values of S-metolachlor and the racemate toward *D. magna* were 51.2 and 69.4 mg/L, respectively, suggesting that the S-enantiomer was a little more toxic than the racemate.⁵⁹ The 48-h LC₅₀ values for R-metalaxyl and the racemate toward *D. magna* were 41.9 and 51.5 mg/L, respectively.⁶⁰

The effects of S-metolachlor and the racemate on the enzyme activities of fifth-instar silkworm larvae were studied. When treated with *rac*-metolachlor, the catalase activity levels in silkworms was much lower than those treated with the S-enantiomer. The midgut alkaline phosphatase activity of silkworms exposed to S-enantiomer was small, and the effect of the racemate was 46% lower than that of the control group. This evidence suggests that the racemate poses a more potent effect than S-metolachlor toward economically important silkworms.⁶¹

3.1.2. Chronic Toxicity. For *rac*-metolachlor, the LOEC and NOEC toward *D. magna* were 0.01 and 0.001 mg/L, respectively, whereas those of S-metolachlor were 0.5 and 0.1 mg/L, respectively. The racemate and S-metolachlor did not affect the brood day of *D. magna*. Longevity and the number of broods per female were affected significantly by 1.0 mg/L *rac*-metolachlor but were not significantly affected by S-metolachlor at concentrations of less than 10 mg/L. The racemate and S-metolachlor affected brood length at the same concentration. When the racemate concentration was above 0.01 mg/L, the number of broods per female was reduced significantly but was not significantly reduced by S-metolachlor at concentrations of less than 0.5 mg/L. These observations suggested that the racemate was significantly more potent toward *D. magna* than S-metolachlor.⁵⁹

The LOEC and NOEC values of racemic metalaxyl toward *D. magna* in a 14-day chronic test were 2 and 1 mg/L, respectively, whereas those of R-metalaxyl were 1 and 0.1 mg/L, respectively. Number of broods per female, days-to-first-brood, and body length were affected significantly ($p < 0.05$) by

R-metalaxyl at over 1.0 mg/L and were affected by *rac*-metalaxyl at ≥ 2.0 mg/L.⁶⁰

3.1.3. Phytotoxicity. Racemic metolachlor and the S-enantiomer have different toxic effects on *Chlorella pyrenoidosa*. The 1-, 2-, 3-, and 4-d EC₅₀ values of *rac*-metolachlor were 196, 241, 177, and 152 μ g/L, respectively; the values were greater than those for S-metolachlor, which were 116, 106, 81, and 68 μ g/L, respectively. This result suggests that the S-metolachlor is more toxic than the *rac*-metolachlor to *C. pyrenoidosa*. The chlorophyll a and b concentrations in *C. pyrenoidosa* treated by the racemate were higher than those in algae treated by the S-enantiomer. In general, the CAT activity of *C. pyrenoidosa* exposed to S-metolachlor was higher than that of algae exposed to the *rac*-metolachlor. The CAT activity decreased in both herbicide treated condition at high concentrations. Transmission electron microscopy was used to observe the ultrastructure of the cells in the presence of the two herbicides. Lipid droplets accumulated, indicating the abnormal metabolism or synthesis of lipids in algae. The cell wall separated from the membrane, suggesting that the binding of the herbicide to the enzyme caused the synthesis of very long chain fatty acids, thus disrupting the fatty acid composition of the cell plasma membranes, resulting in the loss of cell rigidity and permeability. Starch granules accumulated in the chloroplast.⁶²

3.2. Imazethapyr (IM). Imazethapyr (IM) (Figure 8) poses enantioselective toxicity toward the roots of maize seedlings

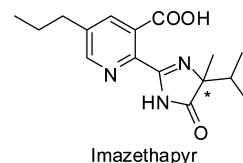


Figure 8. Chemical structures of imazethapyr. *Asymmetric atom.

(*Zea mays* L.). The R-enantiomer was more potent than S-IM at disturbing maize growth, as indicated by more obvious chlorosis, the lower dry weight, and shorter shoot and root length at equal concentrations. During the treatment, compared to the shoot, the root was more sensitive to IM. Ultrastructural characteristics showed that R-IM caused the most pronounced damage.⁶³ Zhou et al.⁶⁴ used computational molecular docking studies (Figure 9) to investigate the molecular interactions between IM enantiomers and the abundance of acetolactate synthetase (ALS). The R-(–)-enantiomer was more potent

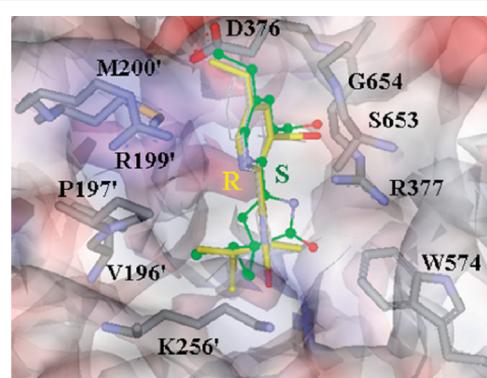


Figure 9. Binding modes of R-(–)- (carbon atoms shown in yellow) and S-(+)-IM (carbon atoms displayed in green) with ALS.

than the *S*-(+)-enantiomer in suppressing the activity of ALS in maize leaves *in vitro* and *in vivo*.

The IM enantiomers caused significantly different effects ($p < 0.05$, ANOVA) on antioxidant enzymes, morphology, gene transcription, and oxidant markers in rice xiushui 63 seedlings. The maximal root growth inhibition rates were 73.5%, 80.4%, and 67.0% for the racemate, R-IM, and S-IM and at 0.5 mg/L, respectively, and the maximal inhibition rates of shoot growth were 61.7%, 77.7%, and 26.9%, respectively. When the plants were treated with 0.5 mg/L R-enantiomer, the activities of SOD, POD, and CAT and the content of MDA increased; these values are 1.8, 3.3, 1.4, and 2.2 times those measured following S-IM treatment, respectively. These results imply that R-IM poses a more toxic effect toward rice growth than S-IM.⁶⁵

The same group⁶⁶ investigated the phytotoxicity of the IM enantiomers toward rice, and the results indicated that R-IM inhibited ALS activity more potently than S-IM, which reduced the synthesis of branched-chain amino acids (BCAAs). Enantioselective effects on the synthesis of other amino acids were also observed. At the cellular level, R-IM was more toxic than the S-enantiomer toward protoplasts. R-IM regulated more genes more strongly than did S-IM. The results suggest that the R-enantiomer is more toxic than the S-enantiomer. This toxicity results not only from changes in targeted enzyme activity and amino acid synthesis but also from the effect of gene transcription on other metabolic pathways, either directly or indirectly, and this toxicity is enantioselective.

A study on enantioselective phytotoxicity of IM as measured using the antioxidant system response and starch metabolism in *Arabidopsis thaliana* indicates that the R-enantiomer inhibited plant growth more potently than S-IM, by enantioselectively acting on ALS and by causing an imbalance in the antioxidant system and disturbing the carbohydrate metabolism. The R-enantiomer induced ROS generation powerfully, yet reduced enzyme activity and transcription of the antioxidant gene drastically, which resulted in oxidative stress. Treatment with R-IM resulted in the accumulation of sucrose, maltose, and glucose in the cytoplasm and chloroplasts and destroyed the utilization of carbohydrates.⁶⁷

Qian et al.⁶⁸ further reported that IM enantioselectively promotes flowering in *Arabidopsis thaliana*. These authors described a possible mechanism by which IM promotes flowering and found that the photoperiod pathway might play an important role in propagating the IM stress signal. IM enantiomers decreased the amplitude of the core oscillators (CIRCADIAN CLOCK ASSOCIATED 1 and LATE ELONGATED HYPOCOTYL) and utilized the up-regulation of the GIGANTEA-(CONSTANS)-FLOWERING LOCUS T pathway to induce floral gene APETALA1 overexpression enantioselectively; this treatment ultimately caused early flowering. These findings provide new insight into the method by which plants control reproductive timing in response to herbicide stress. Flowering time in crops is an important trait and affects the life cycles of pollinator species. The persistence of herbicides in the biosphere alters plant life cycles and diversity.

3.3. Phenoxypropanoic Acids. Phenoxypropanoic acids introduced in the 1940s and 1950s are postemergence herbicides. In agriculture, industrial establishments, pastures, and lawns, these herbicides are applied to control broadleaf weeds. Diclofop and dichlorprop (DCPP) are representatives of this class (Figure 10).

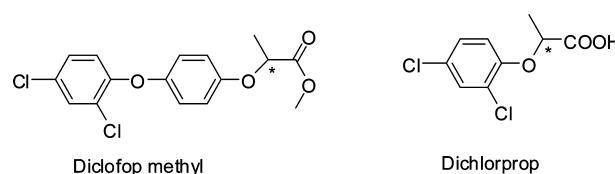


Figure 10. Chemical structures of diclofop methyl and dichlorprop
*Asymmetric atom.

3.3.1. Diclofop. Herbicidally inactive S-diclofop-methyl and S-diclofop were similar to or more toxic than the R-enantiomers toward algae.⁶⁹ The R-enantiomers significantly inhibit the growth of oats. The inverse toxic effects of diclofop enantiomers indicated that the interaction modes may be different among biological systems. Thus, the less herbicidally active enantiomers will significantly contribute to the environmental toxicity. Under the same conditions, the two enantiomers exerted different effects on cell permeability. Both R-diclofop and *rac*-diclofop decreased cell permeability of the algae, and R-diclofop exhibited more potent inhibition than the racemate. For both *C. pyrenoidosa* and *C. vulgaris*, low levels of S-diclofop at 1.0–5.0 mg/L enhanced the permeability of the cell. This compound decreased algal cell permeability at concentrations above 10 or 20 mg/L, but the decrease in permeability caused by the S-enantiomer was lower than those caused by the R-enantiomer and the racemate. Algal cell permeability increased with contact time when treated by diclofop, and the increase in permeability caused by S-diclofop was more rapid than that caused by R-diclofop.

Diclofop poses enantioselective phytotoxicities toward seedlings of rice xiushui 63. The toxicities (72-h EC₅₀ values) of *R*-, *S*-, and *rac*-diclofop acid to seedlings and the Hill reaction activities of chloroplasts indicated great differences between the two enantiomers. The *S*-enantiomer inhibited the Hill reaction activity significantly, and significant differences were observed between the two enantiomers at 1 mg/L. At 0.5 and 1 mg/L, the Hill reaction activities were significantly different between the two enantiomers. ($p < 0.01$).⁷⁰

Diclofop methyl and acid pose enantioselective physiological effects on *Microcystis aeruginosa*.⁷¹ Growth curves, protein content, and ultrastructural changes in thylakoids, glycogen, gas vacuoles, cyanophycin granules, polyhedral bodies, polybetahydroxybutyrate, and lipids suggested that different toxicity patterns exist among diclofop methyl, racemic-, R-, and S-diclofop acid. R-Diclofop acid most likely acts as a proton ionophore which shuttles protons across the plasmalemma, while the S-enantiomer did not demonstrate such an action. Toxicity of the molecules was ranked as follows: S-enantiomer < R-enantiomer < diclofop methyl < racemate.

Diclofop acid also causes enantioselective oxidative stress in *Microcystis aeruginosa*.⁷² R-, S-, and racemic diclofop acid induced the generation of ROS, increased the MDA concentration, enhanced the SOD activity and induced toxin release in *M. aeruginosa* to different degrees. The increase in SOD activity and MDA concentration happened soon after treatment with racemic diclofop acid than when the cyanobacterium was treated with either the R- or the S-enantiomer. Besides, an enantioselective toxic effect was observed. The S-enantiomer triggered lower SOD activity, ROS generation, and toxin synthesis and release in *M. aeruginosa* than R-diclofop acid. Diclofop acid and the R-enantiomer might destroy the cell membrane and collapse the transmembrane proton gradient via lipid peroxidation and free

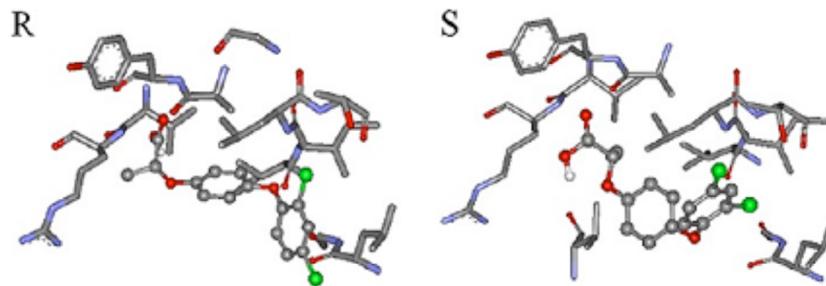


Figure 11. Schematic drawing of the interactions between diclofop acid and the carboxyltransferase domain of ACCase from yeast.

radical oxidation, but S-diclofop acid did not show such an action. The findings suggest that detailed research is required to evaluate the environmental safety of diclofop acid and that workers who apply chiral herbicides need more direct supervision. Besides, the lifecycle analysis of chiral pesticides should be studied in greater detail to aid in the environmental assessment of chiral pesticides.

The enantioselective phytotoxicity of diclofop acid, which is mediated by oxidative stress and the key enzyme ACCase in the fatty acid synthesis system, was investigated by Zhang et al. in the model plant *Arabidopsis thaliana*.⁷³ The two enantiomers exhibited significant differences in phytotoxicity, based on growth inhibition, oxidative damage, and alterations in the expression of key ACCase genes; the R-enantiomer exhibited higher toxicity to *Arabidopsis thaliana* than the S-enantiomer. Molecular docking studies suggested that the R-enantiomer exhibited greater affinity for ACCase, possibly causing the enantioselectivity of phytotoxicity of diclofop (Figure 11).

3.3.2. DCPP. The enantioselective interaction between *Penicillium expansum* alkaline lipase and dichlorprop (DCPP) was studied. The results showed that R-DCPP interacted more strongly with lipase, followed by rac-dichlorprop; S-dichlorprop interacted most weakly with lipase. Hydrophobic interactions appear to play an important role in the interaction. In the endothermic reaction, R-DCPP required less energy, and rac-DCPP and S-DCPP required higher energy to drive the reaction. In addition, the catalytic hydrolysis of fluorescein diacetate (FDA) with lipase and the binding constants between DCPP and lipase suggested that R-DCPP was the most effective form at inhibiting lipase, perhaps because R-DCPP bound more strongly and exhibited a higher enantioselectivity for lipase than rac- or S-DCPP.⁷⁴

The same group⁷⁵ studied the changes in bioavailability of DCPP to *Chlorella pyrenoidosa* caused by the presence of chitosan. The degradation of the R-DCPP in *Chlorella pyrenoidosa* culture solution without chitosan was slower than that of the S-DCPP, whereas R-DCPP dissipated more rapidly than S-DCPP when chitosan was added. Toxicity of S-DCPP toward *Chlorella pyrenoidosa* was less than that of R-DCPP in the absence of chitosan. Conversely, S-DCPP posed a more potent effect than R-DCPP in the presence of chitosan. The findings indicate that chitosan reversed the enantioselective bioavailability of DCPP. Fluorescence spectroscopic analysis suggested that the interaction between chitosan and the DCPP enantiomers relied largely on the steric structure of DCPP, explaining the reason for the addition of chitosan altered the enantioselective degradation of DCPP in *Chlorella pyrenoidosa*. The study indicates that the enantioselective behaviors of chiral chemicals may be altered when interactions with other chiral receptors coexist.

Wen et al.⁷⁶ further studied the impacts of copper (Cu) on the enantioselective ecotoxicity of DCPP toward *Scenedesmus obliquus* and found that the presence of Cu and DCPP, both in combination and individually, induced the generation of ROS. This thereafter enhanced the response of antioxidant defenses, impaired subcellular structure and physiological function, and finally resulted in inhibition of cell growth. Without copper, ROS production after treatment with the S-DCPP was lower than that after exposure to R-DCPP. When Cu and DCPP were simultaneously added to algae, ROS production was preferentially induced by R-DCPP. However, the enantioselectivity in inducing ROS production was reversed when DCPP was mixed with copper for 24 h before addition into algae. The generation of ROS, the growth inhibition rate, and the antioxidant response in alga were all preferentially induced by the R-enantiomer. The results indicated that ROS plays an important role in chemical contaminant toxicity.

A chiral perturbation strategy involving DCPP as a perturbation factor was applied by Chen et al.⁷⁷ Differences in GSH content of algal cells treated with the R-enantiomer or S-enantiomer were correlated with the different ROS production. When R-DCPP or S-DCPP was added to the algal solution along with Cu(II), exposure to R-DCPP-Cu caused a decline in GSH content in algal cells, whereas exposure to S-DCPP-Cu caused an increase in GSH content. The GR activity and GSH/GSSG ratio exhibited similar enantioselectivities. The results provide indirect evidence that the ROS-induced cell toxicity of Cu causes the glutathione redox cycle response. The results also imply that it would be better if the role of ROS production and the glutathione redox cycle were explained before sustainable detoxification strategies for heavy metal pollutants are proposed. Therefore, a chiral perturbation strategy might be a good method.

3.4. Hexachlorocyclohexane (HCH). HCHs increased the activities of CAT and POD but decreased the activity of SOD. The photosynthetic efficiency of PSI and PSII was all down-regulated by HCH in the photosynthesis system. The results obtained with both systems indicated that δ-HCH was the most toxic form in *Arabidopsis thaliana* and that α-HCH was the least toxic form.⁷⁸

4. FUTURE OPPORTUNITIES

The enantioselective environmental toxicology of chiral pesticides is a common issue that cannot be neglected. Demands for the enriched- or single-enantiomer insecticides and herbicides will probably increase because the agrochemical industries develop more complex pesticides that contain a greater number of chiral centers. The development of further synthetic methods for enriched- or single-enantiomer compounds is increasingly conscious of green chemistry, which can

reduce the environmental loading of the inactive enantiomer (the enantiomer with less or no effect on the target organisms, which may be active to nontarget organisms).

Evaluating effects in toxicity studies requires the separation of enantiomers, which requires more time and equipment. It is also necessary to explore low-cost routes to separate the enantiomers of pesticides, which might demand the development of more effective biological or chemical catalysts for synthesizing enantio-pure isomers.

Most toxicity effect end points, such as lethality (LC_{50}) and death (LD_{50}), reflect acute toxicity. Other end points include the rates of metabolism by enzymatic oxidation and hydrolysis, the cholinesterase inhibition, and relative enantiomer receptor binding. These tests provide usable information regarding the toxicity mechanisms and the selective effects of enantiomers. However, the development of modern and sophisticated microarray analysis, proteomics, and metabolomics is needed. Such tools can be used to investigate activities of enantiomers at the molecular level and to provide additional insight into enantiomer toxicity pathways.

The ultimate goal of this research should be the ability to predict enantioselectivity, such that science can guide producers to provide more enriched- or single-enantiomer insecticides and herbicides. These pesticides can reduce lots of unnecessary pesticides that can pose adverse effects on nontarget organisms.

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ABBREVIATIONS

POPs, persistent organic pollutants; SPs, pyrethroids; Ops, organophosphates; BF, bifenthrin; PM, permethrin; LCT, lambda cyhalothrin; FV, fenvalerate; CP, cypermethrin; CF, cyfluthrin; LOEC, lowest observed effective concentration; NOEC, no-observed-effect concentration; ROS, reactive oxygen species; MAPK, mitogen-activated protein kinase; ERKs, extracellular regulated kinases; JNKs, Jun-N-terminal kinases; ER, estrogen receptor; VTG, vitellogenin; EDCs, estrogenic endocrine disruptive chemicals; CD, circular dichroism; AChE, acetylcholinesterases; BChE, butyrylcholinesterases; MCP, O,S -dimethyl- N -(2,2,2-trichloro-1-methoxyethyl)-phosphoramidothioate; ICP, isocarbophos; AF, acetofenone; DDT, dichlordiphenyltrichloroethane; ALS, acetolactate synthetase; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; MDA, malondialdehyde; IM, imazethapyr

REFERENCES

- (1) Crosby, D. G. (1998) *Environmental Toxicology and Chemistry*, Oxford University, New York.
- (2) Williams, A. (1995) Opportunities for Chiral Agrochemicals, in *Symposium on Chirality in Crop Protection Chemistry*, pp 3–9, John Wiley & Sons Ltd., London, England.
- (3) Liu, W. P., and Tang, M. L. (2011) Enantioselective Activity and Toxicity of Chiral Herbicides, *Herbicides-Mechanisms and Mode of Action*, pp 63–80, InTech, Rijeka, Croatia.
- (4) Ye, J., Zhao, M. R., Liu, J., and Liu, W. P. (2010) Enantioselectivity in environmental risk assessment of modern chiral pesticides. *Environ. Pollut.* 158, 2371–2383.
- (5) Ma, Y., Gan, J., and Liu, W. P. (2011) Chiral Pesticides and Environmental Safety, in *Chiral Pesticides: Stereoselectivity and Its Consequences*, pp 97–106, ACS Symposium Series, American Chemical Society, Washington, DC.
- (6) Liu, H. J., Cai, W. D., and Liu, W. P. (2011) Enantioselective Toxicity of Chiral Pesticides in Aquatic Systems, in *Chiral Pesticides: Stereoselectivity and Its Consequences*, pp 107–120, ACS Symposium Series, American Chemical Society, Washington, DC.
- (7) Wang, C., Zhang, Q., Zhao, M. R., and Liu, W. P. (2011) Enantioselectivity in Estrogenic Potential of Chiral Pesticides, in *Chiral Pesticides: Stereoselectivity and Its Consequences*, pp 121–134, ACS Symposium Series, American Chemical Society, Washington, DC.
- (8) Zhou, Q. Y., and Liu, W. P. (2011) Phytotoxicity and Environmental Fate of Chiral Herbicides, in *Chiral Pesticides: Stereoselectivity and Its Consequences*, pp 135–150, ACS Symposium Series, American Chemical Society, Washington, DC.
- (9) Zhao, M. R., and Liu, W. P. (2011) Enantioselective Cytotoxicity and Molecular Mechanisms of Modern Chiral Pesticides, in *Chiral Pesticides: Stereoselectivity and Its Consequences*, pp 153–165, ACS Symposium Series, American Chemical Society, Washington, DC.
- (10) Xu, C., and Liu, W. P. (2011) Evaluating in Vivo Toxicity of Chiral Pesticides Using the Zebrafish (*Danio rerio*) Embryo Model, in *Chiral Pesticides: Stereoselectivity and Its Consequences*, pp 167–179, ACS Symposium Series, American Chemical Society, Washington, DC.
- (11) Garrison, A. W. (2006) Probing the enantioselectivity of chiral pesticides. *Environ. Sci. Technol.* 40, 16–23.
- (12) Lin, K. D., Xu, C., Zhou, S. S., Liu, W. P., and Gan, J. Y. (2007) Enantiomeric separation of imidazolinone herbicides using chiral high-performance liquid chromatography. *Chirality* 19, 171–178.
- (13) Zhou, Y., Li, L., Lin, K. D., Zhu, X. P., and Liu, W. P. (2009) Enantiomer separation of triazole fungicides by high-performance liquid chromatography. *Chirality* 21, 421–427.
- (14) Ye, J., Wu, J., and Liu, W. P. (2009) Enantioselective separation and analysis of chiral pesticides by high-performance liquid chromatography. *Trends Anal. Chem.* 28, 1148–1163.
- (15) Liu, W. P., Qin, S. J., and Gan, J. Y. (2005) Chiral stability of synthetic pyrethroid insecticides. *J. Agric. Food Chem.* 53, 3814–3820.
- (16) Chen, Z. M., and Wang, Y. H. (1996) Chromatographic methods for the determination of pyrethrin and pyrethroid pesticide residues in crops, foods and environmental samples. *J. Chromatogr. A* 754, 367–395.
- (17) Liu, W. P., Gan, J. Y., Schlenk, D., and Jury, W. A. (2005) Enantioselectivity in environmental safety of current chiral insecticides. *Proc. Natl. Acad. Sci. U.S.A.* 102, 701–706.
- (18) Liu, W. P., Gan, J. J., and Qin, S. (2005) Separation and aquatic toxicity of enantiomers of synthetic pyrethroid insecticides. *Chirality* 17, S127–S133.
- (19) Liu, W. P., Gan, J. Y., Lee, S., and Werner, I. (2005) Isomer selectivity in aquatic toxicity and biodegradation of bifenthrin and permethrin. *Environ. Toxicol. Chem.* 24, 1861–1866.
- (20) Miyamoto, J. (1976) Degradation, metabolism and toxicity of synthetic pyrethroids. *Environ. Health Perspect.* 14, 15–28.
- (21) Xu, C., Wang, J. J., Liu, W. P., Sheng, G. D., Tu, Y. J., and Ma, Y. (2008) Separation and aquatic toxicity of enantiomers of the pyrethroid insecticide lambda-cyhalothrin. *Environ. Toxicol. Chem.* 27, 174–181.
- (22) Ma, Y., Chen, L. H., Lu, X. T., Chu, H. D., Xu, C., and Liu, W. P. (2009) Enantioselectivity in aquatic toxicity of synthetic pyrethroid insecticide fenvalerate. *Ecotoxicol. Environ. Saf.* 72, 1913–1918.
- (23) Liu, W. P., Gan, J. J., Lee, S. J., and Werner, I. (2004) Isomer selectivity in aquatic toxicity and biodegradation of cypermethrin. *J. Agric. Food Chem.* 52, 6233–6238.
- (24) Leicht, W., Fuchs, R., and Londershausen, M. (1996) Stability and biological activity of cyfluthrin isomers. *Pestic. Sci.* 48, 325–332.

- (25) Zhao, M. R., and Liu, W. P. (2009) Enantioselectivity in the immunotoxicity of the insecticide acetofenate in an in vitro model. *Environ. Toxicol. Chem.* 28, 578–585.
- (26) Miyamoto, J., Kaneko, H., and Okuno, Y. (1986) A Novel Lipophilic Cholesterol Ester Conjugate from Fenvalerate, *Xenobiotic Conjugation Chemistry*, pp 268–281, American Chemical Society, Washington, DC.
- (27) Liu, H. G., Zhao, M. R., Zhang, C., Ma, Y., and Liu, W. P. (2008) Enantioselective cytotoxicity of the insecticide bifenthrin on a human amniotic epithelial (FL) cell line. *Toxicology* 253, 89–96.
- (28) Liu, H. G., Xu, L. H., Zhao, M. R., Liu, W. P., Zhang, C., and Zhou, S. S. (2009) Enantiomer-specific, bifenthrin-induced apoptosis mediated by MAPK signalling pathway in Hep G2 Cells. *Toxicology* 261, 119–125.
- (29) Wang, L. M., Liu, W., Yang, C. X., Pan, Z. Y., Gan, J. Y., Xu, C., Zhao, M. R., and Schlenk, D. (2007) Enantioselectivity in estrogenic potential and uptake of bifenthrin. *Environ. Sci. Technol.* 41, 6124–6128.
- (30) Zhao, M. R., C. F., Xu, C., Wang, C., Liu, W. P., and Gan, J. Y. (2010) Integrative assessment of enantioselectivity in endocrine disruption and immunotoxicity of synthetic pyrethroids. *Environ. Pollut.* 158, 1968–1973.
- (31) Wang, C., Zhang, Q., Zhang, X. F., Liu, J., and Liu, W. P. (2010) Understanding the endocrine disruption of chiral pesticides: The enantioselectivity in estrogenic activity of synthetic pyrethroids. *Sci. China, Ser. B: Chem.* 53, 1003–1009.
- (32) Liu, J., Yang, Y., Zhuang, S. L., Yang, Y., Li, F. X., and Liu, W. P. (2011) Enantioselective endocrine-disrupting effects of bifenthrin on hormone synthesis in rat ovarian cells. *Toxicology* 209, 42–49.
- (33) Zhao, M. R., Zhang, Y., Zhuang, S. L., Zhang, Q., Lu, C. S., and Liu, W. P. (2014) Disruption of the hormonal network via estrogen receptors and the enantioselectivity of bifenthrin: Maternal-fetal health risk of chiral pesticides. *Environ. Sci. Technol.* 48, 8109–8119.
- (34) Jin, Y. X., Wang, W. Y., Xu, C., Fu, Z. W., and Liu, W. P. (2008) Induction of hepatic estrogen-responsive gene transcription by permethrin enantiomers in male adult zebrafish. *Aquat. Toxicol.* 88, 146–152.
- (35) Jin, Y. X., Chen, R. J., Sun, L. W., Wang, W. Y., Zhou, L., Liu, W. P., and Fu, Z. W. (2009) Enantioselective induction of estrogen-responsive gene expression by permethrin enantiomers in embryo-larval zebrafish. *Chemosphere* 74, 1238–1244.
- (36) Jin, M. Q., Zhang, Y., Ye, J., Huang, C. J., Zhao, M. R., and Liu, W. P. (2010) Dual enantioselective effect of insecticide bifenthrin on locomotor behavior and development in embryo-larval zebrafish. *Environ. Toxicol. Chem.* 29, 1561–1567.
- (37) Jin, Y. X., Liu, J. W., Wang, L. G., Chen, R. J., Zhou, C., Yang, Y. F., Liu, W. P., and Fu, Z. W. (2011) Permethrin exposure during puberty has the potential to enantioselectively induce reproductive toxicity in mice. *Environ. Int.* 42, 144–151.
- (38) Kurihara, N., and Miyamoto, J. (1998) *Chirality in Agrochemicals*, John Wiley & Sons, Chichester, England.
- (39) Wen, Y. Z., Li, C. D., Fang, Z. H., Zhuang, S. L., and Liu, W. P. (2011) Elucidation of the enantioselective enzymatic hydrolysis of chiral herbicide dichlorprop methyl by chemical modification. *J. Agric. Food Chem.* 59, 1924–1930.
- (40) McKone, T. E., Castorina, R., Harnly, M. E., Kuwabara, Y., Eskenazi, B., and Bradman, A. (2007) Merging models and biomonitoring data to characterize sources and pathways of human exposure to organophosphorous pesticides in the Salinas Valley of California. *Environ. Sci. Technol.* 41, 3233–3240.
- (41) Liu, W. P., Lin, K. D., and Gan, J. Y. (2006) Separation and aquatic toxicity of enantiomers of the organophosphorus insecticide trichloronate. *Chirality* 18, 713–716.
- (42) Miyazaki, A., Nakamura, T., Kawaradani, M., and Marumo, S. (2002) Resolution and biological activity of both enantiomers of methamidophos and acephate. *J. Agric. Food Chem.* 50, 835–837.
- (43) Wang, Y. S., Tai, K. T., and Yen, J. H. (2004) Separation, bioactivity, and dissipation of enantiomers of the organophosphorus insecticide fenamiphos. *Ecotoxicol. Environ. Saf.* 57, 346–353.
- (44) Lin, K. D., Zhang, F., Zhou, S. S., Liu, W. P., Gan, J., and Pan, Z. Y. (2007) Stereoisomeric separation and toxicity of the nematicide fosfiazate. *Environ. Toxicol. Chem.* 26, 2339–2344.
- (45) Lin, K. D., Liu, W. P., Li, L., and Gan, J. (2008) Single and joint acute toxicity of isocarbophos enantiomers to *Daphnia magna*. *J. Agric. Food Chem.* 56, 4273–4277.
- (46) Zhou, S. S., Lin, K. D., Li, L., Jin, M. Q., Ye, J., and Liu, W. P. (2009) Separation and toxicity of salithion enantiomers. *Chirality* 27, 922–928.
- (47) Lin, K., Zhou, S. S., Xu, C., and Liu, W. P. (2006) Enantiomeric resolution and biotoxicity of methamidophos. *J. Agric. Food Chem.* 54, 8134–8138.
- (48) Yen, J. H., Tsai, C. C., and Wang, Y. S. (2003) Separation and toxicity of enantiomers of organophosphorus insecticide leptophos. *Ecotoxicol. Environ. Saf.* 55, 236–242.
- (49) Wang, C., Zhang, N., Li, L., Zhang, Q., Zhao, M. R., and Liu, W. P. (2010) Enantioselectivity interaction with acetylcholinesterase of an organophosphate insecticide fenamiphos. *Chirality* 22, 612–617.
- (50) Zhou, S. S., Lin, K. D., Yang, H. Y., Li, L., Liu, W. P., and Li, J. (2007) Stereoisomeric separation and toxicity of a new organophosphorus insecticide chloramidophos. *Chem. Res. Toxicol.* 20, 400–405.
- (51) Zhou, S. S., Wang, L. M., Li, L., and Liu, W. P. (2009) Stereoisomeric separation and bioassay of a new organophosphorus compound, O,S-dimethyl-N-(2,2,2-trichloro-1-methoxyethyl)-phosphoramidothioate: some implications for chiral switch. *J. Agric. Food Chem.* 57, 6920–6926.
- (52) Zhang, A. P., Sun, J. Q., and Liu, W. P. (2014) Enantioselective interaction of acid α -naphthyl acetate esterase with chiral organophosphorus insecticides. *J. Agric. Food Chem.* 62, 1477–1481.
- (53) Liu, H. G., Liu, J., Li, L., Zhou, S. S., and Liu, W. P. (2010) Enantioselective cytotoxicity of isocarbophos is mediated by oxidative stress-induced JNK activation in human hepatocytes. *Toxicology* 276, 115–121.
- (54) Xu, C., Zhao, M., Liu, W., Chen, S., and Gan, J. (2008) Enantioselectivity in zebrafish embryo toxicity of the insecticide acetofenate. *Chem. Res. Toxicol.* 21, 1050–1055.
- (55) Tang, M. L., Zhao, M. R., Zhou, S. S., Chen, K., Zhang, C. L., and Liu, W. P. (2014) Assessing the underlying breast cancer risk of Chinese females contributed by dietary intake of residual DDT from agricultural soils. *Int. Environ.* 73, 208–215.
- (56) Wang, L. M., Zhou, S. S., Lin, K. D., Zhao, M. R., Gan, J. Y., and Liu, W. P. (2009) Enantioselective estrogenicity of o,p'-dichlorodiphenyltrichlor in the MCF-7 human breast carcinoma cell line. *Environ. Toxicol. Chem.* 28, 1–8.
- (57) Zhao, M. R., Wang, C., Zhang, C. L., Wen, Y. Z., and Liu, W. P. (2012) Enantioselective cytotoxicity profile of o,p'-DDT in PC 12 Cells. *PLoS One* 7, e43823.
- (58) Wang, C., Li, Z. Y., Zhang, Q., Zhao, M. R., and Liu, W. P. (2013) Enantioselective induction of cytotoxicity by o,p'-DDD in PC12 cells: implications of chirality in risk assessment of POPs metabolites. *Environ. Sci. Technol.* 47, 3909–3917.
- (59) Liu, H. J., Ye, W. H., Zhan, X. M., and Liu, W. P. (2006) A comparative study of rac- and S-metolachlor toxicity to *Daphnia magna*. *Ecotoxicol. Environ. Saf.* 63, 451–455.
- (60) Chen, S. W., and Liu, W. P. (2008) Toxicity of Chiral Pesticide Rac-Metalaxyl and R-Metalaxyl to *Daphnia magna*. *Bull. Environ. Contam. Toxicol.* 81, 531–534.
- (61) Zhan, X. M., Liu, H. J., Miao, Y. G., and Liu, W. P. (2006) A comparative study of rac- and S-metolachlor on some activities and metabolism of silkworm, *Bombyx mori* L. *Pestic. Biochem. Physiol.* 85, 133–138.
- (62) Liu, H. J., and Xiong, M. Y. (2009) Comparative toxicity of racemic metolachlor and S-metolachlor to *Chlorella pyrenoidosa*. *Aquat. Toxicol.* 93, 100–106.
- (63) Zhou, Q. Y., Xu, C., Zhang, Y. S., and Liu, W. P. (2009) Enantioselectivity in the phytotoxicity of herbicide imazethapyr. *J. Agric. Food Chem.* 57, 1624–1631.

- (64) Zhou, Q. Y., Zhang, N., Zhang, C., Huang, L. D., Niu, Y. F., Zhang, Y. S., and Liu, W. P. (2010) Molecular mechanism of enantioselective inhibition of acetolactate synthase by imazethapyr enantiomers. *J. Agric. Food Chem.* 58, 4202–4206.
- (65) Qian, H., Hu, H., Mao, Y., Ma, J., Zhang, A., Liu, W., and Fu, Z. (2009) Enantioselective phytotoxicity of the herbicide imazethapyr in rice. *Chemosphere* 76, 885–892.
- (66) Qian, H. F., Wang, R. Q., Hu, H. J., Lu, T., Chen, X. L., Ye, H. Q., Liu, W. P., and Fu, Z. W. (2011) The enantioselective phytotoxicity of the herbicide imazethapyr and its effect on rice physiology and gene transcription. *Environ. Sci. Technol.* 45, 7036–7043.
- (67) Qian, H. F., Lu, T., Peng, X. F., Han, X., Fu, Z. W., and Liu, W. P. (2011) Enantioselective phytotoxicity of the herbicide imazethapyr on the response of the antioxidant system and starch metabolism in *Arabidopsis thaliana*. *PLoS One* 6, e19451.
- (68) Qian, H. F., Han, X., Peng, X. F., Lu, T., Liu, W. P., and Fu, Z. W. (2014) The circadian clock gene regulatory module enantioselectivity mediates imazethapyr-induced early flowering in *Arabidopsis thaliana*. *J. Plant Physiol.* 171, 92–98.
- (69) Cai, X. Y., Liu, W. P., and Sheng, G. Y. (2008) Enantioselective degradation and ecotoxicity of the chiral herbicide diclofop in three freshwater alga cultures. *J. Agric. Food Chem.* 56, 2139–2146.
- (70) Ye, J., Zhang, Q., Zhang, A., Wen, Y., and Liu, W. (2009) Enantioselective effects of chiral herbicide diclofop acid on rice Xiushui 63 seedlings. *Bull. Environ. Contam. Toxicol.* 83, 85–91.
- (71) Ye, J., Wang, L. M., Zhang, Z. J., and Liu, W. P. (2013) Enantioselective physiological effects of the herbicide diclofop on cyanobacterium *Microcystis aeruginosa*. *Environ. Sci. Technol.* 47, 3893–3901.
- (72) Ye, J., Zhang, Y., Chen, S. W., Liu, C. N., Zhu, Y. Q., and Liu, W. P. (2014) Enantioselective changes in oxidative stress and toxin release in *Microcystis aeruginosa* exposed to chiral herbicide diclofop acid. *Aquat. Toxicol.* 146, 12–19.
- (73) Zhang, Q., Zhao, M. R., Qian, H. F., Lu, T., Zhang, Q., and Liu, W. P. (2012) Enantioselective damage of diclofop acid mediated by oxidative stress and acetyl-CoA carboxylase in non-target plant *Arabidopsis thaliana*. *Environ. Sci. Technol.* 46, 8405–8412.
- (74) Wen, Y. Z., Yuan, Y. L., Shen, C. S., Liu, H. J., and Liu, W. P. (2009) Spectroscopic investigations of the chiral interactions between lipase and the herbicide dichlorprop. *Chirality* 21, 396–401.
- (75) Wen, Y. Z., Yuan, Y. L., Chen, H., Lin, K. D., and Liu, W. P. (2010) Effect of chitosan on the enantioselective bioavailability of the herbicide dichlorprop to *Chlorella pyrenoidosa*. *Environ. Sci. Technol.* 44, 4981–4987.
- (76) Wen, Y. Z., Chen, H., Shen, C. S., Zhao, M. R., and Liu, W. P. (2011) Enantioselectivity tuning of chiral herbicide dichlorprop by copper: Roles of reactive oxygen species. *Environ. Sci. Technol.* 45, 4778–4784.
- (77) Chen, H., Chen, J., Guo, Y. A., Wen, Y. Z., Liu, J., and Liu, W. P. (2012) Evaluation of the role of the glutathione redox cycle in Cu(II) toxicity to green algae by a chiral perturbation approach. *Aquat. Toxicol.* 120, 19–26.
- (78) Zhang, Q., Zhou, C., Zhang, Q., Qian, H. F., Zhao, M. R., and Liu, W. P. (2013) Stereoselective phytotoxicity of HCH mediated by photosynthetic and antioxidant defense systems in *Arabidopsis thaliana*. *PLoS One* 8, e51043.