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Environmental behavior of the chiral insecticide fipronil: Enantioselective toxicity, distribution and transformation in aquatic ecosystem

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ABSTRACT

The enantioselective environmental behaviors of the chiral insecticide fipronil and its metabolites in lab-scale aquatic ecosystems were studied and the toxicity of fipronil enantiomers and the metabolites to non-target organisms *Lemna minor* (*L. minor*) and *Anodonta woodiana* (*A. woodiana*) was also investigated in this work. Water-sediment, water-*L. minor*, water-*A. woodiana*, and water-sediment-*L. minor*-*A. woodiana* ecosystems were set up and exposed to fipronil through a 90-day period. The results showed fipronil could be degraded significantly faster (half-life of 4.6 days) in the complex water-sediment-*L. minor*-*A. woodiana* ecosystem. *A. woodiana* played a crucial role in the dissipation of fipronil, and the microorganisms in the sediment also made great contribution to the degradation of fipronil in aquatic ecosystems. All the three metabolites fipronil desulfinyl, fipronil sulfide and fipronil sulfone were detected in the ecosystems and were more persistent than fipronil. Enantioselective degradation of fipronil was observed with S-fipronil being preferentially degraded in sediment and *L. minor*, while R-fipronil was metabolized preferentially in *A. woodiana*. EC_{50} for *L. minor* was obtained using 7-day exposure, and for *A. woodiana* was obtained using 72-h exposure. S-fipronil was more toxic to *A. woodiana*, while R-fipronil showed higher toxicity to *L. minor*. Moreover, the three metabolites were found more toxic than fipronil indicating significant environment risks due to their persistence. The present study might have important implications for the risk assessment of fipronil and its metabolites in real aquatic environment.

1. Introduction

Pesticides are routinely applied for agricultural, industrial, and home use to control or prevent invasive insects, diseases, and unwanted plant growth. About thirty percent of the known registered pesticides are chiral, including the most frequently used pyrethroid insecticides, phenoxypropionic acid herbicides and organophosphorus insecticides. Most of chiral pesticides have been applied to agriculture as racemic forms (Garrison, 2006). The enantiomer specific profiles of chiral pesticides have become important topics at the forefront of chemistry and toxicology research (Mattina et al., 2002). Enantiomers have the same physical and chemical properties, but in most cases, behave differently in biochemical processes (Kodama et al., 2002; Williams, 1996; Konwick et al., 2006). In order to reduce the amount of pesticides applied and the adverse impacts, European countries have decreed the use of only single enantiomer of some pesticides (Williams, 1996). Fipronil is a phenylpyrazole insecticide developed by Aventis (Paris, France), used for control against a wide range of soil and foliar insects, such as rice grasshoppers, rice skippers, vine weenil, termites and black ants in agriculture, forestry and urban environments (Bobe et al., 1998; Valerio et al., 1998), which is chiral with two enantiomers. It works by disrupting the central nervous system of the insects and

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blocking the channels of chloride ions through the gamma-aminobutyric acid (GABA) receptor (Zhao et al., 2003; Islam and Lynch, 2012). The degradation of fipronil results in the formation of the metabolites fipronil desulfinyl, fipronil sulfide and fipronil sulfone through abiotic and biotic processes in aquatic environment (Fenet et al., 2001; Ramesh and Balasubramania, 2009; Zhu et al., 2004). Recent research has indicated fipronil has high toxicity to aquatic organisms such as fish, aquatic invertebrates (Stehr et al., 2006; Li et al., 2010) and the metabolites were more toxic against some non-target organism (Hainzl and Casida, 1996).

Lab-scale aquatic ecosystem means an aquatic ecosystem maintained under constant laboratory conditions and attempts to simulate the physical and chemical environment of the natural system. Ecosystems have been widely used to investigate the fate, risk assessment and ecological effect of pesticide because of its advantages such as authenticity, flexibility, and security (Laabs et al., 2007; Cuppen et al., 2002; Van den Brink et al., 2009; Colombo et al., 2013). Fipronil has been widely used in rice field and may cause impacts on aquatic ecosystem. Few studies have been conducted on the environmental behaviors of fipronil and metabolites in complex aquatic systems. Lab-scale ecosystem is a good way to evaluate the pollution mechanism and assess the risk for complex natural systems. To assess the potential risk of fipronil, it is important to understand the distribution, accumulation, degradation, and the formation of any metabolites that may have detrimental effects as well.

L. minor is very prevalent in aquatic ecosystem, which plays a very important role in maintaining aquatic community and biodiversity since it is an important primary producer in aquatic ecosystem and the direct or indirect food of aquatic animals. Recently, the enantioselective environmental behaviors such as toxicity, sorption and degradation of pesticides in *L. minor* have been investigated (Caux et al., 2011; Doganlar, 2012). *A. woodiana*, as one of macroinvertebrates, is ubiquitous in aquatic ecosystems, which has an intimate contraction with the solid phase in the overlying water. Due to the ability to accumulate pollution as well as the biological effect endpoints which can be measured, *A. woodiana* has been used in risk assessment studies (Jacomini et al., 2011; Liu et al., 2007; Lopes et al., 1992).

In this work, the fate of the widely used insecticide fipronil and its metabolites in aquatic systems was investigated using lab-scale aquatic ecosystems. The toxicity of fipronil and the metabolites to non-target aquatic organisms *L. minor* and *A. woodiana* was evaluated. The enantioselective distribution, bioaccumulation and degradation and toxicity of the two enantiomers of fipronil was also determined. This work may supply some information to elucidate the environmental behavior and assess the potential risks of fipronil and its metabolites in the aquatic environment.

2. Materials and methods

2.1. Reagents

Standards of racemic fipronil (rac-fipronil, 96.5%) was obtained from China Ministry of Agriculture Institute for Control of Agrochemicals. The three metabolites fipronil desulfinyl, sulfide, and sulfone were purchased from AccuStandard, Inc., with purities of 97.9%, 99.4% and 98.6%, respectively. The individual R- and S-fipronil enantiomers were prepared using a Chiralcel OD chiral column (Daicel Chiral Technology Co., Ltd.) on HPLC, with purities of 99.5% and 99.4%, respectively. All the solvents used were chromatograph grade from Thermo Fisher Scientific, New York, USA. Stock solutions of fipronil and the metabolites were prepared and stored in amber bottles at 4 °C before use.

2.2. L. minor and A. woodiana

L. minor and *A. woodiana* were purchased from Xi Yuan Aquaculture Market (Beijing, China), and were reared in 30-L glass aquariums containing 10 L of deionized water at 25 ± 1 °C with 12 h light/12 h darkness. The water was continuously aerated and allowed to acclimatize for 1 week prior to the experiments.

2.3. Sediment

The sediment was collected from Shang Zhuang reservoir (Beijing, China), which had not received fipronil application for at least the prior 10 years. The physicochemical properties of the sediment were as follows: organic matter, 8.05%; clay, 30%; sand, 22.95%; silt, 39%; and pH, 7.18. The tests were performed with both natural sediment and sterile sediment. The sterile sediment were prepared by autoclaving at 120 °C for 30 min over three consecutive days.

2.4. Experimental protocol

The determination of the toxicity to *L. mionr* was designed according to the standard method (ISO, 20079). Twelve healthy *L. mionr* was incubated in a 250-mL beaker containing 150 mL of growth medium spiked with fipronil or the metabolites. After 7 d of incubation, the frond numbers, root length and dry weight were recorded. In the control group, *L. minor* were incubated in the same amount of growth medium and exposed to blank acetone without fipronil or the metabolites. The EC_{50} values were determined using linear regression analysis of inhibition percentage versus control.

The acute toxicity test to *A. woodiana* was carried out according to the published Standard guide for conducting laboratory toxicity tests with freshwater mussel of American Society for Testing and Materials (ASTM, 2006). The test compounds were dissolved in acetone, and the working standard solutions were prepared by serial dilution at concentrations of 0.01–15.0 mg L^{-1} spiked in beaker with 100 mL of deionized water. Twenty replicates for each treatment were conducted. *A. woodiana* spiked with 10 μL acetone solution but without the toxicant as control group was performed. Mortality was recorded after incubation for 72 h, and LC_{50} values were calculated using SPSS Version 18.0 (SPSS lnc, Chicago, USA).

The ecosystems were designed according to the US EPA OPPTS 850.1900 for Generic Freshwater Ecosystem Test, Laboratory (USEPA, 1996). Four kinds of ecosystems were set up: water-sediment (WS), water-*L. minor* (WL), water-*A. woodiana* (WA), and water-sediment-*L. minor*-*A. woodiana* (WSLA). A summary of the ecosystem design and the detailed information were shown in Table S1. To examine the influence of the microorganisms in the sediment on the bioaccumulation, degradation and transformation, two water-sediment ecosystems were designed: water with natural sediment (WS1) and water with sterile sediment (WS2). Rac-fipronil was spiked in the water of the ecosystems at a concentration of 0.5 mg L^{-1}. The distribution, accumulation and degradation characteristics of fipronil and its metabolites in the ecosystems were determined throughout a 90-d exposure period. During the experiment, temperature and lighting conditions were set at 25 ± 1 °C with 12 h light/12 h darkness. The samples of water, sediment, *L. minor*, and *A. woodianas* were collected on days 0, 1, 3, 5, 7, 11, 16, 21, 31, 43, 60, and 90, and stored at −20 °C prior to pretreatment. The experiments were performed in triplicate.

2.5. Extraction and cleanup

Ten milliliter of water or 5 g of sediment were added into a 50-mL polypropylene centrifuge tube and 10 mL of acetic ether (for water) or 10 mL of acetonitrile with 2 g of sodium chloride (for

sediment) was added. The tube was vortexed for 3 min and then centrifuged at 4000 rpm for 5 min. The extraction was repeated twice and the organic phase was combined and evaporated to dryness and dissolved in 1 mL of *n*-hexane for GC analysis.

L. minor was ground and 3 g was transferred to a 50-mL polypropylene tube. After addition of 10 mL of acetic ether, the tube was vortexed for 3 min and then centrifuged at 4000 rpm for 5 min. The organic phase was evaporated to dryness and dissolved in 1 mL of acetic ether. A silica SPE cartridge (Thermo Fisher Scientific, New York, USA) was used for clean up. The sample was loaded and eluted with 6 mL of acetic ether/*n*-hexane (2/8, v/v). The eluent was evaporated to dryness under a stream of nitrogen and dissolved in 1 mL of *n*-hexane for GC analysis.

A. woodianas were homogenized using a Waring (Torrington CT) stainless steel blender, and 5 g was transferred into a 50-mL polypropylene centrifuge tube. The same extraction procedure as the same as *L. minor* was used. A SPE procedure was applied for clean up with a florisil cartridge (Thermo Fisher Scientific, New York, USA). The sample was loaded and eluted with 7 mL of acetic ether/*n*-hexane (1/9, v/v). The eluent was collected and evaporated to dryness under a stream of nitrogen and dissolved in 1 mL of n-hexane for GC analysis.

2.6. Instrumental and statistical analysis

The quantitative determination of fipronil and the metabolites was carried out on an Agilent 7890 GC with electron-capture detector (ECD) with a HP-5 column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies). The detector temperature was 310 °C, and the inlet temperature was 250 °C. The column was initially held at 60 °C, heated to 180 °C at 10 °C/min increments, then to 205 °C at 3 °C/min increments and held for 4 min and then heated to 280 °C at 20/min increments and held for 7 min.

The enantiomeric analysis of fipronil was performed on Agilent 7890 GC-ECD with a BGB-172 column (30 m × 0.25 mm × 0.25 μm, BGB Analytik AG, Switzerland). The detector temperature was 310 °C, and the inlet temperature was 250 °C. The column was initially held at 60 °C, heated to 160 °C at 15 °C/min increments and held for 1 min, then heated to 230 °C at 2 °C/min and held for 70 min.

The limits of quantification (LOQs) for both fipronil enantiomers and the metabolites were 1.0 ng mL^{-1}. Recoveries were estimated at 3 levels (1.0 ng mL^{-1}, 100 ng mL^{-1}, and 1000 ng mL^{-1}). The average recoveries were found ranging from 78.2% to 96.6%. Differences between individual treatments were analyzed using one-way analysis of variance (one-way ANOVA). The values in the text were presented as means ± standard deviations (SD).

Enantiomeric fraction (EF) used to give a description of enantioselectivity of enantiomers in chiral analysis is defined as the proportion of R-fipronil to the sum of R-fipronil and S-fipronil.

2.7. Environmental risk implication

To characterize the aquatic ecosystem distribution of compounds from water to organisms, Distribution Factor (DF) was calculated as the ratio of chemical level in organisms to that in water ($C_{organisms}/C_{water}$).

The environmental risks were assessed based on the risk quotient (RQ) method (S.S. Ma, 2006). The RQ values were calculated using the formula below:

$$RQ = L(E)C_{50}/MEC$$

Where MEC was the measured environmental concentration. According to the calculated RQ values, the environmental risks were classified into 4 levels: no risk (RQ > 100), low risk (>5), medium risk (0.5–5), and high risk (<0.5) levels.

3. Results and discussion

3.1. Enantioselective toxicity of fipronil enantiomers and the metabolites to non-target aquatic organisms

The LC_{50} values of rac-fipronil, R-fipronil, S-fipronil, and the three metabolites for *A. woodianas* were listed in Table 1. Enantioselective acute toxicity of the racemate and single enantiomers of fipronil to *A. woodianas* was found. S-fipronil was the most toxic with the LC_{50} (72 h) value of 0.63 mg L^{-1}, which was about 2 times more toxic than rac-fipronil (1.21 mg L^{-1}) and 5 times more toxic than R-fipronil (3.27 mg L^{-1}). Similarly, a previous study indicated that S-fipronil was three times more toxic to *C. dubia* than its antipode (Konwick et al., 2005; Overmyer et al., 2007). For the toxicity of the metabolites, it was found the three main metabolites were more toxic to *A. woodianas* than the parent compound with LC_{50} values of 1.19 mg L^{-1}, 0.32 mg L^{-1}, and 0.24 mg L^{-1} for desulfinyl, sulfide and sulfone respectively.

The EC_{50} (72 h) values of fipronil enantiomers and the metabolites for *L. minor* were shown in Table 2. Enantioselective toxicity of the enantiomers and racemate of fipronil to *L. minor* was also found. The EC_{50} values of rac-, R- and S-fipronil in *L. minor* were 9.36, 8.51 and 10.14 mg L^{-1}, indicating the R-enantiomer was more toxic than S-fipronil and racemate. As for the three metabolites, desulfinyl were of the similar toxicity compared with fipronil, while sulfide and sulfone were more toxic than the parent compound to *L. minor* with EC_{50} values of 10.47 mg L^{-1}, 6.45 mg L^{-1}, and 7.88 mg L^{-1} for fipronil desulfinyl, fipronil sulfide and fipronil sulfone respectively.

3.2. Enantioselective distribution and degradation in water-sediment ecosystem

Results of control experiment confirmed that fipronil was stable in water because about 18% dissipated after 90 days and no enantioselectivity occurred. To examine the influence of the microorganisms in the sediment on transformation, two water-sediment ecosystems were designed: water with natural sediment (WS1) and water with sterile sediment (WS2). In the water-sediment ecosystem (WS1), the half-life of fipronil in water was approximately 11.8 days, and about 62% degraded during the total exposure period of 90 days (Fig. 1A). The EF values in water changed gradually from the initial 0.49 to 0.44 after 90 days (Fig. 4B), suggesting slight enantioselectivity with the R-fipronil being preferentially transformed. In the sediment, a rapid distribution of fipronil during the

Table 1
Median lethal concentration (LC50) values of fipronil enantiomers and metabolites to *A. woodianas*.

Spiked compound	72-h LC50 (mg L^{-1})	R^2[a]	Confidence intervals[b]	P[c]
Rac-fipronil	1.21**	0.945	[0.94–1.65]	0.010
R-fipronil	3.27*	0.961	[3.08–3.62]	0.008
S-fipronil	0.63*	0.911	[0.35–1.03]	0.020
Fipronil desulfinyl	1.19**	0.925	[0.97–1.50]	0.012
Fipronil sulfide	0.32**	0.947	[0.21–0.55]	0.010
Fipronil sulfone	0.24**	0.986	[0.12–0.41]	0.001

* Represents significant difference between the two enantiomers and ** represents significant difference between the rac-fipronil and metabolites. (associated with the SNK test).
[a] Represents the correlation coefficient.
[b] 95% confidence intervals surrounding each estimated LC50 are bracketed.
[c] A *P* value smaller than 0.05 indicates that significant differences among fipronil enantiomers and metabolites.

Table 2
Median effective concentration (EC50) values of fipronil enantiomers and metabolites to *L. minor*.

Spiked compound	7-d EC50 (mg L^{-1})	R^{2a}	Confidence intervals[b]	P^{c}
Rac-fipronil	9.36**	0.993	[7.25–10.34]	0.025
R-fipronil	8.51*	0.981	[6.01–10.76]	0.032
S-fipronil	10.14*	0.976	[7.46–12.82]	0.017
Fipronil desulfinyl	10.47	0.959	[7.32–13.11]	0.054
Fipronil sulfide	6.50**	0.994	[5.15–8.29]	0.015
Fipronil sulfone	7.88**	0.967	[5.41–10.18]	0.029

* Represents significant difference between the two enantiomers and ** represents significant difference between the rac-fipronil and metabolites. (associated with the SNK test).

[a] Represents the correlation coefficient.

[b] 95% confidence intervals surrounding each estimated EC50 are bracketed.

[c] A *P* value smaller than 0.05 indicates that significant differences among fipronil enantiomers and metabolites.

first 16 days was found, and the highest concentration of fipronil detected in the sediment was 86.5 ng g^{-1}, after that, a decline tendency was observed (Fig. 1B). The EFs in the sediment deviated from 0.5 to 0.62 during the exposure period (Fig. 4B). All the three metabolites were detected in the water and sediment in the ecosystem. The highest levels of sulfide (5.6 ng mL^{-1} at the 16th day), desulfinyl (3.3 ng mL^{-1} at the 7th day), and sulfone (4.9 ng mL^{-1} at the 11th day) in water were detected, and they were undetectable after 60 days of exposure (Fig. 1A). The concentrations of metabolites in the sediment were higher than that in water correspondingly (Fig. 1B). The result indicated sediment plays an important role in the degradation and enantioselectivity of fipronil in this ecosystem, in which microorganism might be a crucial factor. Previous study also showed that the microbial population was important for the degradation of fipronil in soil and sediment (Tan et al., 2008; Jones et al., 2007).

In the WS2 ecosystem, fipronil was relative stable in the water with only about 32% being degraded after 90 days (Fig. 1C). Fipronil could also transfer to the sterile sediment rapidly, and the highest concentration was about 139 ng g^{-1} after 11 days of exposure. However, there was no significant decline after that (Fig. 1D). EF values did not apparently deviate from 0.5 in both water and sediment, indicating no enantioselectivity occurred (Fig. 4C). The three metabolites in the water and sediment were undetectable or at very low level during the 90-day period. Therefore, the results showed that microorganisms in the sediment could accelerate the degradation of fipronil and contribute to the enantioselectivity. The results showed that microorganisms in the sediment could accelerate the degradation of fipronil and contribute to the enantioselectivity.

3.3. Enantioselective bioaccumulation, distribution and degradation in water- L. minor ecosystem

In the water-*L. minor* ecosystem, the degradation of fipronil in water was slow and only about 36% disappeared after 90 day (Fig. 1E). The concentration of fipronil in *L. minor* increased to 14 ng g^{-1} at the 16th day and then decreased to undetectable level after 60 days (Fig. 1F). EF values in water were around 0.5 (Fig. 4D), suggesting no enantioselectivity, however, the EF values in *L. minor* changed gradually from the initial 0.53 to 0.63 indicating that R-fipronil was preferentially accumulated or S-fipronil was preferentially degraded by *L. minor* (Fig. 4D). The metabolites were not detected in both water and *L. minor* after 90 days.

3.4. Enantioselective bioaccumulation, distribution and degradation in water- A. woodianas ecosystem

In the water-*A. woodianas* ecosystem, the half-life of fipronil in water was 6.8 days (Fig. 1G) and about 78% was degraded after 90 days. EFs increased from 0.5 to 0.59 (Fig. 4E) with R-enantiomer being enriched. All the metabolites were detected in water, and the highest levels of fipronil desulfinyl, fipronil sulfone and fipronil sulfide reached 5.1 ng mL^{-1} at the 5th day, 11.5 ng mL^{-1} at the 11th day and 8.3 ng mL^{-1} at the 31st day respectively. Fipronil in *A. woodianas* increased to about 260 ng g^{-1} in the initial 16 days, and then decreased gradually (Fig. 1H). The EFs deviated from 0.5 significantly during the exposure period (Fig. 4E) showing S-enantiomer was preferentially accumulated or R-enantiomer preferentially degraded by *A. woodianas*. The concentrations of the three metabolites in *A. woodiana* were relatively high. As shown in Fig. 1H, fipronil sulfone was main metabolite in *A. woodiana*, and it reached a maximum level of 42 ng g^{-1} at 31st day, and decreased to 28 ng g^{-1} at the end of exposure. The results showed *A. woodiana* had strong ability for the metabolism of fipronil.

3.5. Enantioselective bioaccumulation, distribution and degradation in water-sediment-L. minor-A. woodianas ecosystem

In the water-sediment-*L. minor-A. woodianas* ecosystem, the half-life of fipronil in the water was determined to be approximately 4.6 days and more than 90% loss were observed after a total exposure period of 90 days (Fig. 2A). It was the best ecosystem for the remediation of fipronil residue in water because of a combination contribution of sediment, *L. minor* and *A woodianas*. EFs in water increased from 0.5 to 0.55 (Fig. 4A) showing slight enantioselectivity. In the sediment, a rapid distribution of fipronil during the first 7 days was observed, and the highest concentration reached 106 ng g^{-1} at days 21 (Fig. 2B). The EF was 0.57 at the end of the exposure, which was lower than that in WS1 (Fig. 4A). Fipronil was also accumulated in *L. minor*, and approximate 9.2 ng g^{-1} of fipronil was detected after 7 days exposure (Fig. 2C). EFs in *L. minor* increased from the initial 0.49 to 0.72 at the 11th day, and decreased gradually after that. In *A. woodiana*, fipronil was accumulated reaching a maximum concentration of 214 ng g^{-1} at the 21st day. Thereafter, it gradually declined. Eventually, the concentration decreased to 30 ng g^{-1} at the end (Fig. 2D). S-fipronil was preferentially accumulated by *A. woodiana*, with EF values of 0.35 after 90 days.

Fipronil loss was associated with the formation of fipronil sulfide, sulfone and desulfinyl, indicating that reductive, oxidized and photolytic transformations were the main pathways. Fipronil metabolites could be observed in water after 1 day, in which fipronil sulfone was detectable through the whole exposure period, ranging from 2.8 to 13 ng L^{-1} (Fig. 2A). Fipronil sulfide was also found while the concentrations through the exposure period were 2.2–13.5 ng g^{-1}. The concentration of fipronil desulfinyl was lower than the other two metabolites. All the three metabolites were found in sediment (Fig. 2B). The level of fipronil sulfone in sediment was higher than that of the other two metabolites, and the highest concentration was 22.6 ng g^{-1} at the 21st day. Fipronil sulfide from 4.0 to 13.0 ng g^{-1} and desulfinyl from 2.6 to 7.5 ng g^{-1} were also observed. At the same time, fipronil sulfide was found in *L. minor* from 7 to 43 days and the concentrations were between 2 and 5 ng g^{-1} (Fig. 2C). Fipronil sulfone and desulfinyl were not detected in *L. minor* samples. *A. woodiana* could rapidly metabolize fipronil and the levels of fipronil derivatives in *A. woodiana* were significantly higher than in other parts of this ecosystem (Fig. 2D).

3.6. Environmental risk implication

The enantioselective distribution, degradation and transformation of fipronil in the aquatic ecosystems have been studied in this work. As shown in Table 4, the derived DFs of fipronil in simple

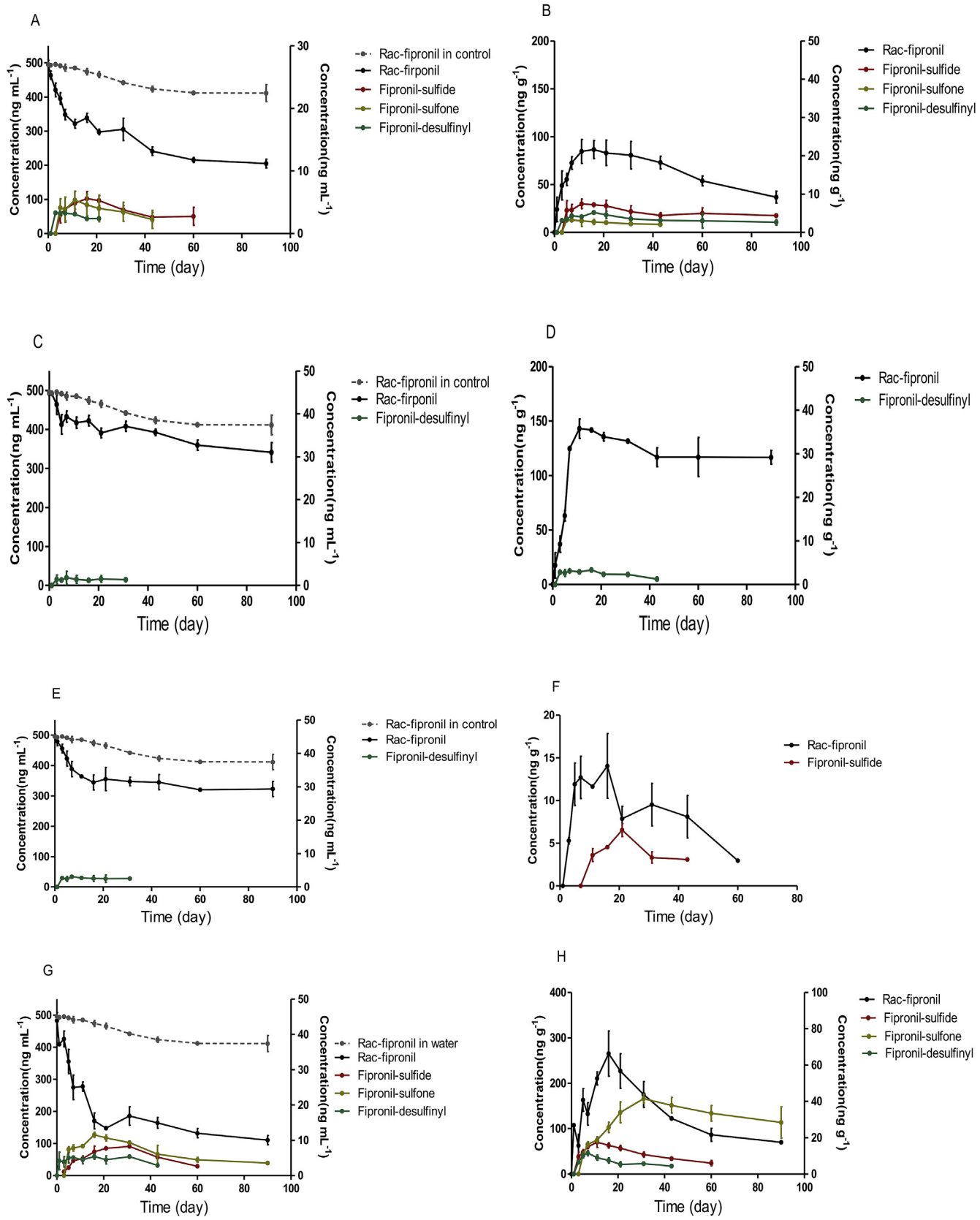

Fig. 1. The degradation of fipronil enantiomers and the formation of the metabolites in ecosystems. (A) fipronil and the metabolites in water in the WS1 ecosystems; (B) fipronil and the metabolites in sediment in the WS1 ecosystems; (C) fipronil and the metabolites in water in the WS2 ecosystems; (D) fipronil and the metabolites in sediment in the WS2 ecosystems; (E) fipronil and the metabolites in water in the WL ecosystems; (F) fipronil and the metabolites in *L. minor* in the WL ecosystems; (G) fipronil and the metabolites in water in the WA ecosystems; (H) fipronil and the metabolites in *A. woodianas* in the WA ecosystems. Bars are standard errors.

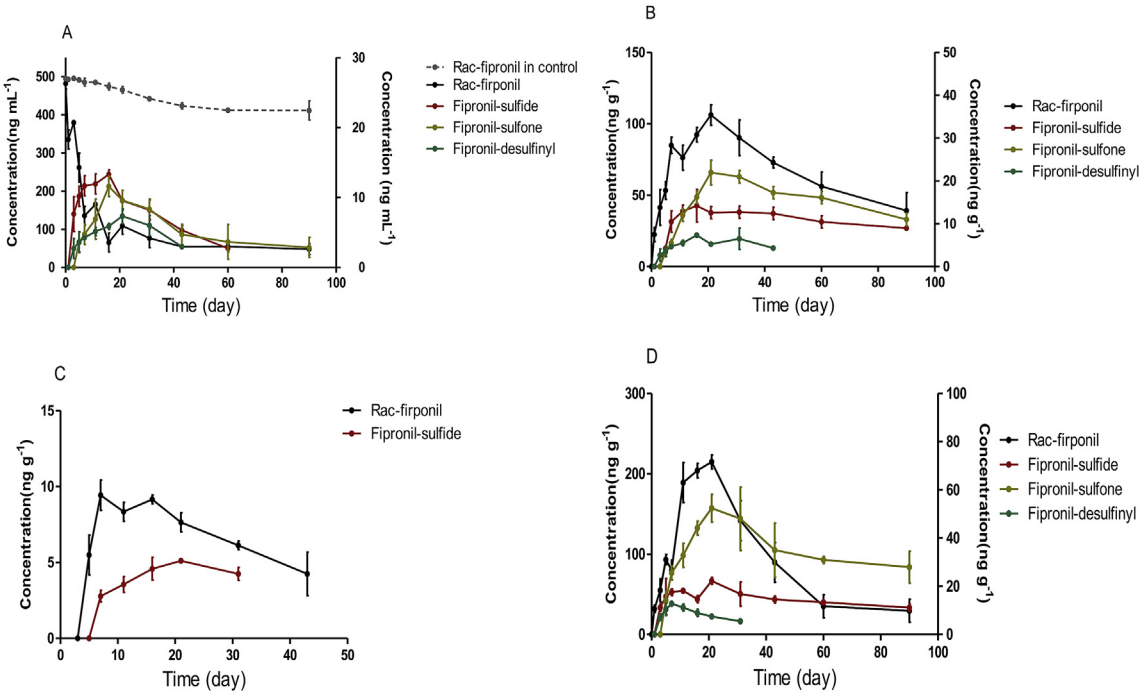

Fig. 2. The degradation of fipronil enantiomers and the formation of the metabolites in WSLA ecosystem. (A) fipronil and the metabolites in water; (B) fipronil and the metabolites in sediment; (C) fipronil and the metabolites in *L. minor*; (D) fipronil and the metabolites in *A. woodianas*. Bars are standard errors.

ecosystem was lower than that in complex ecosystem, demonstrating relative poor transformation of fipronil from aqueous phase to solid phase in simple ecosystem. However, the DF values of fipronil metabolites in simple ecosystem were higher than those of fipronil in complex ecosystem, indicating a higher tendency for the metabolites to distribute into sediment than fipronil. The possible reason might be metabolic fate of the compounds in the aquatic organisms, contributing to the different distribution patterns.

After a rough calculation, about 90% of fipronil was degraded in the WSLA ecosystem after 90 days, and the residue in the whole ecosystem, as shown in Fig. 3A, was distributed mainly in water (87%), sediment (4%), *A. woodiana* (8%) and *L. minor* (less than 1%). *A. woodiana* had the strongest capability for the remediation of fipronil pollution in aquatic ecosystem. Sediment was also important for the dissipation because of microorganism dependent degradation. However, *L. minor* was not effective for the clean up of fipronil. The three metabolites were all observed, in which fipronil sulfone and fipronil sulfide were the main metabolites suggesting

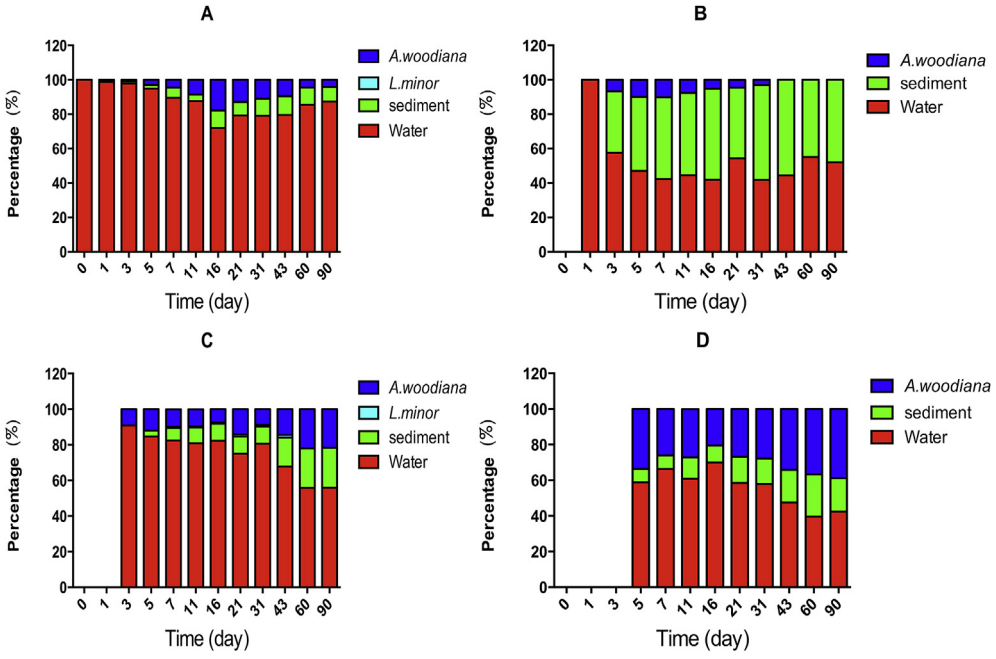

Fig. 3. The distribution of fipronil and the metabolites in WSLA ecosystem. (A) fipronil; (B) desulfinyl; (C) sulfide; (D) sulfone.

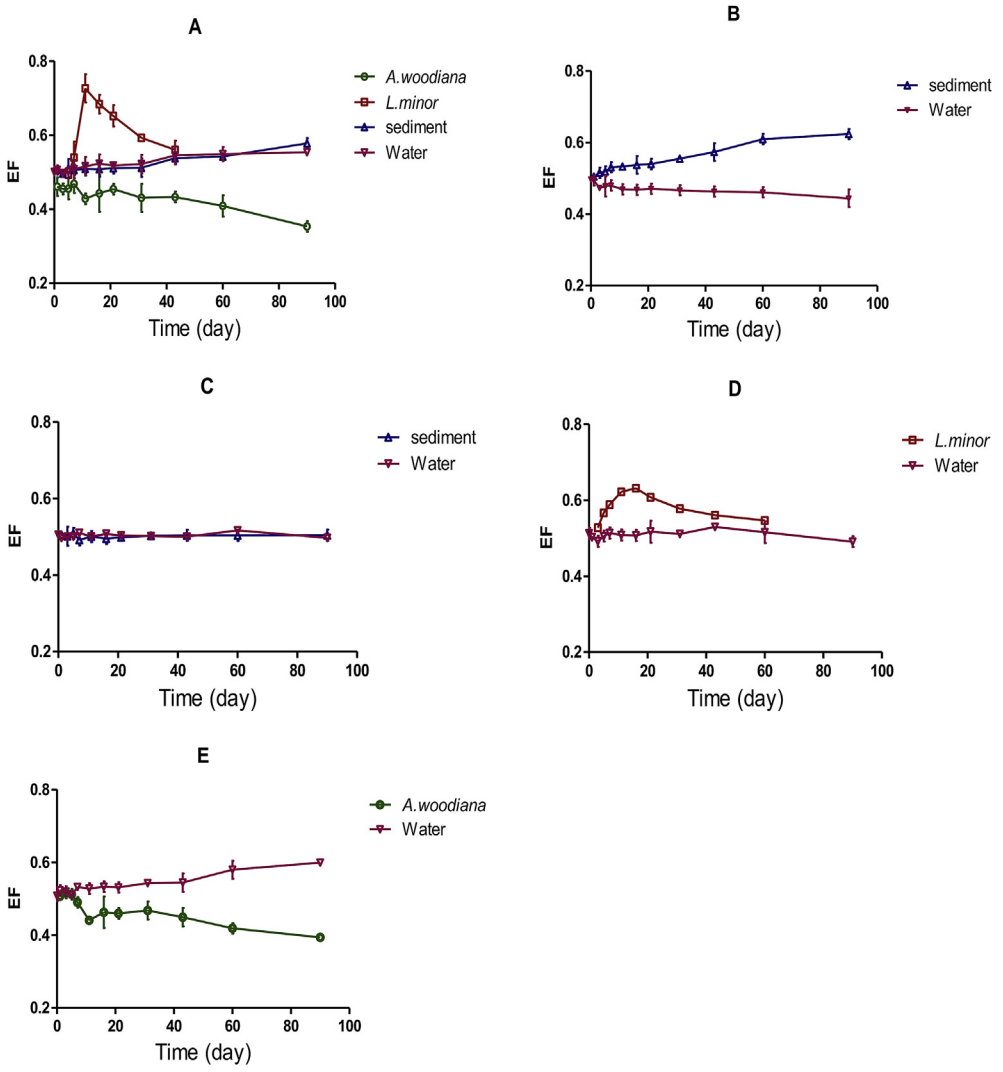

Fig. 4. Enantiomeric fractions (EFs) of rac-fipronil in ecosystems. (A) in WSLA ecosystem; (B) in WS1 ecosystem; (C) in WS2 ecosystem; (D) in WL ecosystem; (E) in WA ecosystem. Bars are standard errors.

Table 3
Concentration profile and risk quotients of the target compounds in aquatic ecosystems water.

Organisms	Compounds	WA		WL		WSLA	
		MEC (mg L^{-1})	RQ	MEC (mg L^{-1})	RQ	MEC (mg L^{-1})	RQ
A. woodiana	Fipronil	0.110–0.482	2–11	/	/	0.004–0.485	2–302
	Desulfinyl	0.003–0.005	238–396	/	/	0.002–0.007	170–595
	Sulfide	0.001–0.006	53–320	/	/	0.003–0.013	24–106
	Sulfone	0.003–0.011	21–80	/	/	0.003–0.011	21–80
L. minor	Fipronil	/	/	0.323–0.491	19–29	0.004–0.485	19–2340
	Desulfinyl	/	/	0.002–0.003	3490–5235	0.002–0.007	1495–5235
	Sulfide	/	/	/	/	0.003–0.013	500–2170
	Sulfone	/	/	/	/	0.003–0.011	716–3940

oxidation and reduction might be the main pathways. The distribution of the metabolites in the ecosystems was different from that of fipronil (Fig. 3). About 48% of fipronil desulfinyl was residues in the sediments, the rest was distributed in water and rarely fipronil desulfinyl was detected in *A. woodiana* (Fig. 3B). About 39% of fipronil sulfone was detected in *A. woodiana*, about 19% was found in the sediment, less than 42% was detected in water (Fig. 3D). About 23% and 21% of fipornil sulfide were detected in the sediment and *A. woodiana* at the end of the exposure period (Fig. 3C). The research also revealed the metabolites had relatively long half-lives and were more easily accumulated by sediment and organisms compared to fipronil. It was reported that the long-term toxicity of fipronil probably resulted from the environmental persistence of the metabolites (Schlenk et al., 2001; Walse et al., 2004). There have not been much researches about the effects of its metabolites on the environment and non-target organisms.

RQs (Table 3) were estimated based on the L(E)C_{50} values of firponil and metabolites to *A. woodiana* and *L. minor* (Tables 1 and

Table 4
Distribution Factor (DF) Values for fipronil and metabolites in different aquatic ecosystems at 0.5 mg L^{-1} after endpoint.

Organisms	Ecosystem	Fipronil	Desulfinyl	Sulfide	Sulfone
A. woodiana	WA DF	0.63 ± 0.03	1.56 ± 0.02	2.28 ± 0.22	7.96 ± 0.47
	WSLA DF	0.83 ± 0.17	0.94 ± 0.08	0.54 ± 0.07	1.02 ± 0.20
Sediment	WS1 DF	0.17 ± 0.01	1.81 ± 0.07	1.74 ± 0.41	0.98 ± 0.26
	WS2 DF	0.34 ± 0.01	0.98 ± 0.24	/	/
	WSLA DF	0.80 ± 0.09	0.71 ± 0.07	0.80 ± 0.04	0.39 ± 0.05
L. minor	WL DF	0.01 ± 0.01	/	/	/
	WSLA DF	0.07 ± 0.01	/	0.53 ± 0.03	/

2). The RQs for fipronil and the metabolites in most ecosystems were above 5, exhibiting low environment risk to *A. woodiana* and *L. minor*. Fipronil showed media or low risk with RQ of 2–11 in WL ecosystem, and low or no risk in WLSA ecosystem. The results also indicated that the complex water-sediment-*L. minor*-*A. woodianas* ecosystem could reduce the risks. Meanwhile, it is worth noting that the environment is usually exposed to a mixture of contaminants, so the combined risks should be evaluated (Backhaus et al., 2003; Silva et al., 2002).

4. Conclusion

In the current work, the enantioselective toxicity, degradation and transformation of fipronil in aquatic ecosystems have been studied. S-fipronil was more toxic than R-fipronil against *A. woodianas*, while R-fipronil was more toxic for *L. minor*. The metabolites fipronil sulfide and fipronil sulfone were more toxic to *A. woodianas* than the parent fipronil. Enantioselective degradation of fipronil was observed in sediment and *L. minor* with S-fipronil being degraded faster, while R-fipronil was metabolized preferentially by *A. woodiana*. The complex water-sediment-*L. minor*-*A. woodianas* ecosystem was the most effective for the remediation of fipronil and the metabolites. Fipronil sulfone and fipronil sulfide were the main metabolites revealing oxidation and reduction might be the main pathways. Furthermore, the metabolites were relatively more persistent indicating they might be more hazardous and more risk evaluations should be conducted.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2016.08.063.

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