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Enantioselective bioaccumulation of soil-associated fipronil enantiomers in *Tubifex tubifex*

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ABSTRACT

Enantioselective behavior of chiral pesticides in the aquatic environment has been a subject of growing interest. In this study, the enantioselective bioaccumulation of fipronil enantiomers in *Tubifex tubifex* (Oligochaeta, Tubificida) was detected in both spike–water and spike–soil systems, respectively. For the spike–water treatment, a 9-day exposure experiment was employed and the enantiomer fraction in tubifex tissue was maintained approximately at 0.58 during the experiment. In addition, a 14-day bioaccumulation period was chosen for the spike–soil treatment and a more significant deviation of enantiomer fraction from 0.5 in tubifex tissue was detected, with concentrations of the *R*-form higher than that of the *S*-form. Therefore, the bioaccumulation of fipronil was enantioselective in tubifex tissue for the two treatments and the magnitude of enantioselectivity may be influenced by different exposure conditions. For the spike–soil treatment, the concentrations of fipronil in verlying water and soil were also determined. With the presence of tubifex worms, higher concentrations of fipronil in overlying water and lower concentrations in soil were detected than that in the absence of tubifex treatment during the whole 14-day exposure period. This means that tubifex has positive functions in fipronil's diffusion from soil to overlying water and in the degradation of the soil-associated fipronil.

1. Introduction

Fipronil,(*R*,*S*)-5-amino-1-(2,6-dichloro-a,a,a-trifluoro-*p*-tolyl)-4-trifluoromethyl-sulfinylpyrazole-3-carbonitrile, discovered in 1987 by the French company Rhône-Poulenc Agro [1], has a chiral center in its molecular structure, so it is one member of the chiral pesticide family which accounts for more than 25% of all agrochemicals used. As is well known, chiral compounds consist of at least two non-superimposable mirror images called enantiomers, structural mirror images were designated as *R* or *S*. Based on their rotation of plane-polarized light relative to a 1:1 (±, racemic) mixture of the two, enantiomers are also identified as (+) and (−). Fipronil molecules consist of a pair of enantiomers (Fig. 1), *R*-enantiomer with left optical (−) rotation and *S*-enantiomer with right optical (+) rotation [2,3], because of the presence of an asymmetric sulfoxide in the chemical structure. Previous studies have shown that the enantiomers of a chiral pesticide have identical physical and chemical properties but they may perform different behavior in the processes of absorption, accumulation and degradation when confront with chrial environment [3–7]. This makes it necessary to study the behavior of the two enantiomers of fipronil individually. It has been proved that the direction and magnitude of enantioselectivity of fipronil varied across species [2,4–7], highlighting the need of examinations on a wider range of nontarget species, including aquatic vertebrates. This helps people to understand the effects of fipronil more comprehensively on the environmental safety and public health.

Fipronil classified as a phenylpyrazole insecticide has greatly increased in popularity in recent years. It has a broad-spectrum for control of insect pests in both agricultural and domestic settings, such as lepidopterous pests, coleopterous larvae, termite, flea, and fire ant [8]. Due to insect resistance and restrictions on organophosphate (OP) insecticides, fipronil became a promising substitute products [9,10]. The other reason for fipronil's popularity is that it has a high degree of selectivity between insect and mammalian nerve cells [11]. Fipronil elicits its toxicity by hindering the GABA-gated chloride channel in the nervous system, resulting in loss of neuronal signaling, hyperexcitation, spasm, and ultimately death [12]. Distinction in receptor-binding affinities between vertebrates and invertebrates lead to a high degree of selective toxicity of fipronil to insects [13–15], with inhibitory effects reported to be more than 500 times greater in invertebrates compared to mammalian targets [15].

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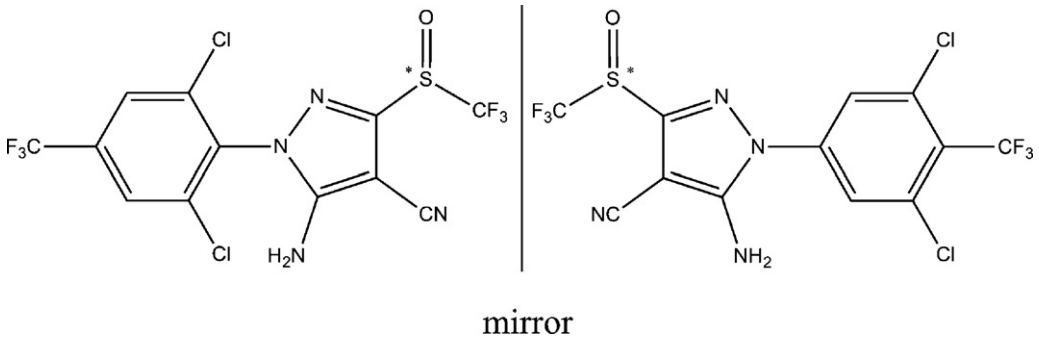

Fig. 1. Structure of fipronil enantiomers. '*' Indicate the chiral center.

Like other agrochemicals, fipronil can enter into aquatic system via direct spraying, rain wash and surface runoff. With the wide use of fipronil, it has been increasingly detected in aquatic systems [16]. Because of fipronil's relatively high hydrophobicity, with a log octanol–water partition coefficient ($\log K_{OW}$) of 3.9–4.1 and an organic carbon partition coefficient (K_{OC}) of approximately 800, there is a tendency for fipronil residue to be associated with the bed sediment once in a surface water body [17]. The sorption coefficient K_d of fipronil to sediments was detected to increase with increasing organic carbon contents and contact time [18], potentially reducing toxicity and risk to aquatic species such as fish. But when taking benthic organisms into consideration which use sediment and organic matter as a food resource and habitat, the increase of fipronil in sediment may potentially be more risky. Meanwhile benthic organisms may accumulate these sediment-associated chemicals and then pose a risk to higher trophic level organisms via food chain [19] and help these compounds input into environment again through bioturbation. In order to evaluate the environmental risk of fipronil comprehensively, it is necessary to consider that it has an effect on benthic organisms.

Tubifex tubifex is an aquatic oligochaete (Tubificidae), discovered in ecosystems contaminated by organic matter with a low level of oxygenation. These worms are very widely distributed and frequently dominant in freshwater benthic communities [20]. They have an intimate contraction with the solid phase and the pore water of the sediment, burrowing the anterior part in the sediment and undulating the posterior part in the overlying water. Thus this worm is particularly exposed to environmental pollutants, via sediment, pore water, and water column, through ingestion and/or epidermal contact. Previous studies have shown that tubifex exhibited a high level of resistance to hostile environment, especially organic pollution associated with severe hypoxic treatments [20]. Because of the endobenthic lifestyle and stress resistance, *T. tubifex* has been designated as a representative freshwater benthic infauna for aquatic system bioassays. Several papers have been reported that *T. tubifex* was utilized in both sediment toxicity and bioaccumulation study [21–23]. But data about enantioselective bioaccumulation of fipronil enantiomers in *T. tubifex* has not been reported.

The aim of this experiment was to study the differences in bioaccumulation behavior of individual enantiomers of fipronil in tubifex tissue. In this paper, a method for extraction, cleaning, and detection of fipronil enantiomers in tubifex tissue was developed. We compared the influence of two different contamination sources, including spike–water and spike–soil, on bioaccumulation of fipronil, and the results showed that bioaccumulations of fipronil were both enantioselective in the two contamination treatments. In addition, we evaluated the effects of the tubifex on diffusion and degradation of fipronil.

2. Materials and methods

2.1. Chemicals and reagents

The analytical standard of *rac*-fipronil (>99.0% purity) was provided by the China Ministry of Agriculture Institute for Control of Agrochemicals. 2-Propanol and *n*-hexane were obtained from Fisher Scientific (Fair Lawn, NJ) and were of HPLC grade. Ethyl acetate, acetone, and acetonitrile (analytical grade) were purchased from commercial sources.

2.2. Origin and maintenance of the worms

T. tubifex was obtained from Beijing Da Senlin Flower Market (Beijing, China). Worms were maintained in 2 L plastic tanks containing uncontaminated soil and deionized water at 21 ± 1 °C with 12 h light:12 h darkness. The water was continuously aerated and 75% replaced weekly. The worms were fed with tetraMin Flakes (Tetra Werke, Melle, Germany) weekly. For the experiments, adult *T. tubifex* (aged 5–7 weeks) was used. Before the worms were introduced to the treatments, they were allowed to live in these kinds of uncontaminated environment for 1 week to acclimate.

2.3. Soil collection, handling, spiking

The experimental substrate was a terrestrial soil collected from BaiWang Forest National park northwest of Beijing, China, that had not been treated with fipronil in the last 5 years. After the superficial layer of 1–2 cm was removed, the soil was collected to a depth of 10 cm, and then sieved through 500 μm mesh and air-dried at room temperature keeping in the dark until used within a few days. Physicochemical properties of the soil were as follows: organic carbon (OC), 2.79 ± 1.46%; moisture content (MC), 1.66%; clay, 3.35 ± 0.02%; sand, 60.47 ± 0.25%; silt, 36.19 ± 0.22%; pH, 6.6 ± 0.2.

To disperse the test substances fipronil homogeneously within the 100 g dry wt soil, we did the dilution spike procedure in steps [24]. First, racemic fipronil was dissolved in acetone to make a stock solution at a concentration of 1000 mg/L. And then, 5 mL of acetone solution was added dropwise into the dry soil (100 g), meanwhile, the mixing manually continued for about 5 min with a stainless-steel lab spoon, giving a nominal fipronil concentration of 50 mg/kgdwt. The spiked soil was left in a fume cupboard overnight. After complete evaporation of the solvent, the contaminated soil (100 g dry wt) was transferred to a 500 mL beaker and rehydrated with 100 g deionized water, the height of bottom substrate was 2–3 cm and the overlying water was 2–3 cm. The test vessels were incubated for a 24-h equilibration period. Prior to adding tubifex, four 50 mL samples of wet soil were weighed, dried

at room temperature until the weight did not change any longer and then reweighed to estimate the moisture content.

2.4. Bioassay procedure

In order to examine the influence of different uptake pathways of fipronil on the total bioaccumulation in tubifex, two types of uptake kinetics were examined resulting from spike–water and spike–soil treatment, respectively. The first scenario was called {+Tub + water}, in which fipronil was accumulated from spiked water. For each individual experiment, acclimated tubifex (5 g) were placed into beakers (twenty four beakers, eight sampling points, triplicates for one sampling point) and the spiking solutions were made by adding fipronil dissolved in acetone to deionized water so that the final concentration was 10 mg/L. After an exposure period (1, 2, 3, 5, 6, 7, 8 and 9 days) worms (5 g) were removed from the beaker, gently passed through a 500 μm sieve to a clean pan, and then washed with deionized water three times. At last, the peripheral water of the worm samples were dried using absorbent paper and samples were weighed before stored at −20 °C. In the present study, uptake from aqueous phase only was studied for 9-day exposure, and this treatment was carried out in semi-static conditions with renewing the water every day.

A second scenario included tubifex, water and spiked soil was called {+Tub + soil}, in which fipronil may be accumulated from overlying water, pore water, and ingestion of soil particles. Acclimated tubifex (5 g) were added to the test beaker containing unspiked water and spiked soil (twenty seven beakers, nine sampling points, triplicates for one sampling point). For the treatment {+Tub + soil}, test organisms, overlying water and soil were also sampled after an exposure period (1, 2, 3, 4, 5, 7, 9, 11, and 14 days). At each sample point, overlying water was gently poured and sampled firstly. Then the beakers were placed on the ice for 2 h, in this period tubifex climbed to the soil surface and intertwined together slowly, at this moment worm aggregation was sampled with forceps and rinsed in deionized water. Water on the surface of the worms was dried by absorbent paper cautiously. Soil samples, overlying water and tubifex sampled from each beaker were weighed and frozen at −20 °C.

To compare with the second scenario, a separate experiment (negative control) only included water and spiked soil was carried out (twenty seven beakers, nine sampling points, triplicates for one sampling point), which was called {−Tub + soil}. The sampling times were also 1, 2, 3, 4, 5, 7, 9, 11, and 14 days, respectively. At these sampling points, overlying water and aliquots of 10 g soil (base on dry weight) were removed from each beaker and transferred into 50-mL plastic centrifuge tube for extraction and analysis.

For the treatment {+Tub + soil} and {−Tub + soil}, the test beakers were weighed daily, and the loss of water resulting from evaporation was compensated by addition of deionized water. All of the beakers were cultured in the dark environmental chamber; temperature was controllable to 20 ± 2 °C. Each treatment beakers were arranged in a randomized block design.

2.5. Samples abstraction

The overlying water samples (25 mL) were extracted by fifty milliliters of ethyl acetate in glass separatory funnels after getting rid of solid matters by vacuum filtration. This extraction was repeated twice using fresh solvent. The combined solvent phase was filtered through 5 g of anhydrous sodium sulfate for dehydration, transferred to a pear-shaped flask, and then evaporated to dryness at 35 °C by vacuum rotary vaporator. The dry extract was re-dissolved to 1.0 mL with 2-propanol for analysis on liquid chromatography.

The wet soil samples were extracted by ethyl acetate, and interfering substances were cleaned up by Alumina-N-solid-phase extraction (SPE) (500 mg) on a cartridge (6 mL). Briefly, soil samples (16.7 g_{wwt} per sample), twenty-five milliliters of ethyl acetate and 2 g of anhydrous sodium sulfate were added to a 50 mL polypropylene centrifuge tube. The tube was capped, vortex-mixed for 3 min, and centrifuged at 3500 rmp for 5 min. The extract was filtered through 5 g of anhydrous sodium sulfate for dehydration, and transferred to a pear-shaped flask. The remaining part was extracted again following the same extraction step. And then the combined extract concentrated to dryness on a vacuum rotary evaporator at 35 °C. The SPE cartridge was preconditioned by eluting with 5 mL of ethyl acetate followed by 5 mL of *n*-hexane and equilibrated with 10 mL of 1:4 ethyl acetate: *n*-hexane. The sample of dry extract was recovered in 2 mL of 20% ethyl acetate in *n*-hexane, and then, the solution was passed through the SPE cartridge. The cartridge was eluted with additional 8 mL of 1:4 ethyl acetate: *n*-hexane. The eluates were combined with the loading eluates. The combined 10 mL of eluates was collected in a glass tube, evaporated to dryness under a stream of nitrogen, and diluted to 1.0 mL with 2-propanol after passed through a filter membrane (pore size, 0.45 μm).

The samples of tubifexs were thawed for about 15 min at room temperature. Twenty milliliters of ethyl acetate was added to a 50 mL polypropylene centrifuge tube containing 5 g worms. The mixture was homogenized with Ultra-Turrax T18 homogenizer for 30 s, vortex-mixed for 5 min, and then separated by centrifugation at 3500 rpm for 5 min. The upper organic phase was passed through a funnel with about 5 g of anhydrous sodium sulfate to a pear-shaped flask. The extraction was repeated two more times. The combined extracts were evaporated to dryness at 35 °C, reconstituted in 5 mL acetonitrile, and then 3 mL × 5 mL of *n*-hexane was added for liquid–liquid partition to extract most of lipid. The upper layer of *n*-hexane was discarded, and the layer of acetonitrile was evaporated to dryness by vacuum rotary evaporator. The purification process was the same as the soil samples described above.

2.6. Chemicals analysis

Chiral analysis was performed on an Agilent 1200 Series HPLC, equipped with G1322A degasser, G1311A pump, G1329A ALS and G1314B VWD. Column temperature was controlled by AT-930 heater and cooler column attemperator (Tianjin Automatic Science Instrument Co., Ltd., China). The two enantiomers of fipronil were separated on cellulose-tri-(3,5-dimethylphenylcarba- mate)-based chiral column (CDMPC-CSP, provided by the Department of Applied Chemistry, China Agricultural University, Beijing). The chiral column 250 mm × 4.6 mm (I.D.) was prepared by our group according to the procedure described in the literature [25]. Racemic fipronil was ideally baseline separated on the CDMPC-CSP that reported in the previous reports [3]. The HPLC method we employed was successful to analyze enantiomers of fipronil in overlying water, soil and tubifex samples. A mixture of *n*-hexane and isopropanol (98:2, v/v) was used as mobile phase and the flow rate was 1.0 mL/min. The injection volume was 20 μL, and the UV detection wavelength was 230 nm. The column attemperator was performed at 20 °C. No enantiomerization was observed for fipronil under this analytical condition. The average recoveries for both enantiomers at levels between 0.5 and 25 mg/kg ranged between 91 and 105% in overlying water, between 81 and 93% in tubifex tissue and between 84 and 96% in soil with SD below 10% ($n = 3$ for each sample type). The limit of detection (LOD) for both enantiomers, defined as the concentration that produced a signal-to-noise ratio of 3, was 0.1 mg/kg both in overlying water and soil, and 0.4 mg/kg in tubifex tissue.

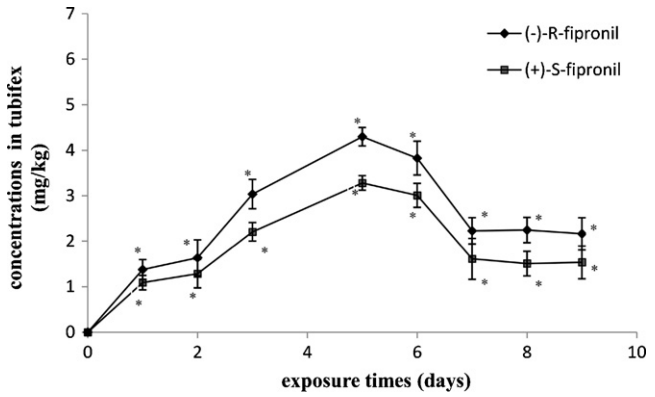

Fig. 2. Accumulation curves for fipronil enantiomers in tubifex tissue in spike–water treatment (bars are standard error). '*' Indicates significant difference between the two enantiomers at the same time point ($P < 0.05$, Duncan's multiple range test).

2.7. Data analysis

The enantiomer fraction (EF) was used to measure the enantioselectivity behavior of fipronil in our experiment. The EF values defined range from 0 to 1, with EF = 0.5 representing the racemic mixture. EF was expressed as follows: EF = peak area of (−)/[(−) + (+)], where (−) is the first eluted chromatograph peak of (−)-*R*-fipronil and (+) is the second eluted peak of (+)-*S*-fipronil.

Date presented corresponds to means ± standard deviations of three independent experiments ($N = 3$). Statistical analysis for the enantioselectivity of fipronil enantiomers was performed using SPSS 16.0. A one sample *t*-test was used to compare the means of the EF values in tubifex and soil samples with EF = 0.5. The concentrations and AFs of the two enantiomers of fipronil were analyzed using one way analysis of variance (one-way ANOVA), and pair wise multiple comparison procedure (Duncan's multiple range test) were used to compare results at $P < 0.05$.

3. Results and discussion

3.1. Enantioselective bioaccumulation detection in spike–water treatment

For the treatment {+Tub + water}, in which water was the sole source of contamination and living environment, concentrations of the two enantiomers of fipronil in tubifex tissue were detected. The accumulation curves of the two enantiomers in tubifex were shown in Fig. 2, and peak-shaped accumulation curves were observed for both enantiomers, Concentrations of the two enantiomers both reached the highest level on 5th day. But a decrease of concentration was observed between 5 and 7 days of exposure. It may be concerned with a development of detoxification in tubifex tissue to metabolize and excrete fipronil. Finally, the concentrations tended to stability after 7 days. In addition, a significant difference was observed between the concentrations of two enantiomers in tubifex tissue at the same sample point, with concentrations of the *R*-(−)-fipronil higher than that of the *S*-(+)-fipronil. Therefore, enantioselectivity occurred when the two enantiomers of fipronil were accumulated by tubifex. Meanwhile the EF values were calculated, and the data were showed in Table 1. The EF values in tubifex tissue were observed to deviate from 0.5 in the bioaccumulation experiment. A one sample *t*-test was carried out to compare the means of the EF values in tubifex with EF = 0.5, and a significant difference ($P < 0.005$) between EF values and 0.5 were detected. So the bioaccumulation of fipronil in tubifex tissue for this treatment was enantioselective.

In this work, we used AF (accumulation factor) to express the bioaccumulation of fipronil in tubifex tissue. AF is a function of the relative sorptive capacities of the organism versus the surrounding environmental, and for this treatment ({+Tub + water}) it was defined as:

$$\mathrm{AF} = \frac{C_{\mathrm{worm}}}{C_{\mathrm{water}}}$$

where C_{worm} and C_{water} are concentrations of fipronil enantiomers in tubifex and water, respectively. The AF value at each sampling date was plotted against time. As shown in Fig. 3A, the AF values of *R*-(−)-enantiomer were larger than that of *S*-(+)-enantiomer, indicating that the *R*-(−)-enantiomer was preferentially accumulated over the *S*-(+)-enantiomer in tubifex tissue, and a significant difference was observed between the two enantiomers. It can be concluded that the concentration of fipronil in tubifex tissue accumulated via single skin exposure was enantioselective.

3.2. Enantioselective bioaccumulation detection in spike–soil treatment

For the treatment {+Tub + soil}, spiked soil acted as the initial contamination source. In this group, tubifex can accumulate fipronil via the skin and ingestion exposure routes. The concentrations of fipronil enantiomers in tubifex tissue were detected, and a significant difference was observed (Fig. 4). The concentrations of *R*-(−)-enantiomer in tubifex tissues were higher than that of *S*-(+)-enantiomer during the whole exposure course. Through a one sample *t*-test that compared the means of EF values in tubifex with EF = 0.5, a significant ($P < 0.001$) deviation of EF values from 0.5 was detected, as shown in Table 2. The deviation of EF values from 0.5 in tubifex tissue showed that bioaccumulation of fipronil was enantioselective. In addition, the accumulation model in this group was more complicated than that in the spike–water treatment. Concentrations in tubifex tissue reached the highest level on 4th day. After a short-time decrease, the concentrations increased gradually and got the maximum values again on 9th day. Thereafter, concentrations declined and reached steady state as the duration of exposure increased.

For this treatment {+Tub + soil}, the AF was defined as:

$$\mathrm{AF} = \frac{C_{\mathrm{worm}}}{C_{\mathrm{soil}}}$$

where C_{worm} and C_{soil} are concentrations of fipronil enantiomers in tubifex and soil, respectively. In Fig. 3B, we plotted the AF values against time. Data analysis based on a Duncan revealed a significant difference between the AF values of the two enantiomers, resulting in relative enrichment of the *R*-(−)-form. Furthermore, the AF values in {+Tub + water} treatment were lower than that in this treatment during the whole exposure period. The higher AF values may result from the different uptake pathways. For

Table 1
EF (EF = *R*/(*R* + *S*), mean ± SD) of fipronil accumulated in tubiex tissue in spike–water treatment.

	Exposure time (days)							
	1	2	3	5	6	7	8	9
Enantiomer fraction EF	0.56 ± 0.01	0.56 ± 0.02	0.58 ± 0.02	0.57 ± 0.01	0.56 ± 0.02	0.58 ± 0.01	0.60 ± 0.01	0.59 ± 0.02

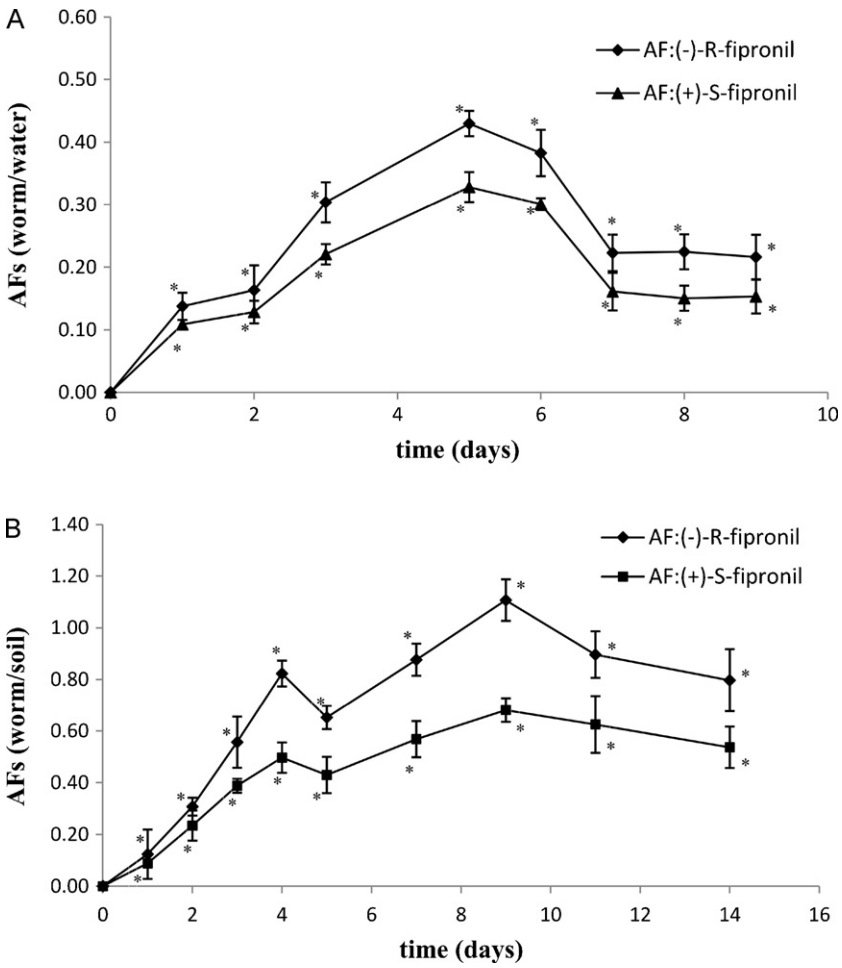

Fig. 3. The Calculated accumulation factors (AFs) for the two enantiomers of fipronil, (bars are standard error). '*' Indicates significant difference between the two enantiomers at the same time point ($P < 0.05$, Duncan's multiple range test).

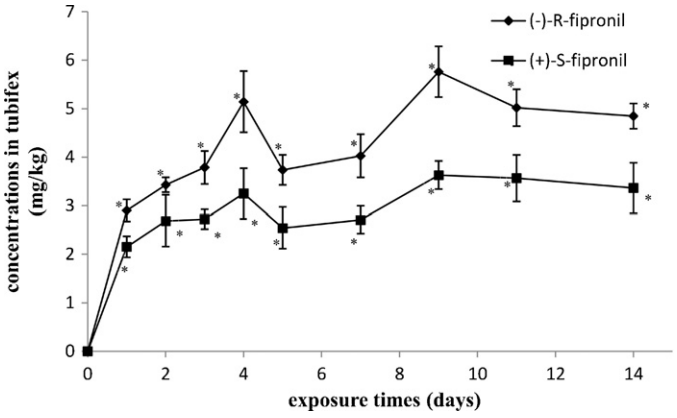

Fig. 4. Accumulation curves for fipronil enantiomers in tubifex tissue for spike–soil treatment (bars are standard error). '*' Indicates significant difference between the two enantiomers at the same time point ($P < 0.05$, Duncan's multiple range test).

{+Tub + water} treatment, the exposure route was contraction of epidermis. While for {+Tub + soil} treatment, bioaccumulation of fipronil may be through overlying water, pore water, and ingestion of soil particles.

3.3. Influence of bioturbation

The concentrations of fipronil in overlying water samples for {+Tub + soil} and {−Tub + soil} treatments were shown in Fig. 5. The concentrations of fipronil were almost constant during the experimental period for the two treatments respectively, and no enantiosective phenomenon of fipronil enantiomers appeared in the two treatments, but a significant difference was discovered with respect to concentrations of *rac*-fipronil between the worm-free and worm-present groups. The explanation could be that fipronil passed through the spiked soil into the overlying water by diffusion processes alone in {−Tub + soil} treatment, while the concentrations of fipronil in the overlying water was the conjunction of diffusion and bioturbation processes for {+Tub + soil} treatment. Therefore, bioturbation has a power-assisted effect when fipronil

Table 2
EF (EF = $R/(R+S)$, mean ± SD) of fipronil accumulated in tubiex tissue in spike–soil treatment.

	Exposure time (days)								
	1	2	3	4	5	7	9	11	14
Enantiomer fraction EF	0.57 ± 0.02	0.56 ± 0.01	0.58 ± 0.03	0.61 ± 0.05	0.59 ± 0.03	0.60 ± 0.04	0.613 ± 0.05	0.58 ± 0.03	0.59 ± 0.01

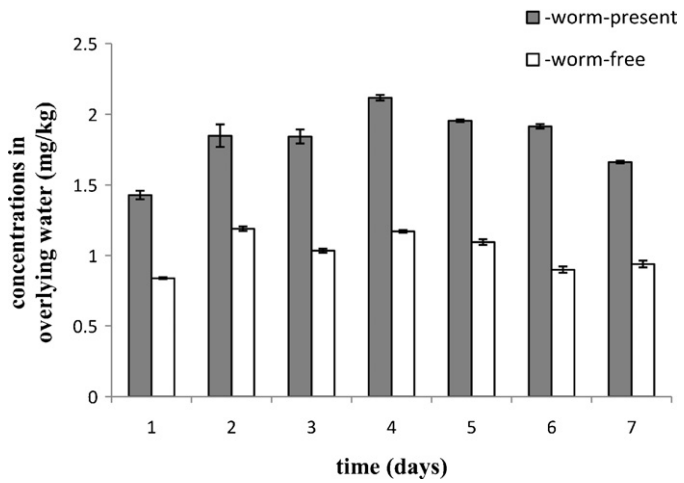

Fig. 5. Concentrations of fipronil in the overlying water (bars are standard error).

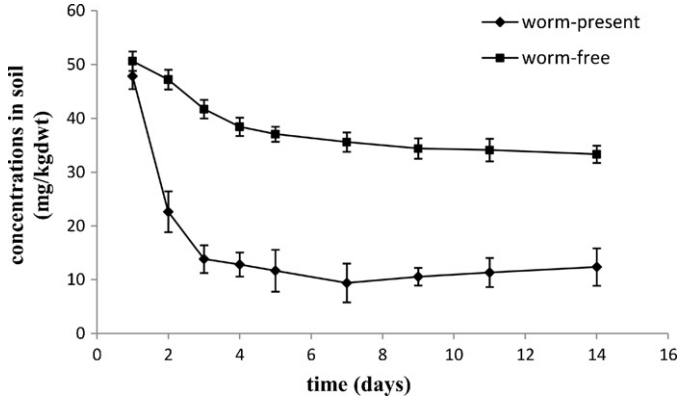

Fig. 6. Degradation curves of fipronil in spiked soil (bars are standard error).

diffused from spiked soil into overlying water and altered the partitioning of fipronil, resulting in the increase of bioavailablility to other aquatic organism. This result was in agreement with previous work on Cd, with the presence of meiofauna such as benthic *harpacticoid copepods* or *foraminiferans meiofauna*, the amounts of Cd became larger in the pore water [26].

3.4. Degradation of fipronil in soil

The decline of fipronil concentration in soil over time was plotted in Fig. 6 for worm-free and worm-present treatments ({+Tub + soil} and {−Tub + soil}), respectively. No enantioseletive behavior was detected in the degradation of the fipronil in soil for the two treatments. Under the {−Tub + soil} experimental conditions, fipronil displayed high persistence in soil, with 34.16% of fipronil was degraded after incubated for 14 days. For the {+Tub + soil} treatment, the existence of tubifex may be an important factor on influencing fipronil's degradation in soil, with 80.42% of fipronil was degraded after incubated for 14 days. Degradation of fipronil under worm-present condition was consistently much faster than that, under worm-free condition. So tubifex play an important role in refining the contaminated soil.

4. Conclusion

In this study, we found that enantioselectivity occurred in both {+Tub + water} and {+Tub + soil} treatments when chiral compound fipronil was accumulated in tubifex tissue. The results showed that the *R*-(−)-fipronil was preferentially accumulated over the *S*-(+)-fipronil in tubufex tissue, and significant differences were observed between the two enantiomers in both two treatments. However, because of the difference in exposure routes, the deviation level of EF, equilibrium time and AF were distinct in these two groups. The concentration of fipronil in overlying water was higher in {+Tub + soil} treatment than in {−Tub + soil} treatment. In other words, the function of bioturbation was significant during the process of fipronil's diffusion from soil to overlying water. Moreover, the degradation rate of fipronil in {+Tub + soil} treatment was higher than that in {−Tub + soil} treatment.

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