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The toxicity, bioaccumulation, elimination, conversion of the enantiomers of fipronil in *Anodonta woodiana*

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HIGHLIGHTS

- The bioaccumulation and degradation of fipronil in *A. woodianas* on an enantiomeric level were studied.
- Enantioselective impacts of fipronil and the three metabolites on *A. woodianas* were determined.
- Enantiomeric conversion of R-fipronil to S-fipronil was found by *A. woodianas.*

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ABSTRACT

The enantioselective bioaccumulation and elimination of fipronil in *Anodonta woodiana* (*A. woodiana*) were studied and the main metabolites fipronil desulfinyl, fipronil sulfide and fipronil sulfone were determined. The acute toxicity of the enantiomers of fipronil and the three metabolites were also investigated. In the bioaccumulation process, fipronil in *A. woodiana* reached equilibrium after 11 days with BCF value of 0.2, and the enantiomeric fraction (EF) values showed that the bioaccumulation was enantioselective with enantioenrichment of S-fipronil. The degradation of fipronil in *A. woodiana* fitted first-order kinetics model with half-lives of the enantiomers were 5.8 d for R-fipronil and 7.6 d for S-fipronil, and the EF values decreasing from 0.5 gradually indicating the R-enantiomer was preferentially degraded. The degradation of single enantiomers was also performed and the results revealed a fast conversion of R-fipronil to S-fipronil by *A. woodiana*. The three metabolites were all detected in *A. woodiana*-water system, in which fipronil sulfone and fipronil sulfide had higher concentration levels. According to the 72-h LC_{50} values, S-fipronil was much more toxic than the racemate and R-fipronil. Moreover, the metabolites were more toxic than the parent fipronil. The results suggested the individual enantiomers of chiral pollutants and the metabolites should be considered in the risk assessments.

1. Introduction

Enantiomers of chiral compounds commonly differ with respect to their biological properties. This may concern not only a desired biological activity but also unintended, human or environmental adverse effects [1,2]. Most chiral pesticides are still applied as racemic mixtures. According to the European regulation 1107/2009, to address enantiosepecific characteristics in the risk assessment of enantiomers in environment, data are needed regarding the toxicological endpoints of individual enantiomers, the enantioselectivity of pesticide metabolism in aquatic environment, and the enantiomers composition of the residues as well [3].

Fipronil is a chiral, highly active, broad spectrum insecticide from the phenylpyrazole family. In agricultural applications, this pesticide has been used on pests of a wide variety of food crops [4–6]. Fipronil bioactivity is attributed to its ability to act at the γ-aminobutytic acid (GABA) receptor as a noncompetitive blocker of the GABA-gated chloride channels of neurons in the central nervous system [7–9]. Moreover fipronil has an asymmetric center of sulfur atom resulting in the chemical structure and thus has a pair of enantiomers that have been identified as S-fipronil and R-fipronil, in which R-fipronil is more active than S-fipronil against some target insects [10]. Due to its frequent application, fipronil has been detected in surface water at concentrations presenting a potential threat to aquatic organisms. Besides, the introduction of chiral chemicals into aquatic ecosystems and their subsequent

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enantioselective transformation may result in changes in the enantiomeric composition and toxicity, since the enantiomers of the same compound may differ in their degradation rates, biological activity and toxicity. Previous studies have shown fipronil exerted acute toxicity to aquatic invertebrates at very low concentrations, for example the LC_{50} (96 h) for clam (*Mercenaria mercenaria*) was 177.00 $\mu g\,L^{-1}$ and EC_{50} (96 h) for phytoplankton (*Dunaliella tertiolecta*) was 631.20 $\mu g\,L^{-1}$ [11]. Recently, increasing attention has been paid to the behaviors of fipronil enantiomers in the environment. Researches have shown S-fipronil is more toxic to *Procambarus clarkia* and *Ceriodaphnia dubia* than R-fipronil [11,12]. Other studies concerning enantioselective of sorption, degradation [13], and toxicology as well as biotransformation [12,14,15] of fipronil in fish or sediments have been reported.

Fipronil undergoes extensive photolysis to fipronil desulfinyl derivative in aquatic environments [16]. Moreover, fipronil can undergo biological oxidation or reduction to its respective sulfone and sulfide metabolites (fipronil-sulfone and fipronil-sulfide). The metabolites have also been detected in surface water such as in the Sakura River (Ibaraki perfecture, Japan) and showed equal or more toxic potential and more stable during environmental processes [16,17]. Fipronil sulfide is mainly formed in soil [5] and sediment [18] and is likely a result of microbial transformation. Fipronil sulfone was found to be the main metabolite in rats and mice [19]. In addition, fipronil desulfinyl was reported to be two times more lethal to daphnids than fipronil and more persistent in environment [20,21]. Hence, the long-term side effects of fipronil may partly owe to the metabolites.

Anodonta woodiana is a widespread aquatic organism, which could be exposed to wide range of pollutants [22,23]. Becker et al. used bivalves as sentinel organism to assess the contamination of freshwater ecosystems by organotin tributyltin and its degradation product triphenyltin [24]. Alves et al. also reported that the herbicide 2,4-Dichlorophenoxyacetic acid could be accumulated by *Anodonta cygnea* and affected the shell maintenance and growth [25]. Although metabolism and environmental fate of fipronil have been extensively studied, data related to enantiospecific biodegradation/biotransformation of fipronil in environments and aquatic biota is still limited. Understanding fipronil's fate and metabolites formation, especially the enantiospecific toxicology to non-target aquatic organism is essential for risk assessment, thus further investigation of the enantioselective environmental behaviors of this insecticide across a wider range of non-target aquatic species were required.

The main objective of this work is to 1) investigate the bioaccumulation, elimination and biotransformation of racemic fipronil and the individual R- and S-enantiomer of fipronil in *A. woodiana*-water system with respect to their stereoselective process, 2) compare the toxicities of parent compound and metabolites, and so as for the first time, enantiomeric conversion was studied by single-enantiomer exposure. The results will be useful to enrich the research of chiral insecticides for more accurate risk assessment of this pesticide.

2. Materials and methods

2.1. Chemicals and animals

Rac-fipronil (96.5% pure) was provided by China Ministry of Agriculture Institute for Control of Agrochemicals. Fipronil desulfinyl (97.9% pure), sulfide (99.4% pure), and sulfone (98.6% pure) were purchased from AccuStandard, Inc. R-fipronil (99.5%) and S-fipronil (99.4%) were prepared by a preparative HPLC equipped with a Chiralcel OD chiral column (Daicel Chiral Technology Co., Ltd.). HPLC analysis in this work was performed using Agilent 1200 series HPLC (Agilent Technology). All the reagents were of analytical grade and purchased from Beijing Chemical Reagent Co., China.

A. woodiana was obtained from Beijing Xi Yuan Aquaculture Market (Beijing, China), and reared in glass aquariums (60 cm × 45 cm × 30 cm) containing 5 L of deionized water at 25 ± 1 °C with 12 h light/12 h darkness. The water was continuously aerated. The *A. woodianas* were fed with freshwater algae (*Scenedesmus acuminatus*) and allowed to acclimatize for 1 week prior to the experiments.

3. Experimental design

3.1. Acute toxicity determination

The acute toxicity test 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) [26]. The toxicity of rac-fipronil, R-fipronil, S-fipronil and the three metabolites fipronil desulfinyl, fipronil sulfide and fipronil sulfone to *A. woodiana* was conducted. 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. The concentration of 0.01 $mg\,L^{-1}$ was stand for the maximum non lethal dose of compound to *A. woodiana*, and the concentration of 15.0 $mg\,L^{-1}$ was stand for the minimumlethal dose of compound to *A. woodiana* during the toxic test. Twenty replicates for each treatment were performed. *A. woodiana* spiked with 10 μL acetone solution but without the toxicant as control group was set up. Mortality was recorded after incubation for 72 h, and LC_{50} values were calculated using SPSS Version 18.0 (SPSS Inc, Chicago, USA).

3.2. Bioaccumulation

For the bioaccumulation experiment, the concentration of rac-fipronil in water was 100 $\mu g\,L^{-1}$ throughout a 16-d exposure period. Water was replaced daily to maintain a constant concentration (100 $\mu g\,L^{-1}$). Ten *A. woodianas* were raised in glass aquariums with 20 L of water, and each aquarium was used as one sample point. Three *A. woodianas* (about 10 g in total) were randomly collected and 10 mL of water were sampled at days 0, 1, 3, 5, 7, 11, 16 and each sampling event was conducted in triplicate. All the samples were stored at −20 °C before analysis.

3.3. Elimination

After a 16-day bioaccumulation, the water and compound were not replaced in order to evaluate the degradation of the fipronil enantiomers. Samples were collected in the same way in 3.2 at days of 17, 19, 21, 23, 27, 32, 38, 46. The degradation of rac-fipronil in water (without *A. woodianas*) was also conducted under identical conditions to verify the stability of fipronil. The samples were frozen at −20 °C until analyzed.

3.4. Enantioselective degradation of fipronil and enantiomeric transformation

Ten of *A. woodianas* were kept in 20 L of water in glass aquariums and exposed to rac-, individual single enantiomer at 100 $\mu g\,L^{-1}$ respectively. The water and compound were not replaced during the whole period. Three *A. woodiana* and 10 mL of water were sampled at days of 0, 0.5, 1, 3, 5, 7, 11, 16, 22, 30, 43, 60. The collected samples were frozen at −20 °C until analyzed. Exposure experiments were performed in duplicate. A control experiment was also