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Comparative toxicity and bioaccumulation of fenvalerate and esfenvalerate to earthworm *Eisenia fetida*

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HIGHLIGHTS

- FV and ESFV exhibited enantioselective toxicity to earthworms.
- Enantiospecific induction in oxidative stress was observed in earthworms exposed to FV and ESFV.
- The bioaccumulation of FV and ESFV in earthworm tissues was enantioselective.

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ABSTRACT

More attention is being paid to the enantioselective toxicity of chiral pesticides. However, limited investigations have been done to assess the ecological risks of chiral pesticides to soil community. Fenvalerate (FV), an extensively used synthetic pyrethroid, is a typical chiral pesticide. The most insecticidally active enantiomer of FV, esfenvalerate (ESFV), also has been marketed and widely used. In this study, the toxicological sensitivity and bioaccumulation of FV and ESFV in earthworms were assessed. The results showed that FV was less toxic than ESFV, but more accumulated in earthworms. ESFV was at least 4 times more toxic to earthworms than FV according to the filter paper contact toxicity test and the artificial soil test. Enantiospecific induction in oxidative stress was observed in earthworms exposed to FV and ESFV. The bioaccumulation of FV and ESFV in earthworm tissues was also enantioselective, preferentially accumulating FV. The uptake of ESFV by earthworms was lower than that of FV, so that the biota to soil accumulation factor (BSAF) value of ESFV was lower than that of FV. Our findings suggest that the enantioselective toxicity and bioaccumulation of chiral pesticides should be considered for evaluating ecological risks of these compounds to non-target organisms.

1. Introduction

Chiral substances exist as two mirror image isomers called enantiomers. Up to 25% of the members of pesticides in the worldwide market are chiral [1]. Nowadays, chiral pesticides account for more than 40% of pesticides usage currently in China [2]. As often as not one enantiomer of chiral pesticides is target-active, while other enantiomers are inactive or less active [3,4]. Chiral pesticides are generally produced and marketed as mixtures of enantiomers or racemates. However, the enantiomers show different activities in biological systems because of the difference in the bioaffinity of the enantiomers to a common site on enzymes or other biomolecules [5–7]. This difference may result in enantioselective degradation, bioactivity, toxicity and bioaccumulation of the enantiomers of chiral pesticides [8–10]. Therefore, the determination of the enantioselective toxicity of chiral pesticides is important for accurate assessment of their safety with respect to the environment and public health.

Fenvalerate (FV) is an extensively used pyrethroid insecticide applied to agricultural, residential and public health protection sites since 1976. FV is a typical chiral compound with two chiral centers and therefore has four enantiomers, i.e. αS-2S-FV, αR-2S-FV, αS-2R-FV, αR-2R-FV [11]. FV was first introduced into the market as racemate. The most insecticidally active enantiomer, αS-2S-FV, named as esfenvalerate (ESFV), was marketed in the United States since 1992. Previous studies have shown the enantioselective toxicity of FV to aquatic organisms [12]. According to the LC50

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values of *Daphnia magna* and zebrafish, the order of toxicity of enantiomers was as follows: αS-2S-FV (EFSV) > αR-2S-FV > αS-2R-FV > αR-2R-FV [12]. The residue of FV has been detected in the soil of agricultural areas with the concentrations range from 1 to 22 μg/kg [13]. Degradation of FV and ESFV in alkaline soil was reported to be enantioselective with half-lives of 26.1 and 27.3 days, respectively [14].

Earthworms, as one of the most prevalent organisms in soil ecosystem, are at risk of being exposed to pesticides residues since they live in close contact with soil particles [15,16]. Earthworms have been widely used to evaluate the impacts of pesticides and organic contaminants in soil. It has been shown that long-term exposure of earthworms to FV inhibited cocoon production and stimulated CYP3A4 enzyme activity [17]. The 7 days LC50 of FV for earthworm *Eisenia fetida* (*E. fetida*) in soils was reported to range from 43.49 mg/kg to 72.8 mg/kg [17,18]. Another recent study indicated that the 28 days LC50 value of ESFV was 36.0 mg/kg for earthworm *E. fetida* in a typical agricultural soil in Norway [19]. A limited number of studies have assessed the ecological risks of chiral contaminants to earthworms and demonstrated enantioselective toxicity and/or bioaccumulation of some chiral pesticides, such as α-cypermethrin, fipronil, furalaxyl, metalaxyl and benalaxyl [20–24]. However, there is still a dearth of enantiomer-specific toxicity and bioaccumulation data of racemic FV and its αS-2S-isomer ESFV on earthworms.

E. fetida is the most widely used earthworm species for ecological risk assessment according to the OECD guidelines [25]. In this study, the toxicological sensitivity of FV and ESFV to *E. fetida* was evaluated using contact filter paper toxicity and soil toxicity bioassays. The bodyweight change and oxidative damage in *E. fetida* exposed to FV and ESFV were determined. The enantioselective bioaccumulation of FV and ESFV in earthworm from soil was further assessed.

2. Materials and methods

2.1. Chemicals, reagents, and soils

FV and ESFV (≥99.5% and 98.8% purity, respectively) were purchased from Sigma, USA. Technical FV and ESFV (≥98.0% purity) were purchased from Nanjing Rongqin Chemical Co., Ltd. All the solvents were HPLC grade and purchased from Aladdin. Other chemicals were analytical grade and purchased from commercial sources.

2.2. Earthworms

E. fetida was provided by the active central earthworm breeding farm of Zhejiang University. The earthworms were maintained in an incubation chamber at 20 ± 1 °C with a relative humidity of 80–85% and a moisture content of 35% in soil. Healthy adult earthworms with well-developed clitellum and weighing 200–300 mg were selected for toxicity tests. Before exposure to chemicals, selected earthworms were rinsed with deionized water and kept on damp filter paper in petri dishes with a depuration period of 24 h in the dark.

2.3. Filter paper contact test

The filter paper contact test was performed to determine the acute toxicity of FV and ESFV to earthworm, as described in the OECD guideline 207 [25]. The test concentrations of FV and ESFV were 0 (solvent control), 0.1, 0.5, 1 and 5 μg/cm^2. One milliliter of corresponding concentration of exposure solution or acetone alone as solvent control was added to a piece of filter paper that was placed in a flat-bottomed glass vial (8 cm in length, 4 cm in diameter). After a 3 h evaporation period of acetone, 1 ml of deionized water was added to each vial to remoisten the filter paper and one mature earthworm was placed on it. Each treatment contained ten replicates. All vials containing earthworms were maintained at 20 ± 1 °C under 80–85% relative humidity. The mortality was assessed after incubation for 24 or 48 h and LC50 values were determined.

2.4. Artificial soil test

The artificial soil was prepared by mixing 10% peat, 20% kaolinite clay, 70% silica sand and moderate $CaCO_3$ to adjust the pH to about 7.0 according to OECD guideline 207 [16,25]. The concentrations of FV and ESFV range from 0 (solvent control), 0.1, 1.0, 10 and 100 mg/kg dry soil according to a pilot trial to determine the concentrations resulted in 0–100% mortality. For each tested concentration, FV and ESFV were dissolved in acetone and mixed into 10 g silica sand at first. After a 3 h evaporation period of acetone, the pesticide-treated silica sand was mixed to 490 g artificial soil. Then deionized water was added to the artificial soil to adjust the water content at 35%. After a depuration period of 24 h, 10 adult earthworms were placed in each container. Each concentration contained three replicates. All containers were maintained at 20 ± 1 °C in 80–85% relative humidity. The mortality was assessed at 2, 7, 14, 28 and 42 days after treatment. A total of 75 vessels (5 concentrations × 5 time points × 3 replicates) for each compound were applied in the mortality test. The LC_{50} values were determined by further statistical analysis.

2.5. Earthworm body weight assessment and sample collection

According to the LC50 values in filter paper contact and artificial soil tests, earthworms were treated with FV or ESFV at sublethal nominal concentrations of 0, 1, 10, 50 and 100 μg/kg in artificial soil as described above. Each treatment group contained three containers and each container contained 10 adult earthworms. A total of 75 vessels (5 concentrations × 5 time points × 3 replicates) for each compound were applied in the sublethal test. Samples of earthworms and soils were collected at 2, 7, 14, 28 and 42 days and the earthworms were weighed after each collection. Five live earthworms in each treatment group were collected and stored at −80 °C for following antioxidant enzyme assays. Five live earthworms and 10 g of the artificial soils from each vessel of 100 μg/kg groups at 5 time points were collected and stored at −20 °C for following chemical analysis.

2.6. Antioxidant enzyme assays

The earthworm samples were homogenized in Tris-HCl buffer (100 nmol/l, pH = 7.5) with the volume of four times of their weight, and centrifuged at 9000*g* at 4 °C for 30 min, as previously described [16]. The supernatant was used for the determination of the content of superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) by the commercial kits (Jiancheng Bioengineering Institute, Nanjing, China) as described previously [26,27].

2.7. Chemical analysis

After homogenization, earthworm or soil sample was mixed with 8 ml acetonitrile in a 50 ml Teflon centrifugal tube. After vortexing for 5 min and ultrasonic treatment for 20 min, the sample was centrifuged at 4500 rpm for 10 min. The extraction was repeated twice and the organic phase was collected in a new tube. The extract was concentrated to dryness by rotary evaporation and the residue was reconstituted in 2 ml hexane. After filtering through the column containing anhydrous Na_2SO_4, aluminium oxide, silica