Journal of Hazardous Materials 192 (2011) 1072–1078

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Enantiomer-specific toxicity and bioaccumulation of alpha-cypermethrin to earthworm *Eisenia fetida*

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ARTICLE INFO

Article history:
Received 18 April 2011
Received in revised form 31 May 2011
Accepted 6 June 2011
Available online 12 June 2011

Keywords:
Alpha-cypermethrin
Enantioselectivity
Toxicity
Bioaccumulation
Earthworm

ABSTRACT

Alpha-cypermethrin, a synthetic pyrethroid, is highly effective against a wide range of chewing and sucking insects in crops, and it is a racemic mixture of two enantiomers ((+)-*1R-cis-αS* + (−)-*1S-cis-αR*). Studies about the toxicity of alpha-cypermethrin to non-target organisms are mainly focused on aquatic organisms, whereas information regarding terrestrial organisms is relatively much less. Very little report about its enantioselective toxicity is known, so the present study tested the enantiomer-specific acute toxicity to earthworm *Eisenia fetida*. Experiment about bioaccumulation of two enantiomers in soil was conducted, peak-shaped accumulation curves were observed for both enantiomers, and the calculated biota to soil accumulations factor (BSAF) have significant difference between the two enantiomers. It was obvious that earthworm can uptake alpha-cypermethrin enantioselectively, preferentially accumulating (−)-(*1S-cis-αR*)-enantiomer. Great difference in toxicity to earthworm between two enantiomers was found, and the calculated LC_{50} values for (+)-(*1R-cis-αS*)-, (−)-(*1S-cis-αR*)-, and *rac*-alpha-cypermethrin were 49.53, 1663.87 and 165.61 ng/cm^2, respectively. The acute toxicity of alpha-cypermethrin enantiomers was enantioselective.

1. Introduction

Synthetic pyrethroid insecticides have been used for more than 30 years to control insect pests in various crops, since the first photostable potent, permethrin, was announced by Elliott and coworkers in 1973 [1]. Due to the existence of multiple asymmetric carbon positions, many pyrethroids contain four or eight optical isomers [2], and a good example is cypermethrin. Cypermethrin [(*RS*)-a-cyano-3-phenoxybenzyl(*1RS*)-cis-trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate], have three chiral carbon atoms at 1C and 3C in the cyclopropane carboxylic acid moiety and αC in the alcohol component and therefore consists of eight enantiomers, with two *cis* diastereomers of (−)-*1R-cis-αR* + (+)-*1S-cis-αS* and (+)-*1R-cis-αS* + (−)-*1S-cis-αR* and two *trans* diastereomers of (−)-*1R-trans-αR* + (+)-*1S-trans-αS* and (+)-*1R-trans-αS* + (−)-*1S-trans-αR*. In cypermethrin, it is known that (+)-*1R-cis-αS* and (+)-*1R-trans-αS* are the only isomers with insecticidal activity [3]. Alpha-cypermethrin consists of two of the four cis-isomers in cypermethrin, (+)-*1R-cis-αS* and (−)-*1S-cis-αR* (Fig. 1), and it was marketed as the racemic product, although the insecticidal activity is almost entirely from the (+)-*1R-cis-αS* [3]. Alpha-cypermethrin is highly effective against a wide range of chewing and sucking insects (particularly Lepidoptera, Coleoptera, and Hemiptera) in crops [4]. It is also active against mosquitoes, flies, and other insect pests in public place and animal houses [5]. Historically, it has been regarded that alpha-cypermethrin is nontoxic to birds but is highly toxic to fish and aquatic invertebrates [6]. This is mainly because it is metabolised and eliminated significantly more slowly by fish than mammals or birds [7–9]. However, the high degree of toxicity to aquatic organisms observed in standard laboratory studies is less likely to be found under field conditions [10]. The reason for the reduction of toxic effects in exposure is that alpha-cypermethrin rapidly and extensively sorb to suspended particulate matter, sediments, and aquatic plants [11,12]. Although the adsorption mitigates the detrimental effects on aquatic organisms significantly, it raises concerns about the potential influence of those chemical residues adsorbed to soil and sediment. Alpha-cypermethrin is commonly used in agriculture in China, and a large portion of application ends up as residuals in soil, posing potential threats to soil ecosystem. Earthworms live in close contact with soil particles, may represent up to 80% of the total soil biomass, and therefore an important testing species. *Eisenia fetida* is the most common species used for acute and chronic ecotoxicity assays according to the OECD guidelines [13], and also is useful as a sentinel organism to survey the quality of the terrestrial environment [14].

Studies about the toxicity of alpha-cypermethrin are mainly focused on aquatic organism, whereas few information regard

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doi:10.1016/j.jhazmat.2011.06.010

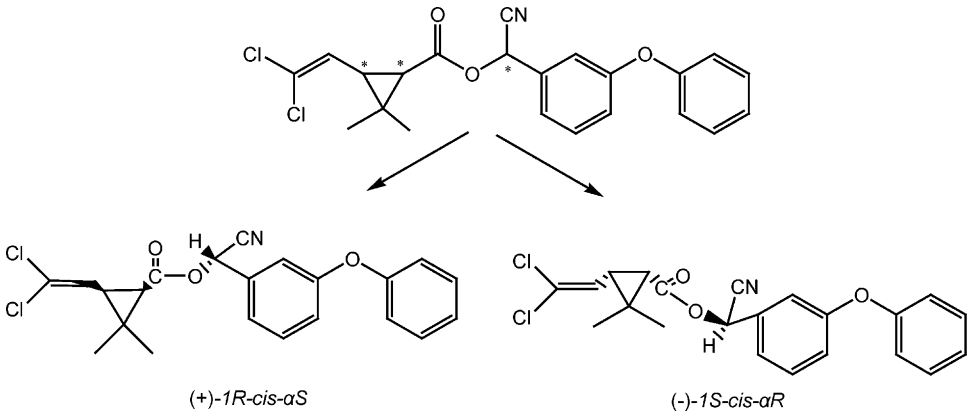

Fig. 1. Chemical structures of two enantiomers from alpha-cypermethrin.

terrestrial organisms. Toxicity of alpha-cypermethrin to aquatic non-target organisms, such as guppy (*Poecilia reticulata*), tilapia (*Oreochromis niloticus* L.) larvae and *Daphnia magna* (*Ceriodaphnia dubia*) has been studied [15–17]. Alpha-cypermethrin is one of chiral pesticides that are manufactured and applied to agro-ecosystems as racemic forms, although the individual enantiomers may show differences in bioactivity, toxicity, metabolism, bioaccumulation and degradation behaviors [18–20]. Therefore, enantioselective behavior assessment of alpha-cypermethrin is crucial. In previous study, Hartnik et al. [21] reported the toxicity of alpha-cypermethrin to earthworm *E. fetida*, but this study provided no information on the enantioselectivity of alpha-cypermethrin. The lack of information on the earthworm toxicity and bioaccumulation of each enantiomer of alpha-cypermethrin is an important knowledge gap.

Enantioselective bioaccumulation and toxic effects in earthworm have been observed for various chiral pesticides. For example, the bioaccumulation of benalaxyl was enantioselective in earthworm tissue, and the calculated LC_{50} of *S*-(+)-benalaxyl enantiomer was about 2 times of that for *R*-(−)-benalaxyl after 72 h of exposure [22]. In the present study, our purposes were to (i) establish a suitable method to detect amounts of two enantiomers of alpha-cypermethrin in earthworms and soil samples, (ii) assess the enantioselective bioaccumulation potential of alpha-cypermethrin in soil, and (iii) generate enantiomer-specific acute toxicity assay of alpha-cypermethrin to earthworm using paper contact test under laboratory condition.

2. Materials and methods

2.1. Chemicals and reagents

The insecticide of alpha-cypermethrin (≥98.5% purity, enriched in (+)-*1R-cis-αS* + (−)-*1S-cis-αR*) was provided by Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA, Beijing, China). Water was purified by a Milli-Q system. *n*-hexane (HPLC grade) and isopropanol (HPLC grade) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals and solvents were analytical grade and purchased from commercial sources.

2.2. Earthworms

Mature redworms (*E. fetida*) purchased from northern suburbs farm, Beijing, were maintained in a wooden breeding box (50 cm × 50 cm × 20 cm) containing a mixture of soil and cattle manure. The worms were active when being introduced in the experiment.

2.3. Enantiomer-specific acute toxicity

To obtain the individual enantiomers of alpha-cypermethrin for acute toxicity assay, the resolved enantiomers were prepared automatically on an Agilent 1200 HPLC system with a preparatory chiral column (250 mm × 10 mm I.D., provided by the Department of Applied Chemistry, China Agricultural University, Beijing) based on cellulose tri-(3,5-dimethylphenyl-carbamate) (CDMPC) chiral stationary phase (CSP). The elution orders of right and left-rotation enantiomers of alpha-cypermethrin were measured by CHIRALYSER–MP optical rotation detector produced by IBZ MESSTECHNIK Company (Germany). The optical signals were received and processed by a N2000 SP1 chromatographic workstation obtained from Zhejiang University Zhida Information Engineering Co., Ltd. (Hangzhou, China). The result showed the first eluted enantiomer was dextro isomer defined as (+)-enantiomer, and that the second eluted enantiomer was laevo isomer defined as (−)-enantiomer using hexane/isopropanol (98:2 by volume) as the mobile phase (Fig. 2). The relationship between absolute configurations and optical rotations of alpha-cypermethrin enantiomers were already reported in previous study [2]. Correspondingly, the first eluted enantiomer was (+)-(*1R-cis-αS*)-alpha-cypermethrin, while the second one was (−)-(*1S-cis-αR*)-alpha-cypermethrin in our study. The purity for both enantiomers was >99%, and the concentrations for the resolved isomers were determined by comparison with racemic solution of known concentration according to the previously established methods [23].

In accordance with the OECD guideline 207, a paper contact toxicity assay was used to test the acute toxicity of *rac*-alpha-cypermethrin, (+)-(*1R-cis-aS*)-enantiomer and (−)-(*1S-cis-aR*)-enantiomer to earthworms [13]. A range of known concentrations of test substances was prepared with acetone as the solvent. After the depuration period of 24 h on wet filter paper under dark conditions to evacuate the earthworms' gut content, earthworms were rinsed in tap water and dried by absorbent paper cautiously. One milliliter of solutions was pipetted and added to the filter paper (5.5 cm × 11.5 cm) placed in flat-bottomed glass vial (3.6 cm in diameter, 8 cm in length). The concentrations of *rac*-alpha-cypermethrin on filter papers were 1.58, 15.81, 79.05, 158.10, 474.31, 632.41, and 790.51 ng/cm^2; the concentrations of (+)-(*1R-cis-aS*)-enantiomer on filter papers were 0.79, 15.81, 79.05, 158.10, 316.21, 474.31, and 632.41 ng/cm^2; the concentrations of (−)-(*1S-cis-aR*)-enantiomer were 31.62, 158.10, 316.21, 1264.82, 1897.23, 3794.47, and 5059.29 ng/cm^2. After drying of the solvent under a stream of compressed air, 1 mL of deionized water was added to each vial. Controls were also run in parallel with the carrier solvent alone. Ten replicates for each treatment and each vial containing one worm were done. Each vial was sealed with plastic film

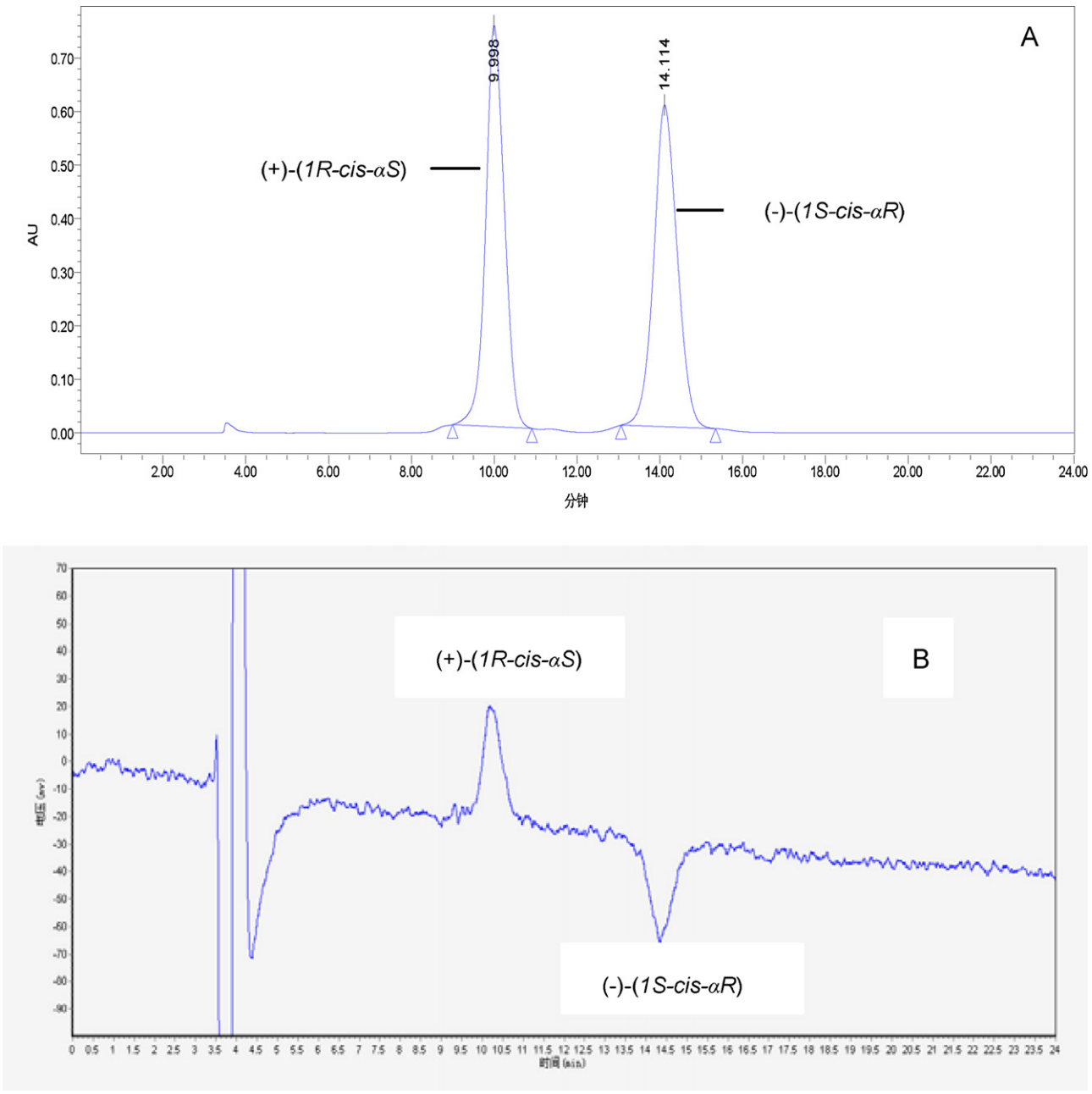

Fig. 2. Optical detection of two enantiomers of alpha-cypermethrin. Panel A shows UV detection (Waters 2695 HPLC with 2998 UV detector); panel B shows optical detection (CHIRASLYSER optical detector) [flow rate 0.5 mL/min, UV = 230 nm, n-hexane/isopropanol = 98/2].

with several ventilation holes. After that all the vials were placed in a room at 20 ± 2 °C, and mortality of earthworms was observed after incubation for 48 h. The LC_{50} values were determined from the survival data using a probit equation with SPSS 16.0.

2.4. Soil collection and earthworm exposures

The site to collect soil is a grassland area 20 km northwest of Beijing, China, which has not received any pesticide applications for 10 years at least. After removing the surficial vegetation, the top soil (0–10 cm) was collected. The soils were sieved (2 mm) and air-dried at room temperature and kept in the dark until used within a few days. Physicochemical properties of the soil were listed in Table 1.

We did the procedure in steps (dilution spike) to ensure that 250 g_{dwt} of medium was spiked homogeneously with *rac*-alpha-cypermethrin [24]. First, 12.5 mg of the chemical was dissolved in 10 mL of acetone, and then the acetone solution was slowly added to dry soil (50 g) while mixing continued for about 5 min. The spiked soil was left in a fume cupboard over-night after which the acetone had evaporated. Next, the contaminated dry soil (50 g_{dwt}) was

Table 1
Properties of soil used in bioaccumulation study.

Parameter	Soil
Soil type	Sandy loam
Organic matter, g/100 g_{dwt}	2.13 ± 0.12
Clay (<2 μm), %	4.7 ± 0.1
Silt (<20–2 μm), %	41.0 ± 1.2
Sand (2000–20 μm), %	54.3 ± 1.4
pH (water, ratio 1:2.5)	7.6 ± 0.2

mixed thoroughly with 200 g_{dwt} of uncontaminated medium, and then the contaminated soil (250 g_{dwt}) was transferred to a 500 mL glass jar. The final concentration of alpha-cypermethrin was at 50 mg/kg_{dwt} soil. Ninety gram tap water was added to each jar to restore the 36% water content.

In this experiment, the earthworms weighed between 200 mg and 300 mg. Before the worms were introduced, they were allowed to live in that kind of uncontaminated soil for one week to acclimate. After evacuating their gut contents on moist filter paper for 3 h at 20 °C, 5 g_{wwt} earthworms were exposed to the chemical in each jar containing 340 g_{wwt} contaminated soil. The jars containing contaminated soil and worms were weighed, and the loss of water by evaporation was compensated by addition of tap water every two days. All the jars were placed in dark in environmental chamber controllable to 20 ± 2 °C.

For the uptake experiment, worms were collected after different exposure periods (0.5, 1, 3, 5, 7, 10, 14, 20, 28, and 40 days), rinsed in tap water, and allowed to depurate most of their gut contents on moist filter paper for 3 h. Water on the surface of the worms was dried by absorbent paper cautiously, and then the worms were weighed and frozen at −20 °C (in 50 mL of polypropylene tubes). Soil samples (6.8 g_{wwt}) from each jar were also stored at −20 °C. All the incubations were carried out in triplicate at each sample point.

2.5. Chemical analysis

To determine the residue of alpha-cypermethrin enantiomers, analytical methods were conducted for the treatments. Earthworms and soils were extracted and the extracts were further fractionated on high-performance liquid chromatography (HPLC).

All the samples were thawed for about 15 min at room temperature. Soil samples were mixed with 5 g of anhydrous sodium sulfate and 25 mL of ethyl acetate in a 50 mL polypropylene centrifuge tube. The tube was stirred for 3 min on a vortex mixer, exposed to ultrasonic vibration for 10 min, and then centrifuged at 3500 rpm for 5 min. The extraction was repeated again following the same step, and the liquid phase was combined. The combined extracts were filtered through 5 g of anhydrous sodium sulfate for dehydration and evaporated to dryness on a vacuumed rotary at 45 °C. The residue was reconstituted in 1 mL of isopropanol and filtered through a 0.22 μm filter prior to HPLC analysis.

For analysis of the earthworms, the samples were blended with 25 mL of ethyl acetate and homogenized with Ultra-Turrax T18 homogenizer for 30 s. The mixture was vortex-mixed for 3 min, exposed to ultrasonic vibration for 10 min, and then centrifuged at 3500 rpm for 5 min. The same extraction step was repeated again with 25 mL of ethyl acetate, and the upper organic phase was passed through a funnel with about 10 g of anhydrous sodium sulfate to pear shaped flask. Next, it was evaporated to dryness at 45 °C and reconstituted in 5 mL acetonitrile, and then 3 × 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. Thereafter the residue was purified by Alumina-N-SPE column (1000 mg, 6 mL, Agilent SampliQ Products). The column was preconditioned by rinsing with 5 mL of ethyl acetate followed by 5 mL of n-hexane and equilibrated with 5 mL of 1:9 ethyl acetate:n-hexane. The sample of dry extract was dissolved in 2 mL of 10% ethyl acetate in n-hexane, and then the solution passed through the column. The SPE column was eluted with additional 8 mL of 1:9 ethyl acetate:n-hexane. The elutes were combined with the loading elutes. The combined 10 mL of elutes were collected in a glass tube, evaporated to dryness under a stream of nitrogen, and diluted to 1.0 mL with isopropanol.

The HPLC analysis were performed using UV detection on an Agilent 1200 Series HPLC (Agilent Technology) equipped with G1322A degasser, G1311A pump, G1314B VWD and G1329A ALS. AT-930 heater and cooler column attemperator (Tianjin Automatic Science Instrument Co. Ltd., China) was used to control column temperature. The signal was processed by Agilent chemstation software. A CDMPC-CSP (150 mm × 4.6 mm I.D., provided by the Department of Applied Chemistry, China Agricultural University, Beijing) was used to separate alpha-cypermethrin in this study. A mixture of n-hexane and isopropanol (98:2, v/v) was used as mobile phase at a rate of 0.5 mL/min. The injection volume was 20 μL, and the UV detection wavelength was 230 nm. The chromatographic separation was conducted at 20 °C. No enantiomerization was observed for alpha-cypermethrin under this analytical condition. The average recoveries for both enantiomers at levels between 0.1 and 25 mg/kg ranged between 75.2 and 81.9% in earthworm tissue and soil (n = 3 for each concentration). The limit of detection (LOD) for both enantiomers, defined as concentration produced a signal-to-noise ratio of 3, was 0.05 mg/kg both in earthworm tissue and soil. Blank determinations of the soil and earthworm sample prior to fortification revealed no alpha-cypermethrin present.

2.6. Data analysis

The data of the residual concentrations of the two enantiomers were used for estimating the enantiomer fraction (EF) values during these experiments. EF was used to measure the enantioselectivity during the experiment, and the EF values defined range from 0 to 1, with EF = 0.5 representing the racemic mixture. EF was expressed as follows:

$$\mathrm{EF} = \text{area of } (+)/[(-)+(+)] \quad (1)$$

where (+) is the first eluted chromatograph peak of (+)-(*1R-cis-aS*)-enantiomer and (−) is the second eluted peak of (−)-(1S-cis-aR)-enantiomer.

Data of bioaccumulation experiment presented corresponds to means ± standard deviations of three independent experiment (n = 3). Statistical analysis for the enantioselectivity of alpha-cypermethrin enantiomers was performed using SPSS 16.0. A one sample t-test was used to compare the means of the EF values in earthworm and soil samples with EF = 0.5. The concentrations and BSAFs of the two enantiomers of alpha-cypermethrin were analysed using one way analysis of variance (one-way ANOVA), and pair wise multiple comparison procedure (Duncan's multiple range test) was used to compare results at $p < 0.05$. In acute toxicity assay, the 50% lethal concentration (LC_{50}) and associated 95% confidence intervals were estimated from the survival data using a probit equation.

3. Results and discussion

3.1. Bioaccumulation of alpha-cypermethrin to earthworm

The concentrations of the two enantiomers of alpha-cypermethrin in earthworm tissue and soil were determined. In soil samples, the initial concentrations of two enantiomers were both 25 mg/kg_{dwt} at dose of 50 mg/kg_{dwt}. Fig. 3 shows the degradation of the two enantiomers during the period of 40 days of incubation, and a significant difference was observed between the two enantiomers. At the end of exposure, the concentrations for both enantiomers decreased to approximately sixty percent. In this experiment the degradation of alpha-cypermethrin is faster than that reported by Hartnik and Styrishave [25]. This difference could be due to the different soil properties, biota species, or incubation conditions. The EF values of the two enantiomers in soil samples were shown in Fig. 4. A one sample t-test was carried out to compare the means of the EF values in spiked soil with EF = 0.5, and the result showed that the difference between EF

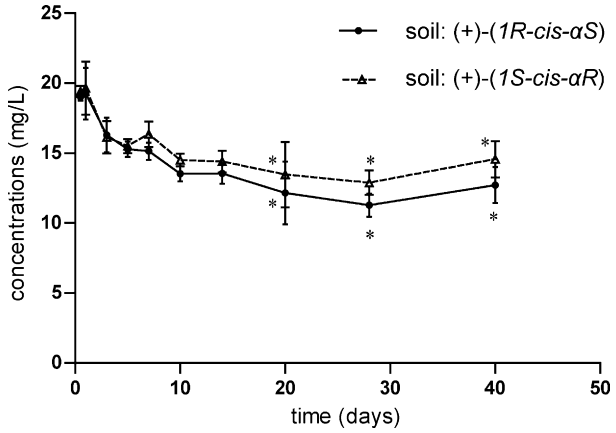

Fig. 3. Concentrations of two enantiomers of alpha-cypermethrin in soil samples (bars are standard error). *Significant difference between the two enantiomers at the same time point ($p < 0.05$, Duncan's multiple range test).

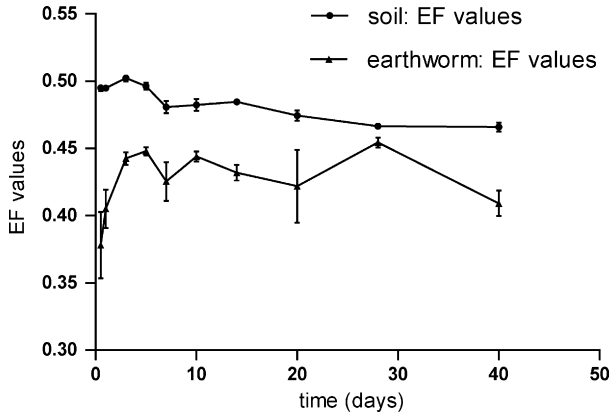

Fig. 4. Calculated enantiomer fraction (EF) values in soil and earthworm samples.

values and 0.5 was significant ($p < 0.005$). Therefore, the degradation of alpha-cypermethrin in this soil was enantioselective, and (+)-(*1R-cis-aS*)-alpha-cypermethrin degraded faster than (−)-(*1S-cis-aR*)-alpha-cypermethrin, resulting in relative enrichment of the (−)-(*1S-cis-aR*)-form.

The accumulation of two enantiomers of alpha-cypermethrin in earthworm during exposure was shown in Fig. 5, and a significant difference was observed between the two enantiomers. During the whole uptake period, the concentrations of (−)-(*1S-cis-aR*)-enantiomer in earthworm tissues were higher than that of (+)-(*1R-cis-aS*)-enantiomer. A one sample *t*-test was carried out to

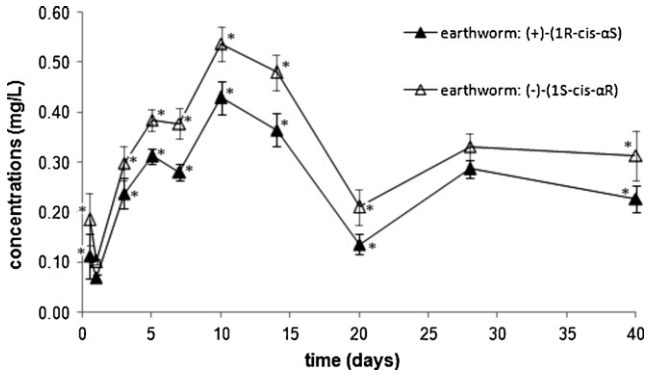

Fig. 5. Concentrations of two enantiomers of alpha-cypermethrin in earthworm samples (bars are standard error). *Significant difference between the two enantiomers at the same time point ($p < 0.05$, Duncan's multiple range test).

compare the means of EF values in earthworm with EF = 0.5. As a result the EF values deviated from 0.5, as shown in Fig. 4, and theses deviation were significant ($p < 0.001$). The deviation of EF values from 0.5 in earthworm tissue showed that enantioselective bioaccumulation of alpha-cypermethrin to earthworm occurred.

The rapid uptake of alpha-cypermethrin was observed, and on the first sample point (0.5 day), the concentrations of (+)-(*1R-cis-aS*)-enantiomer and (−)-(*1S-cis-aR*)-enantiomer were 0.113 and 0.184 mg/kg$_{wwt}$. From the first sample point until the tenth day, the concentrations for both enantiomers kept increasing, and reached highest level, 0.430 mg/kg$_{wwt}$ for (+)-enantiomer and 0.537 mg/kg$_{wwt}$ for (−)-enantiomer, respectively. After 10 days of exposure, the concentrations declined and reached the steady state levels as the duration of exposure increased. This model of peak shaped bioaccumulation curves in earthworm was different from the model reported by Hartnik and Styrishave, in which concentrations of alpha-cypermethrin in earthworms increased during the whole exposure period of 28 days (for Steinskogen soil) or 50 days (for Askov soil) without reaching a steady state [25]. Different feeding behaviors of the earthworms and sorption of alpha-cypermethrin to soil particles might caused the different bioaccumulation models.

In this study, the relative accumulation of alpha-cypermethrin enantiomers was expressed as biota to soil accumulation factor (BSAF, kg dry kg^{-1} wet weight). In order to compare with other chemicals bioaccumulated in earthworm, the activities were normalized to lipid content of earthworm and organic carbon (OC) of soil, assuming a lipid content of 1% and a factor of 1.7 between organic carbon (OC) and organic matter (OM) [24]. The equations are as follows:

$$\text{BSAF(kg dry kg}^{-1}\text{ wet weight)} = \frac{C_{EW}}{C_S} \quad (2)$$

$$\text{BSAF}\left(\frac{\text{kg}_{oc}}{\text{kg}_{lip}}\right) = \frac{C_{EW}\,F_{OM}(\text{soil})}{1.7 C_S\,F_{lip}(\text{earthworm})} \quad (3)$$

where C_{EW} and C_S are concentrations of alpha-cypermethrin enantiomers in earthworm and soil, respectively, F_{OM} (soil) is fraction of organic matter in soil and F_{lip} (earthworm) is fraction of lipid in earthworm. The BSAF values of (−)-(*1S-cis-aR*)-enantiomer were larger than that of (+)-(*1R-cis-aS*)-enantiomer, indicating that the (−)-(*1S-cis-aR*)-enantiomer was preferentially accumulated over the (+)-(*1R-cis-aS*)-enantiomer in earthworm tissue, and a significant difference was observed between the two enantiomers (Fig. 6). It can be assumed that the bioaccumulation of alpha-cypermethrin was enantioselective.

After 40 days of exposure, some earthworms were transferred to the alpha-cypermethrin free soil for the elimination test. Because earthworms stopped feeding of alpha-cypermethrin spiked soil, alpha-cypermethrin in earthworm tissue was degraded completely after 7 days, and this elimination rate constant translates into a half-life ($t_{1/2}$) of less than 1 day.

3.2. Acute toxicity assay

In the filter paper contact test, the acute toxicity was measured for individual enantiomer and racemate of alpha-cypermethrin. The mortality increased with increasing concentrations of individual enantiomer and racemate of alpha-cypermethrin (Fig. 7), and the LC_{50} values for individual enantiomer and *rac*-alpha-cypermethrin were calculated and shown in Table 2. The LC_{50} increased in the order (+)-*1R-cis-αS*- (49.53 ng/cm^2) < *rac*- (165.61 ng/cm^2) < (−)-*1S-cis-αR*-alpha-cypermethrin (1663.87 ng/cm^2). Significant difference was observed in LC_{50} between the (+)- and (−)-enantiomer, with (+)-enantiomer being at least 30 times and 3 times more toxic than the

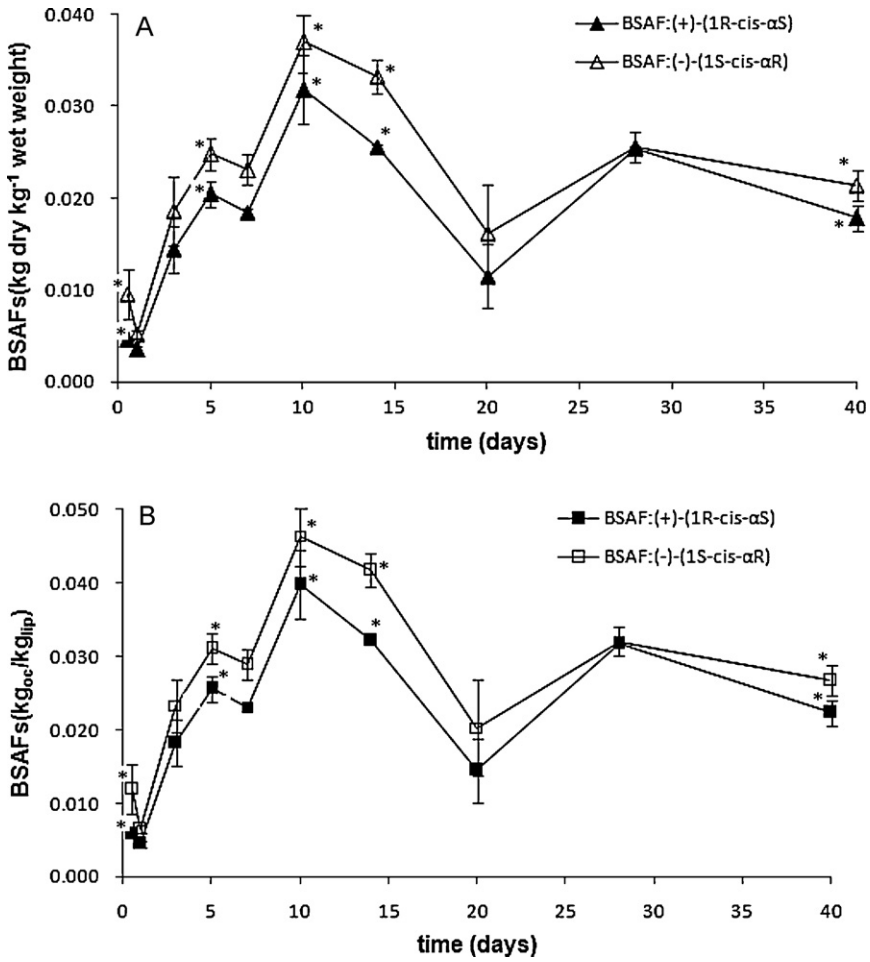

Fig. 6. Calculated biota to soil accumulation factors (BSAFs) for the two enantiomers of alpha-cypermethrin (bars are standard error). The unit of BSAFs in panel A and panel B is kg dry kg^{-1} wet weight and kg_{oc}/kg_{lip}, respectively. *Significant difference between the two enantiomers at the same time point ($p < 0.05$, Duncan's multiple range test).

(−)-enantiomer and racemate, respectively. These results indicated that the acute toxicity of alpha-cypermethrin to earthworm was almost entirely attributed to (+)-*1R-cis-αS* enantiomer. Therefore, it may be concluded that enantioselectivity in earthworm toxicity coincided with that in insecticidal activity for alpha-cypermethrin enantiomers. This phenomenon is agreement with the toxicity of alpha-cypermethrin to *C. dubia*, in which the (+)-*1R-cis-αS* was 10 times greater than the other enantiomer [2]. It is common for the acute aquatic toxicity of chiral pesticides to be associated primarily with only one of the enantiomers. A 50-fold difference response for *D. magna* was observed for isomers of isocarbophos [26] and the R-(−)-lactofen exhibited acute toxicity to *D. magna* was about 47 times of that for the herbicidally active *S*-(+)-enantiomer [27].

Some studies reported the enantiomer selectivity of several synthetic pyrethroid insecticides in aquatic toxicity. Liu et al. [2] found great differences in the acute toxicity to *C. dubia* or *D. magna* between enantiomers from bifenthrin, permethrin, cypermethrin and cyfluthrin. In *cis*-bifenthrin and *cis*-permethrin, the *1R-cis* enantiomers were much more active than the *1S-cis* enantiomers, and in the *trans*-permethrin, the *1R-trans* enantiomer was significantly more toxic than the *1S-trans* enantiomer. In addition, in cypermethrin and cyfluthrin, the *1R-cis-αS* and *1R-trans-αS* enantiomers were also more toxic than the other six enantiomers. Based on the previous studies and our result, we can concluded that the *1R-cis-αS* enantiomer from alpha-cypermethrin may have

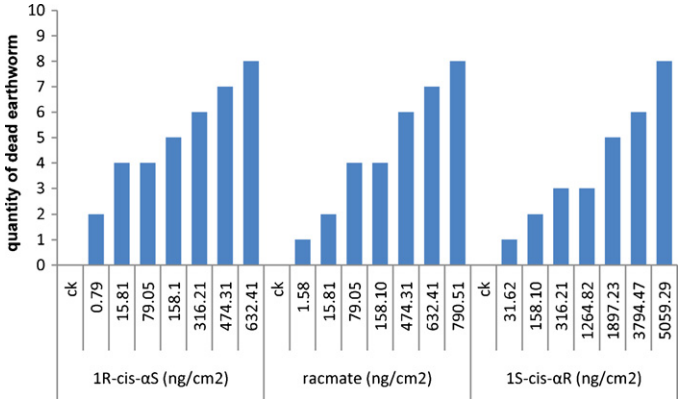

Fig. 7. The quantity of dead earthworm observed in different concentrations during exposure to (+)-, (−)- and rac-alpha-cypermethrin at 48 h by filter paper contact test.

Table 2
Calculated LC_{50} values of enantiomers from alpha-cypermethrin for earthworm *E. fetida*.

Chemicals	48-h-LC_{50} (ng/cm^2)	95% Confidence intervals	*p*	R^2
(+)-(*1R-cis-αS*)	49.53	3.29–227.67	3.97×10^{-4}	0.933
Racemate	165.61	52.53–528.20	3.28×10^{-4}	0.939
(−)-(*1S-cis-αR*)	1663.87	673.87–6582.90	1.87×10^{-3}	0.878

R^2 represents the correlation coefficient.
p represents the probability (associated with the *t*-test). A *p*-value smaller than 0.05 indicates that the correlation of linear equation is significant.

much more adverse effect on some non-target species, such as aquatic invertebrate *C. dubia* [2] and soil invertebrate *E. fetida*.

For pyrethroid insecticides with a cyclopropane ring in the acid portion, the *1R* isomers contribute more to the insecticidal activity [28]. This could be because the pyrethroid esters derived from (1S)-cyclopropanecarboxylic acids are lowest active than those derived from (*1R*)-cyclopropanecarboxylic acids [29]. The insecticidal activity of cycloprothrin with the R-acid moiety for the larvae of *Mythimaseparata* and *Aphismedicagini* was found to be much higher than that with S-acid moiety [30]. Anderson et al. have investigated that the stereochemistry of the alcohol moiety of cypermethrin is also important for biological activity [31]. For cypermethrin, the combination of *1R* and αS shows higher insecticidal activity than the corresponding combination of *1R* and αR [32].

4. Conclusions

Methods to determine the concentrations of two enantiomers of alpha-cypermethrin in soil and earthworm were developed by HPLC based on CDMPC. We found that alpha-cypermethrin was taken up by earthworm rapidly and enantioselectively, preferentially accumulating (−)-(*1S-cis-*αR)-alpha-cypermethrin, and in test soil, (+)-(*1R-cis-aS*)-alpha-cypermethrin degraded faster than (−)-(*1S-cis-aR*)-form, therefore, the degradation of alpha-cypermethrin in test soil was enantioselective. Moreover, filter paper contact acute toxicity test showed that (+)-(*1R-cis-*αS)-alpha-cypermethrin was at least 30 times more toxic for earthworm than (−)-(*1S-cis-*αR)-form.

Acknowledgments

This work was supported by fund from the National Natural Science Foundation of China (Contract Grant number: 20877100) and Innovation Fund for Graduate Student of China Agricultural University.

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