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*

Jinling Diao [1], Peng Xu [1], Donghui Liu, Yule Lu, Zhiqiang Zhou *

*Department of Applied Chemistry, China Agricultural University, Beijing 100193, PR China*

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

* Corresponding author at: Department of Applied Chemistry, China Agricultural University, Yuanmingyuan West Road 2, Beijing 100193, PR China.
Tel.: +86 10 62733547; fax: +86 10 62733547.
*E-mail address:* zqzhou@cau.edu.cn (Z. Zhou).

[1] These authors contributed equally to this paper.

0304-3894/$ – see front matter 
doi:10.1016/j.jhazmat.2011.06.010

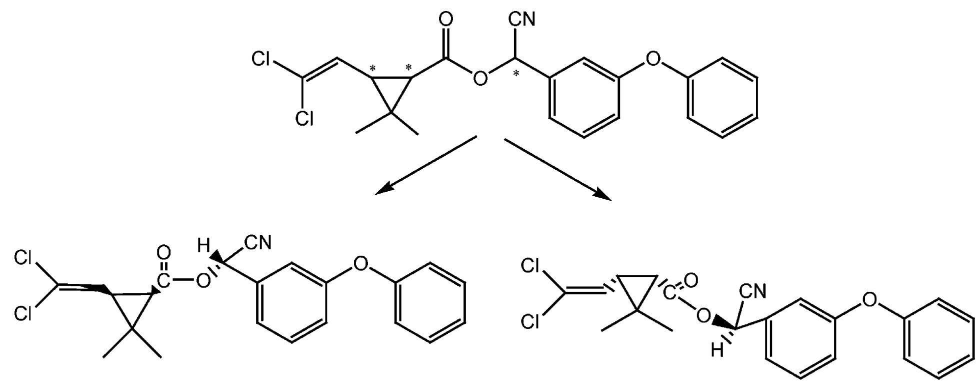


**Fig. 1.** Chemical structures of two enantiomers from alpha-cypermethrin.

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