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Enantioselective environmental behavior and cytotoxicity of chiral acaricide cyflumetofen

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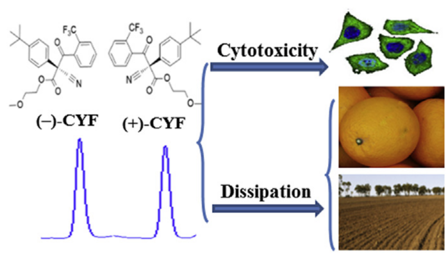
HIGHLIGHTS

- CYF enantiomers achieved similar half-live and enantioselective dissipation.
- Significant difference of cytotoxicity was found between two enantiomers.
- Significant difference of oxidative stress was found between two enantiomers.

GRAPHICAL ABSTRACT



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ABSTRACT

Enantioselective dissipation behavior of the new acaricide cyflumetofen (CYF) in citrus and soil, and its cytotoxicity to human liver hepatocellular carcinoma (HepG2) cells was investigated for the first time. The results of degradation experiment showed that roughly similar half-lives of (−)-CYF and (+)-CYF were achieved in citrus (16.5 and 19.8 d) and soil (6.37 and 6.99 d), respectively. EF values varied from 0.50 to 0.42 in citrus and from 0.49 to 0.48 in soil, indicating that slightly enantioselective degradation happened during experiment period. Moreover, indexes of MTT, LDH, ROS, MDA, SOD, and CAT were used to evaluate enantioselective cytotoxicity and oxidative stress of CYF enantiomers to HepG2 cells. Dose- and structure form-dependent phenomenon was observed with toxicity orders of (−)-CYF > rac-CYF > (+)-CYF. Despite the similar environmental degradation behavior, the toxicities of CYF enantiomers showed great difference, suggesting that (+)-CYF might be developed as potential substitute of rac-CYF for safety consideration.

1. Introduction

Cyflumetofen, [2-methoxyethyl (RS)-2-(4-*tert*-butylphenyl)-2-cyano-3-oxo-3-(α, α, α-trifluoro-*o*-tolyl) propionate], is a novel benzoyl acetonitrile acaricide developed by Otsuka AgriTechno Co., Ltd. It was registered in Japan in 2007 for the first time and now being used in 15 countries to control mites in plants of fruits, vegetables, tea and so on (Takahashi et al., 2012). This acaricide has showed great efficiency to several strains of mites, such as *Tetranych usurticae*, *Tetranychus kanzawai* and *Panonychus citri*. The mode of action was proved as inhibition mitochondria complex II in mites (Takahashi et al., 2012; Hayashi et al., 2013).

As a chiral pesticide, CYF is a racemic mixture containing a carbon atom chiral center. The structures of its two enantiomers were shown in Fig. 1. Dramatical differences in environmental fate,

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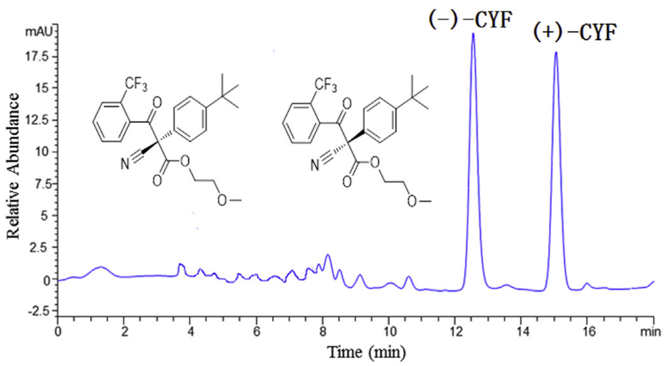

Fig. 1. Chemical structures and HPLC chromatograms of CYF enantiomers.

toxicity, bioactivity of chiral enantiomers had been reported by literature due to their different mode of actions with enzymes and other molecules in organisms (Liu et al., 2005, 2015; Yao et al., 2015). The enantioselectivity or stereoselectivity dissipation behaviors and toxicities of many chiral pesticides had been reported. R-flutriafol was found to exhibit shorter half-live than its antipode S-form in tomato. However, its fungicidal activity and acute toxicity to *Eisenia fetida* and *Scenedesmus obliquus* was 1.49–6.23 times higher than its antipode (Zhang et al., 2015). Dong et al. found that difenoconazole enantiomers displayed different enrichment behaviors in different plant species. Moreover, the two enantiomers exhibited 1.33–24.2 times differences in bioactivity towards pathogens and 1.04–6.78 times differences in toxicity to aquatic organisms (Dong et al., 2013). Therefore, using racemates to evaluate the environmental risk of chiral pesticides might not be accurate. Instead, individual enantiomer should been taken into consideration when assessing the risk of a chiral pesticide.

Until now, research on this new acaricide CYF is mainly focus on its toxicity towards mites and detection method development (Li et al., 2012; Marcic, 2012; Li, 2013; Ullah and Gotoh, 2013; Hu et al., 2014; Wang et al., 2014; Duan et al., 2015; Van Leeuwen et al., 2015). All the research aspects at present are not stereoselective ones and all values mentioned as “CYF” have considered as “sum of isomers”. The degradation behavior in environment and toxicity of each enantiomer to non-target organisms was not investigated in present studies. It is proposed to limit the residue definition for monitoring “CYF (sum of isomers)” only, as parent compound appears to be a significant marker of the total residues in plants and environment (EFSA, 2012). Therefore, it is essential to know the environment behavior and toxicity to non-target organisms of each CYF enantiomer to improve our understanding of its safety and make a comprehensive assessment of its environment fate and toxicity.

HepG2 cells, derived from the liver tissue of human being, had been widely used as model cell in toxicological or pharmacological research (Ahamed et al., 2015). The main cause of cytotoxicity from pesticide is to develop oxidative stress conditions in different tissues followed by its exposure (Banerjee et al., 1999; Giray et al., 2001). Oxidative stress is defined as the imbalance between the production of free radicals and body's antioxidant defense system (Hu et al., 2010). Many studies demonstrated that pesticides can induce oxidative stress in cells or bodies, leading to the generation of oxygen and other free radicals, altering antioxidants enzyme system, and causing liquid peroxidation (Perez-Maldonado et al., 2005; Qiao et al., 2005). High concentrations of reactive oxygen species (ROS) in organisms may damage the biomolecules of protein, lipids, or DNA. Moreover, ROS could change the activities of antioxidant enzymes in organisms, such as superoxide dismutase (SOD), catalases (CAT), and glutathione S-transferase (GST) which are known as the first line of the enzymatic defense against ROS (Roch, 1999). Although CYF had been widely registered and used, little information about its enantioselectivity in the toxicological effects on non-target organisms is available.

In order to make a clear understanding of enantioselective degradation behavior of CYF, experiments were conducted in citrus and soil under the field conditions. The enantioselective cytotoxicity of CYF was also studied in an effort to understand the role of oxidative stress in enantiomer-specific and CYF-induced cytotoxicity to HepG2 cells. To achieve a better understanding of the mechanisms involved, the generation of ROS and MDA, and activities of SOD and CAT antioxidant enzymes were measured. Procedures developed in this study may be useful for understanding behaviors of CYF enantiomers in environment and their toxicities to non-organisms, and also be helpful for the safe usage and accurate risk evaluation of this new pesticide.

2. Materials and methods

2.1. Chemicals and reagents

Standard of rac-CYF (purity of 98.0%) was purchased from Dr. Ehrenstorfer GmbH (Augsberg, Germany). A stock solution of CYF (1000 mg L^{-1}) was prepared in hexane. Anhydrous magnesium sulfate ($MgSO_4$, dried at 500 °C for 5 h before use), sodium chloride (NaCl) and formic acid at analytical grade were purchased from Chuandong Chemical Co., Ltd. (Chongqing, China). Graphitized carbon black (GCB), primary secondary amine (PSA), and HPLC grade of acetonitrile, isopropanol and hexane were purchased from CNW Technologies GmbH (Düsseldorf, Germany). Water was purified by a Milli-Q system (Bedford, MA, USA). The reagent kits of thiazolyl blue (MTT) solution, lactate dehydrogenase leakage (LDH), reactive oxygen species (ROS), malondialdehyde (MDA), super oxide dismutase (SOD), and catalase (CAT) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Dulbecco's modified eagle medium (DMEM) and phosphate-buffered saline (PBS) were purchased from Hyclone Laboratories Inc. (Logan, UT, USA). Fetal bovine serum (FBS) was supplied from Sijiqing Biological Engineering Material Co. Ltd. (Hangzhou, China). A varioskan flash spectral scanning multimode reader was used to detect fluorescence intensity (Thermo Fisher Scientific, America).

2.2. Enantiomer separation and quantitative analysis

Enantiopure isomer was prepared via enantiomeric resolution of rac-CYF by HPLC. In this step, CYF of known quantity was injected into a chiral HPLC system, and mobile phase fractions were corresponding collected manually by observing their UV signals. The pure enantiomer used for cell toxicity was collected into different vials at HPLC outlet. They were evaporated to dryness under a nitrogen stream, and re-dissolved in ethanol.

2.3. Field experiments

Field experiments were conducted at Beibei (Chongqing, China) during August to October in 2014. CYF suspending agent, purchased from Fumeishi Ltd. (Jiangsu, China), was dissolved in water and spayed at dosage of 0.26 a.i mg kg^{-1} for citrus fruit application and dosage of 0.1 a.i g m^{-2} for soil application. Citrus and soil samples were collected at 0 (2 h), 1, 3, 5, 7, 10, 14, 21, 30, 45 d after treatment (DAT). Soil was sampled to a depth of 0–10 cm in each plot using a tube auger. The soil proprieties were as follows: pH of 6.25, total organic carbon (TOC) of 1.45%, moisture content of 21.50%, potassium of 0.083%, phosphorus of 0.077%, and nitrogen of 1.90%. All