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Enantioselective toxic effects of cyproconazole enantiomers against *Chlorella pyrenoidosa*

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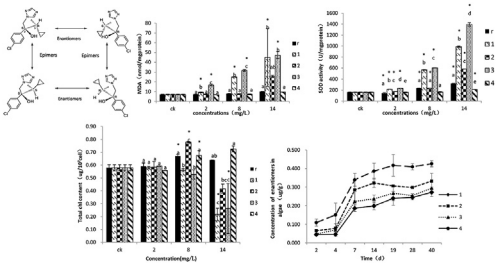
HIGHLIGHTS

- The acute toxicity of cyproconazole against *Chlorella pyrenoidosa* was enantioselective.
- Some biomarkers of four cyproconazole enantiomers to algae were different.
- Enantioselective biodegradation occurred in the algae exposed to racemate.
- There was enantioselective bioaccumulation in the rac-treated algae.

GRAPHICAL ABSTRACT



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ABSTRACT

Enantioselectivity in ecotoxicity, digestion and uptake of chiral pesticide cyproconazole to *Chlorella pyrenoidosa* was studied. The 96h-EC_{50} values of rac- and the four enantiomers were 9.005, 6.616, 8.311, 4.290 and 9.410 mg/L, respectively. At the concentrations of 8 mg/L and 14 mg/L, the contents of pigments exposed in rac-, enantiomer-2 and 4 were higher than that exposed in enantiomer-1 and 3. The superoxide dismutase (SOD) and catalase (CAT) activity of algae exposed to enantiomer-1 and 3 was higher than that exposed to the rac-, enantiomer-2 and 4 at three levels. In addition, the malondialdehyde (MDA) concentrations in algae disposed with enantiomer-1 and 3 were increased remarkably at three levels. For the digestion experiment, the half-lives of four enantiomers in algae suspension were 28.06, 19.10, 21.13, 15.17 days, respectively. During the uptake experiment, the order of the concentrations of cyproconazole in algae cells was enantiomer-4, 2, 3 and 1. Based on these data, we concluded that ecotoxicity, digestion and uptake of chiral pesticide cyproconazole to *C. pyrenoidosa* were enantioselective, and such enantiomeric differences must be taken into consideration when assessing the risk of cyproconazole to environment.

1. Introduction

Organic contaminants in ecosystems such as pesticides and their residues have become environmental concerns because amounts of research revealed that some of agricultural chemicals were detected in lakes, rivers, underground streams, or even coastal sea waters (Gunes, 2008; Chopra et al., 2011). Many research reported the fate, environmental behavior and ecotoxicity of pesticides (Wong, 2006; Daam and Van den Brink, 2010; Li et al.,

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2010). The residual of pesticides in aquatic ecosystems may threaten to microorganism, fish, and other marine life, even to the people's health at last (van der Werf, 1996; Kaur et al., 2008; Katagi, 2010; Li et al., 2010). Therefore, it is necessary to explore the effect of pesticides on aquatic organisms. Microalgae, as the dominant primary producers in many aquatic systems, and non-target effects on phytoplankton communities may likely impact higher trophic levels (Wong, 1995). Besides, it is considered as a sensitive indicator of aquatic environment (McCormick and Cairns, 1994), and pigments, antioxidant enzyme activities and lipid peroxide in algae may change with levels of pesticides or organic compounds in natural waters (Geoffroy et al., 2002).

About 25% pesticides nowadays in use are chiral chemical, and the number is increasing with the large-scale development of agrochemical synthesis (Williams, 1996). Chiral pesticides are chemicals consisting of one or two enantiomers with the identical physicochemical properties, and they are primarily commercialized as racemates. However, increasing researches confirmed some enantiomers exhibit significant differences in bioactivity, toxicity, excretion, metabolism and effects on beneficial and non-target organisms (Liu et al., 2005b). In some cases, only one enantiomer can effectively influence on target organisms, and the other possesses less effective or even inactive biological effects. Therefore, the assessment of racemates can not comprehensively reveal the fate of environment and ecotoxicological risks (Stanley and Brooks, 2009). Although the reasons for enantioselectivity were uncleared, it is required for comprehensive risk assessments to evaluate enantiomer specificity.

Cyproconazole, developed in 1987, is a kind of triazole fungicide with the effect of antibacteria and plant growth regulator. It is typically applied as foliar sprays to restrain diseases caused by basidiomycetes, ascomycetes, and deuteromycetes in foliage and cereal (Tomlin, 1997). It inhibits fungal steroid demethylation, which is the primary site of action the cytochrome P-450 (Buchenauer, 1987). This compound, with solubility and stability in water (Yahiat et al., 2011), comprises two asymmetrically substituted carbon atoms, and consists of four stereoisomers, two diastereomeric pairs of enantiomers (Buerge et al., 2006) (Fig. 1a). Each of enantiomeric in Fig. 1a has two chiral carbons, which are attached to four different moieties. But they differ in the arrangement of four different moieties in space. Although surface water fate factor of cyproconazole (0.85) was lower than soil fate factor (4.3), there were many research about the occurrence of this fungicide in water, at the range of 0.003–0.39 μg/L (Margni et al., 2002; Hladik et al., 2008; Verro et al., 2008; Wightwick et al., 2012).

Buerge et al. (2006) reported the influence of pH on the stereoselective digestion of the fungicides cyproconazole in soils. The four stereoisomers of cyproconazole were also degraded at different rates (overall half-lives 78–184 d)in the various soils, but only the stereoselectivities between epimers showed some correlations with pH, whereas enantioselectivities did not. Besides, there were some research about enantioselective toxicity and metabolism of triazole fungicide. The stereoselectivity of four isomers of triadimenol in aquatic toxicity were studied, and significant differences in their acute toxicity to *Daphnia magna* were observed among the isomers (Li et al., 2014). Moreover, the assessments of four stereoisomers of triazole fungicide difenoconazole were reported, including stereoselective toxicity toward aquatic organisms (*Scenedesmus obliquus*, *Daphnia magna*, and *Danio rerio*), and there was 1.04–6.78-fold differences in toxicity (Dong et al., 2013). Some research have shown the toxicity of racemic cyproconazole (rac-cyproconazole) to algae and rats (Machera, 1995; Durjava et al., 2013), but there is less data regarding enantioselective toxicity and metabolism of four stereoisomers. So it makes senses to invest impact of stereoisomers of cyproconazole to the aquatic organisms.

In this study, we assayed the 96 h-acute toxicity of *rac*- and four individual stereoisomers to *Chlorella pyrenoidosa*. The Chinese National Environmental Protection Agency recommends green algae Scenedesmus obliquus and *C. pyrenoidosa* as ecological indicators for toxic tests because of their high sensitivity to the compounds (Chinese, 1990). Some study reported *C. pyrenoidosa* is more sensitive to pesticides than S. obliquus (Ma, 2002). What's more, *C. pyrenoidosa* is a green unicellular algae and an important primary producer commonly found in many small ponds. *C. pyrenoidosa* has been widely used to evaluate the impacts of pesticides and organic compounds in natural waters. Thus, we choose *C. pyrenoidosa* as testing organism. Different biomarkers such as the EC_{50} (the effective pesticide concentration that reduces population growth rate by 50%), chlorophyll *a* and chlorophyll *b* contents, antioxidant enzyme activities, including superoxide dismutase (SOD) and catalase (CAT), and malondialdehyde (MDA), were detected to compare their enantioselective toxicity. Also, enantioselectivity of cyproconazole during its uptake and digestion in algae was studied here to better understand the effect of enantioselective toxic effects of cyproconazole enantiomers against *C. pyrenoidosa*.

2. Materials and methods

2.1. Chemicals

The analytical standard of cyproconazole (>98.0% purity) was from Jiangsu Seven Continent Green Chemical. All analytical grade reagents in this study was purchased from Yili Fine Chemicals (Beijing, China). Mobile phase reagents distilled and filtered through a 0.45 μm filter membrane prior to use. Water was purified by a Milli-Q system (Millipore Purification Systems). The enantiomers of cyproconazole (purity of four enantiomers $\geq$ 95.0%) were prepared on an Agilent HPLC with a preparatory chiral column (250 × 10 mm (I.D.), provided by the Department of Applied Chemistry, China Agricultural University, Beijing).

2.2. The algae culture

C. pyrenoidosa came from the Institute of Hydrobiology of Chinese Academy of Sciences. It was cultured in sterile 250-mL flasks containing 100 mL liquid BG-11 medium, and stored in the climatic cabinet with standard temperature and lighting conditions (12:12 light:dark cycle; 70 μmol photons/m^2/s; 25 °C).

2.3. Growth inhibition test

The algae growth inhibition test was conducted to determine the effective pesticide concentration that reduces population growth rate by 50% (EC_{50}), according to updated OECD guideline 201 for freshwater algae and cyanobacterial growth inhibition test (OECD, 2006). Cyproconazole and its four enantiomers were dissolved in acetone, and the final stock solutions were added to sterile 250-mL flasks, containing liquid BG-11 medium and exponentially growing algae cells, to achieve a series of exposed concentration. The range of concentration was from 2 to 14 mg/L for *rac*-, from 5 to 11 mg/L for the enantiomer-1, from 7 to 18 mg/L for enantiomer-2, from 2 to 11 mg/L for enantiomer-3 and from 7 to 20 mg/L for enantiomer-4. The initial density of algae cell was 200,000 cells/mL. Each assay was conducted in triplicate. All the flasks were cultivated in the condition mentioned before, and repositioned and shaken three times daily in order to prevent clumping of cells. Cell density was monitored at OD650 (optical density of algae suspension at 650 nm) at 48, 72, and 96 h, and the inhibition of algae growth was calculated by normalizing the data to the results of control cultures.