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Chiral fungicide triadimefon and triadimenol: Stereoselective transformation in greenhouse crops and soil, and toxicity to *Daphnia magna*

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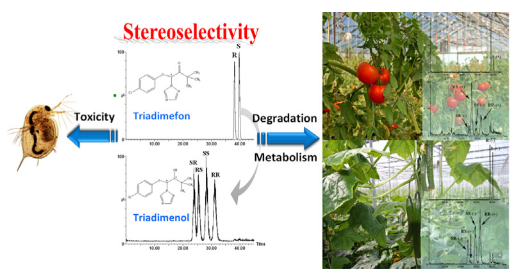
HIGHLIGHTS

- The stereoselective toxicity of triadimefon and triadimenol was first studied.
- The enantioselective metabolism of triadimefon in vegetables was first investigated.
- The enantioselective transformation was conducted under two different uptake-route.

GRAPHICAL ABSTRACT



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ABSTRACT

Various chiral pesticides are used in greenhouses to ensure high crop yields. However, detailed knowledge on the environmental behavior of such chiral contaminants with respect to enantioselectivity in the greenhouse has received little attention so far. Here, the widely used fungicide triadimefon was chosen as a "chiral probe" to investigate its enantioselective degradation and formation of triadimenol in greenhouse tomato, cucumber, and soil under different application modes. In addition, the stereoselectivity of individual isomers of triadimefon and triadimenol in aquatic toxicity were first studied. Significant differences in their acute toxicity to *Daphnia magna* were observed among the isomers. Under foliage application or soil irrigation application, *S*-(+)-triadimefon was preferentially degraded, resulting in relative enrichment of the more toxic *R*-(−)-enantiomer in tomato, cucumber, and soil. Further enantioselective analysis of converted triadimenol showed that the compositions of the four product stereoisomers were different and closely dependent on environmental conditions: the most toxic *RS*-(+)-triadimenol was the most preferentially produced isomer in tomato under foliage treatment, while the *RR*-(+)-triadimenol was proved to be the highest amount of metabolite isomer in cucumber and soil under both treatment modes and in tomato under soil treatment.

1. Introduction

Greenhouse crop systems that protect crops from adverse meteorological conditions and allow the production of high-value vegetables during the entire season are expending worldwide. The greenhouse production of vegetables is characterized by high planting density under high temperature and humidity conditions. These conditions cause the appearance of more pests and diseases in greenhouse crop systems than in open fields [1]. Thus, a large amount of pesticide is used in greenhouses to maintain high crop yield [2]. A major side effect of the use of pesticides is the potential risk they can cause to humans and the environment health. Concerning pesticides, it was estimated that chiral pesticides may account for >40% of currently used pesticides in China [3]. It is well

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known that enantiomers/stereoisomers may show different transformation in plants, humans, and the environment, and they may have different toxicity to humans and nontarget organisms [4–9]. Being primary producers in an ecosystem, plants are responsible for producing all the energy in the ecosystem [10]. Since large quantities of chiral pesticides applied in the greenhouse, the physiological changes due to chiral chemicals may affect the food chain and further ecosystems [11]. Clearly, understanding the specific environmental behavior of chiral pesticide enantiomers in greenhouse crops is essential to assess the risk of these chiral contaminants to ecological health and food safety. However, to date, studies on this subject are still limited and obviously lagging behind laboratory and open field studies [12].

Triadimefon (Fig. 1) is a broad spectrum systemic 1,2,4-triazole fungicide that has been extensively used to control powdery mildews and fungi in greenhouse crops by inhibiting steroid demethylation [13,14]. Triadimefon has a single chiral center and consists two enantiomers, *S*-(+) and *R*-(−) [15]. In plants, soils, and animals, triadimefon is known to undergo carbonyl reduction to a more fungi-active metabolite, triadimenol, which is also registered as an agricultural fungicide [16,17]. The metabolic transformation involves the reduction of the carbonyl group to an alcohol, resulting in the formation of a second chiral center in triadimenol (Fig. 1) [18]. Therefore, triadimenol has two diastereomers: A [enantiomers A1 (1*R*,2*S*) and A2 (1*S*,2*R*)] and B [enantiomers B1 (1*R*,2*R*) and B2 (1*S*,2*S*)], giving a total of four stereoisomers. Interestingly, the fungicidal activities of these stereoisomers differ greatly, and the 1*S*,2*R* isomer is up to 1000-fold more fungicidally active than the other three [19]. In addition, triadimenol diastereomer A is 10 times more acutely toxic to rats (oral LD_{50}) than diastereomer B [16].

Since its first introduction as triazole fungicide, triadimefon has been used as a racemic mixture. An increasing number of studies have shown that many chiral pesticide residues occur non-racemically and can be metabolized enantioselectively, such as dichlorprop [20,21], fipronil [22,23], metalaxyl [24,25], diclofop [26], fenbuconazole [27], and beflubutamid [28]. Although environmental transformation of pesticides usually results in less harmful products, such products may be more harmful to the biological systems or the environment than parent pesticide; this has been shown, for example, with DDT [29]. However, so far chiral selectivity of their metabolites have been largely ignored. In view of the differences in fungicidal activity and toxicity among the stereoisomers, the possible stereoselective formation of triadimenol is an important issue for both human health and ecological risk assessment. Enantioselective transformation of triadimefon has been studied in laboratory soils and animals [13,16,17]. Results indicated that the direction and degree of the observed enantioselectivity often differ across various soils. Studies have also shown that different triadimenol stereoisomer compositions could be produced depending on soil type. However, little is known about the stereoselective nontarget toxicity of triadimefon and triadimenol, as well as the stereoselective biotransformation of triadimefon in plants, especially under greenhouse conditions, although an earlier study has shown that triadimefon was more persistence in greenhouse than in field conditions [14].

In the present study, the stereoselectivity of triadimefon and triadimenol isomers in acute toxicity to *Daphnia magna* were evaluated. The occurrence of stereoselective transformation of triadimefon was further investigated in two common greenhouse vegetables (tomato and cucumber) and soil. The investigation was conducted under two application modes (foliage treatment and soil treatment) to understand the differences in enantioselectivity under different uptake routes. Results of the research provide more comprehensive insights into the environmental and human risks posed by chiral pesticides.

2. Materials and methods

2.1. Chemicals and reagents

Analytical standards of triadimefon (99.4% purity), triadimenol A isomer (racemate of *RS* enantiomer and *SR* enantiomer, 99.9% purity), and triadimenol B isomer (racemate of *RR* enantiomer and *SS* enantiomer, 99.9% purity) were kindly provided by the Bayer CropScience, Germany. The two triadimefon enantiomers and four triadimenol stereoisomers were prepared by normal chiral HPLC with a Chiralpak AD-H column (Daicel, Japan). Briefly, racemic triadimefon, triadimenol A, and triadimenol B of known quantities (1000 mg/L) were injected into the chiral HPLC system. The mobile phase fractions corresponding to the purer enantiomers were collected manually by observing their UV signals. The purity of each separated stereoisomer (enantiomer), checked with chiral HPLC using the same system, was >98%. HPLC-grade methanol, hexane, and 2-propanol were purchased from Sigma–Aldrich (Steinheim, Germany). Analytical-grade sodium chloride (NaCl), anhydrous magnesium sulfate ($MgSO_4$), and acetonitrile (ACN) were purchased from Beihua Fine-Chemicals Co. (Beijing, China). Ultra-pure water was obtained from a Milli-Q system (Bedford, MA, USA). Primary secondary amine (PSA, 40 μm) and graphitized carbon black (GCB, 40 μm) sorbents were obtained from Agela Technologies Inc. (Tianjin, China). The mobile phase solvents were filtered through a 0.22 μm pore size filter membrane (Tengda, Tianjin, China) before use.

2.2. Aquatic toxicity bioassays

Stereoselectivity in aquatic toxicity was evaluated by 48 h acute toxicity assays using *D. magna* as the test species. Stock organisms were obtained from the Chinese Academy of Protection and Medical Science (Beijing, China). Prior to testing, a sensitive test for Daphnia to potassium dichromate ($K_2Cr_2O_7$) was performed as a positive control, and the LC_{50} (24 h) value was about 1.38, in the range of 0.6–1.7 mg/L [30]. Toxicity tests were performed as previously described [31]. Briefly, five neonates were transferred into glass breakers filled with 20 mL of blank or test solutions of various concentrations. The test solutions with the highest concentrations were prepared by adding a known concentration of the stereoisomer (or racemate) to the dilution water. Subsequent dilutions were prepared from solutions with the highest concentrations. The maximum content of acetone in the final test solutions was <0.1% (by volume), which had no effect on the survival of the test species. Seven concentrations [ranging from 2 mg/L to 22.8 mg/L and 3 mg/L to 34.17 mg/L for stereoisomers (or racemates) of triadimefon and triadimenol, respectively] and two controls (a test water control and a test acetone control) for each compound were tested. Four replicates were preformed for each treatment. The test animals were not fed and were incubated at 22 ± 1 °C for 48 h. Mortality of the Daphnia was observed after incubation for 48 h. The concentration that caused 50% mortality of the test population (LC_{50}) was determined from the survival data by a probit equation with SPASS 18.0. Tests were considered to be valid if control mortality was <10%.

2.3. Plant care and fungicide application in greenhouse

The field experiments were conducted during March and April 2012. Tomato (*Lycopersicon esculentum*) seeds and cucumber (*Cucumis sativus*) seeds purchased from Beijing Baofeng Seeds Co. (Beijing, China) were cultivated to tomato and cucumber seedlings in greenhouse. Sixteen plots of working areas for tomato and cucumber were chosen at the experimental field, located at the experimental base of Institute of Plant Protection, Chinese Academy