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Chiral bioaccumulation behavior of tebuconazole in the zebrafish (*Danio rerio*)

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ABSTRACT

Tebuconazole is an effective chiral fungicide, and previous studies have demonstrated that tebuconazole enantiomers exhibit enantioselective toxicity to non-target aquatic organisms. Thus, the aim of the present study was to investigate the chiral bioaccumulation behavior of tebuconazole in zebrafish (*Danio rerio*). Two exposure concentrations (0.107 and 1.07 mg/L) of tebuconazole were used. The uptake experiments lasted for 8 days, and subsequently, the zebrafish were transferred to another clean tank containing water without tebuconazole for depuration experiments (up to 14 days). A significant trend in enantioselective bioaccumulation was observed in these zebrafish with the preferential accumulation of (−)-*R*-tebuconazole at two dose levels. The results of the depuration experiments indicated that the degradation of (−)-*R*-tebuconazole in zebrafish was slower than that of (+)-*S*-tebuconazole. The BCF_k values for (+)-*S*-tebuconazole and (−)-*R*-tebuconazole in a low dose of this chemical were 11.22 and 16.25, respectively, while at a high dose, these values were 9.79 and 10.31, respectively. The enantiomer fraction of tebuconazole in zebrafish and water ranged from 0.31-0.49. Hence, future research should focus on the fate of tebuconazole in the aquatic environment at the enantiomer levels.

1. Introduction

Tebuconazole (Fig. 1) is a broad-spectrum chiral triazole fungicide used to control many plant diseases. As one of the most salable fungicides worldwide, this chemical has been extensively used in agricultural production. However, the US Environmental Protection Agency (EPA) has classified tebuconazole as a potential human carcinogen (Hu et al., 2007). An abundance of tebuconazole was detected through routine environmental monitoring. Particularly in stream water, the occurrence of tebuconazole has increased (Montuelle et al., 2010). For example, one study reported that its concentration in surface water has reached 175–200 μg/L (Zubrod et al., 2010). Tebuconazole comprises two enantiomers, reflecting the presence of a single chiral center in the structure of this molecule. Studies have indicated that the activity of (−)-*R*-tebuconazole is higher than that of (+)-*S*-tebuconazole (Li et al., 2012), which exhibited high toxicity to aquatic non-target organisms (*Scenedesmus obliquus, Daphnia magna, and Danio rerio*) (Li et al., 2015). Moreover, (−)-*R*-tebuconazole degraded slower than (+)-*S*-tebuconazole in aerobic and anaerobic soils (Li et al., 2015). However, the traditional risk assessment did not distinguish enantiomer differences, leading to an inaccurate environmental risk evaluation (Qu et al., 2014). Hence, it is imperative to evaluate the risk assessment of tebuconazole at the enantiomer levels.

The Organization for Economic Cooperation and Development (OECD) has used zebrafish (*D. rerio*) as model organisms to assess the potential biological effects of chemicals released into the environment (Landaluze et al., 2015). However, the exposure of tebuconazole alters thyroid hormone levels and gene transcription in zebrafish larvae from fertilization to 120 h post-fertilization (Yu et al., 2013). Studies have shown that lipid and carbohydrate metabolism and several enzymatic activities are affected in zebrafish after 7 and 14 days of exposure to tebuconazole (Sancho et al., 2010). Thus, it is necessary to monitor tebuconazole enantiomers in zebrafish and the surrounding environment to increase the accuracy of the risk assessment of chiral pesticides.

Bioaccumulation is the increased concentration of a test substance in or on an organism (and specified tissues thereof) relative to the concentration of this test substance in the surrounding medium. Organisms can accumulate these chemicals through any route, including inhalation, ingestion, or direct contact (ElAmrani S. et al., 2012). The pesticides in aquatic ecosystems can be transferred through phytoplankton to fish and ultimately to humans (Toni et al., 2011). Previous studies have reported that the

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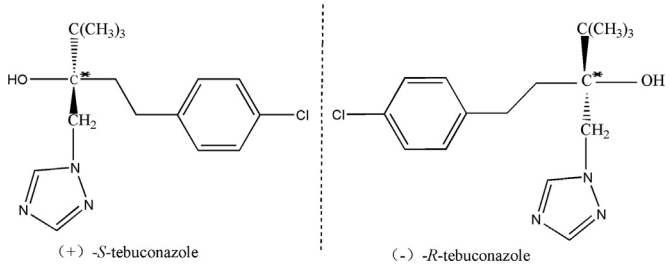

Fig. 1. The chemical structures of tebuconazole stereoisomers.

bioconcentration factor of *rac*-tebuconazole in zebrafish at steady-state is 38.80 L kg^{-1} (Andreu-Sanchez et al., 2012), which is a concentration that is dangerous to fish and humans. However, rare information concerning differences between (+)-*S*-tebuconazole and (−)-*R*-tebuconazole during bioaccumulation has also been reported. Therefore, it is necessary to investigate the enantioselective behavior of tebuconazole during uptake and depuration phases. In addition, information concerning the toxicity and bioconcentration of tebuconazole would be a useful addition to the database of aquatic organisms.

In the present study, zebrafish were used to conduct acute toxicity and bioaccumulation tests under laboratory conditions. The objectives of the present study were to (1) determine the differences in the bioaccumulation behavior of tebuconazole enantiomers in zebrafish and (2) evaluate the fate of tebuconazole enantiomers during the depuration phase.

2. Materials and methods

2.1. Chemicals and reagents

Racemic tebuconazole (98.7% purity) was obtained from China Standard Material Center (Beijing, China). Technical material for tebuconazole (97.8% purity) was obtained from Shenyang Kechuang Chemicals CO., Ltd (Shenyang, Liaoning, China). High-purity CO_2 (≥ 99.999%) and N_2 (≥ 99.999%) were purchased from Haike Yuanchang Gas (Beijing, China). HPLC-grade methanol was purchased from Fisher Scientific (Shanghai, China). Ultra-pure water was obtained from a Milli-Q system (Bedford, MA, USA). Analytical grade NaCl, $MgSO_4$ and ACN were purchased from Beihua Fine Chemicals Co. (Beijing, PRC). The Florisil Sorbent (120–400 mesh size) were obtained from Agela Technologies Inc. (Newark, DE, USA). The mobile phase solvents were distilled and filtered through a 0.22 μm pore size filter membrane (Tengda, Tianjin, China) prior to determination.

Standard stock solutions (100 mg/L) of racemic tebuconazole were prepared in the pure acetonitrile. Standard working solutions of *rac*-tebuconazole at 0.01, 0.05, 0.1, 0.5, 1.0 and 5.0 mg/L were prepared in pure acetonitrile from the stock solution using serial dilution. Accordingly, the concentrations of each enantiomer were 0.005, 0.025, 0.05, 0.25, 0.5 and 2.5 mg/L. All solutions were protected against light with aluminum foil and stored in a refrigerator at 4 °C until further analysis. No degradation was observed for the standard working solutions for 3 months.

2.2. Experimental animals and acclimation

Actively moving zebrafish juveniles (total length of 2.0 ± 1.0 cm, weight of 0.1 ± 0.05 g) were purchased from a commercial fish supplier (Gaofeng Aquarium Supermarket, Beijing, China). The zebrafish were acclimatized to laboratory conditions for one week in a 200-L aquarium prior to the experiments according to the guidelines of the OECD (OECD, 1996). The zebrafish were fed twice a day with commercial mixed feed (Aquatic feed, Zhongshan president enterprises CO. Ltd., China). The aquarium was equipped with aeration device and activated carbon filter. The feces and uneaten food were cleared through the aquarium filter. Tap water was available through aeration for more than 48 h. The water temperatures ranged from 23 to 25 °C with a pH of 7.5 ± 0.5 and dissolved oxygen content of 8.0 ± 0.5 mg/L during acclimation. All zebrafish were healthy. During acclimation, the zebrafish mortality rate was less than 2%. All experiments and animal handling were approved through the Ethics Committee of Shenyang Agricultural University.

2.3. Acute toxicity assays

The acute toxicity of *rac*-tebuconazole to zebrafish was realized according to OECD guideline 203 (OECD, 1992). Lethality was the endpoint assessed in the zebrafish (OECD, 1992). A range of known concentrations of test substances was prepared using acetone as the solvent. The test concentrations for lethality were 9.0, 10.8, 13.2, 15.6 and 18.6 mg/L for *rac*-tebuconazole. Ten zebrafish were exposed to each series of test solutions (10 L) containing *rac*-tebuconazole at the concentrations described above. The solutions were changed daily to maintain the concentrations at a constant level. The fish mortality was recorded at 24-h intervals for a total of 96 h. The solutions and dechlorinated and aerated tap water in the tanks were renewed every 24 h to ensure a consistent exposure concentration during the test (maximum accepted variation, ± 20% of the nominal concentration). The water temperatures ranged from 23 to 25 °C with a pH of 7.5 ± 0.5 and dissolved oxygen content of 8.0 ± 0.5 mg/L during the test. The concentration of *rac*-tebuconazole causing 50% mortality in the test population (LC_{50}) was determined for each sample. Moreover, controls and additional controls were used. The additional control was exposed to acetone at the highest dose used in the test concentrations. The results indicated that 96 h LC_{50} for tebuconazole was 10.74 mg/L (Table 1). According to the classification standards of the European Chemicals Bureau (EC, 1996) and the Organization for Economic Cooperation and Development (OECD, 2001) for the toxicity to fish, tebuconazole is harmful to aquatic organisms at an LC_{50} between 10 and 100 mg/L, and the pollutant is toxic at an LC_{50} between 1 and 10 mg/L.

2.4. Bioaccumulation experiment

According to the national standard of the People's Republic of China (2010), the zebrafish were exposed at concentrations of 1/10 and 1/100 *rac*-tebuconazole acute asymptotic LC_{50}. The uptake experiments were performed in duplicate in 20 L glass aquariums. Each tank was filled with 15 L of dechlorinated and aerated tap water containing *rac*-tebuconazole. The *rac*-tebuconazole solution was dissolved using acetone and dispersed into the aquarium water. A semi-static test was conducted for the bioaccumulation experiments. The solutions and dechlorinated and aerated tap water in the tanks were renewed every 96 h to ensure a consistent exposure concentration during the entire uptake stage (maximum

Table 1
Calculated LC_{50} values for *rac*-tebuconazole.

Exposure time (h)	LC_{50} (mg/L)	95% Confidence intervals	R^2
24	11.94	11.12–12.81	0.97
48	11.56	10.98–41.04	0.91
72	11.29	10.52–12.43	0.92
96	10.74	10.08–12.07	0.93

R^2 represents the correlation coefficient.