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Stereoselective metabolism, distribution, and bioaccumulation brof triadimefon and triadimenol in lizards

Yinghuan Wang, Dingyi Yu, Peng Xu, Baoyuan Guo, Yanfeng Zhang, Jianzhong Li, Huili Wang*

Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 18 Shuangqing Road, Haidian District, Beijing 100085, PR China

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ABSTRACT

In this research, Chinese lizards (*Eremias argus*) were chosen as laboratory animal to evaluate the stereoselectivity in the processes of metabolism, distribution, and bioaccumulation of triadimefon. A validated chiral high-performance liquid chromatography coupled with triple quadruple mass spectrometry (HPLC–MS/MS) method was developed for determining enantiomers' residues of parent compound triadimefon and its metabolite triadimenol in lizard blood and tissues. Pharmacokinetic results of single-does exposure suggested that S-(+)-triadimefon was metabolized easier than R-(−)-triadimefon, and RR-(+)-triadimenol was the main metabolic product of triadimefon. During the continuous exposure of two dose (40 mg/kg^{bw} · d and 200 mg/kg^{bw} · d), enantiomers of triadimefon and triadimenol were detected in all body compartments, with the highest triadimefon concentrations in brain. However, the triadimenol concentrations were not significantly different among the compartments. The concentrations of RS-(+)-triadimenol were negative correlated with concentrations of RR-(+)-triadimenol both in blood ($r=-0.775$, $p=0.024$) and liver ($r=-0.834$, $p=0.02$) in 200 mg/kg^{bw} · d group, which indicates that chiral conversion between enantiomers of triadimenol might exist in the metabolic process of triadimefon. In all the processes, the enantiomer fractions (EFs) of R-(−)-triadimefon and RR-(+)-triadimenol were significantly different from their natural ratios, 0.5 and 0.1, respectively, which proved that metabolism, bioaccumulation, and distribution of triadimefon and triadimenol in lizards were enantioselective. These results help enrich and supplement the knowledge of the stereoselective behaviour of triadimefon and triadimenol in reptile.

1. Introduction

Pesticides are widely adopted in agriculture to control pest damage to crops and raise production. However, there is proof that the residues of some pesticides and their metabolites cause environment problems (Randhawa et al., 2014). Triadimefon and triadimenol are two registered broad-spectrum systemic fungicides, and extensively used in the field (Crowell et al., 2011). Triadimefon (TF) [(RS)-1-(4-chlorophenoxy)-3, 3-dimethyl-1-(1*H*-1, 2, 4-triazol-1-yl) butan-2-one, CAS NO: 43121-43-3] has been commonly used for the control of mildews and fungi on food crops, turf grasses, shrubs, and trees (Roberts and Hutson, 1999). Triadimenol (TN) [(1RS, 2RS; 1RS, 2SR)-1-(4-chlorophenoxy)-3, 3-dimethyl-1-(1*H*-1, 2, 4-triazol-1-yl) butan-2-ol, CAS NO: 55219-65-3], a metabolite of triadimefon, has greater fungicidal activity than TF (Liang et al., 2013). TF and TN enter into the soil ecosystem because of direct spraying on the soil surface during agriculture application, which poses potential danger to the animals living in the soil. Previous studies showed that TF and TN have clear teratogenic effects on the bronchial arches and cranial nerves of rat embryos (Menegola et al., 2000). TF and TN are also harmful to mammalian central nervous system and are neurotoxins in rats, mice, and rabbits (U.S. Environmental Protection Agency (U.S. EPA), 2006; Walker et al., 1990). These two fungicides have been categorized as "possible human carcinogens" (U.S. Environmental Protection Agency (U.S. EPA), 2006).

Pesticides have been considered as one of the major factors contributing in the global decline of reptiles (Gibbon et al., 2000). Past attention was focused on measuring body burdens of various pollutants to wild reptiles which gave an understanding of historical exposure of given populations (Buono et al., 2007; Holem et al., 2008; Mann et al., 2007; Moss et al., 2009; Trinchella et al., 2006; De Falco et al., 2007; Keller et al., 2006; Simoniello et al., 2010; van de Merwe et al., 2010). However, the population-level effects and actual risks of pollutants on reptiles are still generally understudied (Weir et al., 2010). In soil, TF is easily decomposed and converted to TN with a half-life of 6–30 days (Petrovic et al., 1993; Singh, 2005). As a comparison, TN exhibits a much longer persistence (soil half-life > 240 days)

* Corresponding author. Fax: +86 1 62849790.
E-mail address: huiliwang@rcees.ac.cn (H. Wang).

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(Bromilow et al., 1999). Because the abuse of triazole pesticides in China (Lin et al., 2008), these two triazole pesticides may be transported from cultivated soils in significant amounts especially TN, which post threats to the life in soils. However, to our knowledge, there is no known research on the toxicity of these two compounds to reptiles. Reptiles are remaining as the least studied vertebrates in ecotoxicology (Hopkins, 2000).

In order to fill the gap in this research area, Chinese lizards (*Eremias argus*) were used in this study to evaluate the acute and chronic effects of TF and TN on reptiles. Lizards are regarded as one of the significant ecological animals in agriculture systems because they prey on crop pests. *E. argus* are widely distributed in the north of Yangtze River including North China Plain and Northeast China Region, the main agricultural areas in China. Excessive usage of TF and TN in these areas (Lin et al., 2008) has been a direct threat to the *E. argus*.

About 30 percent of the organic agrochemicals are chiral compounds and consist of two or more enantiomers, which have identical physic-chemical properties (Ulrich et al., 2012). Nevertheless, the bioactivity, toxicity, metabolism, excretion, distribution, and bioaccumulation of these enantiomers may be entirely different (Williams, 1996). The enantiomers of chiral pesticides could be different when binding to structure-sensitive biological receptors and naturally occurring chiral biomolecules because of their different molecular configurations (Dong et al., 2013). Stereoselectivity in the processes of absorption, distribution, metabolism, and bioaccumulation in organisms may result in ecotoxicological effects that are beyond the traditional knowledge that treat them as single compounds in risk assessment (Buser et al., 2002; Dong et al., 2013; Wong, 2006). TF has one chiral center and consists of an equimolecular mixture of the two enantiomers while TN has two chiral centers and consists of four stereoisomers including two pairs of diastereomers. The biotransformation of TF into TN is stereoselective and the biological response of each TN stereoisomer is different (Deas et al., 1986; Garrison et al., 2011; Spindler and Fruh, 1998) (Fig. 1). The stereoselective formation of TF and TN is an important issue in ecological risk assessment when considering the difference in fungicidal activity and toxicity among the enantiomers (Li et al., 2014). Stereoselective metabolism of TF to TN has been observed in soil microorganisms and rainbow trout (Garrison et al., 2011; Kenneke et al., 2010; Li et al., 2011). Unfortunately, the stereoselectivity of TF and TN in reptiles have never been studied.

To describe actual environmental fate and ecological risks from a complete view, and understand environmental impact of these pesticides, it is crucial to study the stereoselective behaviors of chiral pesticides. In this study, the experiments were conducted to obtain a better understanding of the biological fate of the TF and TN enantiomers in lizard blood on a single acute exposure and assess the stereoselective metabolism, distribution and bioaccumulation of TF and TN in different tissues in lizards on the continuously exposure. The results complement the knowledge of the stereoselectivity of chiral pesticides in reptiles, and are instructive for future TF and TN toxicity study on reptiles.

2. Materials and methods

2.1. Chemicals and reagents

Racemic TF (99.55 percent) and TN (99.1 percent), analytical standards of TN-A (racemate of RS- and SR-enantiomer, 99.9 percent purity) and TN-B (racemate of RR- and SS-enantiomer, 99.9 percent purity) were kindly provided by College of Science, China Agricultural University (Beijing, China) (Liang et al., 2012). Stock solutions of TF and TN were prepared in methanol (HPLC grade, Dikma, USA) at 1000 mg/L and kept in dark at −20 °C.

2.2. Dosing

Dosing was prepared according to those described previously with modifications (Di Renzo et al., 2009; McFarland et al., 2011, 2009; Suski et al., 2008). TF was first dissolved in the ethanol (analytical grade, Beijing Chemical Reagent Co. Ltd, China) then dispersed in corn oil (ethanol:corn oil was 1:9, v/v). Test preparations were stored in dark at 4 °C until use to prevent compound degradation. The corn oil–ethanol lactescence were warmed to room temperature (25 °C) and continually mixed by magnetic stirring apparatus (90-1, Shanghai ZhenRong Scientific Instrument Co. Ltd, China) before dosing. The micro injector was used to oral inject a volume of 20–50 μL corn oil or corn oil–ethanol lactescence.

2.3. Test lizard and culture conditions

The juvenile *E. argus* were collected in Abag Banner, Inner Mongolia (China) and maintained in laboratory since July 2009. Lizards were kept in 5 × 1.2 × 0.4 m solid bottom indoor aquarium covered with 10 cm mollisol and fallen leaves. The

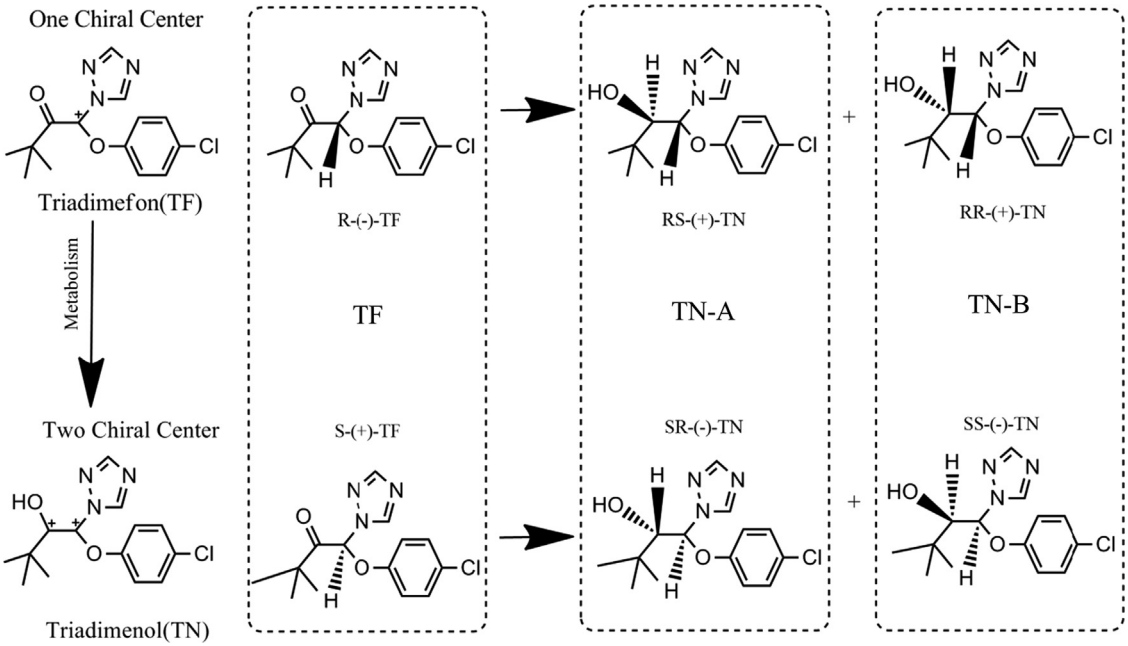

Fig. 1. The metabolic transformation of TF to TN and the enantiomers of TF and TN.