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The metabolism distribution and effect of dinotefuran in Chinese lizards (*Eremias argus*)

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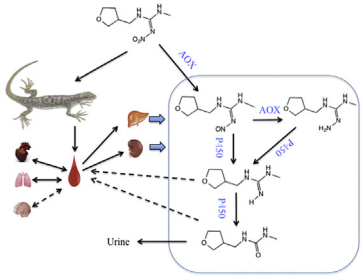
HIGHLIGHTS

- Molting and transurethral exclusion are the main routes for the elimination of DIN and its metabolites.
- AOX plays a major role in the nitro-reduction process of DIN.
- CYP3A4 and CYP2C19 play a crucial role in the metabolism of DIN.
- DIN may pose a risk of damaging the oxidative stress system in liver.

GRAPHICAL ABSTRACT



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ABSTRACT

The Chinese lizards (*Eremias argus*) were used to evaluate the metabolism, distribution and effect of dinotefuran following oral exposed. The HPLC equipped with Q Exactive focus was used for metabolite identification and concentration analysis. After single oral administration, the time-concentration curves of dinotefuran and its metabolites were tissue-dependent. The liver and kidney were the major metabolic organs. Percutaneous and urinary excretions were the main ways for lizards to eliminate dinotefuran, and the urine output was the limiting factor. Nitro-reduction was an important process of the metabolism of dinotefuran that was dominated by aldehyde oxidase, and P450 enzymes were involved. The CYP3A4 and CYP2C19 played a crucial role in the other metabolic pathways of dinotefuran. The mRNA expressions of GST family were severely inhibited in liver, which showed dinotefuran might pose a risk of damaging the oxidative stress system in liver. Prolonged residuals of dinotefuran and its demethylation metabolite might enhance the risk of dinotefuran to brain. The results enrich and supplement the knowledge of the environmental fate of dinotefuran in reptiles.

1. Introduction

Neonicotinoids have become the fastest growing insecticide in the world due to their excellent chemical and biological properties such as broad spectrum, low application rates, high efficiency, and rapid uptake in plants (Wakita et al., 2005; Sparks and Nauen, 2015; Rahman et al., 2017). Due to higher affinity for insect nicotinic acetylcholine receptors than mammalian, neonicotinoid insecticides display outstanding potency and safety in the environment (Morrissey et al., 2015; Mori et al., 2017). Dinotefuran ((RS)-1-methyl-2-nitro-3-(tetrahydro-3-furylmethyl) guanidine) (Fig. 1) is the third-generation of neonicotinoid insecticides which has a characteristic (7)-tetrahydro-3- furylmethyl moiety but not the

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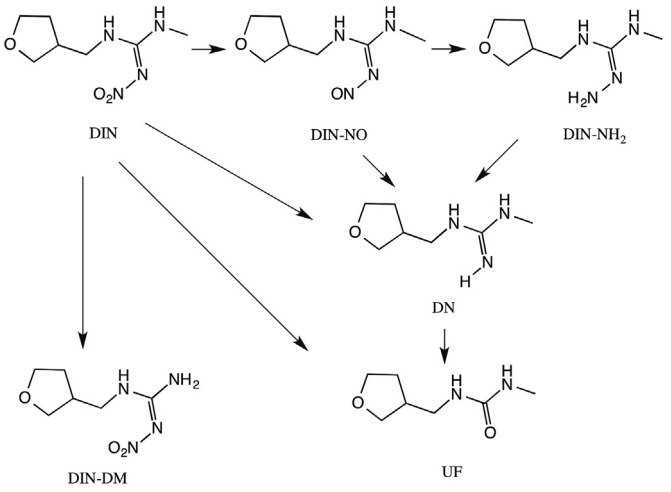

Fig. 1. Structure of the dinotefuran and its metabolites.

pyridine-like moiety of other neonicotinoids (Wakita et al., 2003; Chen et al., 2015). Dinotefuran is widely used and accounting for more than 25% of the pesticides used around the world (Sluijs et al., 2013). It is not only effective applied to prevent and control planthoppers, stink bugs, sucking and biting insects and *Nephotettix* cincticeps (Watanabe et al., 2011; Hem et al., 2012; Rahman et al., 2015), but also used in the areas where mosquitoes are resistant to pesticides for the disease vector control (Corbel et al., 2004). Dinotefuran is relatively stable in the soil with a half-life of 50-100d (Morrissey et al., 2015). Unreasonable use of dinotefuran inevitably results in residues in the environment and grains, which might harm to the soil organisms and human health (Li et al., 2017).

Although neonicotinoid pesticides are considered to be low toxicity to mammals, the metabolites of neonicotinoids may show more virulence than the parent compound (Honda et al., 2006). For example, the desnitro and descyano metabolites of imidacloprid and thiacloprid are more toxic to mammalian nAChRs than insects (Tomizawa and Casida, 2003, 2005). The demethylation of thiamethoxam is a potential carcinogen to mice (Green et al., 2005). It is therefore crucial to figure out the metabolic processes and metabolite forms of neonicotinoid pesticides in vivo to further assess their toxicological risk. As a relatively new pesticide, the studies on dinotefuran were focused on the residues of dinotefuran and its metabolites on environmental media and crops (Watanabe et al., 2011; Rahman et al., 2015; Mu et al., 2016; Li et al., 2017). However, the metabolism and distribution of dinotefuran in animals had rarely been reported.

Reptiles could be directly exposed to pesticides by inhalation, food intake and skin penetration (Amaral et al., 2012a). Pesticides have been identified as one of the major contributing factors in the global decline of reptiles (Buhlmann, 2000; Ram et al., 2013). Lizard is regarded as one of the important animals in agro-ecosystem (Wang et al., 2014b) and is considered as a good model for toxicological studies of reptile (Amaral et al., 2012b). Due to the unreasonable use and long persistence in soil, dinotefuran could be transported from cultivated soils in significant amounts, which post threats to lizards living in farmland. The metabolism of dinotefuran had been reported in mice. Previous literature reported on the types of metabolites of DIN in mice and summarized their trends in brain, liver and plasma (Ford and Casida, 2006). However, the metabolism of exogenous substances in living organisms is species-dependent because of structural differences of metabolic enzymes. To our knowledge, there is no known research on the metabolism of dinotefuran in lizards.

To fill the gap in this research area, Chinese lizards (*Eremias argus*) were used in this study to evaluate the metabolism, distribution and effect of dinotefuran on reptiles. *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 (Wang et al., 2014a; Shen et al., 2017). The extensive use of pesticides has become a huge threat to the survival of lizards in the area.

To describe actual environmental fate and ecological risks of dinotefuran from a comprehensive perspective, the experiments were conducted to better understand the biological fate of the dinotefuran in lizard blood and to assess the metabolism and distribution of dinotefuran and its metabolites in different tissues. The mRNA expressions of metabolic enzymes in liver, kidney and brain were used to find out the enzymes that play a key role in the metabolism of dinotefuran which were further demonstrated in vitro enzyme inhibitor experiments. The results complement the knowledge of metabolism, distribution and effect of dinotefuran in reptiles, and are instructive for future dinotefuran and its metabolites toxicity study on reptiles.

2. Materials and methods

2.1. Reagents

Dinotefuran (DIN, 95.0% purity), 1-methyl-3-(tetrahydro-3-furyl methyl)urea (UF, 97.0% purity), and (1-methyl-3-tetrahydro-3-furylmethyl) guanidine (DN, 97.9% purity) were provided by Institute for the Control of Agrochemicals, Ministry of Agriculture. All solvents of acetone, acetonitrile, methanol, ethanol, n-hexane, and isopropanol were HPLC grade and purchased from Dikma (Beijing, China). Nootkatone, sulfaphenazolum, 2,4-Dinitrochlorobenzene, quinidine, omeprazole, ketoconazole and estrogen (analytical grade) were purchase on Sigma-Aldrich (Beijing, China).

2.2. Animals and husbandry

The sexually mature *E. argus* (3–3.5 g) were obtained from our breeding colony in Changping district, Beijing, China. The selected lizards were half female and male. Lizards were kept in $5 \times 1.2 \times 0.4$ m solid bottom indoor aquarium covered with 10 cm mollisol and fallen leaves. The temperature and humidity were maintained at 25–30 °C and 30–60%. Daylight lamps (100 W) were set to a 14:10-h light: dark photoperiod to provide enough light and maintain the temperature. Lizards were fed with mealworms (*Tenebrio molitor*) twice a day. The water was sprayed every other day and the excreta and residues were cleaned twice a week.

Animal welfare and experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006). The animal care and use procedures were approved by Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences.

2.3. Exposure experiment and sampling

DIN was first dissolved in the ethanol then dispersed in corn oil. The amount of ethanol should be less than 10%. The testing dose was 20 mg/kg ^{bw}. The corn oil-ethanol lactescence were continually mixed on the magnetic stirring apparatus before dosing. The microinjector was used to deliver a volume of 20–30 μL corn oil or corn oil-ethanol lactescence into the oral cavity of each lizard according to the body weight.

After oral administration, lizards were euthanized at 1, 3, 6, 10, 12, 16 and 24 h. Three lizards were selected randomly at each sampling point. Each lizard's blood, brain, heart, lungs, liver, kidney,