Environmental Pollution xxx (2017) 1–10

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Stereoselective degradation and thyroid endocrine disruption of lambda-cyhalothrin in lizards (*Eremias argus*) following oral exposure☆

Jing Chang [a, b], Weiyu Hao [a, b], Yuanyuan Xu [c], Peng Xu [a], Wei Li [a], Jianzhong Li [a], Huili Wang [a, *]

[a] *Research Center for Eco-Environmental Science, Chinese Academy of Sciences, Shuangqing RD 18, Beijing 100085, China*
[b] *University of Chinese Academy of Sciences, Yuquan RD 19 a, Beijing 100049, China*
[c] *Binzhou People's Hospital ICU, China*

A R T I C L E I N F O

Article history:
Received 2 April 2017
Received in revised form
19 September 2017
Accepted 20 September 2017
Available online xxx

Keywords:
Pyrethroid pesticide
Eremias argus
Hypothalamus-pituitary-thyroid axis
Enantioselectivity

A B S T R A C T

The disturbance of the thyroid system and elimination of chiral pyrethroid pesticides with respect to enantioselectivity in reptiles have so far received limited attention by research. In this study, bioaccumulation, thyroid gland lesions, thyroid hormone levels, and hypothalamus-pituitary-thyroid axis-related gene expression in male *Eremias argus* were investigated after three weeks oral administration of lambda-cyhalothrin (LCT) enantiomers. In the lizard liver, the concentration of LCT was negatively correlated with the metabolite-3-phenoxybenzoic acid (PBA) level during 21 days of exposure. (+)-LCT exposure induced a higher thyroid follicular epithelium height than (−)-LCT exposure. The thyroxine levels were increased in both treated groups while only (+)-LCT exposure induced a significant change in the triiodothyronine (T3) level. In addition, the expressions of hypothalamus-pituitary-thyroid axis-related genes including thyroid hormone receptors (*trs*), deiodinases (*dios*), uridinediphosphate glucuronosyltransferase (*udp*), and sulfotransferase (*sult*) were up-regulated after exposure to the two enantiomers. (+)-LCT treatment resulted in higher expression of *trs* and (−)-LCT exposure led to greater stimulation of *dios* in the liver, which indicated PBA-induced antagonism on thyroid hormone receptors and LCT-induced disruption of thyroxine (T4) deiodination. The results suggest the (−)-LCT exposure causes higher residual level in lizard liver while induces less disruption on lizard thyroid activity than (+)-LCT.

1. Introduction

The value of reptiles as an integral part of natural ecosystems and an indicator organism for environmental equality has been widely recognized (Adams et al., 1995). Reptiles are sensitive to contaminants and accumulate pollutants to levels equal to or greater than those of birds and mammals (De Falco et al., 2007). In recent years, the number of reptiles has dramatically declined, which might be caused by habitat loss, disease, and environmental pollution (Gibbons et al., 2000). The widespread use of pesticides, which could induce endocrine disruption, might be a fundamental cause of the population decline of reptiles (Sun et al., 2009). However, less than 1% of studies in vertebrate ecotoxicology involve reptiles (Sparling et al., 2010).

As in other vertebrates, the thyroid system of reptiles is controlled by the hypothalamus–pituitary–thyroid (HPT) axis (Meyer et al., 2014). The HPT axis regulates the synthesis and secretion of thyroid hormones (THs). THs play a crucial role in the development, growth, and reproduction of reptiles (Jugan et al., 2010). Disruption of the reptilian thyroid system may affect these important functions as well as the thyroid hormone homeostasis, and HPT axis-related gene expression (Rivera and Lock, 2008). The mechanisms by which pesticides disrupt circulating hormone levels are complex and involve direct effects on the pathways of hormone biosynthesis, transport, and metabolism, or indirect effects on feedback mechanisms (Brasfield et al., 2008). Previous studies have shown structural or functional differences in the

☆ This paper has been recommended for acceptance by Eddy Y. Zeng.

* Corresponding author.
E-mail address: huiliwang@rcees.ac.cn (H. Wang).

https://doi.org/10.1016/j.envpol.2017.09.072
0269-7491/

thyroid glands of reptiles after pesticides exposure (Sciarrillo et al., 2008; Bicho et al., 2013). However, few studies have investigated the potential toxic mechanisms of pesticides associated with reptilian HPT axis-related gene expression.

Reptiles are not required test subjects under the minimal requirements of federal regulations. However, a toxicity assay using lizards has been developed as an important model for ecotoxicological studies of reptiles in recent years (Amaral et al., 2012). Lizards have a wide geographic distribution, are small in size and easy to feed in the laboratory. What's more, most lizards are insectivorous (Bishop and Gendron, 1998) and thus likely have high contact with pesticides. A Chinese native lizard species-*Eremias argus* (*E. argus*) is widely distributed in the north of the Yangtze River. We collected juvenile *E. argus* from the wild in the Inner Mongolia Province and have maintained them in our laboratory for more than 4 years. The reproduction method of *E. argus* has also been established, so *E. argus* is regarded as an ideal model for reptilian risk assessment.

Pyrethroids, a class of endocrine-disrupting insecticides, contain one to three chiral centers and are now the fourth most used group of insecticides worldwide (Brander et al., 2016). The estrogenic activity and thyroid disruption of pyrethroid pesticides in mice or aquatic vertebrates have been previously investigated (Jin et al., 2015; Wang et al., 2011; Tu et al., 2016) but little is known about their effects on reptiles (Hopkins, 2000). Pyrethroids have been detected in the sediment of Sacramento-San Joaquin Delta of California (Weston and Lydy, 2010). The application rate of pyrethroid pesticides in field crops ranges from 5 to 15 g/ha, and applications are repeated at intervals of 1–2-week (Velmurugan et al., 2007). The lizards living in cropland will be exposed to these pesticides. The enantiomers of pyrethroids are known to selectively interact with biological systems (Liu et al., 2005) and may show enantioselective bioaccumulation and toxicity. Whether the enantiomers of pyrethroids are enantioselective with respect to their thyroid disruption potential in reptiles is still unclear.

To understand the role of pyrethroids in disruption of the reptilian thyroid axis, two enantiomers of the type II pyrethroid insecticide lambda-cyhalothrin (LCT) (Fig. S1) were orally administered to *E. argus* for three weeks. Thyroid histopathology, THs level and the transcription of HPT axis-related genes in the liver and brain were assessed to elucidate the potential mechanisms of thyroid disruption by different LCT enantiomers. The LCT and its metabolite-3-phenoxybenzoic acid (PBA) concentrations in the liver were also analyzed to determine the dose-response and time-relationship on lizard thyroid system. Our findings are intended to provide new insights into the enantioselective impact of pyrethroids on the thyroid axis of reptiles.

2. Materials and methods

2.1. Animals and husbandry

Immature (1–2 years old, before sexual maturity) male *E. argus* were obtained from our breeding colony in Changping district, Beijing, China. The average body weight and body length were 2.5 g and 35.6 mm respectively. The lizards were kept in a $5 \times 1.2 \times 0.4$ m solid bottom indoor aquarium covered with 10 cm of mollisol and fallen leaves. Ultraviolet lamps were set on a 12 h:12 h light/dark cycle to provide enough light and maintain the necessary temperature. The temperature and humidity were maintained at 25–30 °C, 30–50%, respectively. The lizards were fed with live mealworms twice each day and sprayed with water several times a day. The excreta were cleaned every day.

2.2. Chemicals

LCT (racemate, 98% pure, CAS 91465-08-6) was provided by J&K Chemical Technology (Beijing, China). All solvents of acetone, acetonitrile, n-hexane, and isopropanol were of HPLC grade and purchased from Dikma (Beijing, China).

2.3. Chromatographic separation and concentration analysis

The enantiomers of LCT were separated by HPLC on a CHIRALCEL® cellulose TRIS (3,5-dimethylphenyl-carbamate) (OD) column (Fig. S2). A volume of 20 μL was injected for chiral separation in the normal-phase mode. N-hexane and isopropanol (95:5, v/v) were used with a flow rate of 2.5 mL min^{-1}. The signal at 236 nm was recorded. The resolved enantiomers were manually collected into separate glass vials at the HPLC outlet. All the solvent fractions of the enantiomers were evaporated to dryness and redissolved in enthanol. A stock solution was prepared by dilution in corn oil following Wang et al. described method (Wang et al., 2014).

Acetonitrile was employed to extract the lizard samples. The analysis of LCT concentration was performed on a Thermo-TSQ 8000 GC/MS/MS equipped with an electron-impact ionization (EI) source and column-TR-35MS (0.25 mmΦ × 30 mID × 0.25 mm, Thermo). The two enantiomers of LCT can be separated completely by BGB-172 chiral capillary column (20% *tert*-butyldimethylsilyl-β-cyclodextrin dissolved in 15% diphenyl-polysiloxane and 85% dimethyl-polysiloxane, GBG Analytik, Adliswil, Switzerland) on GC/MS/MS (Fig. S3). The purity for both enantiomers was determined to be $\geq$ 99.9%. The PBA analysis was performed on HPLC/MS/MS equipped with a TSQ Quantum Access MAX triple quadrupole MS, an Accela 600 pump/auto sampler HPLC and a C_{18} column (2.1 mmΦ × 100 mm × 5 μm, Thermo). Blank liver and faeces samples were detected and no target chemicals were found. Detailed protocols for extraction, clean-up, identification and quantification are provided in previous studies (Chang et al., 2016a, 2016b).

2.4. Exposure experiment and sampling

Male lizards were randomly separated into three groups—a control group, a (+)-LCT exposure group, and a (−)-LCT exposure group (total n = 81, n = 27 for each group). Prior to the experiments, each group was allowed to acclimate to the experimental conditions for one week in the experimental glass cages ($60 \times 60 \times 40$ cm). Each glass cage contained 9 lizards and there were 3 cages for each group. The lizards were dosed orally with 10 mg kg^{-1} body weight (bw) of the LCT enantiomers or corn oil once a week. The dosing was operated with a GC instrument injection syringe to deliver a volume of 10–20 μL corn oil-ethanol or corn oil-ethanol LCT enantiomer into the oral cavity of each lizard (Wang et al., 2014). There are no direct toxicity data of LCT in reptiles and birds are usually used as surrogates in risk assessment (Weir et al., 2010). The LD_{50} value of LCT in birds is greater than 2000 mg kg^{-1}, and 0.5% of the LD_{50} value was selected in this study. This dosage is also similar with pyrethroid bioaccumulation studies on earthworm (Chang et al., 2016b) and rabbit (Liu et al., 2011). The every week exposure is to mimic the usual usage of LCT on the farmland. Lizards from each group were euthanized 7, 14, and 21 days after exposure. Three lizards were selected randomly from each cage, and three replicates were prepared. The body weights were measured. The brain and liver were collected, weighed, and frozen at −80 °C with RNA store. A part of liver was left for concentration analysis. The blood was immediately centrifuged at 2500 × g for 10 min, and the plasma was stored at −80 °C for TH analysis. The collected faeces of the lizards from the same