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Enantioselective Elimination and Gonadal Disruption of Lambda-Cyhalothrin on Lizards (*Eremias argus*)

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Supporting Information

ABSTRACT: In this study, the different metabolic pathways of lambda-cyhalothrin (LCT) enantiomers in *Eremias argus* feces and enantioselective disruption on hypothalamus-pituitary-gonad (HPG) system were investigated. After 7 days oral exposure to LCT enantiomers, the concentration of 3-phenoxybenzoic acid (PBA), hydroxylated and sulfated LCT were higher in the (+)-LCT exposure group than that in the (−)-LCT exposure group, which indicated a higher metabolic rate of (+)-LCT than (−)-LCT. Although no significant differences were seen on lizard body weight after enantiomers' exposure, the gonadosomatic index was dramatically decreased. The testicular impacts such as increased seminiferous tubule diameters were only observed in the (+)-LCT exposure group. Consistent with this result, the expression of *ar* gene in the (+)-LCT exposure was significantly higher than that in the (−)-LCT exposure group. In addition, the stronger binding affinity of AR with (+)-LCT further demonstrated the more serious disruption of (+)-LCT on lizard HPG axis than (−)-LCT. This study first elucidated the metabolic pathway and endocrine effects of LCT in lizards at enantiomeric level and provided some evidence for lizard population decline.

KEYWORDS: *pyrethroid pesticides, endocrine disruption, metabolism, enantiomers, reptiles*

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INTRODUCTION

Pyrethroids are now the fourth group of insecticides that are widely used in the world. Because of their lower neurotoxicity to mammals compared with organophosphate pesticides, the use of pyrethroid pesticides has increased significantly over the past 20 years.[1] It is found to be ubiquitous in treated wastewater[2−4] and soil.[5,6] Uncontrolled use of these insecticides resulted in their entry into the food chain, thereby causing toxicity to different organism systems. Compared with dermal exposure, oral and inhalation result in faster systemic exposure.[7,8] Because of its lipophilic properties, pyrethroids tend to deposite in the fat of lizard, rat, and pig.[9−11] Pyrethroids are demonstrated to be accumulated in fish tissues.[12,13] However, the body burden of pyrethroids in terrestrial animals has been seldomly studied.

The metabolic pathways of pyrethroids in rats have been thoroughly studied. According to Kaneko et al., ester hydrolysis is the primary metabolic reaction of pyrethroids via esterase, and the alcohol moiety can be further oxidized to an acid.[14] These metabolites are rapidly metabolized to inactive metabolites via partly conjugated to glucuronide and both the conjugates and free acids can be excreted through the urine.[15] Because of its easy of availability, urine is being most heavily used as a matrix to determine which metabolites are major biomarkers to identify the exposure of its parent compound.[16] However, the metabolite profile of pyrethroids in animal feces has been little studied, which can also reflect the environmental fate of pyrethroids.

In fish and mammals, pyrethriods are regarded as endocrine disrupting compounds (or EDCs), which can interact with hormone receptors to disrupt the endocrine signaling pathways.[17−19] For example, Tu et al. have predicted high binding affinity of lambda-cyhalothrin (LCT) and bifenthrin (BF) to thyroid hormone receptor (TR) protein in zebrafish embryos.[20] Besides, pyrethroids are chiral pesticides containing 1−3 chiral centers and up to 8 optical isomers.[21] These optical isomers also show very different metabolic rate and toxicity in fish or mammals.[22,23] However, the metabolism and endocrine effects of pyrethroids in terrestrial animals have not been detailed studied at enantiomeric level.

Reptiles are one kind of the most common terrestrial animals. Among these reptiles, lizards are considered to be the most sensitive species toward pesticides.[24] Moreover, lizards are the most abundant species among reptiles (http://www.reptile-database.org/db-info/SpeciesStat.html), but their populations are decreasing year by year.[25,26] The influence of EDCs on lizards has been regarded as one of the important reasons for the population decline.[27] As endocrine disruptors, the impact of pyrethroids on endocrine system of lizards is still unknown.

The objective of this study is to elucidate the different metabolic pathways of the enantiomers of a pyrethroid in lizard feces and its enantioselective effects on lizard sexual gland system. We chose a Chinese native lizard species-*Eremias argus* as an ideal model and lambda-cyhalothrin (LCT) as a representative type II pyrethroid. The metabolite screening, body weight, gonadosomatic index, testes injuries, and hypothalamus−pituitary−gonad (HPG) axis associated gene expression of *Eremias argus* were investigated after LCT enantiomers exposure.

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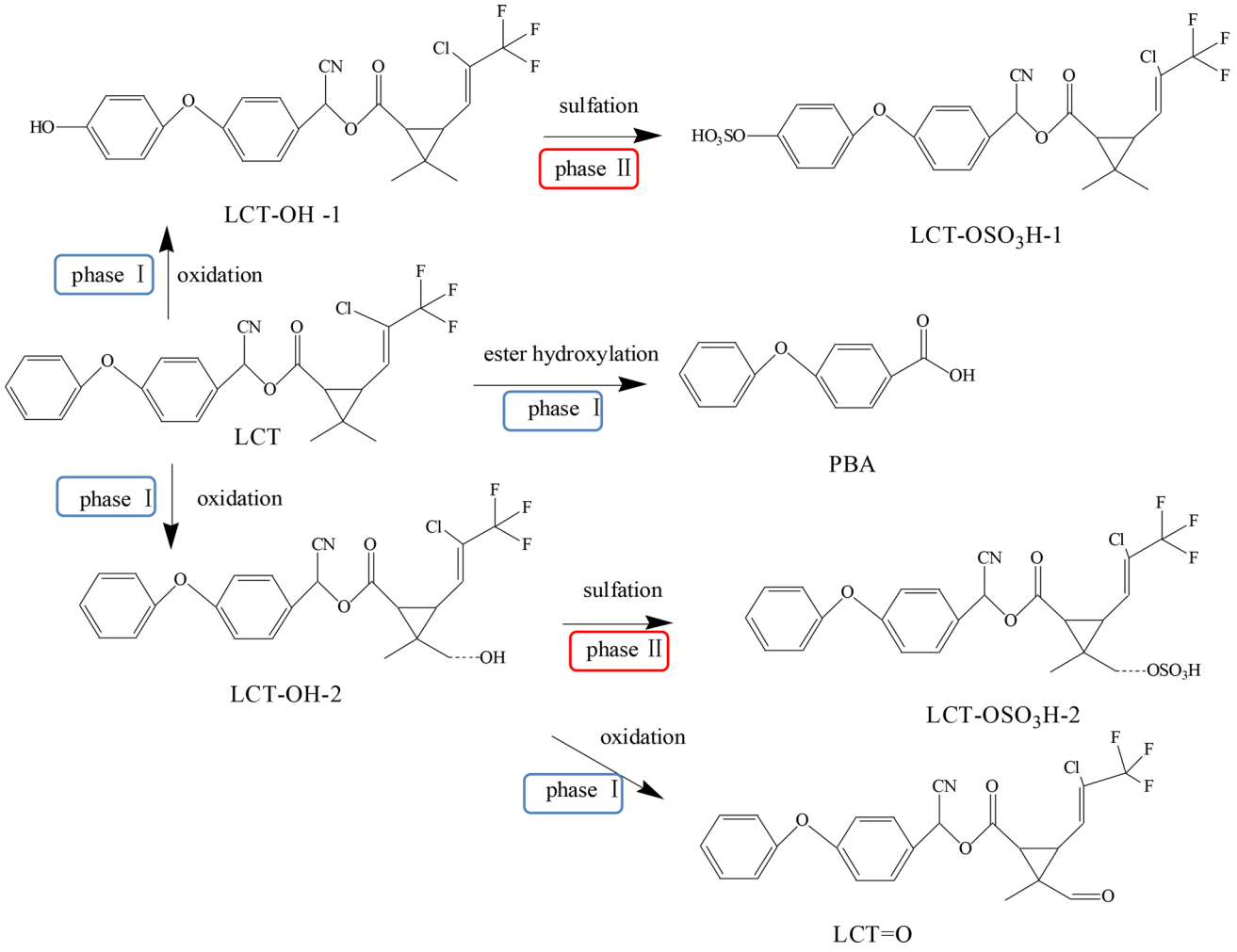

Figure 1. Proposed metabolic pathway of LCT enantiomers in lizard feces.

MATERIALS AND METHODS

Chemicals and Reagents. LCT (racemate, purity >98%, CAS# 91465−08−6) and 3-phenoxybenzoic acid (purity >98%, CAS# 3739−38−4) were purchased from J&K Chemical Technology (Beijing, China). HPLC grade solvents, such as *n*-hexane, acetonitrile, and isopropanol were obtained from Dikma (Beijing, China). TRIzol reagent, reverse transcription (RT) kit, and qPCR SYBR green kit were provided by TIANGEN Biotech (Beijing, China).

LCT Enantiomer Separation. A CHIRALCEL cellulose TRIS (3, 5-dimethylphenyl-carbamate) (OD) column was used to separated the optically pure enantiomers of LCT on HPLC. The separation of enantiomers was operated on normal-phase mode consisting of 95% N-hexane and 5% isopropanol (v/v). The flow rate was 2.5 mL/min. The wavelength of detector was 236 nm. The corresponding enantiomers were manually collected and then evaporated to dryness and redissolved in enthanol. The purity of LCT enantiomers was ≥99.9%, which was measured by GC−MS/MS using chiral column-BGB-172 according to our previous report.[28] LCT enantiomers were dissolved in corn oil with ethanol as carrier solvent.

Exposure Experiment. Immature male *Eremias argus* (1−2 years old, body length from snout to vent was 3.0−3.5 cm) were collected from our breeding colony in Changping District, Beijing, China. The average body weight/body length was 0.7 g/cm. A 5 × 1.2 × 0.4 m^3 indoor aquarium covered with 10 cm of mollisol and fallen leaves was used to keep the collected lizards. To provide enough light, ultraviolet lamps were set to a 12:12 h light/dark cycle. The temperature and humidity were set at 25−30 °C, 30−50%, respectively.

Lizards were allowed to acclimate for 1 week in the experimental cages before experiment. During experiment, lizards were randomly separated into three groups including control group, (+)-LCT and (−)-LCT exposure group (n = 6 for each group). According to the OECD guideline, generally 5%−20% LD50 value is used as highest exposure dosage. The LD50 of LCT to lizards was 916.5 mg/kg.[29] In this study, 1% LD50 was chosen as a mild exposure dosage. This dosage has been used in our previous studies and makes us easy to compare the relevant results.[9,28] LCT enantiomers (10 mg/kg) or enthanol-corn oil (10−20 μL) were orally dosed to lizards once using a GC instrument injection syringe. After 7 days of exposure, lizards in each group were sacrificed. Lizard testes were collected and stored at −80 °C. A part of testes was stored in RNA store for gene expression analysis, and the left part of testes was put into 4% paraformaldehyde for histological analysis. The feces during experiment were collected and stored at −20 °C before analysis.

Chemical Analysis. In this study, lizard feces were collected from 0 to 7 days after the exposure of LCT enantiomers. The extraction protocol is as follows: feces (0.1−0.2 g) were mixed with 15 mL of acetonitrile in a 50 mL polypropylene centrifuge tube. The mixture was vortexed for 2 min, ultrasonic for 10 min, and then centrifuged for 5 min at 8000 rpm. The upper phase was collected, and then the precipitate (lower phase) was extracted again with 15 mL of actonitrile. The combined supernatant was dehydrated with 2 g of anhydrous magnesium sulfate and then evaporated on a vacuum rotary at 35 °C. The dried residues were redissolved in 1 mL of n-haxane and filtered through 0.22 μm filter before GC−MS/MS analysis. For the analysis of LCT metabolite-PBA, the extract was redried and redissolved in 1 mL of acetonitrile.

A Thermo-TSQ 8000 GC−MS/MS equipped with a Thermo column TR-35MS (0.25 mmΦ × 30 mID × 0.25 mm) and an

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electron-impact ionization (EI) source was used to detect the LCT metabolites in lizard feces. The ramped temperature program was operated as follows: held at 50 °C for 1 min, ramped to 200 °C at 20 °C/min for 1 min, ramped to 260 °C at 5 °C/min for 10 min, ramped to 280 °C at 20 °C/min for 2 min. The full scan mode was used with a scan range from 50 to 550 m/z.

The quantification of 3-phenoxybenzoic acid (PBA) level in lizard feces was conducted on HPLC−MS/MS. HPLC−MS/MS was equipped with an Accela 600 pump/auto sampler HPLC and a TSQ Quantum AccessMax triple quadrupole MS. A Thermo Hypersil Gold C_{18} column (200 × 2.1 mm^2, 1.9 μm) was used, and the isocratic mobile phase was composed with acetontrile and 0.05% formic acid in water (80:20, v/v) at a flow rate of 0.2 L/min. Multiple reaction monitoring (MRM) mode was used for PBA detection. The transition from precursor ion (m/z 213.00) to product ion (m/z 93.25) was used for quantification of PBA. The limit of detection (LOD) and limit of quantification (LOQ) for PBA was 0.005 mg/kg and 0.015 mg/kg, respectively.

Testicular Histopathology. The histological sections of lizard testes were prepared following the method described by McFarland et al.[30] Briefly, the samples were trimmed, put in cassettes, embedded in paraffin, cut into 6 μm thick slices, and then stained with hematoxylin and eosin. The slices were observed on light microscope (ZEISS, AXIO).

Isolation of mRNA, cDNA Synthesis, and Real-Time PCR. The isolation of RNA and cDNA synthesis were operated according to the protocol described in our previous paper.[31] Briefly, total mRNA in lizard gonad was extracted by TRIzol reagent. NanoDrop 2000 microvolume spectrophotometer was used to measure the concentration and purity of the extracted RNA. cDNA was synthesized according to the reverse transcription of mRNA using RT kit with gDNase.

The HPG axis related genes including steroid hormone receptors (*erα*, *ar*), cytochrome P450 enzymes (*cyp17*), 17-beta hydroxysteroid (*hsd17β*), and aromatic hydrocarbon receptor (*ahr*) were investigated in this study. The Genebank accession number and their respective primers were listed in Table S1.[32,33]

Quantitative real-time PCR was conducted in a MX3005P real-time qPCR (Stratagene, USA) using the SYBR Green PCR Kit. Relative quantification of each mRNA expression level was measured by normalizing the values against the β-actin mRNA level.

Molecular Docking. The molecular docking method was modified according to Tu et al.[20] The three-dimensional (3D) structure of AR (PDB ID: 2am9) was obtained from Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). The generation of docking input files was performed on the AutoDockTools 1.5.6 package (http://mgltools.scripps.edu). The AutoDock Vina 1.1.2 (http://vina.scripps.edu) was employed to study the molecular docking. For Vina docking, the search grid of AR was set as center_x, 20.404; center_y, 5.308; and center_z, 9.165 with dimension size_x, 54; size_y, 46; and size_z, 66. The exhaustiveness value was 20. On the basis of the Vina docking score, the best-scoring pose was selected and analyzed visually on AutoDockTools 1.5.6.

Data Analysis. Statistical analysis of the data was performed using one-way analysis of variance (ANOVA) with subsequent Tukey test on SPSS (version 13.0; USA). $P < 0.05$ was regarded as statistically significant.

The gonadosomatic index (GSI) was calculated with the formula below:

$$\text{GSI}\ (\%) = \frac{\text{Gonad weight}}{\text{body weight}} \times 100$$

RESULTS AND DISCUSSION

Metabolic Process of LCT Enantiomers in Lizard Feces. The elimination of LCT follows the first-order kinetics and most of the dose is eliminated within the first 1−6 h after absorption in lizard tissues.[9] Pyrethroids are rapidly degraded to inactive metabolites, which are excreted primarily through urine.[34] However, the format of LCT metabolites in feces has not been well studied. In this study, we screened the metabolites of LCT enantiomers in lizard feces after 7 days of exposure. In lizard feces, oxidized (LCT−OH, LCT═O) and sulfated (LCT-OSO_3H) LCT were detected in both (+)-LCT and (−)-LCT exposure groups. The precursor ions for LCT−OH, LCT═O, LCT-OSO_3H were 465, 463, and 545. The metabolic pathway was predicted in Figure 1. At the first metabolic step, oxidation and ester hydroxylation occurred. Depending on the chemical structures, the oxidation reactions could occur on both the acid and alcohol moieties sites.[35] Generally, the oxidation of LCT is preferentially occurred on the methyl group and the 4′-position of phenoxy ring compared with other positions. In that case, two isomers of hydroxylated LCT were detected at 28.80 and 28.88 min (Figures S1 and S2). LCT−OH hydroxylated on methyl group site was further oxidized to LCT═O, which was detected at 27.17 min. The two hydroxylated LCT metabolites could process the phase II reaction, which formed sulfate conjugate in lizard feces. The sulfated LCT was detected at 30.81 and 30.96 min, respectively, as isomers. Study on mammals found that the hydrophilic conjugates of pyrethroids were glucuronides, sulfates, and amino acid conjugates.[14] Only relatively high LCT-OSO_3H concentration was found in lizard feces indicating the unique metabolism system of reptiles compared with mammals.

Semiquantified method (peak area) was used to calculate the concentrations of LCT metabolites, which were varied in different exposure groups (Table S2). The concentration of (+)-LCT metabolites from high to low was LCT−OH > LCT-OSO_3H > LCT═O, while the concentration of (−)-LCT metabolites from high to low was LCT−OH > LCT═O > LCT-OSO_3H. These results indicated that LCT enantiomers preferred to excrete through feces in hydroxylation form. Moreover, the concentration of LCT−OH-2, which was hydroxylated on methyl group, was higher than LCT−OH-1 in the (−)-LCT exposure group, and the result was opposite after the (+)-LCT treatment. The further metabolic pathway of LCT−OH-2 was also different between the (+)-LCT and (−)-LCT exposure group. For (+)-LCT exposure, LCT−OH-2 was mainly metabolized into sulfated LCT, while further oxidation preferred to occur in (−)-LCT exposure group. These results elucidated the different metabolite profiles of LCT after enantiomers' exposure, which may explain the enantioselective residual level of LCT in lizard tissues. Another interesting finding is that LCT-OSO_3H-1 was only detected in the (−)-LCT exposure group. Similar result has been reported that only one isomer produced cholesterol ester conjugate out of four optical isomers of fenvalerate.[36] It demonstrated that chiral pyrethroid may produce different metabolites for different enantiomers.

Ester hydroxylation was also regarded as one of the most important metabolic pathways of pyrethroids. One of metabolites, 3-phenoxybenzoic acid (3-PBA) exhibited higher endocrine activity than its parent compound.[19] If 3-PBA could be excreted through lizard feces, it might have the potential to further influence the environment. In this study, we quantified the concentration of 3-PBA in lizard feces. The residual level of 3-PBA was 0.15 mg/kg in the (+)-LCT exposure group and 0.10 mg/kg after the (−)-LCT treatment, respectively. Combined with the semiquantified results of LCT metabolites, it could be hypothesized that (+)-LCT was metabolized faster

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than (−)-LCT after 7 days of exposure. The phase II reaction and higher hydroxylation rate might accelerate the excretion of (+)-LCT in lizards.

Body Weight and Gonadosomatic Index (GSI). The body weight and GSI of lizards were shown in Table 1 after 7 days of exposure in both control and exposure groups. No significant differences were found on lizard body weight between control and exposure groups ($p > 0.05$, one-way ANOVA). The GSI in exposure groups were significantly decreased when compared with control group ($p < 0.05$, one-way ANOVA). However, there was no significant difference on GSI between (+)-LCT and (−)-LCT exposure ($p > 0.05$, one-way ANOVA with subsequent Tukey test). It can be hypothesized that both the two LCT enantiomers' exposure affected the function of lizard testes, but no enantioselective effects were shown according to GSI.

Table 1. Body Weight and Gonadosomatic Index (GSI) of Lizards (*Eremias argus*) in Both Control and Exposure Groups at 7 Days

groups	body weight (g)	GIS (%)
control	2.58 ± 0.22	1.77 ± 0.13a
(+)-LCT	2.45 ± 0.35	1.35 ± 0.20b
(−)-LCT	2.47 ± 0.24	1.30 ± 0.18b

Groups with different letters indicated significant different from each other ($p < 0.05$, one-way ANOVA with subsequent Tukey test).

Testicular Histology. As the significant decrease of GSI value is a good indicator for the testicular damage, we further investigated the testicular histology. The tissue slices of lizard testes were shown in Figure 2, which elucidated the effect of LCT enantiomers on spermatogenesis. Immature lizard testis in control group presented normal testicular architecture and relatively large lumen of seminiferous tubular (Figure 2A). Lizards exposed to 10 mg/kg (+)-LCT showed significantly larger diameter of seminiferous tubular and more spermatozoa compared with testis in control group (Figure 2B). The increased diameter of seminiferous tubular might be a consequence of altered seminiferous tubule fluid level. As the secretion of seminiferous tubule fluid was influenced by testosterone level,[37,38] the result could be further explained by the altered testosterone level. In addition, more spermatozoa indicated the increased spermatogenesis in lizard testes after the (+)-LCT exposure. It can be hypothesized that the (+)-LCT exposure stimulated the activity of lizard testis and disrupted the normal sexual gland system. In the (−)-LCT exposure group, although no significant changes were found regarding to the diameter of seminiferous tubular compared with testis in control group, more spermatozoa appeared in seminiferous tubular (Figure 2C). This phenomenon also demonstrated the accelerated activity of testis after the (−)-LCT treatment, even though it was not as serious as in the (+)-LCT exposure group. This is an interesting result that seldomly reported in other studies. Studies focusing on the adverse effect of pollutants in mature animals often showed decreased diameter of the seminiferous tubules[39] and inhibition of spermatogenesis.[37,40] In that case, the testicular effect induced by pesticides may be related with the different life stages of nontarget animals.

HPG Axis Related Gene Expressions. The HPG axis related genes including *cyp17*, *hsd17β*, *erα*, *ar*, and *ahr*, regulate the synthesis of steroid hormone and the normal function of sexual gland system. In this study, we quantified the mRNA expression of HPG axis related genes in lizard gonad after LCT enantiomers' exposure (Figure 3). After (+)-LCT or (−)-LCT exposure, the expression of *cyp17*, *hsd17β*, *erα*, *ar* gene was significantly increased compared with that in control group ($p < 0.05$, one-way ANOVA with subsequent Tukey test). However, no significant differences were seen on *ahr* mRNA expression ($p > 0.05$, one-way ANOVA).

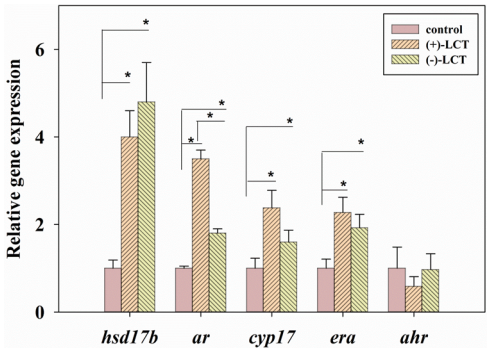

Figure 3. Relative expression levels of *cyp17a*, *hsd17β*, *erα*, *ar*, and *ahr* genes in lizard (*Eremias argus*) gonad after 7 days of exposure. ∗ indicated significant differences between two different exposure groups ($p < 0.05$, one-way ANOVA with subsequent Tukey test).

Cytochrome P450 17a (cyp17) is the key enzyme that catalyzes androstenedione biosynthesis.[41] The further reduction reaction from androstenedione to testosterone is catalyzed

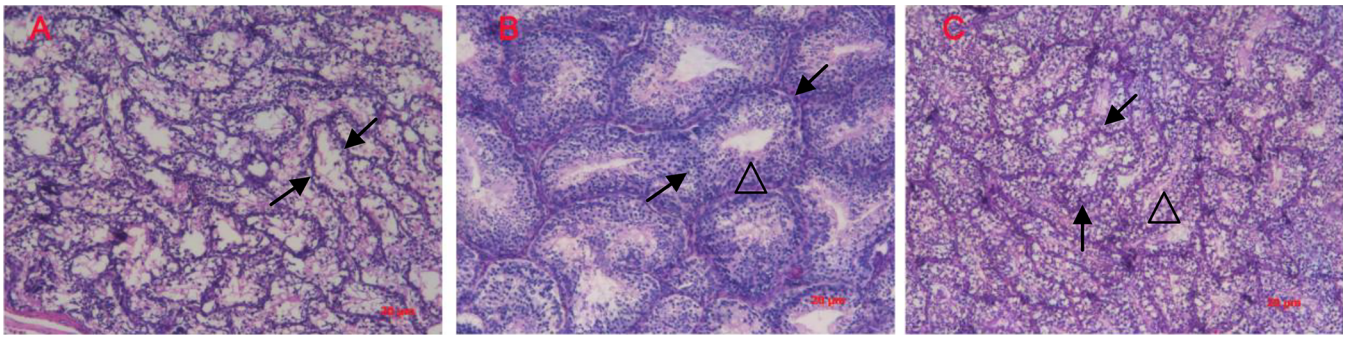

Figure 2. Gonad section of the male lizards (*Eremias argus*). (A) Representative section of the lizard in control group; (B) representative section of the lizard in (+)-LCT exposure group; (C) representative section of the lizard in (−)-LCT exposure group. Black arrow represented seminiferous tubular; triangle represented spermatozoa.

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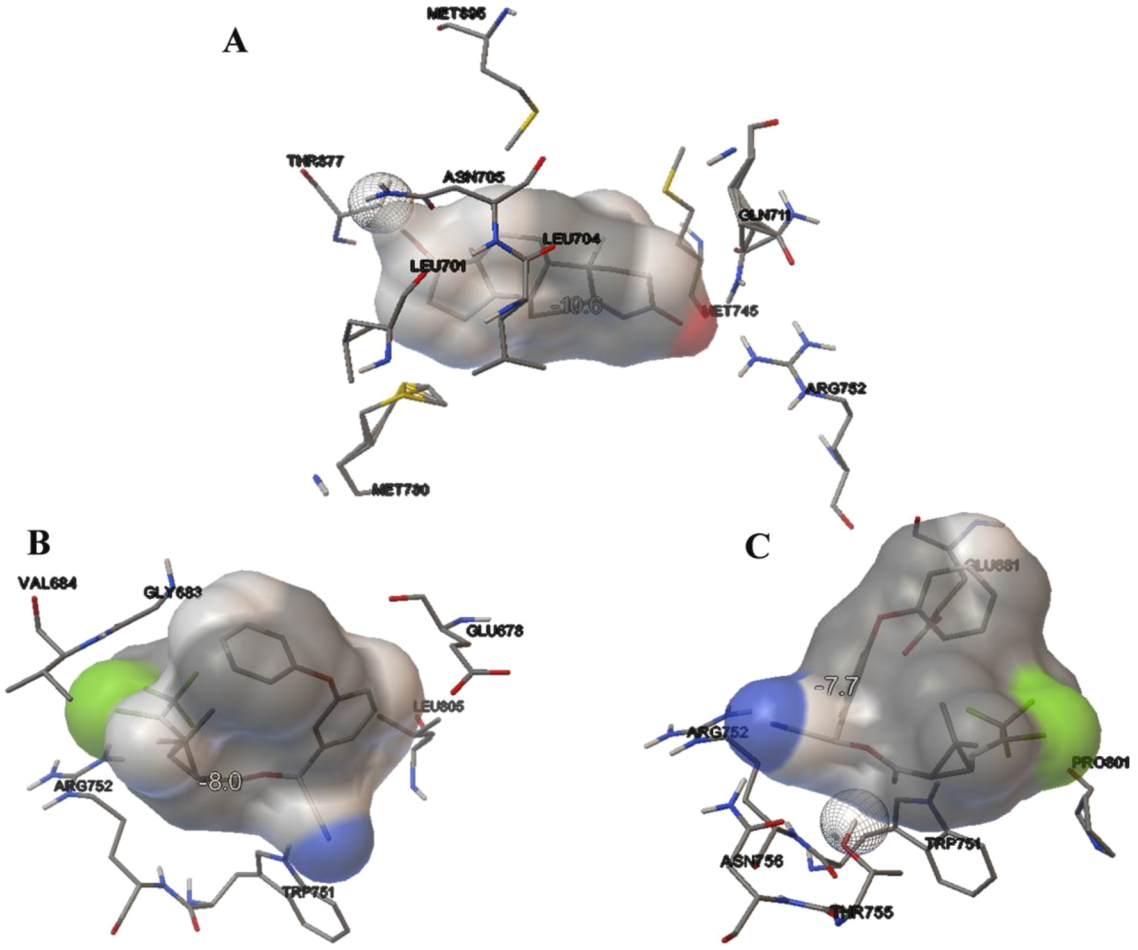

Figure 4. Binding mode of (A) estrodiol, (B) (+)-LCT, (C) (-)-LCT to AR protein in cartoon mode.

by *hsd17β*.[42] Thus, both *hsd17β* and *cyp17* genes were responsible for the synthesis of testosterone. As both *hsd17β* and *cyp17* mRNA levels were significantly up-regulated after enantiomers' exposure of LCT, the testosterone levels might also increase. Moreover, the up-regulation of *cyp17* in (+)-LCT exposure group (2.4-fold) was higher than that in (−)-LCT exposure group (1.6-fold). The results were also consistent with the changes of testicular histology, which indicated higher excretion rate of testosterone in lizard testes after the (+)-LCT exposure. In lizard testes, spermatogenesis and steroidogenesis were two vital processes which were controlled by endocrine system.[43] After LCT exposure, the two important processes in lizard testes were seriously affected.

The steroid hormone receptors (ER and AR) can regulate the transportation of testosterone and estrodiol hormone from gonad to blood and other tissues. The *erα* and *ar* genes modulate the steroid hormone receptors. Thus, the *erα* and *ar* mRNA levels can demonstrate the alteration of steroid hormones to some extent. Since the expression of *erα* and *ar* mRNA was significantly increased in both (+)-LCT and (−)-LCT exposure group and the function of spermatogensis in testes was also stimulated, LCT enantiomers might have the ability to increase the excretion of steroid hormones and accelerate the activity of lizard HPG system. In addition, the expression of *ar* mRNA was higher after the (+)-LCT treatment (3.5-fold) compared with that in the (−)-LCT group (1.8-fold) ($p < 0.05$, one-way ANOVA with subsequent Tukey test). It indicated higher toxicity of (+)-LCT than (−)-LCT on lizard HPG axis combined with the results of *cyp17* gene levels. One of our previous studies also showed that (+)-LCT induced higher disruption on lizard thyroid system than (−)-LCT regarding thyroid hormone and thyroid gland histological changes.[28] We can hypothesize from these results that (+)-LCT has higher potency to disrupt lizard endocrine system than (−)-LCT.

Recent research found that the aryl-hydrocarbon receptor (ahr) is regarded as a target of pyrethroid pesticides in fish.[19] However, the expression of *ahr* gene in lizard testes was not significantly changed after the exposure of LCT enantiomers ($p > 0.05$, one-way ANOVA). These results indicated that other targets of LCT such as androgen receptor (AR) could compete with AHR to show its higher affinity with LCT.

Molecular Docking between Androgen Receptor (AR) and LCT Enantiomers. A previous study has demonstrated an enantiomers-specific affinity of bifenthrin to estrogen receptor (ER).[44] According to our study, only the gene expression of *ar* mRNA was enantioselective after LCT enantiomers' exposure. Thus, the enantioselective binding affinity between LCT and androgen receptor (AR) was investigated. LCT enantiomers docked into the binding pocket of AR was shown in Figure 4.

In the binding pocket of AR (Figure 4A), 17-OH of testosterone interacted with Thr 877 and Asn705 to form hydrogen bond. Moreover, Arg752 and Gln711 were further involved in hydrogen bond with 3-O.

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Both (+)-LCT and (−)-LCT docked into the binding pocket of AR but showed different orientations, as shown in Figure 4B and C. Detailed analysis demonstrated that the oxidibenzene ring of (+)-LCT fitted into the hydrophobic binding pocket of AR surrounded by Leu805 and Glu678. The key hydrogen bond was formed between the nitrogen atom of the isobutyronitrile bond with Trp751. In contrast, the oxidibenzen ring of (−)-LCT was surrounded by Glu681. The nitrogen atom of isobutyronitrile bond with Arg752 and oxygen atom of ester bond with Thr755 formed the hydrogen bond.

The binding affinities of testosterone, (+)-LCT and (−)-LCT with AR were measured using the Vina docking score algorithm. The binding affinity between testosterone and AR was −10.6 kcal/mol. The binding energy obtained for (+)-LCT and (−)-LCT with AR were −8.0, −7.7 kcal/mol, respectively, indicating higher binding affinity for (+)-LCT. As the gene expression of *ar* mRNA in lizard gonad was also higher in the (+)-LCT exposure group than that in the (−)-LCT treatment group, the AR activity may be more seriously blocked by (+)-LCT.

We have reported the enantioselective effects of LCT enantiomers on lizard hypothalamus-pituitary-thyroid (HPT) system. In this study, the enantioselective toxicity of LCT enantiomers on lizard HPG system was further investigated. Although the elimination of (+)-LCT was faster than (−)-LCT, (+)-LCT exposure induced more serious effects on lizard testes and associated gene expression levels. The enantioselective endocrine disruption of LCT was predicted to be partially due to their enantiospecific binding affinity with AR. As LCT enantiomers induced significantly endocrine disorder on lizards, the reproductive effects of LCT on lizard need further study, which may explain the population decline.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b05990.

Primers used for real-time PCR and GenBank accession numbers; concentration of LCT metabolites calculated by peak areas; chromatography and mass spectra of predicted (+)-LCT and (−)-LCT metabolites in lizard feces (PDF)

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

erα, *ar*, steroid hormone receptors; *cyp17*, cytochrome P450 enzymes 17a; *hsd17β*, 17-beta hydroxysteroid; *ahr*, aromatic hydrocarbon receptor; HPG, hypothalamus−pituitary−gonad; GSI, gonadosomatic index; LCT, lambda-cyhalothrin.

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