Unraveling the different toxic effect of flufenoxuron on the thyroid endocrine system of the Mongolia racerunner (*Eremias Argus*) at different stages

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HIGHLIGHTS

- The triiodothyronine level was more seriously disturbed at the resting stage.
- The epithelium cell height was significantly increased at the proliferation stage.
- The HPT axis related genes were more sensitive at the proliferation stage.

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ABSTRACT

Flufenoxuron is a widely used pesticide to inhibit the synthesis of chitin during insect development and its effect on the growth of lizards has been little addressed. The hypothalamus-pituitary-thyroid (HPT) axis plays an important role on the development of lizards. In this study, the lizards at different development stages (proliferation and resting stages) were exposed to flufenoxuron for 21 days. The plasma thyroid hormone levels, thyroid gland histopathology and expression profiles of thyroid hormone receptors (*trα*, *trβ*), deiodinases (*dio1*, *dio2*), and transthyretin (*ttr*) genes were measured to evaluate the toxic effect of flufenoxuron on the HPT axis at different stages. The flufenoxuron exposure showed more serious effect on the triiodothyronine (T₃) level at resting phase than that at proliferation stage. The follicle epithelium cell height in the thyroid was only significantly increased when the exposed male lizards were at proliferation stage. The alteration of HPT axis-related genes expression was gender and tissue dependent after flufenoxuron treatment. The lizards exposed to flufenoxuron showed that the *trα*, *trβ*, *dio1*, *dio2*, and *ttr* genes in the female liver were more sensitive at the proliferation stage than that at the resting stage. In the male brain, the expressions of *trα*, *trβ*, *dio1*, and *dio2* gene were significant decreased at proliferation stage while significant increased at resting stage after flufenoxuron exposure. Therefore, the thyroid endocrine system of lizards could be affected by the flufenoxuron exposure and the different development stage should also be considered when study the toxic effect of contaminants on the lizards.

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1. Introduction

Benzoylurea insecticides are widely used to control the various insect pests through inhibiting the synthesis of chitin during insect development. Although the toxicity to marine species is low, the

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environmental relevant concentrations of benzoylurea pesticides can cause a complete cessation of molting and finally death in the exposed copepods (Macken et al., 2015). Flufenoxuron is one of halogenated benzoylurea pesticides and used as a foliar spray to manage the vegetable insect pest (Hsiao et al., 2013). Due to its slower metabolism and reduced excretion in the larvae, flufenoxuron has showed a priority compared with the early benzoylurea pesticides (Clarke and Jewess, 1990). In China, flufenoxuron is widely used to control desert locust. However, flufenoxuron did not

readily decompose in the environment and prefer to accumulate in the soil (Hsiao et al., 2013). The humans poisoning with flufenoxuron showed severe lactic acidosis, shock, elevation of cardiac enzymes, and global left ventricular hypokinesia (Woo and Lim, 2015). There is no report about the toxic effects of flufenoxuron on the reptiles which can easily contact with the pesticides.

Generally, the hypothalamus-pituitary-thyroid (HPT) axis regulates the synthesis and release of thyroid hormones. The thyroid hormones play important roles in the development and physiological processes in the vertebrates (Dunlap, 1995). For example, thyroid hormones are responsible for the metabolism in the mice liver (Martin et al., 2007), metamorphosis in the *Xenopus laevis* (Fort et al., 2011) and teleost fish (Power et al., 2001). In the reptiles, the tail regeneration, metabolic rate and shedding frequency also have a correlation with the thyroid hormones (Kanaho et al., 2006). Particularly, injection of thyroxine increases the shedding frequency in the lizards (Chiu et al., 1967) while decreases it in the snakes (Chiu and Lynn, 1970). The contaminants exposed to lizards or snakes at different physiological stages (before and after skin shedding) might display different toxic effects on the thyroid endocrine system. The alteration of thyroid hormones may show compensatory mechanism through disrupting the transcription of most target genes, such as thyroid hormone receptors (*trα* and *trβ*), deiodinases (*dio1* and *dio2*), and transthyretin (*ttr*) genes, which are responsible for the biological response, metabolism and transportation of thyroid hormones. The underlying mechanism is still unclear, partly due to the lack of investigation on the window phase of reptiles.

Lizards are one of the most remarkable members consisting of more than 70% reptile species (Gibbons et al., 2000). Besides, lizards are usually characterized by its wide geographic distribution, small size and are easy to feed in the laboratory. Recently, lizards are gradually regarded as an ideal model for reptilian ecotoxicology study (Amaral et al., 2012a). The lizard species are varied in different countries and many researchers use their native species for investigation, which are more sensitive to the local environment (Trinchella et al., 2006; Amaral et al., 2012b). In this study, we chose one of Chinese native lizard species-*Eremias Argus* (*E. argus*) as a representative of reptiles. *E. argus* is one kind of small lizards, widely distributes in the north of Yangtzi River. We collected *E. argus* from the wild in the Inner Mongolia Province and maintained them in our laboratory since 2012. The laboratory breeding and reproduction methods have been well established.

There is no study about the alteration of HPT axis-related genes expression after contaminants exposure on lizards. The aim of our study was to go insight into the disturbance of flufenoxuron on the thyroid endocrine system of lizards at different stages (proliferation and resting stages). The *trα*, *trβ*, *dio1*, *dio2* and *ttr* mRNA levels were determined in different tissues (liver, brain, kidney, gonad, skin and thyroid gland) of lizards. The changes of thyroid hormone levels, thyroid gland lesions and HPT axis-related genes of lizards at different stages were investigated after treatment with flufenoxuron. This study could be a basis of toxicogenomics of thyroid endocrine system in the lizards.

2. Materials and methods

2.1. Chemicals

Flufenoxuron (Fig. S1) (purity 98%) was purchased from J&K Chemical Technology (Beijing, China). The solvents were analytical grade and purchased from Dikma (Beijing, China).

2.2. Lizards husbandry

The mature (2–3 years old) male and female *E. argus* were obtained from our breeding colony at Changping district, Beijing, China. The average body lengths were 48 mm for male and 50 mm for female. The lizards were kept in a 5 × 1.2 × 0.4 m solid bottom indoor aquarium covered with 10 cm of mollisol and fallen leaves. Ultraviolet lamps were set at on a 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 other day. Prior to the experiments, *E. argus* were allowed to acclimate in the experimental conditions for one week in experimental glass cages (60 × 60 × 40 cm).

2.3. Experimental design and sample collection

There is no toxic data of flufenoxuron on the lizards and birds are usually used as a surrogate in the risk assessment for reptiles (Weir et al., 2010). The LD₅₀ value of flufenoxuron in birds is greater than 2000 mg kg⁻¹. The preliminary experiment demonstrated that the 50 mg kg⁻¹ flufenoxuron exposure caused more seriously influence on the body weight of lizards than the 5 or 20 mg kg⁻¹ exposure but no mortality was observed. Thus, the 2.5% of the LD₅₀ value (50 mg kg⁻¹) in birds was selected as the exposure concentration in lizards.

The skin shedding frequency of *E. argus* is 3–4 weeks once for the metabolism and growth. The skin shedding cycle can be divided into resting and proliferation phase (Maderson and Licht, 1967). In this experiment, the lizards were separated into proliferation stage (before skin shedding) and resting stage (after skin shedding). Following acclimation, the lizards at the proliferation stage or resting stage were sorted by body length and distributed evenly into two groups with each group consisting of nine male and nine female lizards (n = 18). The average body weight between the groups was not statistically significant (*p* > 0.05). The treatment groups were administered orally with 0, 50 mg kg⁻¹ flufenoxuron once a week for three weeks. The body weight was measured on 0, 7, 14, and 21 days. Six lizards (three male) were euthanized at 21 days from each group and three replicates were prepared. The lizard liver, brain and thyroid gland were collected, and then frozen at –80 °C. The blood was immediately centrifuged at 2500 × *g* for 10 min, and the plasma was stored at –80 °C for thyroid hormone analysis.

2.4. Thyroid hormone analysis

The plasma samples were collected after 21 days exposure. The T3 and T4 levels in the plasma were analyzed by enzyme-linked immunosorbent assay (ELISA) kit (purchased from Elabscience Biotechnology Co., LTD, Wuhan, China) following the manufacture's instructions. No significant cross reactivity or interference was observed for kit.

2.5. Thyroid gland histology

The lizard thyroid tissue was sampled at 42 days after dosing and stored in 4% paraformaldehyde for histopathological analysis. Thyroid gland was processed for paraffin wax embedding. Sections were cut, stained with hematoxylin and eosin (HE), and then examined by light microscopy (Olympus DP73).

2.6. Isolation of mRNA and quantitative real-time polymerase chain reaction

The tissue samples were homogenized and prepared for total RNA isolation using TRIzol reagent (Life technology, Beijing, China). The cDNA was synthesized by reverse transcription reactions according to the manufacture's instructions. Reverse transcription reaction mixtures contained 22 μ L of total RNA, 2 μ L of Oligo(dT)₁₅ primers, and 4 μ L DNTP. The mixtures were heated to 70 °C for 5 min and quickly chilled on ice. After cooling, 8 μ L 5 × 6 buffer, 2 μ L M-mlv, and 40 units RNAsin (an RNase inhibitor) were added to a total volume of 41 μ L. The mixture was incubated for 50 min at 42 °C and then heated to 95 °C for 5 min to inactivate the reverse transcription reaction.

The HPT axis-related genes selected in this study and their respective primers are listed in Table 1. All the primers were designed by the authors using online National Center for Biotechnology Information (NCBI). Real-time PCR was performed in a MX3005P real-time quantitative polymerase chain reaction system (Stratagene, USA) in a total volume of 20 μ L, including the SYBR Green RealMasterMix, 1 μ M forward primer and 1 μ M reverse primer. The thermal cycle parameters were: 95 °C for 5 min, 40 cycles of 95 °C for 30 s, 54 °C for 40 s and 72 °C for 40 s. All of the samples were analyzed in triplicate and the delta-delta Ct method was used for results analysis. The thyroid hormone receptors (*tr α* , *tr β*), deiodinases (*dio1*, *dio2*), and transthyretin (*ttr*) mRNA expression were normalized by *β -actin* mRNA expression. A dissociation curve analysis was performed for each gene to check the amplification of the untargeted fragments. Only one peak was observed for the amplification, indicating the specific amplification of the target gene. The gene expression data showed changes relative to the control animals for the same treatment period.

2.7. Data analysis

Statistical analysis of the data was performed by the SPSS (version 13.0; USA) using one-way ANOVA, followed by the LSD Multiple Comparison Test. A probability of $p < 0.05$ was considered statistically significant.

3. Results and discussions

3.1. Mortality and body weight

All the seventy-two lizards were survived when exposed with 0 and 50 mg kg⁻¹ flufenoxuron for 21 days. The changes of body mass can be an indicator for the lizard growth (Peig and Green, 2009). In the control group, the body weight of lizards at proliferation stage showed a slight increase especially at 7 days, then

remained at a steady state (Fig. 1a). During 7–21 days, many lizards appeared to slough within 3 days. This phenomenon may suggest that the exposed lizards were at the last stage of proliferation at the beginning, and then entered into the resting stage through sloughing. In contrast, the body weight of lizards at resting stage was relatively stable from 0 to 14 days, but showed a slight increase at 21 days which may suggest a turning point from resting into proliferation stage (Fig. 1b). The lizards (proliferation stage) in the exposure group suggested a progressive loss of body weight during the 21 days exposure ($p < 0.05$). However, the body weight of lizards at resting stage only showed a significantly decrease at 21 days. One of our previous studies suggested that the body weight of lizards was not significantly changed after 7-days lambda-cyhalothrin exposure (Chang et al., 2016). More serious effects of pesticides on the body weight of lizards may appear after long-term exposure. The growth of lizards was modulated by thyroid hormones. The decrease of body weight may be caused by the disturbance of flufenoxuron on thyroid hormone levels.

3.2. Thyroid hormone levels

In the reptiles, thyroid hormones are supposed to affect the tail regeneration, metabolic rate and shedding frequency (Kanaho et al., 2006). Little is known about the influence of flufenoxuron on the thyroid hormone levels of lizards. In this study, the plasma T4 and biological active T3 levels were measured in both control and exposure groups. In the control group, the thyroid hormone levels in the female lizards were higher than that in the male lizards at both proliferation and resting stages (Table 2). It can be explained by the different physiological response between female and male lizards especially at the reproduction period (Dong et al., 2016). At resting stage, the thyroid hormone levels in the control groups showed a decrease compared with that of lizards at proliferation stage. According to the body mass changes at 21 days, the lizards at resting stage turned into proliferation stage may need more thyroid hormones for growth. Thus, the down-regulation of thyroid hormone levels was considered to be a normal physiological phenomenon.

The flufenoxuron exposure showed more seriously effect on the T3 level (53.8 fold in male and 32.8 fold in female plasma compared with the control) at resting phase than that at proliferation stage (47.3 fold in male and 10.2 fold in female plasma compared with the control). Another phenomenon was that the T3 level in the male was more seriously influenced than that in the female lizards when dosed with flufenoxuron. To data, our study and others have determined that the males are more sensitive than females to the oral administration of several contaminants (Simona et al., 2009). This phenomenon may be caused by the different adaptive responses to pollutants between different genders. In the exposure

Table 1
Primers used for PCR and the quantification of the mRNA expression by real-time PCR.

Genes	Sequence (5'-3')	Product size (bp)	Genbank accession no.
<i>β-actin</i>	TCTTCCAGCCCTCATTCCT ACGGTGTGGCATAACAGGT	186	KX459399
<i>trα</i>	GCAGCAATGTTTCGGTGAAA CCTTGCAGCCTTCACAGGTA	135	KX494867
<i>trβ</i>	TGCTGATGAAAGTGACGGA TGACAGACACCCAGTAGTGT	176	KX459400
<i>dio1</i>	TCCCACTCCACCCTTGTA CCCTCATGTCCAGTGGTTGT	120	KX452398
<i>dio2</i>	AGATGCCTACAAACAGGTGAAG GCTGAGCCAAAGTTGACCA	103	KX459401
<i>ttr</i>	AGCAGCAATGGTGTAGTGCC CTCATACTGGAAGGCCCTTGG	100	KX827005

Fig. 1. The changes of body weight when *Eremias Argus* (*E. argus*) were exposed with 0, 50 mg kg⁻¹ flufenoxuron every week. The exposed *E. argus* were at proliferation phase (a) and resting phase (b) at the beginning. **p* < 0.05 represents significantly difference compared to control (one-way ANOVA).

Table 2

The concentration of triiodothyronine (T3) and thyroxine (T4) in the plasma of *Eremias Argus* (*E. argus*) at different stages exposed with 50 mg kg⁻¹ of flufenoxuron.

Concentration/mg·kg ⁻¹	T4 (μg·L ⁻¹)		T3 (ng·L ⁻¹)	
	Male	Female	Male	Female
0 (proliferation)	689.40 ± 72.20	1773.02 ± 214.20	17.45 ± 2.10	26.40 ± 2.83
50	183.76 ± 15.20*	398.25 ± 45.92*	825.55 ± 81.61*	268.40 ± 26.62*
0 (resting)	380.92 ± 42.50	1218.12 ± 150.42	12.81 ± 1.53	17.94 ± 1.82
50	3242.96 ± 405.42*	3157.89 ± 360.72*	689.06 ± 72.62*	588.31 ± 62.15*

The data was expressed as mean ± S.E.(n = 3).

**p* < 0.05 indicates significant difference compared to the control (one-way ANOVA).

groups, the T4 level was significantly down-regulated in the lizards at proliferation stage while significantly up-regulated in the lizards at resting stage. These results suggested that the lizards at different stages exhibited different effect on the thyroid hormone levels after exposed to flufenoxuron. A previous study indicated that the up-regulation of thyroid hormones could shorten the resting stage and increase the sloughing frequency of lizards (Maderson and Licht, 1967). Thus, flufenoxuron has a tendency to disturb the skin shedding of lizards, even though the significant increase of thyroid hormone levels in the exposure groups did not show obvious changes on the sloughing frequency of lizards.

3.3. Thyroid gland histopathology

Structurally, the change of thyroid gland in the lizards indicates the alteration of thyroid activity (Virgilio et al., 2003). The thyroid gland of *E. argus* is a single discrete ribbon like structure that transversely across the middle of the trachea which is similar with other lizards species (Sciarrillo et al., 2008). The follicles are surrounded by epithelium cells and filled with colloids. The changes of the follicular epithelium cell height and the colloid area have a correlation with the functional state of thyroid gland.

Because the plasma T3 levels were more seriously affected by the flufenoxuron in the male lizards than that in the female lizards, the thyroid glands lesions in the male lizards were assessed. Compared to the control group (Fig. 2a), the male lizards at proliferation stage treated with flufenoxuron (Fig. 2b) showed large number of reabsorption vacuoles and obvious colloids vacuolation. In addition, the follicular epithelium height ($0.94 \pm 0.21 \mu\text{m}$) was significantly increased in the treatment group (Table S1). These results suggested that the flufenoxuron stimulated the activity of thyroid gland when the exposed lizards were at proliferation stage. In contrast, at resting stage, both the control and exposure groups showed several reabsorption vacuoles in the colloids and no

significant difference was observed in the epithelium cell height between the two groups (Fig. 2c–d). These results indicated that the thyroid gland was at active phase and the activity of thyroid gland was not significantly influenced by flufenoxuron when the exposed lizards were at resting stage, although the thyroid hormone levels were significantly up-regulated. The active stage of thyroid gland in the lizards further demonstrated that the resting phase lizards turned into the proliferation phase at 21 days. A previous study showed that the male lizards from herbicide exposure locations displayed thyroid follicular lumens with more reabsorption vacuoles and significantly larger follicular area (Bicho et al., 2013). It can be hypothesized that various pesticides can disturb the normal function of lizard thyroid gland and flufenoxuron exposure induced more serious structural influence on the thyroid gland of lizards at proliferation stage.

3.4. Tissue distribution and stage-related expression of HPT-axis related genes

Tissue distribution of *trα*, *trβ*, *dio1*, *dio2*, and *ttr* genes were evaluated in the liver, gonad, brain, kidney, skin and thyroid gland of *E. argus* by real-time PCR (Fig. 3). The *trα* and *trβ* genes were get expressed in all the six analyzed tissues. However, the expression of *trα* mRNA was negligible in the male skin, the *trβ* gene almost not expressed in the male gonad and female thyroid. The relative *dio1* gene levels were the highest in the kidney followed by the liver. The *dio2* mRNA preferred to express in the gonad, kidney and liver of the female lizards while the expression was highest in the brain of male lizards. Similar with the result in the fish, the *ttr* gene was only expressed in the lizard liver (Li et al., 2011). These results showed that the expression of HPT axis-related genes varied in the different tissues and genders of lizards. All the *trα*, *trβ*, *dio1*, and *dio2* genes showed relatively high expression only in the kidney, liver and brain tissue. Thus, the liver and brain is considered to be the main

Fig. 2. The thyroid gland section of the male *Eremias Argus* (*E. argus*). (a) Representative section of the *E. argus* (proliferation stage) treated with corn oil; (b) Representative section of the *E. argus* (proliferation stage) treated with flufenoxuron; note the follicular epithelium was significant higher than that in the control species and the colloid showed a large area of vacuolization (black arrows); (c) Representative section of the *E. argus* (resting stage) treated with corn oil; note several reabsorption vacuoles (white arrows); (d) Representative section of the *E. argus* (resting stage) treated with flufenoxuron; note several reabsorption vacuoles (white arrows).

Fig. 3. Relative expression of thyroid hormone receptors (*tra*, *trb*), deiodinases (*dio1*, *dio2*), and transthyretin (*ttr*) genes in the male (a) and female (b) *Eremias Argus* (*E. argus*) liver, gonad, brain, kidney, skin and thyroid. Values present mean ± S.D. (n = 3).

organ to regulate the HPT axis-related genes.

The expression of *tra*, *trb*, *dio1*, *dio2*, and *ttr* mRNA at different stages was assessed in the lizard liver (Fig. 4). In the male lizards, the expression of *tra*, *trb*, and *ttr* genes were much higher before than that after skin shedding, suggesting that the thyroid hormone receptors and transport protein genes were activated at the proliferation stage. In the female lizards, only the *dio1* and *dio2* genes were expressed more strongly before than after skin shedding, indicating that the deiodinase enzymes may be more sensitive at the proliferation stage. The HPT axis-related genes play different roles between the male and female liver and can be regarded as indicators during the lizard development. A previous study also suggested that the roles of the *tra* and *trb* genes are distinct in the skin tissue both before and after skin shedding (Kanaho et al., 2006). The *tra* and *trb* genes expression were drastically activated at different development stages of *Xenopus laevis* (Shi et al., 1996). Galton et al. (Becker et al., 1997) have proposed that the deiodinase

enzymes modulate the metamorphosis of *Ranacatesbeiana* and these genes are expressed at their highest levels at the time when the tissue was undergoing its greatest changes. However, a previous study showed that the expression of deiodinase genes drops in activity just before metamorphic changes occur in the *Xenopus laevis* (Huang et al., 1999). It can be hypothesized that the expression of HPT axis-related genes during the development of several vertebrate is varied.

3.5. Quantitation of *tra*, *trb*, *dio1*, *dio2*, and *ttr* mRNA in the tissues of lizards at different stages

Because the lizards at different stages (proliferation and resting stages) showed different expression of HPT axis-related genes, the contaminants exposure may also induce different toxic effect.

In this study, the lizards at different stages were exposed to 50 mg kg⁻¹ flufenoxuron in order to compare the different

Fig. 4. Relative expression levels of thyroid hormone receptors (*tra*, *trβ*), deiodinases (*dio1*, *dio2*), and transthyretin (*ttr*) genes in the liver of *Eremias Argus* (*E. argus*) at different stages (proliferation and resting stages). Values present mean $\pm$ S.D. (n = 3). *p < 0.05 indicates significant difference between the two groups (one-way ANOVA).

influence on the HPT axis-related genes (Fig. 5). After 21 days of exposure, the male lizards at resting stage showed significantly down-regulation of *tra*, *dio1*, *dio2*, and *ttr* genes expression in the liver, whereas the *tra*, *trβ*, and *dio1* mRNA levels were significantly increased at proliferation stage (Fig. 5a). In the female liver, the *tra*, *trβ*, *dio1*, *dio2*, and *ttr* genes were more seriously influenced at the proliferation stage than that at the resting stage after flufenoxuron exposure (Fig. 5b). It is presumed that these genes in the liver were more sensitive to the thyroid hormone changes at the proliferation stage. Similar with this result, the grass shrimp at premolt stage were also more sensitive than other stages when exposed to diflubenzuron (Fischer and Hall, 1992). It is also important to note that at the proliferation stage, the main effect of flufenoxuron on the thyroid hormone receptor (*tra* and *trβ*) and deiodinases (*dio1* and *dio2*) genes was opposite for female versus male. It may be explained by the physiological sexual dimorphism in the thyroid hormone receptor and deiodinase activities of the lizard liver.

In the male brain, the expression of *tra*, *trβ*, *dio1*, and *dio2* genes were significantly increased at resting stage while these genes were significantly decreased at proliferation stage (Fig. 5c). The brain is considered to be a dominant organ to regulate the endocrine system. At the proliferation stage, the *tra* and *trβ* genes in the brain were supposed to show feedback effect to the up-regulation of T3 levels while the *dio1* and *dio2* gene was more sensitive to the down-regulation of T4 level. At the resting stage, the expression of thyroid hormone receptor genes (*tra* and *trβ*) may be not thyroid hormone-regulated but modulated by the flufenoxuron. However, the lizards exposed to flufenoxuron at different stages showed no significant difference on the expression of *tra*, *trβ*, and *dio1* genes in the female brain (Fig. 5d). These results indicated that the different stages of lizards should be considered when investigate the toxic effect of contaminants, although the regulatory requirements for toxic assay on the lizards are scarce.

Fig. 5. Relative expression levels of thyroid hormone receptors (*tra*, *trβ*), deiodinases (*dio1*, *dio2*), and transthyretin (*ttr*) genes in the liver (a–b) and brain (c–d) of *Eremias Argus* (*E. argus*) at different stages (proliferation and resting stages) after 21 days of exposure to flufenoxuron. Values present mean $\pm$ S.D. (n = 3). *p < 0.05 indicates significant difference compared to the control (one-way ANOVA).

4. Conclusion

In conclusion, the lizards at different stages showed different sensitive to the flufenoxuron pesticide. The lizards at proliferation stage suggested significant down-regulation on the T4 levels, whereas the T4 level was significant increased at the resting stage after exposure. The flufenoxuron exposure caused more serious structural effect on the thyroid gland of lizards at proliferation stage. The expression of HPT axis-related genes has a correlation with the gender and tissue. In the female liver, the expression of *trα*, *trβ*, *dio1*, *dio2* and *ttr* genes was more seriously affected at the proliferation stage than that at the resting stage after treatment. The sensitivity of the *trα*, *trβ*, and *dio1* genes in the female brain were similar between different stages. Therefore, the flufenoxuron exposure influenced the thyroid endocrine system of lizards and we should also pay attention on the lizards at different development stages when investigate the toxic effect of contaminants on their thyroid endocrine system.

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The authors declare that there are no conflicts of interests.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.chemosphere.2017.01.002>.

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