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The body burden and thyroid disruption in lizards (*Eremias argus*) living in benzoylurea pesticides-contaminated soil

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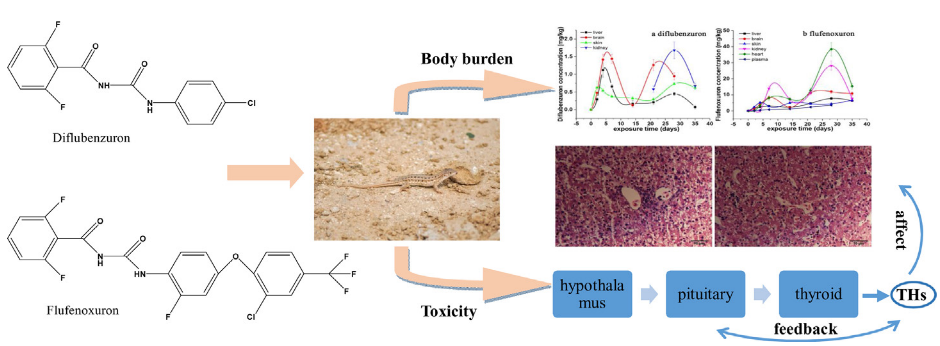
HIGHLIGHTS

- Diflubenzuron degraded faster than flufenoxuron in the soil.
- The SPFs of flufenoxuron were greater than diflubenzuron.
- The body burden of BPUs was related with $LogK_{ow}$ and molecular weight.
- BPUs exposure disturbed both thyroid and metabolism system of lizards.

GRAPHICAL ABSTRACT



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ABSTRACT

Dermal exposure is regarded as a potentially significant but understudied route for pesticides uptake in terrestrial reptiles. In this study, a native Chinese lizard was exposed to control, diflubenzuron or flufenoxuron contaminated soil (1.5 mg kg^{-1}) for 35 days. Tissue distribution, liver lesions, thyroid hormone levels and transcription of most target genes were examined. The half-lives of diflubenzuron and flufenoxuron in the soil were 118.9 and 231.8 days, respectively. The accumulation of flufenoxuron in the liver, brain, kidney, heart, plasma and skin (1.4–35.4 mg kg^{-1}) were higher than that of diflubenzuron (0–1.7 mg kg^{-1}) at all time points. The skin permeability factor of flufenoxuron was more than 20-fold greater than that of diflubenzuron at the end of exposure. However, the liver was more vulnerable in the diflubenzuron exposure group. The alterations of triiodothyronine (T3) and thyroxine (T4) level after diflubenzuron or flufenoxuron exposure were accompanied with the changes in the transcription of target genes involved not only in hypothalamus-pituitary-thyroid (HPT) axis (*sult*, *dio2*, *trα* and *udp*) but also in metabolism system (*cyp1a* and *ahr*). These results indicated that flufenoxuron produced greater body burdens to lizards through dermal exposure, whereas both diflubenzuron and flufenoxuron have the potential to disturb metabolism and thyroid endocrine system.

Abbreviations: BPUs, benzoylurea pesticides; HPT, hypothalamus-pituitary-thyroid; *trα*, thyroid hormone receptor α; *trβ*, thyroid hormone receptor β; *dio1*, deiodinase type 1; *dio2*, deiodinase type 2; *udp*, uridine diphosphate glucuronosyl transferase; *sult*, sulfotransferase; (*cyp1a, cyp2a and cyp3a*), cytochrome P450; (*ahr*), aryl hydrocarbon receptor; T3, triiodothyronine; T4, thyroxine.

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1. Introduction

Benzoylurea pesticides (BPUs) act as a chitin synthesis inhibitor to influence the molting of insects [1]. BPUs are mainly used to control locusts and grasshoppers on rangeland and croplands worldwide [2]. Due to their effectiveness as insecticides and low mammalian toxicity, the commercial development and use in agriculture practice has increased [3]. Diflubenzuron was shown to be persistent on conifer foliage after aerial application [4]. In rat, diflubenzuron was eliminated rapidly from the tissues [5] and was not cytotoxic to the cells [6]. In contrast, fish can accumulate diflubenzuron to concentrations more than 70 times greater than that of the water content when exposed at environmental concentration (2.5 mg L^{-1}) [7]. Moreover, diflubenzuron impairs reproduction in crustaceans, as shown in mysid shrimp [8]. Flufenoxuron is one of the third generation BPUs that is metabolized more slowly than first generation compounds, such as diflubenzuron by insects [9]. The bioconcentration factor (BCF) of flufenoxuron is more than 100-fold higher than that of diflubenzuron in aquatic organisms [3]. Flufenoxuron displayed little or no acute toxicity up to 1000 $\mu g\,L^{-1}$ whereas developmental effects were seen under environmentally relevant concentrations (3.2–320 ng L^{-1}) in exposed copepods [10]. Flufenoxuron has also shown very high toxicity against *Daphnia magna*, and the EC_{50} value is 0.000908 mg L^{-1} [3]. Although the effects of BPUs on mammals or aquatic organisms are well-studied, reptiles remain neglected in ecotoxicology studies. In fact, reptiles are not routinely included in toxicity tests and there is little information about their sensitivity to pesticides.

Lizards account for 59.93% of reptile species [11]. A previous study conducted in European Union (EU) demonstrated that lizards seem to display higher exposure sensitivity than other reptile suborders, such as snakes and tortoises [12]. Lizards primarily inhabit grasslands in Asian countries [13], where BPUs could be applied in order to control massive locusts [14]. However, there is no data about the degradation kinetic of BPUs in lizard tissues and the toxic effects of pesticides on lizards are usually predicted from the toxicity threshold established for birds [15]. Using birds as a surrogate might not be effective because of the lizard's unique combination of physical and life history characteristics [16,17]. For example, lizards were suspected to receive higher relative exposure than birds due to the greater percentage body contact with soil and vegetation if dermal route is explicitly considered [18]. In that case, it would be meaningful to study the body burden and toxicity of BPUs in the lizards under dermal exposure.

Thyroid hormones play an important role in metabolic regulation, growth, protein synthesis, activity of others hormones in mammals [19]. The standard metabolic rate and cardiac muscle mass in lizards also depend on the thyroid function [20]. A field study already showed that lizards exposed to agricultural areas have thyroid disrupting effects that ultimately influenced the male reproduction system [21]. Therefore, the disturbance of thyroid hormone levels by pesticides may affect the normal reproduction and metabolism of lizards. BPUs are known to inhibit the growth of insects through disruption of molting [1] while their effects on lizard development are little known.

In this study, we used a Chinese native lacertid species-*Eremias argus* (*E. argus*) to investigate the effects of diflubenzuron and flufenoxuron on lizard thyroid endocrine system. The bioaccumulation of the two BPUs in lizard plasma and tissues were measured after dermal exposure with environmentally relevant concentration. Furthermore, liver histopathology was conducted, thyroid hormone levels and transcriptional expressions of the target genes were also determined. Our results facilitate understanding of the potential effects of BPUs on lizard thyroid endocrine system.

2. Materials and methods

2.1. Chemicals

Diflubenzuron and flufenoxuron (Purity: 98%, Fig. S1) were purchased from J&K Scientific Ltd. (Beijing, China). The solvents including methanol, acetonitrile, acetone and n-hexane (HPLC grade) were obtained from Dikma (Beijing, China).

2.2. Test lizards and husbandry

Although *E. argus* has been listed as endangered by the Korean Ministry of Environment in 2005 due to its limited distribution and decreased population sizes [22], it is still regard as the most abundant lizard species in China. The species is easily maintained in a laboratory and will readily breed in captivity. We collected juvenile *E. argus* from the natural landscape in the Inner Mongolia Province and fed them in our breeding base from July 2009 till the start of our experiments. We tried to mimic a micro ecosystem for lizards and kept them in enclosures with pesticide-free sand and soil to let them reproduce naturally. The experimental lizards were captured from the enclosures. Females were excluded from the exposure experiment, as they were in the timing of reproduction. As body weight and length are good indicators of the age of lizards [22], the 2–3 years old mature male *E. argus* (progeny of the previous wild caught females, body weight: 3.2–3.8 g, snout-vent length: 3.5–4.5 cm) were picked out according to body weight and length [23]. Prior to the experiment, lizards lived in the uncontaminated soil for two weeks to acclimate in experimental glass tanks (40 × 25 × 15 cm). Ultraviolet lamps (Canada, Exo Terra, UVB 150, 13 W, the intensity is 150 $\mu w\,cm^{-2}$ measured 30 cm beneath the lamp) were set on 12 h light/dark cycles to provide enough light, promote vitamin D protection, and maintain the needed temperature. The heat lamp created a gradient of approximately 26–34 °C in the container when the lamps were on. When the lamps were off, temperatures were maintained at 25 ± 4 °C. Live mealworms (*Tenebrio molitor*) were put in food containers every day to feed lizards. We sprayed water containing mixed vitamin D3 and calcium carbonate (Repti calcium, made in USA; 0.5 g powder dissolved in 1 kg water) four times a day on the tank wall in order to provide enough water for lizards (lizards prefer to lick the water on the wall rather than get water from the provided water containers).

2.3. Exposure experiment and sample collection

Aerial application resulted in deposition level of diflubenzuron on soil surface ranging from 1.07–3.84 mg kg^{-1} [24]. The nominal exposure concentration in both diflubenzuron and flufenoxuron exposure group is 1.50 mg kg^{-1} in sandy soil, which is similar with the environmental concentration. In order to make sure that the 5.5 kg of sandy soil was spiked homogeneously with the chemical, we did the procedure in steps. Firstly, the acetone solution (only the carrier solvents - acetone was used in the control group) was added slowly to the soil (0.5 kg, organic content, 1.0 ± 0.6%; sand (2000–20 μm), 71.2 ± 5.0%; slit (<20–2 μm), 5 ± 2.5%; clay (<2 μm), 23.8 ± 2.5%; pH, 7.5 ± 0.2), and then mixed for 15 min using a mechanical shaker. The contaminated soil was left in a fume cupboard overnight to evaporate acetone. Secondly, 5 kg of uncontaminated medium was mixed thoroughly with the contaminated 0.5 kg soil using the mechanical shaker. The depth of soil in the tanks was 3 cm.

After acclimating, the male lizards were impartially separated into control, diflubenzuron, and flufenoxuron exposure tanks (total 3 groups, a total of 144 lizards were evenly separated into 9 tanks, 3 tanks for each group with 3 replicates). Lizards in both control and exposure groups were fed as usual. Six individuals (two lizards from