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# Distribution, Metabolism and Toxic Effects of Beta-Cypermethrin in Lizards (*Eremias argus*) Following Oral Administration

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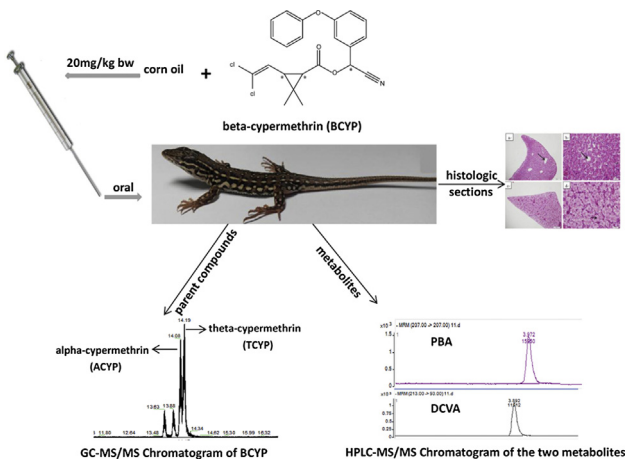
## HIGHLIGHTS

- Neurological effects were found in the beta-cypermethrin(BCYP)-treated lizards.
- The absorption, distribution and elimination of BCYP varied for different tissues.
- The two metabolites, DCVA and PBA, were detected in excreta.
- The metabolic and excretion rate of TCYP was faster than that of ACYP in lizards.
- The liver was main target organs in the BCYP-treated lizards.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Beta-cypermethrin (BCYP), a synthetic pyrethriod (PYR) pesticide which is a mixture of the alpha- and theta- cypermethrin, have been reported various toxicological profiles to non-target organisms. But little is known about assimilation, accumulation and toxic effects of BCYP in reptiles. The present study firstly elucidated absorption, tissue distribution, excretion of BCYP in *Eremias argus* . Treated group were administered orally with BCYP 20 mg/kg body weight (bw) dissolved in corn oil. Neurotoxicity was observed at 24 h after gavage, and the poisoning symptom ameliorated at 72 h. The changes of BCYP concentration depended on degradation time and tissues. Lizards had a strong capacity to eliminate BCYP with different tissue distribution. The tissues concentration of BCYP from high to low were intestine, stomach, heart, kidney, blood, lung, liver and brain. Bimodal phenomena were observed in lung, liver and kidney. These results may be due to the activities of enzymes, circadian rhythm, and enterohepatic circulation in lizards. Based on the results of organ coefficient and histopathology analysis in liver, the liver was confirmed as the main target organ.



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## 1. Introduction

Pyrethroid (PYR) insecticides have been used in agricultural and home formulations for more than 30 years. It has been regarded as alternatives to more toxic or recalcitrant organochlorines and organophosphates due to its high broadspectrum, performance, safety and hypotoxicity to human and livestock [1]. Now it is accounting for approximately 25% of the worldwide insecticide market [2]. Moreover, since the 1980s, Beta-cypermethrin (BCYP), a synthetic PYR pesticide which is a mixture of the alpha and theta forms, has been widely used and comprises more than 50% of the total PYR market production in China [3–5]. Before the commercialization of pesticide, the registration usually requires submitting some toxic data to a regulatory agency. The toxicology objects generally consisted of aquatic fauna, bees, terrestrial mammals and bird, but reptiles were ignored. And, virtually, no information about reptiles was available on sensitivity to pesticides [6]. Cypermethrin (CYP) exerts its neurotoxic effect through voltage-dependent sodium channels and integral protein ATPases in the neuronal membrane [7,8]. In rats, CYP can be accumulated in body fat, skin, liver, kidneys, adrenal glands, ovaries, lung, blood, and heart [9,10]. For aquatic organisms, it shows high toxicity [11–13]. At 2003, U.S. department of health and human services had published toxicological profile for PYRs, but the profile had not mentioned the reptiles. Likewise, some researches of BCYP have no relevant toxicology studies about lizards.

CYP could be rapidly metabolized in vivo, and the main metabolite products (Fig. 1) that DCVA (cis/trans-3-(2, 2-dichloroethenyl)-2, 2-dimethylcyclopropanecarboxylic acid) and PBA (3-phenoxybenzoic acid) were eliminated through feces and urine [14–16]. PYRs residues and their major degradation product 3-phenoxybenzoic acid had been frequently detected in soils, sediments, natural waters and agricultural products [17,18]. The widespread occurrence of BCYP and its degradation products could represent a threat for reptile species. Gibbons et al. reported that many worldwide species of reptiles were decreasing in abundance [19]. Among reptiles, lizards were particularly suitable as pollution biomonitor because they are mostly insectivorous [20], and could contact the soil closely, resulting in the sensitivity to environmental pollution. Meanwhile, within some terrestrial food chains, lizards were important to many food webs [21,22], and reveal high site fidelity [23,24]. So it was important to advance a clear research about lizards to protect them from endangerment. In the present study, basic information on the tissue distribution, excretion, metabolism and histopathology in *Eremias argus*, which was the most abundant lizard specie present in Asia [25,26], following oral administration of BCYP was reported.

## 2. Experimental

### 2.1. Chemicals and reagents

BCYP (96%) was obtained from the China Ministry of Agriculture's Institute for Control of Agrochemicals. DCVA was obtained from Jiangsu Yangnong chemical group co. (Jiangsu, China). PBA and corn oil were purchased from Sigma–Aldrich. Water was purified by a Milli-Q system. Acetonitrile, n-hexane, and acetone (analytical grade) were from Beijing chemical work (Beijing, China). Ethyl acetate (analytical grade) was from Beijing tong guang fine chemicals company (Beijing, China). Iso-octane was from FisherScientific (Fair Lawn, NJ). Heparin sodium (anticoagulant) was purchased from Beijing Chemical Reagent Co. Ltd. (Beijing, China). Trifluoroacetic acid (TFA) was purchased from Beijing xingjin chemical plant (Beijing, China).

### 2.2. Experimental lizards and husbandry

The adult male *Eremias argus* were purchased from Guan Yuan flower and bird insect fish market (Beijing, China). Lizards were maintained under laboratory conditions at a 12 h dark/light cycle, $22 \pm 3\,^{\circ}C$ temperature and 45%–50% relative humidity. Each group was housed in a $25 \times 15 \times 12$ cm clear-plastic cages with a water dish, and the cage's floor was covered with 100 g sand. All lizards were acclimated in the laboratory for seven days prior to experiments. Lizards were fed with live mealworms every day, and water was changed daily. After one week of acclimation, the lizards were assigned randomly to BCYP-treated groups (BCYP and corn oil addition) and control groups (just only corn oil addition). Each group consisted of nine lizards.

### 2.3. Sample preparation

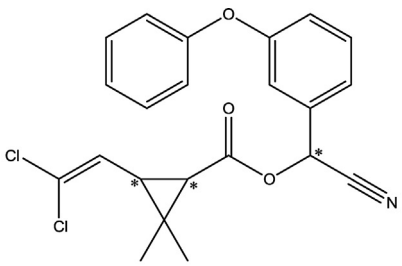
After sampling, whole blood, heart, lung, liver, stomach, intestine and kidney samples were placed into a 15 mL polypropylene centrifuge tube containing 5 mL ethyl acetate with 0.1% trifluoroacetic acid (TFA), respectively, and homogenized for 90 s. The mixtures were extracted by ultrasonication for 10 min, vortex mixer for 3 min in 15 mL of ethyl acetate and centrifuged at 4,500 rpm for 5 min. The organic phase was transferred into test tubes. Re-extracted the samples in the same way and the supernatants were combined. The combined extract was evaporated to dryness with a vacuum rotary evaporator at 35 °C. Subsequently, the residue was reconstituted in 1 mL of acetonitrile, and then washed with n-hexane (1 mL) twice. The acetonitrile layer was re-dried with a vacuum rotary evaporator at 35 °C. The residue was diluted to 1 mL with iso-octane and filtered through a 0.22 μm filter prior to GC–MS/MS analysis.

For determination of the precision and accuracy of the method, native plasma and tissue samples were fortified at three fortification levels with BCYP. The data were calculated as a ratio of the found concentration of BCYP spiked in a blank sample to the predicted concentration. Good linear calibrations were obtained over the concentration range of 3–250 μg/L for BCYP with R2 0.9960 and RSD < 20.71%. The mean recoveries of samples were listed in Table 1.

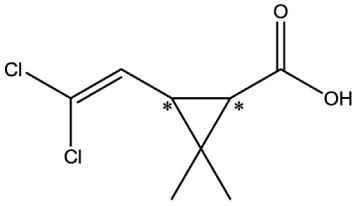
### 2.4. GC–MS/MS and HPLC-MS/MS analysis

GC–MS/MS was used to detect the parent compound, BCYP, and HPLC-MS/MS was used to detect the metabolites, DCVA and PBA.

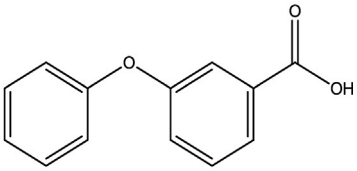
A gas chromatograph hyphenated to triple quadrupole mass spectrometer and attached to a Tri-Plus liquid auto-sampler was used for analysis of samples. Chromatographic separation was achieved with a HP-5 capillary column (30 m × 0.25 mm, 0.25m). High purity helium (>99.999%) was used as the carrier gas with the column flow of 1.0 mL/min. The GC was equipped with a programmable temperature vaporizing (PTV) inlet. Samples were introduced in splitless-injection mode at 260 °C and the initial injection temperature was 90 °C for 0 min. During the evaporation phase, temperature was ramped to 250 °C at a rate of 30 °C/s (held for 0.0 min), further at 1 °C/s to 260 °C (held for 0.0 min). At the cleaning phase, temperature was ramped to 290 °C at a rate of 30 °C/s (held for 1.0 min). At the end, the total run time was 17.33 min. The temperature of the ion source was 250 °C with 50 μA emission current and the state duration time was 5.00 min. The electron energy was 70 eV and the experimental type SRM. For BCYP, transition *m/z* 163–127 was used for quantification, and m/z 181–152 was used for confirmation. The collision energies were 5 eV and 25 eV, respectively. An Agilent system consisting of a membrane degasser, an autosampler, a quaternary gradient pump, and a column oven (25 °C) was used for separation.




CYP




DCVA




PBA

**Fig. 1.** Structures of cypermethrin and two metabolites (* represent chiral center).

**Table 1**
Summary of method recovery data for BCYP from fortified lizard blood and tissues (n = 9)[a].

| Matrix | Fortification | Recovery (%) | Matrix | Fortification | Recovery (%) |
|---|---|---|---|---|---|
| Blood | 0.10 | 110.73 ± 3.07 | Liver | 0.02 | 72.12 ± 0.04 |
| | 2.50 | 120.86 ± 4.21 | | 1.50 | 87.34 ± 2.26 |
| | 15.00 | 126.93 ± 0.50 | | 15.00 | 126.04 ± 11.55 |
| Brain | 0.08 | 119.53 ± 3.40 | Stomach | 2.50 | 108.05 ± 10.72 |
| | 2.50 | 115.83 ± 23.99 | | 250.00 | 70.33 ± 0.47 |
| | 6.50 | 124.35 ± 0.66 | | 625.00 | 78.39 ± 9.80 |
| Heart | 1.00 | 101.94 ± 12.6 | Intestine | 0.50 | 122.35 ± 2.84 |
| | 12.50 | 97.71 ± 10.49 | | 125.00 | 79.62 ± 11.58 |
| | 50.00 | 74.47 ± 8.29 | | 765.00 | 64.89 ± 9.59 |
| Lung | 0.50 | 107.91 ± 6.11 | | 0.50 | 104.57 ± 5.29 |
| | 2.50 | 120.3 ± 14.60 | Kidney | 2.50 | 99.23 ± 2.95 |
| | 20.00 | 122.70 ± 10.23 | | 15.00 | 118.12 ± 0.74 |

n represent the number of lizards.
[a] Values represent the means ± standard deviations.

Sample volumes of 10 μL were injected. The separation was performed on C18 column with eluents of ACN/$NH_3 \cdot H_2O$ (80/20). The flow rate was 0.2 mL/min and the wavelength was 245 nm. Analytes were detected by multiple reaction monitoring (MRM) using electrospray ionization mass spectrometry (ESI–MS). The source temperature was 300 °C. Nitrogen was used both as nebulizing and drying gas. For each compound, two MRM-transitions were monitored. For DCVA, the precursor ion was 213, and product ion was 93. And for PBA, the precursor and product ion were both 207.

### 2.5. Degradation studies

Treated group were administered orally with 20 mg/kg body weight (bw) of BCYP dissolved in corn oil, and the control group received only corn oil orally. We chose 20 mg/kg bw because there is no toxicity data about CYP to *Eremias argus*, and birds are often used as surrogates in risk assessments to represent reptiles [27]. The LD50 of CYP to birds (chickens, acute, oral) is greater than 2000 mg/kg [28], and 1% of the LD50 was selected in this study. And no adverse effects on the survival of lizards at BCYP concentration 20 mg/kg were seen during the whole preliminary experiment. Two days before the experiments, the lizards were fasted, but had free access to water. Treated-group lizards were euthanized at hours 0, 1, 3, 6, 12, 24, 72, 120 and 168 after lizards were dosed once. The control group was sacrificed at 168 h. In the preliminary experiment, three groups (24 h, 72 h and 168 h, respectively) and three control groups (24 h, 72 h and 168 h, respectively) were carried out. No difference was found between the control groups. In addition, the experimental designs also implied that the ethically acceptable option is the one that provides most benefits and involves the least costs ration for animals. So the control group was sacrificed only at 168 h. Individuals of each group were weighted, anesthetized by cooling on ice, killed by decollation, and then dissected at once. This method was designed according to Maria [29]. Considering that the blood volume of one lizard was limited, the blood of lizards of each group was collected in one heparinized tube. The organs including heart, lung, liver, stomach, intestine and kidney were removed and weighted separately from sacrificed lizards. Because the lizards' feces and urine were hard to be collected, the 100 g sand in the cage was collected and tested at hours 24, 72, 120 and 168 after dosing.

### 2.6. Histopathological analyses

The lizards treated by the control and BCYP (20 mg/kg bw) were euthanized at 168 h for histopathology analysis. Tissue samples (liver, lung, stomach, intestine and kidney) were fixed in 10% neutral buffered formalin, dehydrated in ethanol, cleared in xylol, and embedded in paraffin. Serial two-micron sections were stained with hematoxylin and eosin, and examined with a light microscopy.

### 2.7. Data analysis

Organ coefficient expressed as tissue weighting factor elsewhere was calculated according to the equation [organ coefficient = (organ mass/lizard mass) × 100]. The organ coefficients were used to evaluate hyperplasia, swelling, or atrophy of the organs induced by BCYP exposure. The organs (including heart, brain, lung, liver and kidney) were weighted after washed in normal saline solution, sucked dry with normal filters. The difference between the test group (lizards were exposed to 20 mg/kg bw BCYP and killed at 168 h after exposure) and control group was assayed according to analysis of variance (ANOVA). The differences were considered statistically significant at $p < 0.05$.

BCYP was an active synthetic pesticide containing two pairs of enantiomers, which were alpha-cypermethrin (ACYP) and theta-cypermethrin (TCYP). Normally in commercial BCYP, the ratio of TCYP/ACYP (T/A) was about 1.5. In this study, it was $1.58 \pm 0.15$, which was detected by GC–MS/MS. The changes of T/A could response the selective accumulation and metabolism of TCYP and ACYP. All data were analyzed and fitted by using SPASS 21.0 software. All values were represented as mean ± SD, and statistical comparisons were made using one-way ANOVA as appropriate.

## 3. Results and discussion

### 3.1. Neurological effects

Twenty-four hours after dosing, BCYP-induced neurological signs including pawing and burrowing behavior, profuse salivation, abnormal movements, and coarse whole body tremor that progresses to sinuous writhing occurred. From 24 h to 72 h, the other signs improved markedly except that the predation ability was not fully recovered. Subsequently, lizards returned to normal state. Although U.S. EPA guidance on risk assessment of pesticides suggests that birds can be used as surrogates for reptiles [27]. Pauli et al. had studied that reptiles might be more sensitive than homeothermic vertebrates to some pesticides [30]. Because of relatively simple monooxygenases enzyme systems [31] and low metabolic rates of lizards [32], they may not be able to detoxify pesticides at the same high rates as warmblooded birds or mammals. Therefore, risk estimates used surrogate species were convenient, but risk estimates based on bird were not rational [33].

### 3.2. Organ coefficient

The organ coefficients of the control and treat groups were shown in Fig. 2. No significant differences between the control and treat groups were observed in heart, brain, lung and kidney ($p > 0.05$) except the liver coefficient ($p < 0.05$). This phenomenon may indicate that the liver could be more sensitive to BCYP exposure. As shown in Fig. 2, the liver coefficients at treat groups were all significantly higher than the value of the control groups. In order to describe the changes of organ coefficient more clearly, Table A.1 including the coefficient of the organ for each step of sacrifice was added to the appendices.

In mouse and quail, the liver was the principal site of biotransformation, while plasma and intestine were more important sites

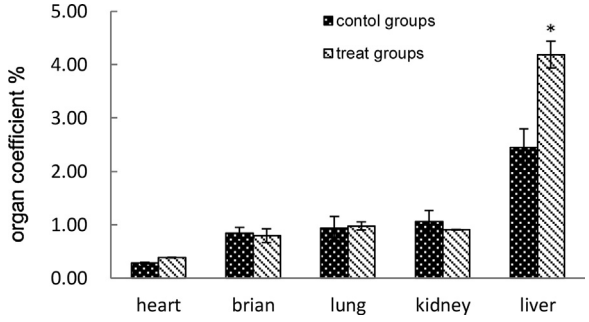



**Fig. 2.** The weights of heart, brain, lung, kidney and liver expressed as a percentage of total body weight between control groups and exposure groups after BCYP treatment. (The bars are standard errors;*represent significant difference).

than liver for the detoxification of trans-cypermethrin in trout. And some of these differences were resulted from variations in liver/body weight ratios [34]. Therefore, the change of liver coefficients would directly affect the degree of detoxification.

### 3.3. Degradation Studies

The concentrations of BCYP in different tissue samples are plotted over time in Fig. 3. And these curves could clearly show the absorption, distribution and elimination of BCYP in different tissue samples. In general, the BCYP residue level was low in most tissues except stomach and intestine. The highest concentration was observed in the intestine, followed by (in decreasing order) stomach, heart, kidney, lung, liver, brain and blood.

At the present experiment, BCYP was exposed to lizards by oral gavage. The lizard individual was small and its esophagus was narrow, so it was difficult to ensure that all compounds were given into the stomach. Moreover, some BCYP solution overflowed along the lavage needle to the esophagus. Consequently, the concentration of BCYP in stomach was not to be highest at 0 h. But the overflowed solution would reach to stomach quickly within 1 h, so we thought the administered dose was still 20 mg/kg bw. After 1 h exposure by oral gavage, a reduced amount of BCYP in stomach was accompanied by an increased amount of BCYP in intestine, and approximately 99% of BCYP in stomach was removed after 24 h exposure (Fig. 3A and B). Furthermore, the concentration of BCYP in stomach reached minimum while it met the maximum in intestine at 24 h. Then the amount of BCYP in intestine rapidly reduced while the amount of BCYP in the sand of the cage reached the maximum (Fig. 3B and A.1a). The BCYP was present after 0.5 h in the blood, heart, lung, liver and kidney, while in the intestine was little. The situation may be due to a direct absorption from the stomach, and Naumann et al. [35] had reported that the pyrethroids can be absorbed by the stomach. Based on these results, after dosing, the BCYP rapidly centralized in stomach, quickly transferred to intestine then partly or mostly were eliminated. Studies [16,36,37] in the fish, mammals, and birds have shown that the metabolites of CYP include DCVA and PBA. Sampling the last three points' sand, the DCVA and PBA were detected (Fig. A.1b). For blood, brain, heart, lung, liver, stomach, intestine and kidney, the DCVA and PBA had not been detected. This may be resulted from the high LOD and LOQ of the metabolites in HPLC-MS/MS.

Generally, the concentration of BCYP in blood was lower than other organs (Fig. 3C). Although the concentration of BCYP was greater after 24 h, the concentration was still lower than 1.29 μg/L. And the concentration of BCYP was maintained in saturation state. The reasons for explaining the lower level include the following reasons: 1. Ingested materials pass through the hepatoenteral circulation before entering the blood stream [38], and the liver might metabolic the parent compounds. 2. PYRs were distributed

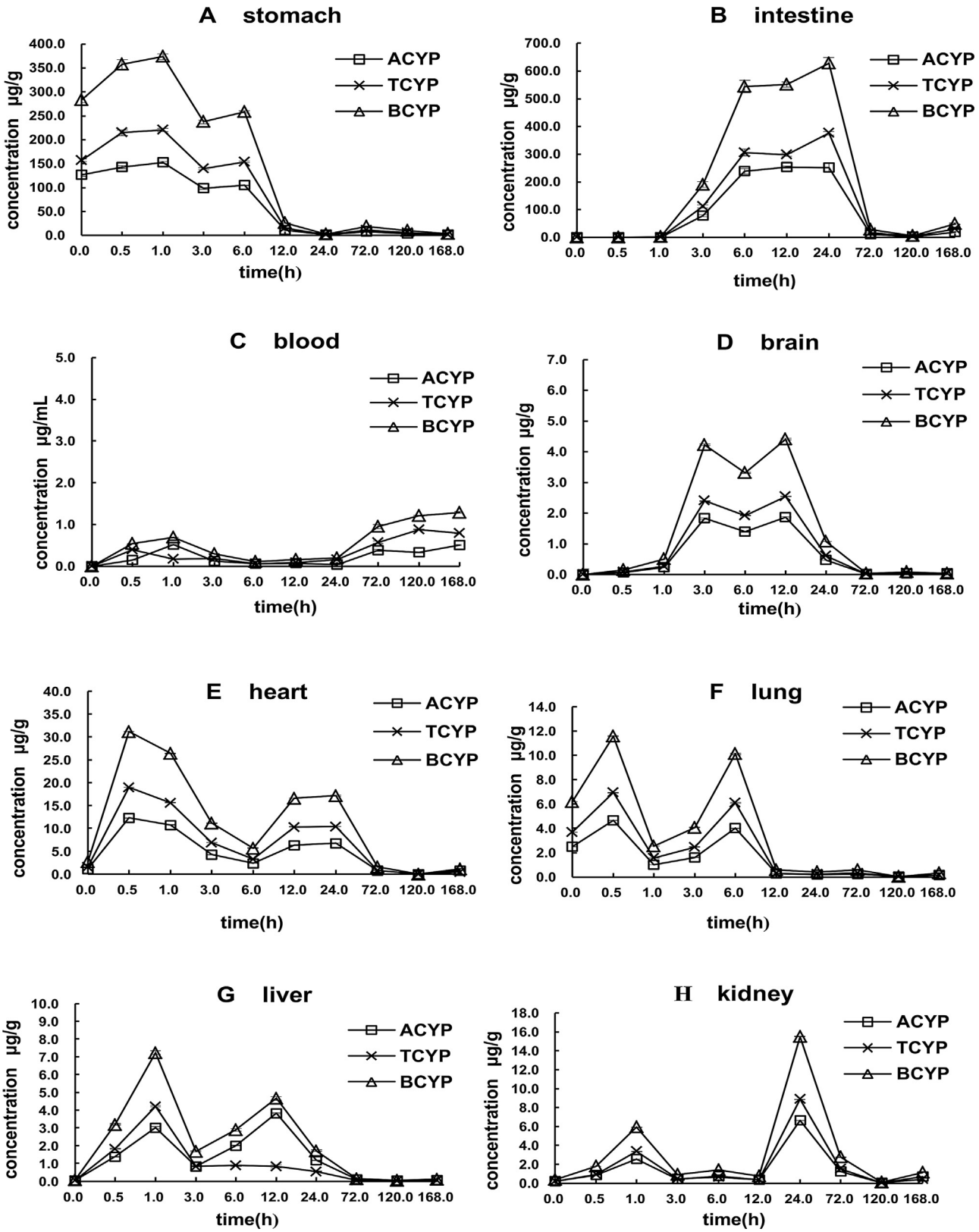



**Fig. 3.** Blood and tissues concentration-time curves of BCYP, TCYP and ACYP in lizards following BCYP administration at 20 mg/kg bw (□ = ACYP, × = TCYP, △ = BCYP; values represent the means ± SD).

to nearly all tissues and were concentrated in tissues with high lipid contents, such as fat and nerve tissue [39]. And the organs of *E. argus* could have a strong ability to absorb and accumulate BCYP. 3. *E. argus* has a small amount of blood (about 100–200 μl per animal). So the ability of transport BCYP was limited, and the BCYP in blood also would quickly reach saturation states.

CYP was involved in the pathogenesis of various neurological disorders. It can cross the blood-brain barrier and exert its effect

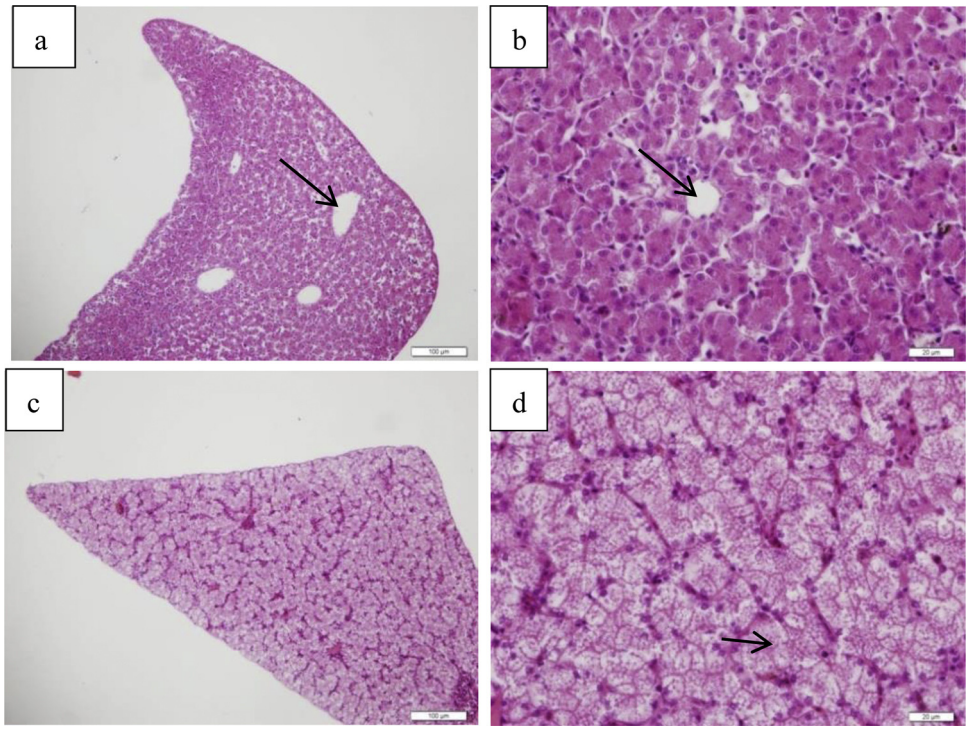



**Fig. 4.** Representative liver sections of *Eremias argus* exposed to BCYP for a period of 168 h. (a) and (b) Liver section of a control animal representing a normal liver (HE 100 and 400, respectively), black arrows show the central vein and hepatic cords, respectively; (c) and (d) Examples of liver sections of individuals treated group (HE 100 and 400, respectively), black arrow show the disappeared nuclei.

on the nigrostriatal system. Compared with the concentration of BCYP between in blood and brain, the concentrations of the BCYP in the brain were relatively higher (Fig. 3C and 3D). This phenomenon was in accordance with the previous study. After guinea pigs were dermally exposed to permethrin, the measured permethrin in brain was 7-fold higher than that in plasma at 24 h [40]. It might be attributed to the high fat content of the brain, resulting in the accumulation in brain. Moreover, the significant symptoms of neurotoxicity were found at 24 h exposure, and then the lizards gradually restored calm. This suggested that the concentration in brain became lower, resulting in the decreased neurotoxicity. A variety of enzymes in the brain might play the role in detoxification. And the BCYP could quickly transport to other organs through the blood. These reasons might contribute to the lower concentration of BCYP in brain.

During the whole degradation experiment, the concentrations of the BCYP were mostly higher 10 μg/g in the heart (Fig. 3E). The trend of BCYP concentrations in heart and in blood was different. Banu Coskun et al. found that the frog heart rate was reduced after CYP-inhalation for 90 and 120 min [heart$^{-1}$] [41]. And it was known that, when the heart rate decreased, the complete cardiac cycle was prolonged [42]. Combined with the above description, it was speculated that the lizard heart rate was reduced after BCYP exposure, and a large amount of BCYP retained in heart and reduced the transportation of BCYP in the blood.

Typical bimodal phenomena were observed in Fig. 3F–H. In lungs, the first and second peak appeared at 0.5 h and 6 h, respectively (Fig. 3F). In liver and kidney, the first peak emerged at 1 h with similar concentration, and the second absorption peak appeared at 12 h and 24 h, respectively (Fig. 3G and H). The second peak in kidney and intestine was appeared at the same time. We speculated that the appearance of the second peak in lung and kidney could be related to the absorption of stomach and intestine. After dosing, the concentrations of BCYP in lung were higher than that in liver and kidney before 12 h. Because the concentrations of BCYP in heart were high, some BCYP could output with venous blood from heart to lung and be accumulated in lung. Available information regarding renal effects in animals was limited. Casida et al. reported that the decreased of kidney weights and tubular degeneration were

**Table 2**
The ratios of T/A in different organs at different sampling times[a].

| time(h) | brain | heart | Lung | liver | stomach | intestine | kidney | excretion |
|---|---|---|---|---|---|---|---|---|
| 0.00 | 1.22 ± 0.05 | 1.23 ± 0.47 | 1.48 ± 0.03 | 1.32 ± 0.11 | 1.49 ± 0.01 | 1.23 ± 0.02 | 1.06 ± 0.11 | – |
| 0.50 | 1.23 ± 0.06 | 1.55 ± 0.02 | 1.49 ± 0.09 | 1.37 ± 0.03 | 1.49 ± 0.01 | 1.31 ± 0.09 | 1.00 ± 0.24 | 1.50 ± 0.05 |
| 1.00 | 1.20 ± 0.08 | 1.45 ± 0.04 | 1.52 ± 0.09 | 1.39 ± 0.02 | 1.45 ± 0.01 | 1.51 ± 0.09 | 1.27 ± 0.03 | 1.44 ± 0.05 |
| 3.00 | 1.30 ± 0.08 | 1.63 ± 0.07 | 1.52 ± 0.04 | 1.02 ± 0.02 | 1.42 ± 0.02 | 1.33 ± 0.04 | 0.97 ± 0.32 | 1.41 ± 0.04 |
| 6.00 | 1.39 ± 0.0 | 1.40 ± 0.08 | 1.53 ± 0.04 | 0.42 ± 0.03 | 1.49 ± 0.01 | 1.30 ± 0.01 | 1.14 ± 0.18 | 1.40 ± 0.04 |
| 12.00 | 1.36 ± 0.09 | 1.64 ± 0.02 | 1.10 ± 0.09 | 0.23 ± 0.03 | 1.39 ± 0.01 | 1.19 ± 0.01 | 0.96 ± 0.13 | 1.41 ± 0.05 |
| 24.00 | 1.29 ± 0.12 | 1.54 ± 0.06 | 1.37 ± 0.19 | 0.49 ± 0.01 | 1.08 ± 0.18 | 1.26 ± 0.09 | 1.35 ± 0.01 | 1.55 ± 0.04 |
| 72.00 | 1.34 ± 0.07 | 1.00 ± 0.19 | 1.35 ± 0.09 | 0.44 ± 0.02 | 1.53 ± 0.01 | 1.44 ± 0.11 | 1.25 ± 0.04 | 1.15 ± 0.05 |
| 120.00 | 1.27 ± 0.10 | 1.53 ± 0.06 | 0.94 ± 0.10 | 0.21 ± 0.03 | 1.44 ± 0.04 | 1.42 ± 0.01 | 0.31 ± 0.04 | 1.13 ± 0.18 |
| 168.00 | 0.95 ± 0.12 | 0.60 ± 0.09 | 0.89 ± 0.03 | 0.56 ± 0.10 | 1.30 ± 0.04 | 1.55 ± 0.04 | 0.62 ± 0.01 | 1.20 ± 0.06 |

– represent no data.
[a] Values represent the mean ± SD.

found in rats consuming pyrethrins 320 mg/kg/day in the diet for 90 days [43].

Lizards were poikilotherm which the activities of enzymes as the ambient temperature changes. And most enzymes were involved in drug metabolism. So the metabolism of BCYP also could be affected by ambient temperature in lizards, and the lower temperature at night slowed down the metabolic rate of BCYP. Furthermore, many studies have showed that hepatic microsomal enzyme have obvious changes of circadian rhythm [44]. Thus, the appearance of second absorption peak in liver was found at 12 h (at ten o'clock at night). Besides the above reasons, the enterohepatic circulation may also be the cause of the second absorption peak.

### 3.4. T/A value

CYP was a mixture of cis- and trans- isomers, with a cis configuration greatly reducing the cleavage rate. In rats and in mice, it was known that ester cleavage was more extensive for trans- PYRs than cis- PYRs [14,45,46]. In treated lizards tissues, the T/A value was fluctuant over time (Table 2), and all of these values were less than or equal $1.58 \pm 0.15$ (the initial value of T/A). This may be explained that TCYP metabolic and excretion rate was faster than ACYP in lizards.

T/A values were various in the different organs. In stomach and intestine, all T/A values were higher than 1.0 and generally between 1.3 and 1.5. It was suggested that the organ of stomach and intestine may not participate in the metabolism of TCYP and ACYP. On the contrary, T/A values in liver were lower than 1.0 after 1 h. It was speculated that ACYP is more stable and may be remained in lizards for longer, leading to the potential toxicity effects. Corcellas et al. also reported a general preference of cis isomers in bioaccumulation [47]. During the experiment, a low level of the parent compound was found in liver. This phenomenon demonstrated that the liver was more sensitive and had higher detoxification ability for BCYP (especially for TCYP). Comparing with other organisms, the liver was the principal site of biotransformation in mouse and quail [48]. But in trout, plasma and intestine were more important sites than liver for the detoxification of trans-CYP [48]. In lung and kidney, the T/A values were lower than $0.94 \pm 0.10$ until 120 h. This could be attributed to the lower metabolic rate of the two organs and lower content of TCYP in vivo at the later stage of experiment. In excreta (feces and urine), T/A values were higher than $1.13 \pm 0.18$ during the experiment, indicating that the most excreta were BCYP rather than the metabolites.

### 3.5. Histopathology analysis

The current study was carried out to determine the alterations of histopathology in the lizards exposed to BCYP 20 mg/kg bw. Macroscopically, tissues from both control and exposed lizards had a normal appearance except liver during necropsy. Likewise, no histological changes were observed in the heart, lung, stomach, intestine and kidney (Fig. A.2–A.6), while the changes in liver histology were significant.

The liver from the control group had a serous membrane integrity, connective tissue underdevelopment, clear central vein and hepatic cords when it was observed at low power. And these presented a normal histological appearance (Fig. 4a). At high magnification, the liver cells row neatly with clear boundary, and nuclei had a distinct outline and distributed evenly on the slides (Fig. 4b).

For the treated group, changes in the liver occurred after 168 h. At low magnification, serous membrane was integrated, but hepatic sinusoid congestion was observed. In the meantime, the structure of central veins and hepatic cords were vague (Fig. 4c). At high power, the characteristic features of liver histology were losing the normal structure of the cell, loose and transparent cytoplasm, nuclei being small or disappear and significant cellular swelling (Fig. 4d). Previous study also had been demonstrated histological changes after a treatment with 50 mg/kg ACYP in rats [49]. Because of the increase of liver index and histopathological lesion in liver, it could be suspected that the liver was main target organs in the BCYP-treated lizards.

## 4. Conclusions

Lizards have been proposed as ideal candidates for bioindicating, as they are plentiful and obvious in many environments [50]. Currently, no adequate data exist for predicting risk from pyrethroids in reptile populations. The present work draws attention to the absorption, distribution and elimination of BCYP in different lizard tissues. The T/A values could explain that the metabolic and excretion rate of TCYP was higher than that of ACYP in lizards. Our results supported a detrimental effect on BCYP-treated lizards, indicating that the neurotoxicity at 24 h and liver histological changes were observed after dosing BCYP. It was suggested that liver was main target organs in the BCYP-treated lizards.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2015.11.053.

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