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Comparative toxic responses of male and female lizards (*Eremias argus*) exposed to (*S*)-metolachlor-contaminated soil☆

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ABSTRACT

Soil contamination caused by the widespread use of pesticides is one of the main environmental problems facing conservation organizations. (*S*)-metolachlor (SM) is a selective pre-emergent herbicide that poses potential risks to soil-related organisms such as reptiles. The present study elucidated the toxic effects of SM (3 and 30 mg/kg soil weight) in *Eremias argus*. The results showed that growth pattern was similar between the sexes in breeding season. For males, both kidney coefficient (KC) and testis coefficient in the exposure group were significantly different from those in the control group, while only KC in the high-dose group was significantly higher for females. Based on histopathological analysis, the livers of female lizards were more vulnerable than those of males in the exposure group. A reduction in total egg output was observed in SM exposed lizards. Accumulation studies indicated that skin exposure may be an important route for SM uptake in *E. argus*, and that the liver and lung have strong detoxification abilities. In addition, the body burdens of the lizards increased with increasing SM concentration in the soil.

1. Introduction

As an important part of the ecological system, soil provides nutrition and moisture for plants and habitats for terrestrial animals. However, soil pollution by various organic (e.g., pesticides, cyanide and synthetic detergent, Cozzolino, 2016; Snousy et al., 2016) and inorganic (e.g., acids, alkalines and heavy metals, Fayiga and Saha, 2016; Ross, 1994; Wang et al., 2016) contaminants represents one of the main environmental problems at the global scale (Ballabio, 2016). These contaminants originate from industrial and agricultural practices through intentional applications, deposition, spills, and disposal. The unreasonable use of pesticides in agriculture could lead to serious pesticide residues (Oliveira-Silva et al., 2001). Meanwhile, in the eco-environmental system, soil acts an important matrix for the storage and transport of pesticides (Guo et al., 2009), and pesticide residues in soils might threaten the survival of many species (Freemark and Boutin, 1995). Reptiles are the most diverse groups of terrestrial vertebrates, and they might be more sensitive to contaminants and climate than birds and mammals (Gibbons et al., 2000; Meng et al., 2016; Pauli et al., 2010; Weir et al., 2010). Lizards might be exposed to environmental pollution through various routes, including the ingestion of contaminated food, polluted water or soil. Although the ingestion of contaminated food is probably one of the most important routes, contaminants could also enter into reptiles through ingestion (Beyer et al., 1994; Rich and Talent, 2009; Sokol, 1971; Sylber, 1988) and dermal exposure (Alexander et al., 2002; Cardone, 2015; Weir et al., 2015) of polluted soil.

Metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-metoxy-1-methylethyl) acetamide), a selective pre-emergent herbicide, is a member of the chloroacetanilide family. Metolachlor acts as a growth inhibitor in target weeds by preventing the synthesis of proteins, chlorophyll, fatty acids, and lipids. Initial marketing focused on the racemic metolachlor product, which consists of four stereoisomers, two (*R*)-enantiomers and two (*S*)-enantiomers, in the same proportions. However, it was found that the herbicidal activity was derived mainly from the two (*S*)-enantiomers of metolachlor (Moser et al., 1982). Thus, (*S*)-metolachlor (SM)

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is currently being used to replace the racemic mixture in regular products and one of the three most used herbicides in the word in the chloroacetanilide class (Blaser et al., 2007; Martins et al., 2007). The replacement of metolachlor by SM allows for the application of 35% less pesticide while providing the same effectiveness in weed control (O'Connell et al., 1998). Although metolachlor can be eliminated from the soil through volatilization, photodegradation and biodegradation (Rivard, 2003; Vryzas et al., 2012; Wondi et al., 2004), the widespread use of metolachlor has led to the detection of the parent herbicide and its metabolites in soil (Aga and Thurman, 2001; Aga et al., 1996; Clark and Goolsby, 2000; Krutz et al., 2004; Miller et al., 1997). Thus, it is important for us to understand the effects of SM-contaminated soils on plants, wildlife and humans. Data on four taxa (plants, invertebrates, birds, and mammals) are being included into the development of soil screening levels (EcoSSLs) for the U.S. Environmental Protection Agency's (USEPA's) Ecological Soil Screening Level Guidance (http://www.epa.gov/superfund/programs/risk/ecorisk/guidance.pdf). However, information on reptiles is missing.

Pesticide contamination might be contributing to reptile declines (Gibbons et al., 2000). However, in research on terrestrial vertebrate ecotoxicology, reptiles remain the least-studied vertebrate group (Sparling et al., 2010). Lizards are commonly found in agricultural areas (Amaral et al., 2012a; Glor et al., 2001) and can be used as a representative reptilian group to determine the toxicity of SM, thus providing the necessary information for the ecotoxicological risk assessment of the pesticide. *Eremias argus* was selected as an experimental model in this study because it is the most abundant lizard species in Asia (Jae-Young, 2010), it is easy to keep in the laboratory, and its preferred habitat brings it in close contact with soil. In addition, its reproductive cycle has been well documented (Deyi et al., 2000). Importantly, *E. argus* is believed to have a high rate of soil ingestion (Xue-Feng, 2008). Given the lack of data on the relationship between contaminated soil and pesticide accumulation in lizards, our study was designed to evaluate the accumulation of SM in lizards. Meanwhile, we also investigated the changes in weight, organ coefficient, histopathology and reproduction of SM-treated female and male lizards.

2. Material and methods

2.1. Chemicals

SM (96%) was obtained from the China Ministry of Agriculture's Institute for Control of Agrochemicals. Distilled water was purified by a Milli-Q system. *N*-hexane and acetone (analytical grade) were from Beijing Chemical Work (Beijing, China). Acetonitrile (chromatographic grade) and ethylacetate (analytical grade) were from Beijing Tong Guang Fine Chemicals Company (Beijing, China). Heparin sodium (anticoagulant) was purchased from Beijing Chemical Reagent Co. Ltd. (Beijing, China).

2.2. Lizards maintenance

Adult *E. argus* lizards were purchased from Guan Yuan Flower and Bird Insect Fish Market (Beijing, China). The lizards were maintained under laboratory conditions with a 12-h dark/light cycle, a temperature of 22 °C ± 3 °C and a relative humidity of 45%–50%. Each group was cultivated in a 48 × 32 × 14 cm clear plastic cage with 5 kg of sandy loam (clay, 26.80% ± 0.42%; silt, 12.00% ± 0.49%; sand, 61.20% ± 0.57%; organic matter, 22.70% ± 0.26 g/kg; moisture content, 4.24% ± 0.02%; and pH, 7.80 ± 0.03) and a water dish. All lizards were cultivated in the laboratory for two weeks to acclimate to the environment. Lizards were fed live mealworms every day, and the water was changed daily. One 25-W incandescent light bulb was positioned over one side of each cage to permit thermoregulation across a temperature gradient. Meanwhile, because the lizards are very sensitive to the surrounding environment and good at hiding themselves, we placed an egg carton on one side of each cage to allow the lizards to hide.

2.3. Bioassay procedure

After one week of acclimation, a total of 108 animals (sex ratio = 1:1) were randomly assigned to three experimental groups: control, low-dose and high-dose. Each group consisted of 36 lizards (sex ratio = 1:1) and was divided into three replicates. The concentrations of SM in the soil (5 kg) were 0 mg/kg (control group), 3 mg/kg (low-dose group) and 30 mg/kg (high-dose group). Based on the recommended application rate of SM in agricultural areas 1200 g a.i./ha.(http://www.chinapesticide.gov.cn/hysj/index.jhtml), the 3 mg/kg, an initial residue of SM in soil, was used to be the low concentration. Individual weights of animals were measured every week, and the changes in weight were calculated. In order to reduce disturbance to the lizards, their weights were measured only once a week. Following the live animal bioassays (60 d after exposure), surviving animals were weighed and then humanely killed (Chen et al., 2016). Two days before the end of the experiment, the lizards were fasted with free access to water. The methods for anesthetizing, killing and dissected the lizards were based on (Amaral et al., 2012b). Given that the blood volume of one lizard was not sufficient to analyze the SM concentration, the blood of male or female lizards in each replicate was collected in one heparinized tube. Blood, brain, heart, lung, liver, kidney, testis, and skin were removed and weighed for biomarker analyses: (1) the change in viscera index (lung, liver, kidney and testis); (2) the accumulation of SM in lizard tissues; and (3) histopathological analysis (liver and testis). Because significant differences existed in the timing of mating and oviposition for the individual females in different groups, there were large differences in the developmental conditions of the ovaries of different females. Therefore, we did not estimate the histopathological changes in the ovaries of the lizards.

2.4. Sample preparation

After sampling, samples were placed into 10-mL polypropylene centrifuge tubes containing 5 mL ethyl acetate and then homogenized for 90 s. The mixtures were extracted by ultrasonication for 10 min, vortex mixed for 3 min in 10 mL ethyl acetate and centrifuged at 3800 rpm for 5 min. The organic phases were transferred into test tubes. The samples were then re-extracted in the same way as above, and the supernatants were combined. The combined extract was evaporated to dryness with a Termovap sample concentrator at 35 °C. Subsequently, the residue was reconstituted in 1 mL of acetonitrile and then washed twice with n-hexane (1 mL). The acetonitrile layer was redried with a vacuum rotary evaporator at 35 °C. The residue was diluted to 0.2 mL with acetonitrile and used for HPLC-MS/MS analysis. The average recoveries are listed in Table 1.

2.5. Trace element analysis

A ThermoFisher TSQ Quantum Access MAX system (Tewksbury, Massachusetts, USA) was used to detect SM. A Hypersil GOLD C18 column (100 mm × 2.1 mm, 3 μm) was used for separation at 25 °C. The injection volume was 5 μL. The mobile phase was composed of water and acetonitrile (10/90, v/v), and the flow rate was 0.3 mL/min. The analytes were detected by multiple reaction monitoring. The electrospray ionization source temperature was 250 °C. Argon

Table 1
Summary of method recovery data for SM from fortified lizard blood and tissues (n = 6).[a]

Matrix	Fortification (mg/kg)	Recovery (%)	Matrix	Fortification (mg/kg)	Recovery (%)
Blood	2.50	83.93 ± 11.01	Liver	0.10	70.38 ± 2.34
	5.00	72.91 ± 0.24		0.50	87.34 ± 2.26
	10.00	78.73 ± 11.73		1.00	74.16 ± 2.29
Brain	0.25	76.31 ± 4.10	Kidney	1.00	108.05 ± 10.72
	1.00	60.41 ± 14.78		3.00	75.97 ± 13.58
	2.00	69.56 ± 2.27		6.00	85.04 ± 2.36
Heart	1.00	76.61 ± 3.30	Skin	1.00	75.40 ± 3.49
	5.00	79.78 ± 5.88		5.00	76.11 ± 12.79
	15.00	85.23 ± 8.29		15.00	103.39 ± 9.09
Lung	0.10	80.06 ± 0.82	Tail	0.50	67.82 ± 8.10
	1.00	86.43 ± 6.56		5.00	76.11 ± 12.79
	5.00	62.02 ± 5.91		15.00	95.58 ± 7.84

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

(99.999%) was used as the collision gas. For SM, the product ions were 251.99 and 176.06, and the respective collision energies were 16 and 25 V.

2.6. Histopathological lesions

Tissue samples (liver and testis) were fixed in 10% neutral buffered formalin, dehydrated in ethanol, cleared in xylol, embedded in paraffin and sectioned. Serial two-micron sections were stained with hematoxylin and eosin. Images were taken using an Olympus BX51 microscope at 400× magnification and using the SPOT Insight image capture system CCD camera. For the histopathological scores, in total 12 areas (at 400×) from each section were examined. For this purpose, tissue (liver and testis) sections were randomly chosen under the light microscope. The score was derived as semi-quantitatively (Amaral et al., 2012b; Topal et al., 2017), considering the number of microscopic areas with lesions and reporting as follows: none: - (no lesion), mild: + (1–4 lesions), moderate: ++ (5–8 lesions) and severe: +++ (>9 lesions).

2.7. Data analysis

Growth is usually defined as the production of new cells. However, growth is typically measured as an increase in mass (Owens et al., 1993). Mass changes are indicators of reproductive stress in interspecific or intersexual comparisons, which also reflects the breeding biology and life history of lizards. Because the initial weights of the lizards varied between different groups and sexes, the relative growth rate (RGR) was used in the present study to reflect growth. RGR was calculated as follows

$$\mathbf{RGR} = \frac{W_{x+1} - W_x}{W_x} \text{ (Yan et al., 2002)}$$

where W_{x+1} and W_x represent the lizards' weights at times T_{x+1} and T_x, respectively, and x (x = 0,1,2,3,4,5,6 and 7) represents the number of weeks.

The viscera index (VSI, %) was used to evaluate the swelling, atrophy, or hyperplasia of the organs due to SM exposure:

$$\mathbf{VSI} = \frac{\textbf{lizard visceral mass (g)}}{\textbf{lizard body mass (g)}} \times 100\%$$

In order to evaluate the effects of SM on reproduction, the egg-laying rate (ELR) and the amount of laying eggs (ALE) were used to reflect the reproductive outputs of female lizards in each group. ELR was calculated as follows:

$$\mathbf{ELR} = \frac{\textbf{the amount of egg laying female lizards}}{\textbf{the total number of females}} \times 100\%$$

Statistical analysis was performed using SPSS 19.0. Differences in accumulation, body weight, ALE and ELR data between the treatments were compared with the control group according to analysis of variance followed by least significant difference test. Differences in the prevalence and intensity of histological changes in tissues between treatments were compared with the control using Pearson's chi-squared test. We set the significance level at $p < 0.05$. The degrees of freedom from the treatments (df_1) and residual degree of freedom (df_2) were also reported.

3. Results

3.1. Mass change

The RGRs of female and male lizards are shown in Fig. 1. The growth patterns were similar in the male and female lizards in breeding season. After the fourth week, three groups were into the spawning period, leading to a lower RGR in succession. At the fourth week, the RGR values of lizards in control group were significantly lower than those of in low- ($df_1 = 2$, $df_2 = 52$; for female, $F = 23.08$, $p < 0.001$; for male, $F = 10.71$, $p = 0.003$.) and high-dose ($df_1 = 2$, $df_2 = 52$; for female, $F = 23.08$, $p < 0.001$; for male, $F = 10.71$, $p = 0.002$.) group. The RGR values of lizards in control group showed the fastest recovery at sixth week, followed by those of the low- ($df_1 = 2$, $df_2 = 52$; for female, $F = 113.27$, $p < 0.001$; for male, $F = 233.60$, $p < 0.001$) and high-dose groups ($df_1 = 2$, $df_2 = 52$; for female, $F = 113.27$, $p < 0.001$; for male, $F = 233.60$, $p < 0.001$).

3.2. Change in VSI

For female lizards, no significant differences were observed between the control and exposure groups in lung ($p = 0.16$ and $p = 0.92$ for low- and high-dose group, respectively; $df_1 = 2$, $df_2 = 52$, $F = 1.54$) and liver ($p = 0.31$ and $p = 0.16$ for low- and high-dose group, respectively; $df_1 = 2$, $df_2 = 52$, $F = 3.11$), with the exception of the kidney coefficient (KC) ($p < 0.001$, $df_1 = 2$, $df_2 = 52$, $F = 249.5$) in the high-dose group (Fig. 2).

For male lizards, the KC ($p = 0.013$ and $p = 0.036$ for low- and high-dose group, respectively; $df_1 = 2$, $df_2 = 52$, $F = 6.75$) and testis coefficient (TC, $p = 0.007$ and $p = 0.038$ for low- and high-dose group, respectively; $df_1 = 2$, $df_2 = 52$, $F = 22.15$) were significantly different between the control and exposure groups. Unlike for female lizards, the KC values of males were significantly higher in the low- and high-dose groups compared with the control. In contrast, TC was significantly higher in the low-dose group than in the control group but significantly lower in the high-dose group.

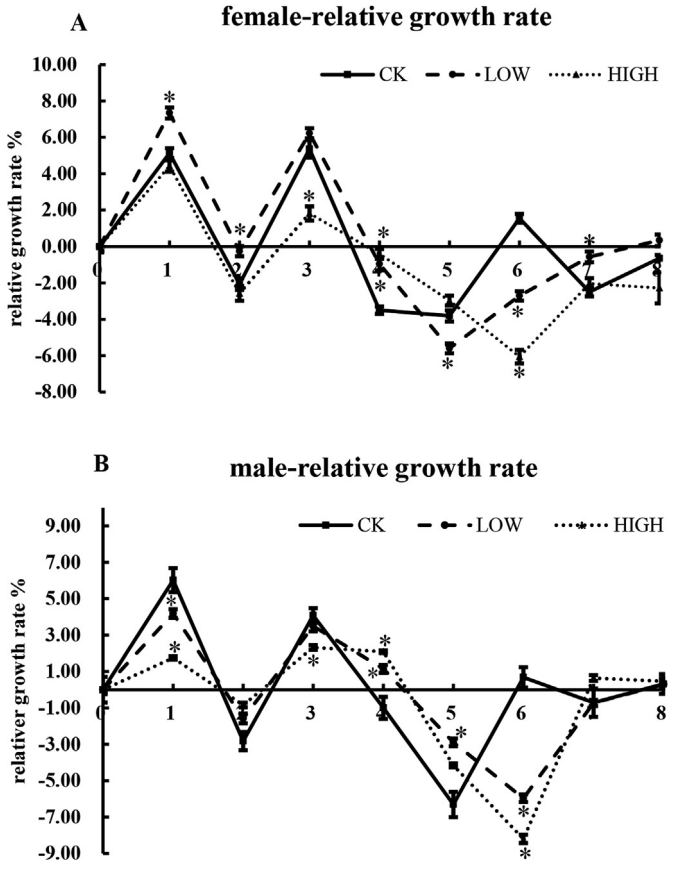

Fig. 1. Change in relative growth rates over time for female (A) and male (B) lizards in the control (CK), low-dose (LOW) and high-dose (HIGH) groups (bars indicate standard error; * represents significant difference between treatments and control group ($p < 0.05$)).

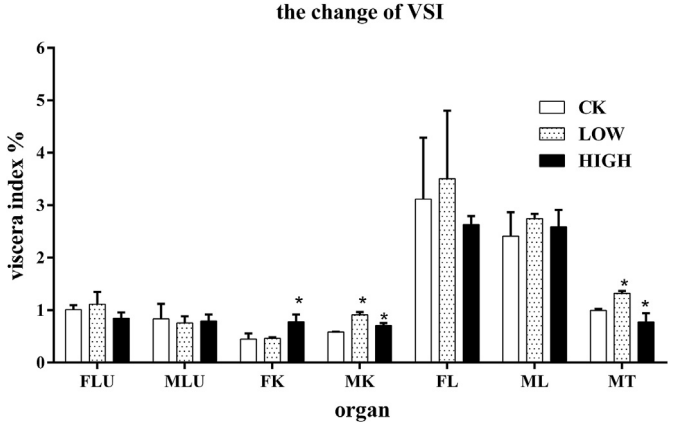

Fig. 2. Weights of lung, liver, kidney, brain and testis expressed as percentages of total body weight for the control group and exposure group (bars indicate standard error; * represents significant difference ($p < 0.05$); CK, LOW and HIGH represent the control, low-dose and high-dose groups, respectively; FLU = female lung, MLU = male lung, FK = female kidney, MK = male kidney, FL = female liver, ML = male liver, MT = male testis).

3.3. Accumulation studies in lizard tissues

The concentrations of SM in different lizard tissues were determined, and the results are listed in Fig. 3. Skin was washed with distilled water prior to extraction and dried by absorbent

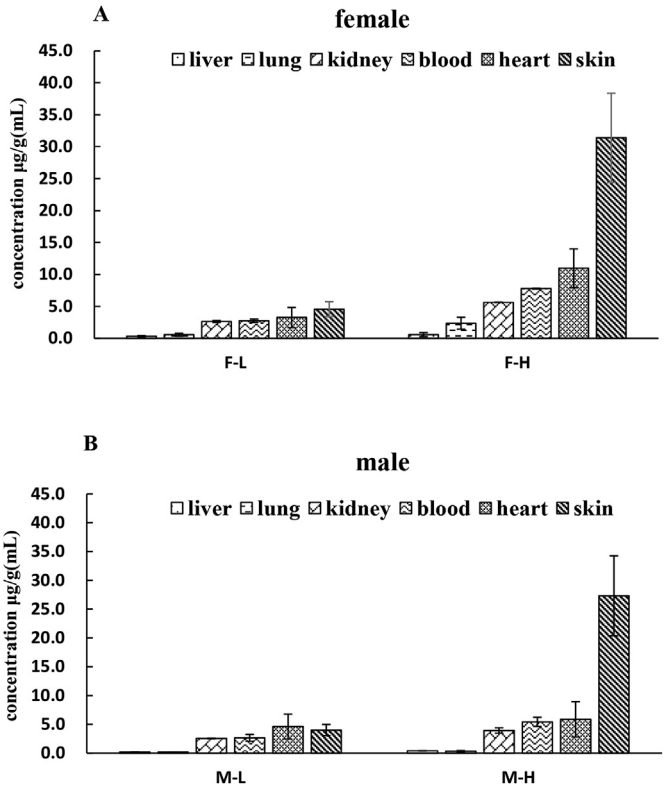

Fig. 3. Residue levels of SM in different tissues and blood for female (A) and male (B) lizards in the low- and high-dose groups (F-L = female low-dose groups, M-L = male low-dose groups, F-H = female high-dose groups, M-H = male high-dose groups; bars indicate standard error).

paper cautiously. For male lizards in both the low- and high-dose groups, the SM concentrations were lowest in the liver and lung. The concentrations of SM in skin were not lower than in other organs in the exposure group. The orders of SM concentrations were as follows: skin ≥ heart > blood ≥ kidney > liver ≥ lung in the low-dose group and skin > heart ≥ blood > kidney > liver ≥ lung in the high-dose group.

For female lizards, the lowest and highest concentrations of SM in the exposure groups were found in liver and skin, respectively. The concentrations of SM were significantly higher ($p = 0.035$ and $p < 0.001$ for low-and high-dose group, respectively; $df_1 = 2$, $df_2 = 52$, $F = 34.34$.) in the lung than in the liver. Furthermore, there were no significant differences ($p = 0.21$, $p = 0.69$ and $p = 0.54$, respectively; $df_1 = 2$, $df_2 = 52$, $F = 1.22$.) among the concentrations among kidney and blood, kidney and heart, blood and heart in the low-dose group. For high-dose group, the order of SM concentration in female tissues was skin > heart > blood > kidney > lung > liver.

3.4. Histopathological lesions

For lizards, the livers from all the groups had serous membrane integrity (Fig. 4 and Fig. 5). Dose dependent comparisons of liver and testis tissues are displayed in Table 2. In the control group, livers were characterized by normal tissue with some signs of lymphocyte infiltration and hepatocyte. Livers of individuals from low- dose groups (Fig. 4B, b and Fig. 5E, e) presented melanin deposition ($df_1 = 1$, $df_2 = 34$; for females, $\chi = 1.52$, $p = 0.22$; for male, $\chi = 0.55$, $p = 0.46$.), congestion ($df_1 = 1$, $df_2 = 34$; for females, $\chi = 0.55$, $p = 0.46$; for male, $\chi = 2.70$, $p = 0.10$.), hepatocyte

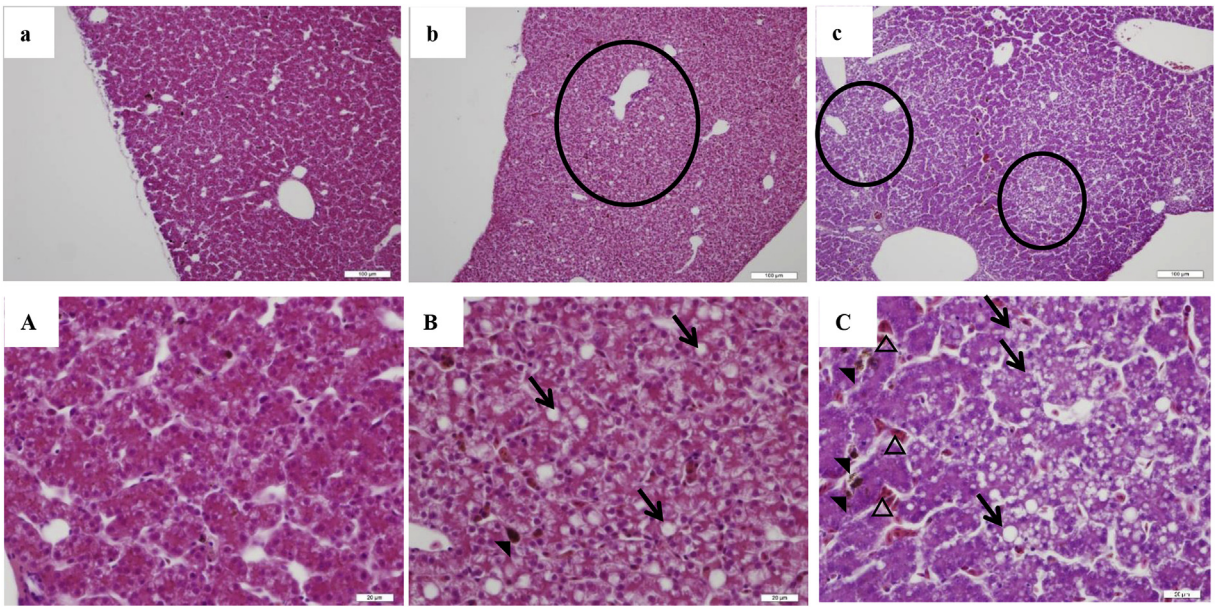

Fig. 4. Sections of livers of female lizards stained with hematoxylin and eosin (HE). (a and A) Liver sections of control groups representing normal liver (100 × and 400×, respectively). (b and B) Liver section of low-dose groups (100× and 400 ×, respectively). (c and C) Liver sections of high-dose groups (100 × and 400 ×, respectively). Circles represent the scope of vacuoles. (→ represents liver vacuoles, ◥ represents Kupffer cells, △ represents sinusoids with congestion).

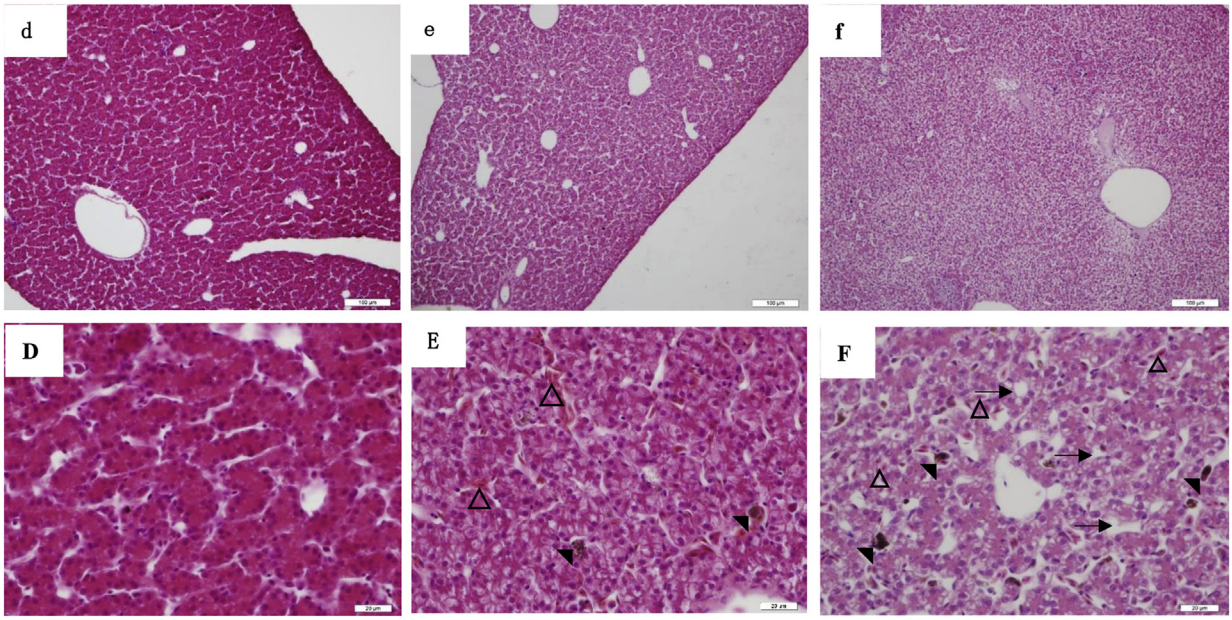

Fig. 5. Sections of livers of male lizards stained with hematoxylin and eosin. (d and D) Liver sections of control groups representing normal liver (100 × and 400 ×, respectively). (e and E) Liver sections of low-dose groups (100 × and 400 ×, respectively). (f and F) Liver sections of high-dose groups (100 × and 400 ×, respectively). (→ represents liver vacuoles, ◥ represents Kupffer cells, △ represents sinusoids with congestion).

vacuoles ($df_1 = 1$, $df_2 = 34$; for females, $\chi = 3.23$, $p = 0.07$; for male, $\chi = 4.69$, $p = 0.03$.) and hepatocyte necrosis ($df_1 = 1$, $df_2 = 34$; for females, $\chi = 2.40$, $p = 0.12$). In general, livers in the high-dose group showed a higher serious histological changes (Fig. 4C, c and Fig. 5F, f). In the high-dose treatment, the incidence of melanin deposition ($df_1 = 1$, $df_2 = 34$; for females, $\chi = 2.00$, $p = 0.16$; for male, $\chi = 1.52$, $p = 0.22$.), congestion ($df_1 = 1$, $df_2 = 34$; for females, $\chi = 4.04$, $p = 0.04$; for male, $\chi = \chi = 6.40$, $p = 0.01$.), hepatocyte vacuoles ($df_1 = 1$, $df_2 = 34$; for females, $\chi = 16.67$, $p < 0.001$; for male, $\chi = 15.61$, $p < 0.001$.), and hepatocyte necrosis ($df_1 = 1$, $df_2 = 34$; for females, $\chi = \chi = 20.17$, $p < 0.001$; for male, $\chi = 24.00$, $p < 0.001$.) approached significance when compared with the control.

For the lizard testes, the integrity of the tunica albuginea, visible sizes of the convoluted seminiferous tubule (CST) and the interstitial tissue, evacuated distribution of cells and thread sample materials in CST were observed in each group (Fig. 6). Meanwhile, the spermatogonia, primary and secondary spermatocytes, spermatids and sperm were also observed in the control and exposure groups. In the high-dose treatment, the incidence of seminiferous tubule damage ($df_1 = 1$, $df_2 = 34$; $\chi = 2.70$, $p = 0.10$) and significant congestion ($df_1 = 1$, $df_2 = 34$; $\chi = 7.26$, $p = 0.007$) were observed when compared with the control.

Table 2
Dose dependent comparison of histopathology in liver, testis of exposed lizards to control and exposure group.

Pathological findings						
Liver	FCK	FLD	FHD	MCK	MLD	MHD
melanin deposition	−	+	++	−	+	+++
congestion	−	+	++	+	+	++
hepatocyte vacuoles	+	++	+++	+	++	+++
hepatocyte necrosis	−	+	+++	−	−	+++
testis	MCK		MLD		MHD	
Congestion	−		+		++	
Seminiferous tubule damage	−		−		+	

FCK represent female lizards in control group; FLD represent female lizards in low-dose group; FHD represent female lizards in high-dose groups; MCK represent male lizards in control group; MLD represent male lizards in low-dose group; MHD represent male lizards in high-dose groups. − represent no lesion, + represent mild lesions, ++ represent moderate lesions and +++ represent severe lesions.

4. Discussion

4.1. Mass change

Body condition was assumed to influence the lizards' fitness and health. Owens (Owens et al., 1993) reported that the body shapes of animals change over time, and the deposition of certain internal muscle components is more extensive in later life. This finding is consistent with our experiments, in which the masses of the lizards changed over time, even in the control groups. The similarity in the growth patterns between the sexes of this species might be indicative of similar life history traits (Ramírez-Bautista et al., 2016). The weights of pregnant lizards increased significantly with successful mating. After ovulation, the weights of female lizards decreased rapidly, and their RGRs became negative in the fourth week, sooner than those of the males.

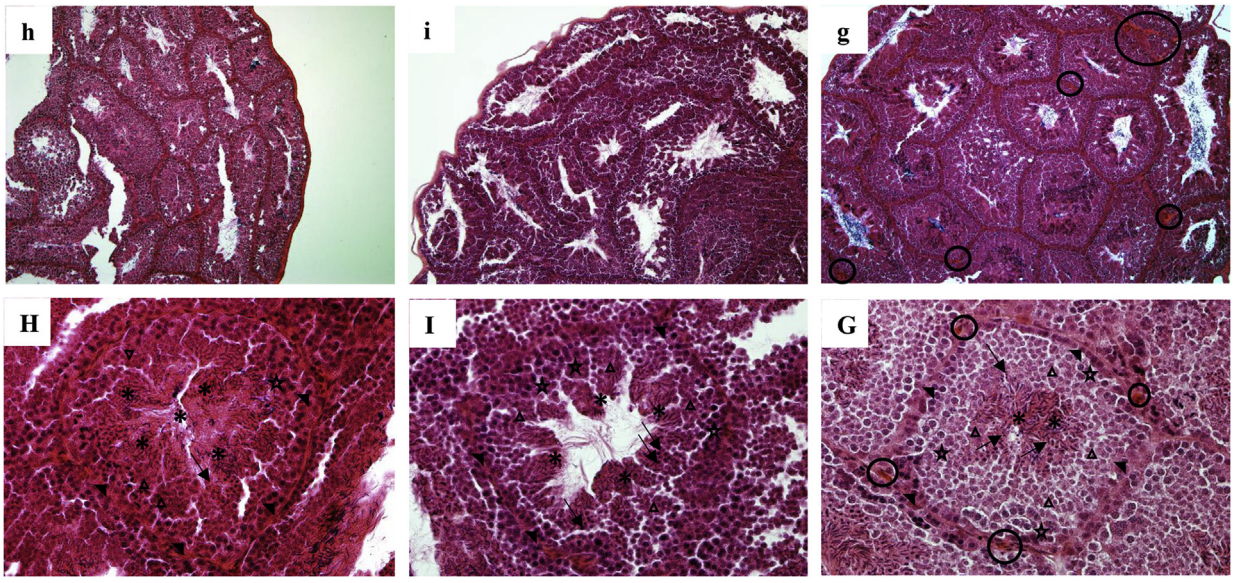

Fig. 6. Sections of testes of male lizards stained with hematoxylin and eosin. (h and H) Testes sections of control groups representing normal testis (HE 100 and 400, respectively). (i and I) Testes sections of low-dose groups (100 × and 400 ×, respectively). (g and G) Testes sections of high-dose groups (100 × and 400 ×, respectively). Circles represent the congestion of blood vessels in the interstitial tissue. (↰ represents spermatogonium, ☆ represents primary spermatocyte, △ represents secondary spermatocyte, → represents spermatid, * represents sperm).

Taking all histopathological results together, the hepatocyte vacuoles in liver and congestion in testis could be one of the most sensitive indicators after SM exposure.

3.5. The values of ELR and ALE

The ELR and ALE values were used to assess the reproductive toxicity of SM in female lizards. The amount of egg laying female lizards were 6.00 ± 0.00, 5.33 ± 0.58 and 3.67 ± 0.58 for each replicate of control, low- and high-dose group. The ELR of the control (100%) and low-dose group (90%) were similar ($p = 0.13$, $F = 19.5$, $df_1 = 2$, $df_2 = 43$.). However, the ELR of the high-dose group (60%) was significantly lower ($p = 0.001$, $F = 19.5$, $df_1 = 2$, $df_2 = 43$.) than control group. The ALE values decreased in the following order: control (33 eggs), low-dose roup (23 eggs) and high-dose group (12 eggs). In the low- and high-dose group, the ALE was significantly lower ($p = 0.004$ and $p < 0.001$, respectively; $F = 25.92$, $df_1 = 2$, $df_2 = 66$.) than the control group.

For female lizards, the higher RGR in the low-dose group might be attributed to the lower SM exposure, which did not have adverse effects on the lizards or promoted growth before the fourth week. As a result of individual differences, the RGR in the low-dose group was higher than in the control group. The lowest RGR of the high-dose group might be due to adverse effects caused by the high dose of SM. Furthermore, the high dose of SM may have led to a low rate of mating or pregnancy, as indicated by decreases in both the number of pregnant lizards and eggs. The immature eggs found in the three groups at the end of the experiment suggest that the female lizards were into the second mating and conception, leading to the increase of RGR at experiment in the late stage. For male lizards, the negative RGR after the fifth week reflected the normal physiological states of the male lizards. For example, at the beginning of the breeding season, the volume of testes in adult male lizards was maximized and then decreased due to mating. The testicular volume reached a minimum at the end of the breeding season.

4.2. Change in VSI

In high dose group, the higher KC indicated that the high concentration of SM could result in increased kidney weights in females. A previous study also investigated the dermal toxicity of SM using New Zealand white rabbits and found that SM led to increased kidney weights in females (EPA, 1995). However, in a one-year study in dogs, the administration of metolachlor resulted in decreased kidney weight at the two highest dose levels (Organization, 2003). These conflicting results might be explained by the different species and pesticide doses. Because different species have unique physiologies, they might have different responses to the same pollutants. Thus, these compounds must be widely tested across species to understand their influence on organisms (Liu et al., 2014). Additionally, we found that the LC values of female lizards had greater error. This might be because the females were in the mating or breeding season, resulting in low individual tolerance and large individual differences. Previous studies have also reported this phenomenon; for example, Beaton et al. reported that animals during the pregnancy period experienced liver and kidney damages when exposed to acute doses of Cd^{2+} (Samarawickrama and Webb, 1981).

For male lizards, the changes in KC suggested that the low concentration of SM had harmful effects on the kidneys of the male lizards. Albino rats that were fed diets containing metolachlor for two years showed testicular atrophy at concentrations of 300 and 3000 mg/kg (Chesters et al., 1989). However, no significant changes in relative testicular weight were found in male Wistar rats exposed to metolachlor (Mathias et al., 2012). This indicates that SM rather than metolachlor has adverse effects on testes. Although there were no statistically significant changes in LC, the mean LC values were higher in the exposure group than in the control group. This result was similar to that of a previous study in which the liver weights of male rats exposed to no-effect levels (100 mg/kg/day) of metolachlor increased during a 21-day study on dermal toxicity in rabbits (EPA, 1995).

4.3. Accumulation studies in lizard tissues

In the exposure groups for both male and female lizards, the concentrations of SM were highest in skin. This might be because the skin was in close contact with the contaminated soil. In this study, skin exposure may have been an important route for the uptake of SM in *E. argus*. Meanwhile, the accumulations of SM in tissues were similar in the exposure groups, with the exception of in skin. This indicated that the higher concentration of SM in soil resulted in more SM uptake in skin, while the abilities of other viscera to accumulate SM were limited. The concentrations of SM were higher in heart and blood than in kidney, lung and liver. These findings might be related to the fact that SM was absorbed from the soil and entered into blood circulation. So we assumed that the SM would be absorbed by the skin and then distribute to viscera followed the blood circulation. Interestingly, the concentrations of SM were low in liver and lung, suggesting that the liver and lung have strong detoxification abilities that allow them to rapidly metabolize and clear the pesticide.

Additional contaminant intake could occur through either incidental or intentional soil ingestion in *E. argus* (Xue-Feng, 2008). Meanwhile, metolachlor has been reported to moderately adsorb to soil. The adsorption of metolachlor increases with increasing soil organic matter or clay content (Rivard, 2003). In this experiment, the organic matter and clay contents of the soil were 22.7% and 26.8%, respectively. The large content of organic matter in the soil would absorb more SM, causing *E. argus* to accumulate more SM via soil ingestion. This indicates that soil ingestion might be another important route for SM uptake in *E. argus*. It is necessary to consider the effect of soil texture on the accumulation of contaminants for reptiles when conducting environmental risk assessments of soil-based contaminates for reptiles. A comprehensive assessment of the relationship between the soil characteristics, pesticide residues and hazards for animals will improve the rational application of pesticide.

4.4. Histopathological lesions

Low concentrations of SM in the liver were found in the exposure group, which reflects the high metabolic ability of the livers of lizards. To explore whether the SM caused liver damage, the histopathological changes in the liver were evaluated. The results also suggested that the livers of female lizards were more vulnerable than those of males in the exposure groups. The vacuoles of the liver cells might indicate that SM induced abnormal lipometabolism and glycometabolism in the lizards.

The USEPA reported that metolachlor can cause testicular atrophy in albino rats (Chesters et al., 1989). This indicates that SM might induce reproductive toxicity. Thus, the histopathologic alterations in the testes of *E. argus* exposed to different SM concentrations were also assessed. The slides of the testes showed that SM did not affect the development of germ cells. However, the vitality of mature sperm was not determined; thus, we cannot be sure if SM has harmful effects on germ cells. As previously described, the fertilizing capacity of spermatozoa was significantly affected after exposure to metolachlor (Mai et al., 2013). Mai et al. also reported that DNA damage in oyster spermatozoa was observed at low concentrations of metolachlor (0.01 μg/L) and sperm toxicity could result in a reduction in the number of offspring or their quality (Mai et al., 2014). Considering SM could induce reproductive toxicity, more detailed research on the reproductive toxicity of SM in male lizards, including studies of sperm vitality, hormone levels and other biochemical indicators should be carried out in the future.

4.5. The values of ELR and ALE

The results of ELR and ALE values indicated that SM induced adverse effects on the egg-laying abilities of the female lizards. The reduction in the number of eggs might cause a reduction in the number of offspring, leading to a decrease in the population density of *E. argus*.

The mechanism on reduction of eggs, such as the transcription of genes and enzymes, could be researched.

5. Conclusions

Pesticide contaminants may be contributing to reptile decline (Gibbons et al., 2000). However, in research on terrestrial vertebrate ecotoxicology, reptiles remain the least studied vertebrate group (Sparling et al., 2010). In general, toxicological data are lacking for predicting risk from pesticides in lizard. Our study thus was designed to evaluate the accumulation and toxic effects (include the changes in weight, organ coefficient, histopathology and reproduction) of SM in lizards. After exposure to SM-contaminated soil for eight weeks, for both male and female lizards, SM was bioavailable, and the concentrations of SM were highest and lowest in skin and liver, respectively. Skin exposure and soil ingestion may be important routes for the uptake of SM in *E. argus*. The significantly different RGR, changes of KC and serious liver pathological lesions suggested that SM influenced the lizards' fitness and health. In the low dose group, the TC was significant increased and the ALE was significant decreased. The significant decrease of TC, ALE and ELR and the congestion of testis were

observed in the high-dose group. These indicated that reproductive output was impaired in lizards exposed to SM, and SM might be harmful on lizards' reproductive system. While an understanding of the mechanism behind these changes has yet to be fully elucidated, more future works such as the sperm quality, hormone levels and the determination of the expression of genes important in regulating reproduction are needed to elucidate the mechanisms of reproductive toxicity caused by SM exposure.

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