Chemosphere 216 (2019) 69–74

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Effects of beta-cypermethrin and myclobutanil on some enzymes and changes of biomarkers between internal tissues and saliva in reptiles (*Eremias argus*)

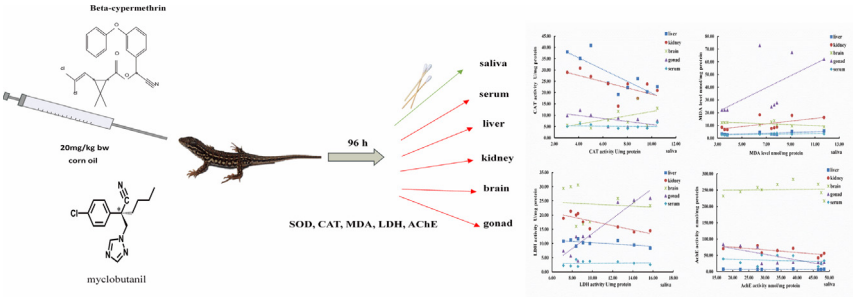
Li Chen [a], Jinling Diao [b], Wenjun Zhang [a], Luyao Zhang [a], Zikang Wang [a], Yao Li [c], Yue Deng [a], Zhiqiang Zhou [a, *]

[a] Beijing Advanced Innovation Center for Food Nutrition and Human Health, Department of Applied Chemistry, China Agricultural University, Yuanmingyuan West Road 2, Beijing 100193, China
[b] Department of Applied Chemistry, China Agricultural University, Yuanmingyuan West Road 2, Beijing 100193, China
[c] College of Plant Protection, China Agricultural University, Yuanmingyuan West Road 2, Beijing 100193, China

HIGHLIGHTS

- Beta-cyperimethrin can cause more oxidative severe damage than myclobutanil.
- Enzymes showed different changes in different organs or tissues.
- Salivary enzymes activities showed sensitivity changes in reptiles.
- Buccal swabs could be used as a tool for saliva sampling in reptiles.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 6 August 2018
Received in revised form
15 October 2018
Accepted 16 October 2018
Available online 16 October 2018

Handling Editor: Willie Peijnenburg

Keywords:
Pesticide
Enzyme
Tissues
Saliva
Reptile

ABSTRACT

Numerous studies suggested that reptiles are sensitive to environmental pollution and the abundance of many species are in decline. Our research is aim to assess the toxic effects of pesticide in reptiles. And we also want to supply some data about nondestructive samples for environmental risk assessment in reptiles. Lizards were orally administered a single-dose of beta-cypermethrin (BCP) or myclobutanil (MC) at the concentration of 20 mg/kg body weight (bw). The results showed that pesticides could induce changes in enzymatic activities (SOD, CAT, LDH, AChE) and MDA levels in organs or tissues of lizards. BCP could cause more severe oxidative damage than that of MC. Salivary enzymes activities showed sensitivity changes to the toxicity of pesticides. We could use saliva to reflect whether the reptiles are toxic by pesticides. We also agree that buccal swabs could be used as a tool for saliva sampling.

1. Introduction

Various group of fungicides and insecticides are widely used in agriculture to improve grain quality and to guarantee the food

* Corresponding author. Beijing Advanced Innovation Center for Food Nutrition and Human Health. Department of Applied Chemistry, China Agricultural University, Yuanmingyuan West Road 2, Beijing 100193, PR China.
E-mail address: zqzhou@cau.edu.cn (Z. Zhou).

https://doi.org/10.1016/j.chemosphere.2018.10.099
0045-6535/

security. The abundance of pesticides may cause adverse effects on the environment and non-target species (Kenneke et al., 2009; Wieczorek et al., 2018). Pyrethroid insecticides and triazole fungicides are famous chemical families of the worldwide pesticide market (Bhhatarai and Gramatica, 2011; Zhuang et al., 2011). Beta-Cypermethrin (BCP), a synthetic pyrethroid compound, can accumulate in the liver, kidneys, testes, lungs, blood, brain and heart (Singh and Singh, 2008; Chen et al., 2016). BCP can induce oxidative stress (Mu et al., 2017), and damages functions of kidney, liver and reproductive system (Zhou et al., 2018). Myclobutanil (MC), a triazole compound, can also accumulate in vertebrate (Chen et al., 2017; Cheng et al., 2017). MC can also disturb P450-mediated detoxification (Han et al., 2018), induce oxidative stress (Huang et al., 2016), and have toxic effects on the liver, kidney, and gonad (Stellavato et al., 2016).

For numerous evaluations of BCP and MC, risk tends to be highest for aquatic animals (Lin et al., 2014; Zhang et al., 2017), birds (Parsons et al., 2010; Liu et al., 2017) and mammals (Berenstein et al., 2017; Zhou et al., 2018). Reptiles remain primarily neglected vertebrate group in assessments of pesticides exposure (Hopkins, 2000; Sparling et al., 2010) and lizards are always chosen as pollution bioindicators of reptiles (Gultekin et al., 2000). The ignored reasons for reptile may be as follows: 1) The lack of sympathy that many people have for reptiles (Rendón-von Osten et al., 2005); 2) No statutory requirements for testing reptiles exist (Ankley et al., 1998); 3) criteria of birds and mammals also be used to reptiles (Urban and Cook, 1986); 4) No standardized tests with reptiles exist (Ankley et al., 1998); 5) Reptiles exhibit long generation times, limited numbers offspring, and challenging to maintain in captivity (Urban and Cook, 1986). With the decreased abundance of global species of reptiles, concerns over the status of reptile populations are increasing. That also stimulates demand for the development of nondestructive sampling techniques used in environmental risk assessments (Hopkins et al., 2001). Saliva is a diagnostic fluid, because of its non-invasive sampling and changes in saliva composition caused by the pathological process (Streckfus and Bigler, 2002).

Hopins et al. evaluated the amount of trace element in nondestructive tissue samples of snake (Hopkins et al., 2001, 2005). Serum "B" esterases as a nondestructive biomarker monitored the exposure of reptiles to pesticides (Sanchez et al., 1997). Mingo et al. use the saliva as a noninvasive sample to detect effects of pesticide in lizards (Mingo et al., 2017), and they also suggested that buccal swabs could be a sampling method.

Pesticides could induce oxidative stress by a production of free radicals and changes in antioxidant defense mechanisms (López et al., 2007). The primary target of reactive oxygen species (ROS) are polyunsaturated fatty acids in membrane phospholipids, and malondialdehyde (MDA) is an end product (Draper and Hadley, 1990). The final membrane damage can lead to confusion of cell structure and role (Patterson and Leake, 1998). ROS is involved in the toxicity of various pesticides (Gultekin et al., 2000). Superoxide dismutase (SOD) and catalase (CAT) are oxygen free radical scavenging enzymes could protect the cellular system from various deleterious effects (Seth et al., 2001). Lactate dehydrogenase (LDH), a metabolic enzyme catalyzed the reaction of lactate production via pyruvate reduction during anaerobic glycolysis has been used as biomarker stress in animals (Vieira et al., 2008; Sangha et al., 2013). Acetylcholinesterase (AChE) activity has been an essential biomarker for neurotoxicity study in wildlife toxicology to diagnose the exposure to environmental contaminants (Rendón-von Osten et al., 2005; Richetti et al., 2011).

In our research, we described the relationships of enzymes among saliva, serum, liver, kidney, brain, and gonad in lizards that had been exposed to BCP and MC after 96 h. For better understanding, the activities of SOD, CAT, LDH, AChE and MDA levels were selected as biomarkers. This study aims to assess the toxic effects of pesticide and buccal swabs whether can be a nondestructive sampling method for environmental risk assessment in reptiles.

2. Material and methods

2.1. Chemicals and reagents

BCP (96%) and MC (97.5%) were from the Institute for the Control of Agrochemicals of the Ministry of Agriculture. Disodium hydrogen phosphate and sodium dihydrogen phosphate were purchased from Beijing Chemical Reagent Co., China. Total protein, SOD, CAT, GST, and MDA of commercial test kits were obtained from Nanjing Jiancheng Bioengineering Institute, Suzhou Comin Biotechnology Co., Ltd. Corn oil, pyruvic acid, NADH, 5,5'-dithiobis-(2-nitrobenzoic acid) and acetylthiocholine iodide were obtained from Sigma-Aldrich. Stock solutions were dissolved in acetone and then diluted with corn oil. A Milli-Q system purified water. Figs. 1–6.

2.2. Animals maintenance

Husbandry information has been reported in our previous article (Chen et al., 2016). After two weeks accumulation period, eighteen male lizards were randomly distributed to a control ($n = 6$) and two exposure groups ($n = 6$). The control group was administered only corn oil orally. Two exposure groups were received orally with 20 mg/kg $_{bw}$ of BCP and MC, respectively. Based on our previous researches (Chen et al., 2016, 2017), the concentrations of these two pesticides were chosen.

2.3. Sample collection

All lizards were euthanized at 96 h after lizards were dosed once. The serum, saliva, and organs including the brain, liver, kidney and testes were removed and weighted. After sampling, samples were homogenized with phosphate buffer ($pH = 7.0$). Then samples were centrifuged at 4 °C with 3000 rpm for 10 min. The supernatant was stored at - 20 °C and used to measure biomarkers.

2.4. Studied biomarkers

The total protein, SOD activities, CAT activities, and MDA content were measured according to the protocol of commercial test kits.

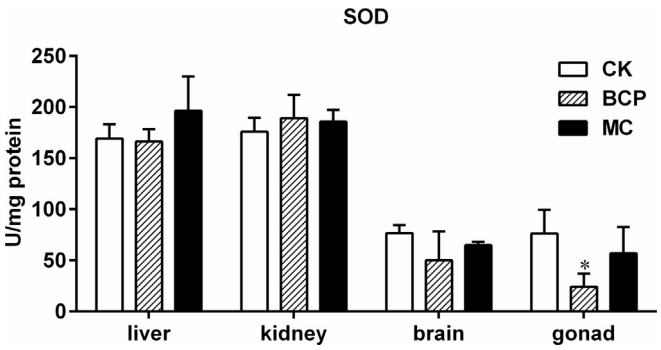


Fig. 1. Effects of 20 mg/kg concentration of beta-cypermethrin (BCP) and myclobutanil (MC) on the activities of SOD in the liver, kidney, brain and gonad of *E. argus*. CK represents control group. * represents $p < 0.05$.

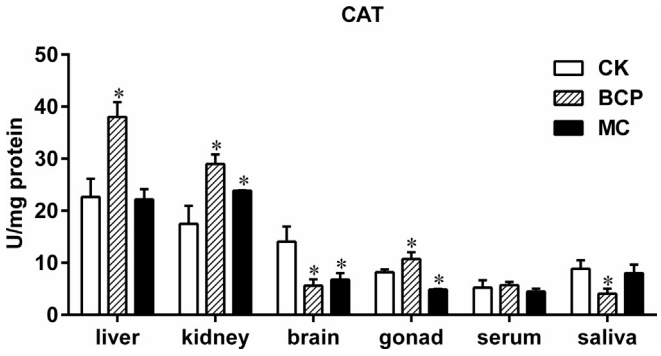


Fig. 2. Effects of 20 mg/kg concentration of beta-cypermethrin (BCP) and myclobutanil (MC) on the activities of CAT in the liver, kidney, brain, gonad, blood and saliva of *E. argus*. CK represents control group. * represents $p < 0.05$.

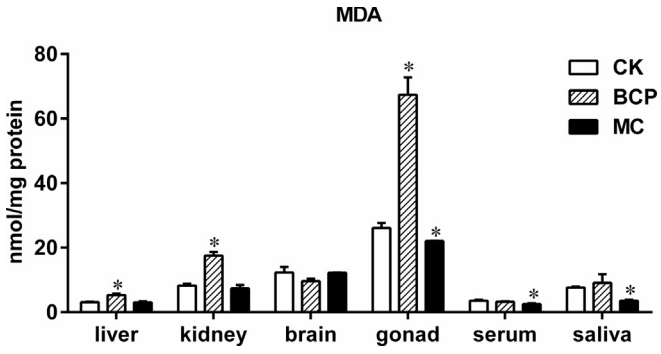


Fig. 3. Effects of 20 mg/kg concentration of beta-cypermethrin (BCP) and myclobutanil (MC) on the activities of MDA in the liver, kidney, brain, gonad, blood and saliva of *E. argus*. CK represents control group. * represents $p < 0.05$.

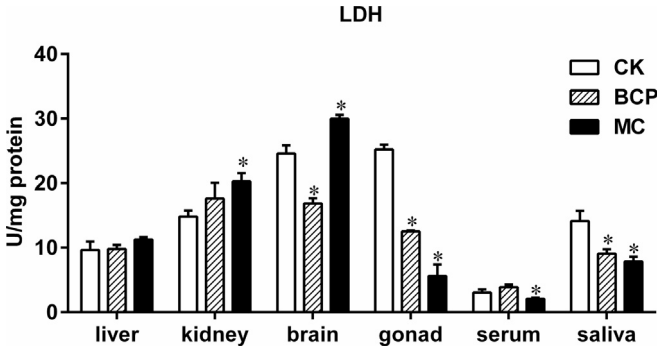


Fig. 4. Effects of 20 mg/kg concentration of beta-cypermethrin (BCP) and myclobutanil (MC) on the activities of LDH in the liver, kidney, brain, gonad, blood and saliva of *E. argus*. CK represents control group. * represents $p < 0.05$.

The CAT activities were measured by the decomposition rate of H_2O_2 at 240 nm. SOD was measured using xanthine oxidase method by monitoring the change of absorbance at 550 nm. MDA content was assayed according to thiobarbituric acid method by monitoring the change of absorbance at 532 nm. The LDH activity was measured according to (Di et al., 2017) by the reaction of homogenate, 2.9 mL pyruvic acid solution (0.98 mM) and 100 μL NADH (5.3 mM) at 340 nm for 3 min. The AChE activity was measured according to (Ellman et al., 1961) by following the increase of yellow color produced from thiocholine when it reacts with dithiobisnitrobenzoate ion at 412 nm.

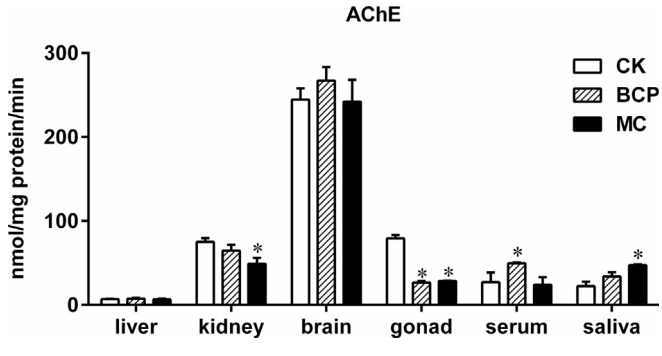


Fig. 5. Effects of 20 mg/kg concentration of beta-cypermethrin (BCP) and myclobutanil (MC) on the activities of AChE in the liver, kidney, brain, gonad, blood and saliva of *E. argus*. CK represents control group. * represents $p < 0.05$.

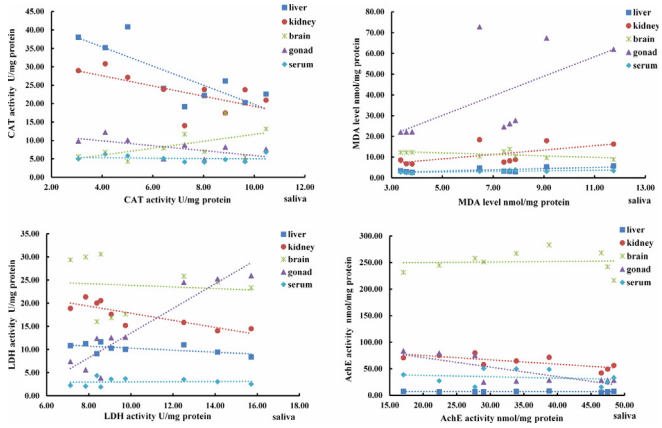


Fig. 6. Scatterplots showing activity in tissue (liver, kidney, brain, gonad and blood) and saliva samples of *E. argus* for CAT, MDA, LDH and AchE.

2.5. Data analysis

SPSS 19.0 was used to analyze data. The method of data analysis generally based on (Mingo et al., 2017). Levene's test and Shapiro-Wilk test were run to examine assumptions of homogeneity of variances and normality distribution of data. We investigated the degree of normality, and all the values of parameters fitted a normal distribution. Spearman's rank correlation were used to calculate the correlation rates between enzyme activities in different tissue samples. For tissues and saliva samples, linear regressions determined the amount of variance explained by each model. We set the significance level at $p < 0.05$. Whenever significant differences could be observed between tested groups, Dunn-Bonferroni tests were used as post-hoc-tests to potentially identify them.

3. Results

In the research, we measured enzyme biomarkers in saliva, liver, kidney, brain, gonad, and serum in lizards that had been exposed to BCP and MC after 96 h. We found SOD activities were failed to be evaluated in serum and saliva. Enzymatic assays for others' biomarkers using saliva and other tissues showed a success. Activity levels of CAT, SOD and lipid peroxidation were differently altered in serum, liver, kidney, brain, and testis of treated lizards at both BCP and MC exposure group.

3.1. Antioxidant enzymes and MDA level

In the experiment, the SOD activities were too little to measure in serum and saliva. And SOD activities were no difference were observed in liver, kidney, and brain after exposure BCP or MC 96 h. But the SOD activity was significant decrease in gonad ($p = 0.027$, $F = 0.584$, $df = 4$) in the BCP group.

In BCP exposure group, significant variations in the activities of CAT were observed in all tissues but serum. Significant increase were found in liver ($p = 0.004$, $F = 0.092$, $df = 4$), kidney ($p = 0.007$, $F = 0.689$, $df = 4$) and gonad ($p = 0.037$, $F = 3.624$, $df = 4$). And the activities were significantly decrease in brain ($p = 0.01$, $F = 2.899$, $df = 4$) and saliva ($p = 0.01$, $F = 0.428$, $df = 4$). After MC exposure, CAT activities were significant increase in kidney ($p = 0.033$, $F = 3.834$, $df = 4$) and decrease in brain ($p = 0.017$, $F = 2.845$, $df = 4$) and gonad ($p = 0.001$, $F = 2.445$, $df = 4$).

After exposure BCP 96 h, in comparation with control group, significant increase in MDA levels were found in liver ($p < 0.001$, $F = 2.56$, $df = 4$), kidney ($p < 0.001$, $F = 1.80$, $df = 4$), although decreased significantly ($p = 0.07$, $F = 2.56$, $df = 4$) in brain. No increase of MDA levels were observed in liver and kidney after exposure MC 96 h. But significant decrease in MDA levels were found in gonad ($p < 0.001$, $F = 3.76$, $df = 4$), serum ($p = 0.004$, $F = 0.01$, $df = 4$) and saliva ($p < 0.001$, $F < 0.001$, $df = 4$).

3.2. LDH activity

After BCP exposure, the activity of LDH in the brain ($p = 0.001$, $F = 0.361$, $df = 4$) and gonad ($p < 0.001$, $F = 2.448$, $df = 4$) increased significantly, while LDH activity in saliva ($p = 0.07$, $F = 1.133$, $df = 4$) decreased significantly. After the exposure of MC, LDH activity was significantly increased in the brain ($p = 0.03$, $F = 0.895$, $df = 4$), while in the gonads ($p < 0.001$, $F = 1.180$, $df = 4$), serum ($p = 0.035$, $F = 1.450$, $df = 4$) and saliva ($p = 0.003$, $F = 1.006$, $df = 4$) showed significant decrease.

3.3. AChE activity

In the BCP exposure group, the activity of AChE in serum ($p = 0.027$, $F = 3.447$, $df = 4$) increased significantly, and the activity of AChE in gonad ($p = 0.035$, $F = 1.450$, $df = 4$) decreased significantly.

In the MC exposure group, the activity of the kidney ($p = 0.006$, $F = 0.353$, $df = 4$) and gonad ($p < 0.001$, $F = 3.580$, $df = 4$) decreased significantly, while the activity in saliva ($p = 0.001$, $F = 2.632$, $df = 4$) increased significantly.

3.4. Correlations between saliva and tissue samples

Based on a Spearman rank correlation test, CAT activities in saliva negatively associated with kidney ($p = 0.016$, rho = - 0.767) and gonad ($p = 0.030$, rho = - 0.717). Furthermore, linear regressions between saliva and kidney showed that the model could explain 43% of the variance; the linear regression could explain 41% of variance between saliva and gonad.

MDA levels from saliva positively correlated with gonad ($p = 0.025$, rho = 0.733) and serum ($p = 0.020$, rho = 0.750). And linear regressions between saliva and gonad showed that 37% of variance could be explained by the model; the linear regression could explain 55% of variance between saliva and serum.

LDH activities form saliva negatively correlated with kidney ($p = 0.004$, rho = - 0.850), while positively correlated with gonad ($p = 0.002$, rho = 0.883). And linear regressions between saliva and kidney showed that 69% of variance could be explained by the model; 86% of variance could be explained by the linear regression between saliva and gonad.

AChE activities from saliva negatively correlated with kidney ($p = 0.030$, rho = - 0.717); 55% of the variance could be explained by the linear regression between saliva and kidney.

According to the above analysis, saliva was more correlated with kidney and gonad. In all enzymes, LDH was with high R^2 and absolute rho value. So saliva could be accurate to predict the LDH activity of liver and kidney.

4. Discussion

4.1. Enzymatic activities after BCP exposure

In all cases, pesticides could induce changes in enzymes activities (Üner et al., 2001; Ding et al., 2010; El-Demerdash and Nasr, 2014). Therefore, measuring changes in enzymes activities are considered to be an effective method of indicating the toxic effects of pesticides (Mu et al., 2017). The increase or decrease of enzymatic activity is respond to the cell damage (Manna et al., 2004). Jin et al. (2011) demonstrated that cypermethrin could cause hepatic mRNA levels for the genes encoding antioxidant proteins (SOD, CAT). Cypermethrin also produced significant high MDA in the liver after single oral exposure (170 mg/kg) (Giray et al., 2001). Rats were administered cypermethrin (25 mg/kg, orally) daily for 28 days that caused evaluated level of MDA in the liver, kidney (Sankar et al., 2012). Increased activity of CAT and increased MDA levels in liver were contradictory. That could suggest that increased activity of CAT was not enough to scavenge hydrogen peroxide and resulted in lipid peroxidation. Our previous pathological changes (Chen et al., 2016) correlated with the altered enzyme activities. A contradictory phenomena also happened in kidney and gonad. That reason could be similar with the liver. However, significantly decreased SOD in gonad could suggest that high concentration of superoxide consumed SOD. In human, the salivary antioxidant system plays an important role in the oral cavity and stomach (Motamedi et al., 2013). Mingo et al. (2017) also suggested to measure activity rates of the antioxidative enzymes (GR and GST) in saliva. In our study, saliva sample failed to satisfy the method of measuring SOD activity. This need to solve in the further research. However, significantly decreased CAT activity could indicate that the existence of oxidative reaction in lizard saliva.

Lactate dehydrogenase (LDH) plays a role in cells and catalyzes the reaction of lactate production. It will be extracellular when cell death (Motamedi et al., 2013). The decreased LDH activities in testes and epididymis of rats were observed after pyrethroid exposure (Srivastava et al., 2006). Guraya et al. also reported a reduced LDH activity in the ovary (Guraya, 2000). Decreased activity of LDH in brain, gonad and saliva could indicate that BCP induced toxic effect in lizards and resulted in a reduced metabolism.

Animal behavior is usually regulated by neurosecretion such as AChE (Rao, 1999). AChE could break acetylcholine into acetic acid and choline (Tiwari et al., 2012) and delay the sodium channel opening (Vijverberg and vanden Bercken, 1990; Narahashi, 1996). Cypermethrin could induce alterations of AChE activity in fish (Kumar et al., 2009). We reported (Chen et al., 2016) that BCP induced neurological effects gradually disappear and returned to normal state at 72 h. And low concentration of BCP in the brain at 96 h could also explain no statistically significant changes of AChE activity. Das et al. (Das and Mukherjee, 2003) reported that cypermethrin inhibits the activity of AChE in the brain of fish. Tiwari et al. (2012) also found that the activities of AChE in the liver and muscle were also significantly inhibited by cypermethrin. In this study, we also found that BCP inhibits the activity of enzyme AChE in the gonad. However, the increased AChE activity in serum

may indicate that the difference of metabolism existed between different tissues and organs.

4.2. Enzymatic activities after MC exposure

Cheng et al. (2017) reported that increased SOD activity and MDA level were observed in tadpole after MC exposure. In our study, no significant changes of SOD activities in all organs may be attribute to the fact that the dose of MC is too low to produce a large amount of $O_2^{\cdot-}$. The decreased MDA levels in gonad, serum, and saliva were observed. These suggest that antioxidant defense system in lizards might be actively responded to the oxidative stress, which in turn enables the lizard being recovered from the oxidative stress. It also could be stated that there was no alternation in oxidative state at 96 h with MDA levels remaining low in all samples. In the kidney, increased CAT activity is consistent with the MDA levels. However, the opposite of the results for CAT activities and MAD levels occurred in the gonad. The decrease in gonad CAT activity is not consistent with the reduction of MDA levels. That would indicate a certain alteration of the cell metabolism rather than a state of oxidative stress (Martinez-Alvarez et al., 2002).

The increased LDH levels were observed in the kidney and brain. Elevation of a release of LDH is indicative of cellular and/or membrane damage (Salama et al., 2005). And it also indicates anaerobic metabolism, which would be resulting in enhanced production of lactic acid (Manna et al., 2004). In saliva, the LDH enzyme has two origins that oral epithelial cells and salivary glands (Avezov et al., 2014). Decreased activity of LDH level in gonad, serum, and saliva could indicate that reduced metabolism occurred in the gonad, blood cells and oral epithelial cells or salivary glands.

Zhu et al. (2014) reported that significant decreases of AChE activities were discerned in fish embryos after MC exposure. We also found the decreases of AChE activities in kidney and gonad. However, the increased AChE activity in saliva may also indicate that the difference of metabolism existed between different tissues and organs.

4.3. Saliva as a minimal-invasive sample for enzyme activity determination

The results of the enzymes activities analyses performed in two pesticides exposure groups. And six tissues from lizards showed that no relevant variations in BCP and MC were detected. Saliva was used to be a non-destructive sample in pesticide biomonitoring (Claus Henn et al., 2006; Mingo et al., 2017). As Mingo et al. (2017) suggested, we measured the correlations in enzyme activity levels among saliva, organs and blood. While different organs or tissues also showed different enzyme changes in the same exposure group. According to our analysis, saliva was more correlated with kidney and gonad. In all enzymes, LDH was with high R^2 and absolute rho value. So saliva could be accurate to predict the LDH activity of liver and kidney. In most cases, salivary enzymes showed significant changes. The sensitivity of the salivary enzymes to pesticides were observed. So buccal swab could be an effective method to collect saliva in reptiles.

5. Conclusion

Numerous toxicological data evaluates beta-cypermethrin (BCP) and myclobutanil (MC), but the adverse effects of BCP and MC in lizards have not been widely reported. According to our results, BCP can cause more oxidative severe damage than MC. And we found that enzymes showed different changes in different organs or tissues after exposure to the same pesticide. Therefore, it is difficult to use saliva to replace internal organs to explain the changes in enzyme activity in reptiles. But salivary enzymes activities did show sensitivity changes to the toxicity of pesticides. Therefore, we could use saliva to reflect whether the reptiles are toxic by pesticides. And we also agree Mingo et al. (2017) proposal. However, there are no standard methods to get saliva samples form reptiles. Further researches need to make the saliva related data to be more reliable. We hope our data will be useful for the non-destructive testing of reptiles.

Acknowledgements

We gratefully acknowledge the financial support from the National Natural Science Foundation of China (grant number: 21577171) and the National Key Research and Development Program of China (grant number: 2016YFD0200202).

References

Ankley, G., Mihaich, E., Stahl, R., Tillitt, D., Colborn, T., McMaster, S., Miller, R., Bantle, J., Campbell, P., Denslow, N., 1998. Overview of a workshop on screening methods for detecting potential (anti-) estrogenic/androgenic chemicals in wildlife. Environ. Toxicol. Chem. 17, 68–87.

Avezov, K., Reznick, A.Z., Aizenbud, D., 2014. LDH enzyme activity in human saliva: the effect of exposure to cigarette smoke and its different components. Arch. Oral Biol. 59, 142–148.

Berenstein, G., Nasello, S., Beiguel, É., Flores, P., Di Schiena, J., Basack, S., Hughes, E.A., Zalts, A., Montserrat, J.M., 2017. Human and soil exposure during mechanical chlorpyrifos, myclobutanil and copper oxychloride application in a peach orchard in Argentina. Sci. Total Environ. 586, 1254–1262.

Bhhatarai, B., Gramatica, P., 2011. Modelling physico-chemical properties of (benzo) triazoles, and screening for environmental partitioning. Water Res. 45, 1463–1471.

Chen, L., Li, R., Diao, J., Di, S., Zhang, W., Cheng, C., Zhou, Z., 2017. Tissue distribution and toxicity effects of myclobutanil enantiomers in lizards (Eremias argus). Ecotoxicol. Environ. Saf. 145, 623–629.

Chen, L., Xu, P., Diao, J., Di, S., Li, R., Zhou, Z., 2016. Distribution, metabolism and toxic effects of beta-cypermethrin in lizards (Eremias argus) following oral administration. J. Hazard Mater. 306, 87–94.

Cheng, C., Di, S., Chen, L., Zhang, W., Diao, J., Zhou, Z., 2017. Enantioselective bioaccumulation, tissue distribution, and toxic effects of myclobutanil enantiomers in Pelophylax nigromaculatus tadpole. J. Agric. Food Chem. 65, 3096–3102.

Claus Henn, B., McMaster, S., Padilla, S., 2006. Measuring cholinesterase activity in human saliva. J. Toxicol. Environ. Health, Part A 69, 1805–1818.

Das, B.K., Mukherjee, S.C., 2003. Toxicity of cypermethrin in Labeo rohita fingerlings: biochemical, enzymatic and haematological consequences. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 134, 109–121.

Di, S., Liu, R., Tian, Z., Cheng, C., Chen, L., Zhang, W., Zhou, Z., Diao, J., 2017. Assessment of tissue-specific accumulation, elimination and toxic effects of dichlorodiphenyltrichloroethanes (DDTs) in carp through aquatic food web. Sci. Rep. 7.

Ding, F., Song, W., Li, Z., Guo, J., 2010. Study on activities of antioxidant enzyme Induced by myclobutanil in Danio rerio. In: Bioinformatics and Biomedical Engineering (ICBBE), 2010 4th International Conference on. IEEE, pp. 1–4.

Draper, H., Hadley, M., 1990. Malondialdehyde Determination as Index of Lipid Peroxidation. Methods in Enzymology. Elsevier, pp. 421–431.

El-Demerdash, F.M., Nasr, H.M., 2014. Antioxidant effect of selenium on lipid peroxidation, hyperlipidemia and biochemical parameters in rats exposed to diazinon. J. Trace Elem. Med. Biol. 28, 89–93.

Ellman, G.L., Courtney, K.D., Andres Jr., V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95.

Giray, B., Gürbay, A., Hincal, F., 2001. Cypermethrin-induced oxidative stress in rat brain and liver is prevented by vitamin E or allopurinol. Toxicol. Lett. 118, 139–146.

Gultekin, F., Ozturk, M., Akdogan, M., 2000. The effect of organophosphate insecticide chlorpyrifos-ethyl on lipid peroxidation and antioxidant enzymes (in vitro). Arch. Toxicol. 74, 533–538.

Guraya, S.S., 2000. Comparative Cellular and Molecular Biology of Ovary in Mammals: Fundamental and Applied Aspects. Science publishers, Inc.

Han, W., Wang, Y., Gao, J., Wang, S., Zhao, S., Liu, J., Zhong, Y., Zhao, D., 2018. Acute toxicity and sublethal effects of myclobutanil on respiration, flight and detoxification enzymes in Apis cerana cerana. Pestic. Biochem. Physiol. 147, 133–138.

Hopkins, W., Roe, J., Snodgrass, J., Jackson, B., Kling, D., Rowe, C., Congdon, J., 2001. Nondestructive indices of trace element exposure in squamate reptiles. Environ. Pollut. 115, 1–7.

Hopkins, W.A., 2000. Reptile toxicology: challenges and opportunities on the last frontier in vertebrate ecotoxicology. Environ. Toxicol. Chem. 19, 2391–2393.

Hopkins, W.A., Snodgrass, J.W., Baionno, J.A., Roe, J.H., Staub, B.P., Jackson, B.P., 2005. Functional relationships among selenium concentrations in the diet, target

tissues, and nondestructive tissue samples of two species of snakes. Environ. Toxicol. Chem. 24, 344–351.
Huang, A.-G., Tu, X., Liu, L., Wang, G.-X., Ling, F., 2016. The oxidative stress response of myclobutanil and cyproconazole on Tetrahymena thermophila. Environ. Toxicol. Pharmacol. 41, 211–218.
Jin, Y., Zheng, S., Pu, Y., Shu, L., Sun, L., Liu, W., Fu, Z., 2011. Cypermethrin has the potential to induce hepatic oxidative stress, DNA damage and apoptosis in adult zebrafish (Danio rerio). Chemosphere 82, 398–404.
Kenneke, J.F., Mazur, C.S., Kellock, K.A., Overmyer, J.P., 2009. Mechanistic approach to understanding the toxicity of the azole fungicide triadimefon to a nontarget aquatic insect and implications for exposure assessment. Environ. Sci. Technol. 43, 5507–5513.
Kumar, A., Rai, D.K., Sharma, B., Pandey, R.S., 2009. λ-cyhalothrin and cypermethrin induced in vivo alterations in the activity of acetylcholinesterase in a freshwater fish, Channa punctatus (Bloch). Pestic. Biochem. Physiol. 93, 96–99.
Lin, C.-H., Chou, P.-H., Chen, P.-J., 2014. Two azole fungicides (carcinogenic triadimefon and non-carcinogenic myclobutanil) exhibit different hepatic cytochrome P450 activities in medaka fish. J. Hazard Mater. 277, 150–158.
Liu, X., Wang, P., Liu, C., Liang, Y., Zhou, Z., Liu, D., 2017. Absorption, distribution, metabolism, and in vitro digestion of beta-cypermethrin in laying hens. J. Agric. Food Chem. 65, 7647–7652.
López, O., Hernández, A.F., Rodrigo, L., Gil, F., Pena, G., Serrano, J.L., Parrón, T., Villanueva, E., Pla, A., 2007. Changes in antioxidant enzymes in humans with long-term exposure to pesticides. Toxicol. Lett. 171, 146–153.
Manna, S., Bhattacharyya, D., Basak, D., Mandal, T., 2004. Single oral dose toxicity study of a-cypermethrin in rats. Indian J. Pharmacol. 36, 25.
Martinez-Alvarez, R., Hidalgo, M., Domezain, A., Morales, A., García-Gallego, M., Sanz, A., 2002. Physiological changes of sturgeon Acipenser naccarii caused by increasing environmental salinity. J. Exp. Biol. 205, 3699–3706.
Mingo, V., Lötters, S., Wagner, N., 2017. The use of buccal swabs as a minimal-invasive method for detecting effects of pesticide exposure on enzymatic activity in common wall lizards *. Environ. Pollut. 220.
Motamedi, M., Mansour-Ghanaei, F., Sariri, R., Vesal, M., 2013. Salivary enzymes in peptic ulcer disease. J. Oral Biol. Craniofacial Res. 3, 83–87.
Mu, X., Shen, G., Huang, Y., Luo, J., Zhu, L., Qi, S., Li, Y., Wang, C., Li, X., 2017. The enantioselective toxicity and oxidative stress of beta-cypermethrin on zebrafish. Environ. Pollut. 229, 312–320.
Narahashi, T., 1996. Neuronal ion channels as the target sites of insecticides. Pharmacol. Toxicol. 79, 1–14.
Parsons, K.C., Mineau, P., Renfrew, R.B., 2010. Effects of pesticide use in rice fields on birds. Waterbirds 33, 193–218.
Patterson, R.A., Leake, D.S., 1998. Human serum, cysteine and histidine inhibit the oxidation of low density lipoprotein less at acidic pH. FEBS Lett. 434, 317–321.
Rao, K., 1999. Pesticide Impact on Fish Metabolism. Discovery publishing house.
Rendón-von Osten, J., Ortiz-Arana, A., Guilhermino, L., Soares, A., 2005. In vivo evaluation of three biomarkers in the mosquitofish (Gambusia yucatana) exposed to pesticides. Chemosphere 58, 627–636.
Richetti, S.K., Rosemberg, D.B., Ventura-Lima, J., Monserrat, J.M., Bogo, M.R., Bonan, C.D., 2011. Acetylcholinesterase activity and antioxidant capacity of zebrafish brain is altered by heavy metal exposure. Neurotoxicology 32, 116–122.
Salama, A.K., Osman, K.A., Saber, N.A., Soliman, S.A., 2005. Oxidative stress induced by different pesticides in the land snails, Helix aspersa. Pakistan J. Biol. Sci. 8, 92–96.
Sanchez, J., Fossi, M., Focardi, S., 1997. Serum “B” esterases as a nondestructive biomarker for monitoring the exposure of reptiles to organophosphorus insecticides. Ecotoxicol. Environ. Saf. 38, 45–52.
Sangha, G., Kaur, K., Khera, K., 2013. Cypermethrin induced pathological and biochemical changes in reproductive organs of female rats. J. Environ. Biol. 34, 99.
Sankar, P., Telang, A.G., Manimaran, A., 2012. Protective effect of curcumin on cypermethrin-induced oxidative stress in Wistar rats. Exp. Toxicol. Pathol. 64, 487–493.
Seth, V., Banerjee, B.D., Chakravorty, A.K., 2001. Lipid peroxidation, free radical scavenging enzymes, and glutathione redox system in blood of rats exposed to propoxur. Pestic. Biochem. Physiol. 71, 133–139.
Singh, P.B., Singh, V., 2008. Cypermethrin induced histological changes in gonadotrophic cells, liver, gonads, plasma levels of estradiol-17beta and 11-ketotestosterone, and sperm motility in Heteropneustes fossilis (Bloch). Chemosphere 72, 422–431.
Sparling, D.W., Linder, G., Bishop, C.A., Krest, S., 2010. Ecotoxicology of Amphibians and Reptiles. CRC Press.
Srivastava, A., Srivastava, M.K., Raizada, R.B., 2006. Ninety-day toxicity and one-generation reproduction study in rats exposed to allethrin-based liquid mosquito repellent. J. Toxicol. Sci. 31, 1–7.
Stellavato, A., Lamberti, M., Pirozzi, A.V.A., de Novellis, F., Schiraldi, C., 2016. Myclobutanil worsens nonalcoholic fatty liver disease: an in vitro study of toxicity and apoptosis on HepG2 cells. Toxicol. Lett. 262, 100–104.
Streckfus, C., Bigler, L., 2002. Saliva as a diagnostic fluid. Oral Dis. 8, 69–76.
Tiwari, S., Tiwari, R., Singh, A., 2012. Impact of cypermethrin on fingerlings of common edible carp (Labeo rohita). Sci. World J. 2012.
Üner, N., Oruç, E.Ö., Canli, M., Sevgler, Y., 2001. Effects of cypermethrin on antioxidant enzyme activities and lipid peroxidation in liver and kidney of the freshwater fish, Oreochromis niloticus and Cyprinus carpio (L.). Bull. Environ. Contam. Toxicol. 67, 657–664.
Urban, D.J., Cook, N.J., 1986. Standard Evaluation Procedure Ecological Risk Assessment. Standard Evaluation Procedure Ecological Risk Assessment. EPA.
Vieira, L., Sousa, A., Frasco, M., Lima, I., Morgado, F., Guilhermino, L., 2008. Acute effects of Benzo [a] pyrene, anthracene and a fuel oil on biomarkers of the common goby Pomatoschistus microps (Teleostei, Gobiidae). Sci. Total Environ. 395, 87–100.
Vijverberg, H.P., vanden Bercken, J., 1990. Neurotoxicological effects and the mode of action of pyrethroid insecticides. Crit. Rev. Toxicol. 21, 105–126.
Wieczorek, M.V., Bakanov, N., Bilancia, D., Szöcs, E., Stehle, S., Bundschuh, M., Schulz, R., 2018. Structural and functional effects of a short-term pyrethroid pulse exposure on invertebrates in outdoor stream mesocosms. Sci. Total Environ. 610, 810–819.
Zhang, J., Liu, L., Ren, L., Feng, W., Lv, P., Wu, W., Yan, Y., 2017. The single and joint toxicity effects of chlorpyrifos and beta-cypermethrin in zebrafish (Danio rerio) early life stages. J. Hazard Mater. 334, 121–131.
Zhou, Y.-j., Wang, J.-h., Wang, L.-q., Xiao, S., Wang, X.-d., Yan, H.-l., Li, C.-f., Zhu, H.-q., 2018. Effect of beta-cypermethrin exposure on embryo implantation in mice. Reprod. Toxicol. 76, 1–11.
Zhu, B., Liu, L., Gong, Y.-X., Ling, F., Wang, G.-X., 2014. Triazole-induced toxicity in developing rare minnow (Gobiocypris rarus) embryos. Environ. Sci. Pollut. Res. 21, 13625–13635.
Zhuang, R., Chen, H., Yao, J., Li, Z., Burnet, J.E., Choi, M.M., 2011. Impact of beta-cypermethrin on soil microbial community associated with its bioavailability: a combined study by isothermal microcalorimetry and enzyme assay techniques. J. Hazard Mater. 189, 323–328.