

# The acetylcholinesterase (AChE) inhibition analysis of medaka (*Oryzias latipes*) in the exposure of three insecticides

Jianping Zhu<sup>1#</sup>, Cheng Huan<sup>2#</sup>, Guiyun Si<sup>1</sup>, Haitang Yang<sup>1</sup>, Li Yin<sup>1</sup>, Qing Ren<sup>1</sup>,  
Baixiang Ren<sup>1</sup>, Rongshu Fu<sup>1</sup>, Mingsheng Miao<sup>1</sup> and Zongming Ren<sup>1\*</sup>

<sup>1</sup>College of Life Science, Shandong Normal University, Ji'nan, PR China

<sup>2</sup>Department of Neurosurgery, Provincial Hospital Affiliated to Shandong University, Jinan, PR China

**Abstract:** The continuous effects on Acetylcholinesterase (AChE) activity of medaka (*Oryzias latipes*) caused by dichlorvos, methomyl and deltamethrin *in vivo* were investigated, and the trends of AChE activity inhibition due to the influence of these insecticides were discussed. The LC<sub>50-24h</sub> of dichlorvos, methomyl and deltamethrin on medaka were 2.3 mg/L, 0.2 mg/L, and 2.9×10<sup>-3</sup> mg/L respectively. The result suggested that at the beginning of the exposure, the AChE activity might increase, and the AChE activity in dead individuals was obviously lower than the live individuals. Though the *de novo* synthesis of AChE in medaka might help the AChE activity recover, the trends during the exposure in different treatments were downward, and it showed both exposure time and concentration dependent. Meanwhile, higher temperature might cause the AChE inhibition earlier due to the higher metabolic rate. Therefore, as a specific biomarker for organophosphate, carbamate pesticides and pyrethroids, the degree of the AChE inhibition with *in vivo* conditions is a good tool in continuous monitoring of insecticides, which may induce the nerve conduction disorders.

**Keywords:** Acetylcholinesterase (AChE), medaka, *in vivo*, AChE inhibition.

## INTRODUCTION

Organophosphate pesticides, carbamate pesticides and pyrethroids have been widely used throughout the world to control pests in agricultural crops, forests, and wetlands (Soderlund and Bloomquist, 1989). These insecticides can exert their toxicity by inhibiting acetyl cholinesterase (AChE) of organisms (Edwards and Fisher, 1991). Discharges of these insecticides in environment, however, may cause unpredictable toxicity to human and numerous biological organisms. As a key enzyme that hydrolyzes the neurotransmitter acetylcholine in cholinergic synapses of both vertebrates and invertebrates, AChE is strongly inhibited by organophosphate, carbamate pesticides and pyrethroids at low concentrations (de la Torre *et al.*, 2002). Meanwhile, for this reason, this enzyme has been widely used as a specific biomarker for these compounds (Guilhermino *et al.*, 2000). The compounds to depress the AChE activity of fish and invertebrates, *in vitro* and/or *in vivo* conditions, have been demonstrated in several studies (Guilhermino *et al.*, 2000), however, hardly any was about the continuous detection of the AChE inhibition in these insecticides with *in vivo* conditions.

Medaka (*Oryzias latipes*), which is listed as a standard test species in the OECD guidelines (OECD, 1999), was used as indicator in this study. Therefore, in order to assess the continuous effects on AChE activity caused by these three kinds of insecticides *in vivo*, exposures of medaka in the treatments of dichlorvos, methomyl and deltamethrin were carried out, and the trends of AChE activity inhibition due to the influence of these insecticides were discussed.

## MATERIALS AND METHODS

### Test species

The individuals of medaka fish were cultured in our laboratory for more than 3 generations. The brood stock was raised in flow-through system with dechlorinated tap water (using active carbon) at a constant temperature of 24±2°C under a photoperiod of 16 h light and 8 h dark. The brood stock was fed with newly hatched brine shrimp in the morning and flake food (Trea®, Germany) in the afternoon. By 15 days later after hatching, medaka was fed 2 times every day. Medaka individuals (7 days after hatching) were selected as the test organisms.

### Chemicals

Dichlorvos, Methomyl, and deltamethrin were purchased from J&K Chemical Ltd (Beijing). All compounds were technical grade (95% purity). Stock solutions were prepared by dissolving the herbicide directly in the water immediately before each experiment.

Acetylthiocholine iodide (ATCh), 5, 5-dithio-2, 2-nitrobenzoic acid (DTNB), Sephadex G-25, Bovine serum albumin (BSA) and TritonX-100 were purchased from Sigma (Sigma-Aldrich Corporation, St. Louis, MO, USA). All chemicals were of analytical grade.

### Experimental setup

Setting the logarithm spacing concentration of insecticide in exposure experiment to measure the LC<sub>50-24 h</sub> (50% Lethal concentration) of medaka. During the experiment, medaka was fed nothing and five parallels with 100

\*Corresponding author: e-mail: zmren@sdu.edu.cn

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individuals of medaka in each parallel. The exposure concentration of 50% (LC<sub>50</sub>) values was calculated by probit analysis.

*In vivo* AChE inhibition tests were performed by exposing medaka to several treatments (0.5×LC<sub>50</sub>, and 2×LC<sub>50</sub>,) with control. 24 h exposures for 0.5×LC<sub>50</sub> and 12 h exposures for 2×LC<sub>50</sub> in different treatments with 24-26° were performed. Take 4 individuals for every parallel once every hour, and every sample it was 20 individuals. Only the live individuals were used to prepare homogenates for AChE determinations.

Homogenates were prepared in ice-cold phosphate buffer (0.1 M, pH 7.4) using a homogenizer. Homogenates were centrifuged at 12,000×g for 20 min at 4° using a centrifuge (Guilhermino, *et al.*, 1996). The supernatant was used as an enzyme source for measuring the activity of AChE.

50 µL enzymes and 50 µL ATCh (5 mM final concentration) were incubated at 30° for 15 min, and then the reaction was stopped by 0.9 mL of 0.125 mM DTNB-phosphate-ethanol reagent as the thiol indicator (Gorun *et al.*, 1978). The color was detected immediately at 412 nm using an ELIASA (Infinite M200).

## RESULTS

### The acute toxicity of insecticides on medaka

The acute toxic effects of dichlorvos, methomyl and deltamethrin on medaka were shown in table 1 with 95% confidence interval. The LC<sub>50</sub>-24 h values of dichlorvos,

methomyl and deltamethrin on medaka were 2.3 mg/L, 0.2 mg/L, and 2.9×10<sup>-3</sup> mg/L respectively. According to the toxicity of chemicals to the aquatic organism, the methomyl and deltamethrin were in a high toxicity grade to medaka, and dichlorvos was in a medium toxicity grade to medaka.

Therefore, in the studies on the *in vivo* AChE activity inhibition by these chemicals, medaka were exposed to the treatments respectively: 1.15 mg/L and 4.6 mg/L for dichlorvos, 0.1 mg/L and 0.4 mg/L for methomyl and 1.45×10<sup>-3</sup> mg/L and 5.8×10<sup>-3</sup> mg/L for deltamethrin with control.

### The AChE Inhibition of medaka

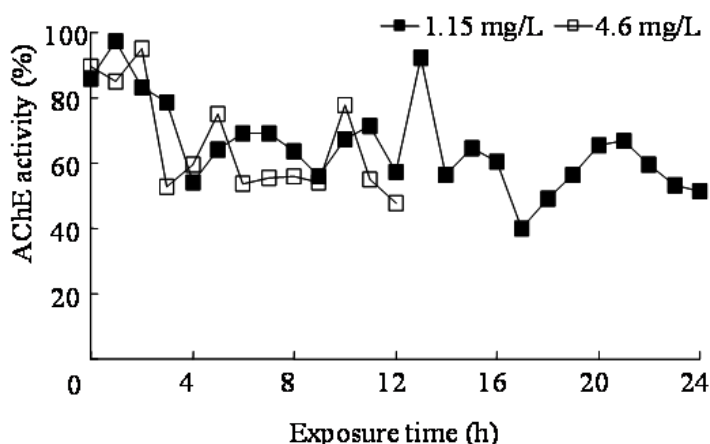
Fig. 1 shows the AChE activity inhibition of medaka after *in vivo* exposure in dichlorvos. In the first 2 h to 3 h in different treatments, the AChE activity of medaka was almost the same as start time (0). After a period of exposure, the AChE activity of medaka was inhibited significantly. Meanwhile, during the exposure, AChE activity showed evident recovery, especially in 1.15 mg/L treatment.

Fig. 2 shows the AChE activity inhibition of medaka after *in vivo* exposure in methomyl. The main tendency was similar to the exposure in dichlorvos. At the end of each exposure, the AChE activity went up and down. But the whole tendency was down.

Fig. 3 shows the AChE activity inhibition of medaka after *in vivo* exposure in deltamethrin. At the beginning of exposure, the AChE activity was same as dichlorvos and

**Table 1:** The acute toxic effects of dichlorvos, methomyl and deltamethrin on medaka

Chemicals	LC <sub>50</sub> (mg/L)	95% Confidence interval (mg/L)
Dichlorvos	2.3	2.01-2.53
Methomyl	0.2	0.17-0.22
Deltamethrin	2.9×10 <sup>-3</sup>	2.63×10 <sup>-3</sup> -3.21×10 <sup>-3</sup>



**Fig. 1:** The AChE activity inhibition of medaka after exposure in dichlorvos.

methomyl. After about 9 h in  $5.8 \times 10^{-3}$  mg/L treatment, it decreased to less than 0.5, which suggested that it was inhibited. The AChE activity of medaka in  $1.45 \times 10^{-3}$  mg/L changed between 100% and 80%.

#### The regularity of AChE inhibition

After first period (about 2 to 6 hours) of the effects on the AChE activity in different treatments, significant inhibition would occur in all the treatments with several recoveries. These results were similar to the effects of three ridge mussel (*A. plicata*) to chlorpyrifos (Doran *et al.*, 2001), which suggested that the significant reductions in ChE activity were observed during the experiments. Due to lower concentration (0.1 to 1.2 mg/L) of chlorpyrifos ( $LC_{50}$  was 2.0 mg/L to *A. plicata*) and lower temperature ( $14-17^\circ$ ), however, the time when the significant reductions in ChE activity occurred was about 20 h. The difference suggested that temperature and the toxicity of chemicals to test organisms may play an important role in the effects of chemicals on the ChE activity. A higher metabolic rate of medaka due to the higher temperature could lead to greater uptake of insecticides and the higher toxicity may strengthen the

process (Cooper and Bidwell, 2006).

## DISCUSSION

The results of AChE Inhibition of medaka in three different chemicals as shown in Fig. 1-3 suggested that: 1<sup>st</sup>, at the beginning of the exposure, the AChE activity might increase; 2<sup>nd</sup>, AChE activity inhibition showed evident recovery; 3<sup>rd</sup>, the inhibition degree in higher concentration treatments were more significant than in lower concentration.

It is clear that organophosphate, carbamate and pyrethroid insecticides possess inhibitory effects on AChE activity of medaka from the results shown in Figs. 1-3 and previous research (Edwards and Fisher, 1991; Guilhermino, *et al.*, 1996). However, the continuous detection of the AChE activity was ignored. Our results suggested that the inhibition of AChE activity based on the in vivo conditions might be different in different exposure time, and sometimes especially at the beginning of the exposure, it showed positive effects on the AChE activity, which was similar to the previous results of Cooper and Bidwell

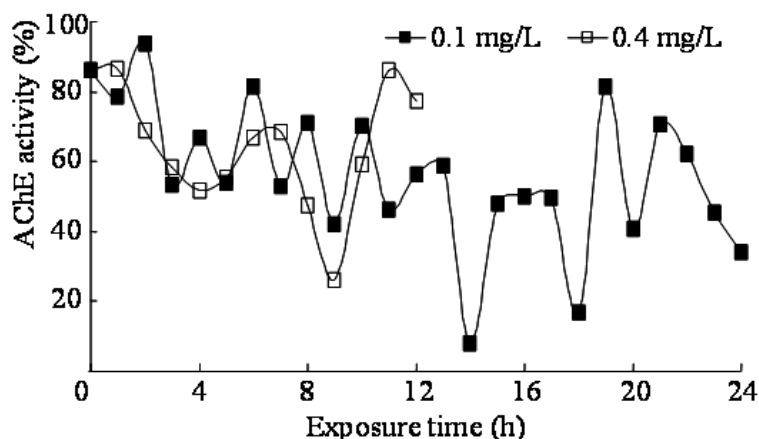


Fig. 2: The AChE activity inhibition of medaka after exposure in methomyl.

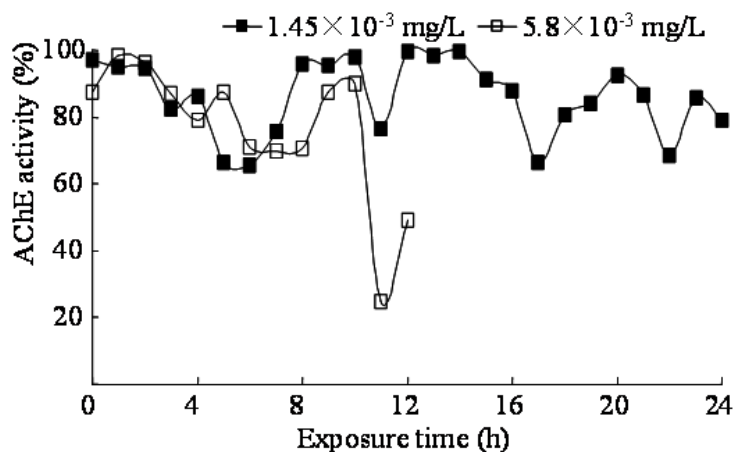


Fig. 3: The AChE activity inhibition of medaka after exposure in deltamethrin.

(Cooper and Bidwell, 2006). Meanwhile, during the exposure in different treatments, the AChE activity sometimes showed evident recover. The reason for the positive effects and the activity recovery might be that these insecticides stimulated the de novo synthesis of AChE in medaka (Morgan *et al.*, 1990).

## CONCLUSIONS

The AChE activity to different insecticides was observed in medaka in vivo. In common, the AChE activity kept consistent in control, and positive and negative effects of different treatments were observed due to different intrinsic mechanisms to the environmental stress. Though the de novo synthesis of AChE in medaka might help the AChE activity recover, the trends during the 12 h exposure in different treatments were downward, and it showed both exposure time and concentration dependent. Meanwhile, higher temperature might cause the AChE inhibition earlier due to the higher metabolic rate. Therefore, as a specific biomarker for organophosphate, carbamate pesticides and pyrethroids, the degree of the AChE inhibition with in vivo conditions is a good tool in continuous monitoring of insecticides which may induce the nerve conduction disorders.

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