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The Use of Acetylcholinesterase Inhibition in River Snails (*Sinotaia ingallsiana*) to Determine the Pesticide Contamination in the Upper Ping River

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ABSTRACT

The purpose of this study was to monitor the organophosphorus and carbamate pesticide contamination in an aquatic environment using the indigenous river snail (*Sinotaia ingallsiana*) as the bioindicator. This study was carried out from October 2006 to March 2007. The river snails were dipped in the Ping River in the Chiangmai area, where agricultural sites are densely located. One day after exposure, the samples were sacrificed for the determination of acetylcholinesterase activity. Results indicated that the AchE activity in the river snails collected from several sites of the Ping River was lower than control specimens kept in laboratory. In rainy season, un-exposed snails exhibited significantly higher AChE activity ($P < 0.05$) than the other three specimens derived from contaminated sites (9.4740, 1.0450, 1.0428 & 1.6002 μmole min^{-1} g^{-1} tissue, respectively). The AChE activity showed seasonal differences with minimum activities during the winter period. Therefore the AChE activity of the river snail could be considered as a good early indicator of pesticide contamination in an aquatic environment. Besides AChE activity, the other biochemical markers as well as histopathology effects, growth, reproduction and survival of aquatic organisms living in these contaminated areas have also been determined.

Key Words: Pesticide; Acetylcholinesterase; Biomarker; Risk assessment; River snail

INTRODUCTION

There are increasing concerns about the potential of pesticides as harmful agents to human health and non-target populations since pesticides are able to enter the waterways from agricultural and urban run-off, movement through soil into water courses and after direct application (Schulz & Leiss, 1999). These pesticides are able to move towards the water column or accumulate in plants, fishes and consequently enter the food chain. Cereal grain; for example, might be contaminated with several pesticides, which can enter in food chain of human consumption with its consequential hazard (Khan *et al*., 2007). The organophosphorous and carbamate pesticides, the most frequently used due to their high insecticidal activity are also acutely neurotoxic. These are effective inhibitors of the enzyme acetylcholinesterase located at neuromuscular junctions in the central and peripheral nervous system of the organisms (Walker *et al*., 2001). As a result, the inhibition of these peripheral enzymes provides a convenient means of monitoring exposure to pesticides. Acetylcholinesterase activity index has been widely used to indicate exposure of both vertebrate and invertebrate species to organophosphorous and carbamate pesticides (Moulton *et al*., 1996; Fulton & Key, 2001; Van Erp *et al*., 2002) including freshwater bivalves (Moulton *et al*., 1996; Doran *et al*., 2001).

Various methods are used to determine pesticide residues in the organisms; however there are several drawbacks, which include the skilled personnel required and the complex and time-consuming treatments of the samples i.e., extraction of pesticides, extract cleaning and solvent substitution. In addition, the concentrations of agricultural pesticides in large rivers and lakes often fall below the instrument detection limit; for this reason, biomarkers may be an alternative way to obtain early-warning signals of environmental risk and they can detect either exposure to or the effects of pesticides.

The river snail, *Sinotaia ingallsiana* had been tested as the bio-indicator, because of its general abundance, the ease with which it could be collected and because it shares some basic life history characteristics with native freshwater organisms in that it is a filter feeder. The goal of this study was to determine if the reduction in enzyme activity of the

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river snails can be used as a biomarker for pesticide contamination in aquatic environments. However, when interpreting AChE activities in relation to pesticide exposure the possible effects of natural factors have to be taken into account, since environmental variables may also have a direct or indirect effect on AChE activity. In this study, the seasonal differences in the AChE activity of river snails were also determined.

MATERIALS AND METHODS

Sample preparation. River snails (*S. ingallsiana*) with average weight of 6.27 ± 1 g were collected from the Maejo University, Chiangmai. Prior to testing, snails were held in 40 L temperature controlled (26 ± 2°C) aerated tanks containing dechlorinated water for ten days to allow acclimation to laboratory conditions.

Exposure conditions. Following acclimation, snails were dipped into Ping River in Chiangmai area, where agricultural sites are densely located (Fig. 1) for 24 h. Three sites including Wangkhampom, Wangpha and Wangplasoi were used in this study. In order to investigate the seasonal course of AChE activity, river snails were dipped in the river in three different seasons. 10 snails were removed from each site and analyses of acetylcholinesterase activity were undertaken. Snails to be used as controls were kept in dechlorinated tap water at the laboratory until biochemical analysis.

Acetylcholinesterase activity. Analysis of AChE activity followed procedures described by Ellman *et al*. (1961) with modifications for multi-well plate readers. Snail tissues were homogenized in 0.1 m phosphate buffer saline (pH 8). Samples were then centrifuged at 3,500 rpm for 10 min. A 40 µL sample of the supernatant was then added to three replicate wells of a 96-well plate (also held on ice) followed by the addition of 200 µL of reagent solution [3 mL buffered Ellman's reagent (17.85 mmol L^{-1} DNTB), 20 µL acetylthiocholine iodide (75 mmol L^{-1}) and 100 µL of 0.1 m phosphate buffer saline]. The plate was read on a GDV Microplate Reader. AChE activity was expressed as µmol min^{-1} g^{-1} tissue.

Pesticide analysis. Water sample extraction and GC analysis were carried out by the Water Quality Management Bureau (2008). Detection limits of Heptachlor, Aldrin, Dieldrin and Endosulfan Sulfate were 0.004, 0.004, 0.008 and 0.012 µg L^{-1}, respectively for water.

Statistical analyses. All data were first tested for normality and homogeneity of variance after which an analysis of variance (ANOVA) and/or Tukey's test (P=0.05) was used to determine, whether there were any significant differences between the controls and treatments in acetylcholinesterase activity.

RESULTS AND DISCUSSION

The average of pesticide concentration (µg L^{-1}) in Upper Ping River of year 2005 – 2007 is shown in Table I, while acetylcholinesterase (AChE) activity of exposed snails is shown in Table II. The AChE activity of unexposed control snails showed higher AChE activity than that in the control group (9.47 against 1.04 – 1.60 µmole min^{-1} g^{-1} tissue, respectively) in rainy season. Results obtained in the Upper Ping River indicated a homogeneous pollution by the AChE inhibitors in these aquatic ecosystems except in summer season. In addition to interpreting AChE activities in relation to pesticide exposure, the possible effects of natural factors have to be taken into account, since environmental variables may also have a direct or indirect effect on AChE activity. In this study, seasonal differences in the AChE activity have been shown, which might be related to seasonal changes in water temperature. Although the possibility of pesticide contamination into the aquatic environment through runoff might be higher in the rainy period, the dilution of toxic substances could result in the reduction of AChE activity. In winter, the AChE activity was lower than the other seasons due to low temperature. This result was consistent with the study of Abdel-Halim *et al.* (2006) who reported the activity of cholinesterase in brain and liver of tilapia fish samples collected from New Damietta drainage canal in winter was lower than the ones in spring and autumn. The AChE activity of control organisms was the lowest in summer periods. This contrasted with Pfeifer *et al.* (2005) who showed that the maximum AChE activities were found during the summer period. This possible explanation relating to low AChE activities in laboratory snails might be due to background pesticide pollution. Therefore, our results will be annually repeated in order to confirm the results and see the trend of

Fig. 1. Three sampling sites (Wangkhampom, Wangpha & Wangplasoi) in the Upper Ping River

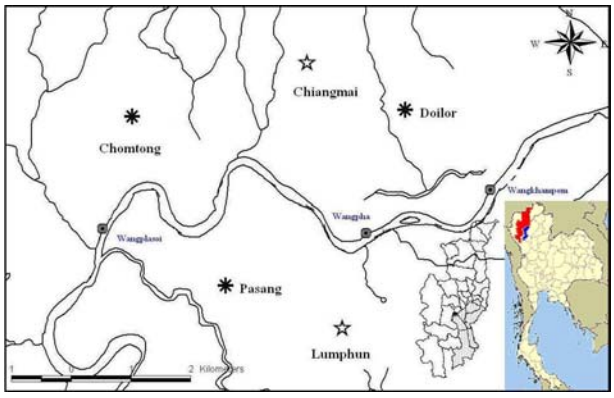

Table I. The Average Pesticide Concentrations (µg L^{-1}) in Upper Ping River during 2005 – 2007 (Water Quality Management Bureau, 2008), ND = Not detectable

Year	Heptachlor	Aldrin	Dieldrin	Endosulfan Sulfate
2005	ND – 0.1	ND – 0.01	ND – 0.01	ND – 0.02
2006	ND – 0.01	ND – 0.01	ND – 0.01	ND – 0.01
2007	ND – 0.01	ND – 0.01	ND – 0.01	ND – 0.01

Table II. Seasonal variations in acetylcholinesterase activities of river snail samples dipped in the Upper Ping River*

Station	AChE Activity (Mean ± SD; μmole min^{-1} g^{-1} tissue)		
	rainy season	winter season	summer season
Control (Lab)	9.47±2.80a	10.298±1.775c	4.839±1.482e
Wangkhampom	1.05±0.04b	0.003± 0.009d	1.305±0.408f
Wangpha	1.04±0.01b	0.001±0.003d	0.321±0.254g
Wangplasoi	1.60± 0.01b	0.001±0.003d	0.489±0.358g
Water temperature	25.39±0.98	22.82 ± 0.55	33.36±1.26

*Different letters in same column indicate significant differences at $P < 0.05$ (ANOVA)

contamination. The concentrations of Heptachlor, Aldrin, Dieldrin and Endosulfan Sulfate in water were shown in Table II.

In accordance with the US Environmental Protection Agency, the inhibition of AChE activity inhibition of 20% or greater indicates exposure to OP pesticides. However, some aquatic animals are able to survive with more than 50% of AChE inhibition. For example, a reduction of about 85% in AChE activity of tilapia exposed to trichlorfon when compared to the control group did not cause the death (Guimaraes *et al.*, 2007). The chronic exposure of the eastern rainbow fish (*Melanotaenia duboulayi*) to sublethal levels of profenofos resulted not only in a 70% reduction in acetylcholinesterase (AChE) activity but also associated decreases in growth rates, food consumption rates, and food conversion efficiency. Future laboratory studies on biochemical and immunological responses in using the river snail should set out the environmental quality classes more accurately as well as, the interference effects of other competitive factors. In addition, the recovery of AChE from exposure to organophosphorous and carbamate pesticides should be further investigated. The selection of endpoints and organisms to be used in risk assessment is still one of the challenging tasks. Additionally, aquatic organisms living in agricultural areas, however, are exposed to a multitude of toxicologically and structurally different pesticides, which are a challenging task for management and regulatory purposes, while most of the published results for pesticide toxicity are assessed from the single substance toxicity. The possible synergism and/or antagonism among the pesticides must be considered.

CONCLUSION

Exposure of *S. ingallsiana* to contaminated aquatic environment produced a significant decrease in AChE activity in their tissues. These responses may be useful as early indicators of pesticides toxicity in river snails, particularly where it is difficult to get contamination information with very expensive analysis. However, the influence of temperature on AChE activity in river snails has to be assessed when applying AChE activity as biomarker to monitor pesticide effects.

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