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Full Length Research Paper

The use of cholinergic biomarker, cholinesterase activity of blue mussel *Mytilus edulis* to detect the effects of organophosphorous pesticides

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The aim of the study was to investigate the effects of organophosphorous pesticide on the cholinesterase activity of different organs of *Mytilus edulis*. The mussels were exposed to serial dilutions of the pesticides (0, 50, 100, 200, 500, and 1000 µg/l) for 96 h. A significant inhibition of the cholinesterase activity from gill occurred at the lowest concentration, which indicated that gill was the most sensitive organ. The moderate sensitive organs were foot and mantle, which were inhibited by trichlorfon at 200 µg/l. The least sensitive organs were hemolymph, posterior adductor muscle and digestive gland were inhibited at 1000 µg/l. After incubation of the trichlorfon-exposed mussels for seven days in clean media, the cholinesterase activities from different organs of mussels were not cured completely. The cholinesterase activities from hemolymph, gill, posterior adductor muscle and digestive gland recovered, while the persistent inhibition of cholinesterase activity from foot and mantle were observed.

Key words: Biomarker, cholinesterase, mussel, trichlorfon.

INTRODUCTION

The extensive use of organophosphorous (OP) pesticides in agricultural and other antrophogenic activities causes increase of discharged pesticides wastes in the environment. Although, the occurrence of organophosphorous pesticides in the environment is thought to be unstable compared to organochlorine pesticides, the persistent effects of the pesticides in non-target organisms and ecological system cannot be ruled out (Scholz and Hopkins, 2006). Once organophosphorous pesticides enter the body of organism, most of them are transformed into metabolites which in many cases are more toxic compounds than the parent compounds or induced directly the target enzymes or organs (Belden and Lydy, 2000). Consequently, the effects of most of the pesticides on acetylcholinesterase (AChE) activity are considered as an irreversible action since the time of re-synthesis of the enzyme are naturally longer than the duration of dissociation of the organophosphorous-complex (Gaglani and Bocquene, 2000). Deteriorate effects of the pesticides become more prominent when de-alkylation or what it called ageing occurs which involves cleavage of an alkyl group of the phosphoryl moiety and the formation of negative charge, which stabilizes the OP-Complex (Ray, 1998). *De novo* synthesis of the enzyme is the only way to recover AChE activity in the synaptic cleft, which goes slower than de-alkylation reaction.

In aquatic ecosystem, the mechanisms of the OP (organophosphorous) actions in aquatic organisms such as *Mytilus* sp in the enzymatic levels, particularly cholinesterase (ChE) activity, provide a comfortable tool as a biomarker for the detection of the pesticide impacts in that ecosystem. Since the works of Grigor'eva et al. (1968), on ChEs from cardiac muscle and hemolymph of *Mytilus edulis* (Moralev and Rozengrat, 2004) and Wachtendonk and Neef (1979) on ChEs from hemolymph, the ChE activity from the mussels has been explored and employed as a biomarker to detect the effects of OP pesticides in laboratory and field studies. Different organs of the mussels have been used to

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evaluate the detrimental effects of the OP pesticides on the ChE (cholinesterase) activity, which showed that gill was more frequently used compared to other organs or whole tissue (Escartin and Porte, 1997; Mc.Henery et al., 1997; Mora et al., 1999a; 1999b; Dizer et al., 2001; Kopecka et al., 2004).

In addition, the correlation between the inhibition of the ChE activity from gill and increasing usage of dichlorvos in marine culture was proved (Mc.Henery et al., 1997). However, compare to the tissue homogenate, hemolymph from *M. edulis* had higher AChE activity (Galloway et al., 2002), and can be used without sacrificing the animals. Hence, the authors claimed that the AChE activity from the mussel hemolymph provides a rapid, relatively cost-effective, reliable, and non-destructive tool to assess the exposure of mussels to OP and carbamate pesticides.

Notwithstanding, transformation of the contaminant induction of ChE activity in hemolymph to ecological levels is more difficult than that in other organs such as gill, mantle, digestive gland, posterior adductor muscle (PAM) and foot. It is because existence of conta-minant-damage in hemolymph is easy to recover due to regeneration of hemolymph in *M. edulis* which occurs quickly (Gosling, 2003). Besides, induction of ChE activity in hemolymph may not be related to nervous system (Brown et al., 2004). Therefore, it is a point of interest to know different responses of different organs of *M. edulis* in term of cholinergic activity which can be useful information to determine which organs of *M. edulis* is sensitive and effective as a target organ in terms of biomarker applications in laboratory and field scales.

The study was carried out to compare the cholinergic responses which are reflected as ChE (cholinesterase) activity in different organs of *M. edulis* after exposure to OP pesticide, trichlorfon. In addition, since induction, adaptation and recovery mechanisms in sentinel organisms are important mechanisms to delineate environmental stress over time (Wu et al., 2005) which are useful factors for applying biomarkers in screening test and field monitoring, the study was also aimed to investigate the recovery of pesticide-induction of ChE activity in the different organs.

MATERIALS AND METHODS

Chemicals

Acethylthiocholine iodide, 5,5'-Dithio-bis-(-2-Nitrobenzoic acid) and γ-globuline were obtained from Sigma, USA. Trichlorfon (PESTANAL®, analytical standard (Riedel-de Haën) was purchased from Sigma-Aldrich, Germany. Bradford-reagent was purchased from BIO-RAD.

In vivo exposure experiment (I)

Mussels, *M. edulis* (6-7 cm) were collected from a clean area of Sylt Island Germany and carried dry to the laboratory. *In vivo* exposure experiment was conducted for 96 h in duplicate by using trichlorfon as a contaminant model. A stock solution was made by diluting trichlorfon with distilled water. Serial dilutions of trichlorfon were made by adding the trichlorfon stock solution into the glass aquarium that contained 4 l of 3% salinity of artificial sea water (ASW) until it reached the final concentrations (0, 50, 100, 200, 500, and 1000 µg/l). Each experiment media was loaded with 12 mussels. The dissolved oxygen in each aquarium was maintained not less than 80% using an aerator. To assure the desired contaminant concentrations in each aquarium, the media and the contaminant concentrations were replaced everyday 2 h after the mussels were fed by the commercial algae.

After 96 h, the exposure experiment was terminated. Six mussels per aquarium were sacrificed and the intended organs namely, hemolymph, gill, foot, posterior adductor muscle (PAM) and digestive gland were collected and treated as outlined below.

In vivo (II) and *in situ* (III) recovery experiments

The recovery experiment in laboratory scale was conducted by placing the trichlorfon-exposed mussels (experiment I) into 4 l of clean artificial sea water media for seven days. Each aquarium had six mussels. After seven days, the experiment was completed and all the mussels were sacrificed and selected organs were collected and treated as described below.

In situ recovery experiment (III) was performed by transplanting the trichlorfon-exposed mussels into the clean original habitat in Sylt Island. Prior to the transplantation experiment, 12 mussels per aquarium in 4 l of ASW were exposed to 0 and 500 µg/l of trichlorfon for 96 h in duplicate. At the end of the exposure, six mussels per aquarium were sacrificed and the rest were sent dry to Sylt Island. In Sylt Island the mussels were transplanted in sea water using the net. After seven days, the mussels were removed from the sea water and sent back dry to the laboratory and sacrificed for dissecting and collection of the intended organs immediately.

Organs collection and preparation

Hemolymphs of six mussels in each aquarium were sucked from posterior adductor muscle (PAM) using 1 ml syringe and 0.4 mm needle. Immediately, 1 ml of hemolymph was centrifuged at 10000 *x* g for 10 min at 4°C for the separation of the hemolymph from the hemocytes. The hemocytes-free hemolymph were then harvested, transferred into 1.5 ml eppendorf tube and kept under -80°C prior to ChE activity determination. The intended organs of mussels (gill, foot, mantle, PAM and digestive gland) were dissected out, dam-dried and weighed. A dounce homogenizer was used to homo-genize 0.3 g of each tissue in 2 ml of potassium phosphate buffer (0.1 M/pH 8.0). The homogenate obtained was centrifuged at 10000 *x* g for 10 min at 4°C. The supernatant was removed into 1.5 eppendorf tube and kept under -80°C before ChE activity measurement was conducted.

Cholinesterase activity measurement

The enzyme activity was measured following the modified Ellman method (Ellman et al., 1961), for a 96-well plate and microplate reading (Herbert et al., 1995; Dizer et al., 2001). The enzyme measurement was carried out by placing 50 µl of the diluted sample into each well of the microplate. A blank was made by adding 50 µl of potassium phosphate buffer into a blank section of the microplate wells. The plate was incubated for 5 min at 25°C with 200 µl of 0.75 mM 5,5'-Dithio-bis-(-2-Nitrobenzoic acid) prior to the start of the reaction by the addition of 50 µl of 3 mM a cethylthiocholine iodide.

Accordingly, the plate was read by using a photometer for microtiter plate (Spectra Thermo TECAN) at an interval of 30 s for 5 min at 405 nm. Protein content measurement was carried out using Bradfort reagent solution. Finally, AChE activity was expressed as nmoles of product developed per minute per mg of protein (nmol/min/mg protein).

Statistical analysis

Parametric or non parametric analysis of variant was used to determine the effects of the pesticide for the assays according to data distribution. Distribution and homogeneity of variant of data set were checked firstly. If the data set was not distributed normally and the variant was not homogenous, the data set were log-transformed. Parametric one-way analysis of variant was used on data set, which demonstrated a normal distribution and homogeneity of variant, both before and after transformation. If the means were different significantly, Bonferroni's multiple comparison test was applied to determine the different means between the treatments. Nevertheless, for the data set, which did not show a normal distribution and homogeneity of variant, a non-parametric Kruskall-Wallis test was used to determine the differences between the medians. If the differences were significant, the median values were compared by Dunn's multiple comparison test. $P < 0.05$ was considered as statistically significant. The data set were analyzed by GraphPad prism software program, which expressed mean and standard deviation.

RESULTS

Cholinesterase activity

Hemolymph

Hemolymph of mussels showed the highest ChE activity with mean value 104.70 ± 34.48 nm/min/mg protein. There was a stimulation of the ChE activity on mussel's hemolymph (12% compared to the control) which were exposed to the lowest concentration (50 µg/l), but it was not significant. The effect of the used pesticide on the ChE activity from hemolymph of mussels occurred only significantly when mussels were exposed to 1000 µg/l (Figure 1). At this concentration, the ChE activity of mussel decreased by 39% compared to the control.

After incubation in the clean ASW media for the seven days, hemolymph of mussels in the control showed a slight decrease of the ChE activity (95.12 ± 25.60 nm/min/mg protein) compared to the previous control which were used for the 96 h exposure experiment. The recovery of the ChE activity from hemolymph of mussels, which were exposed to 1000 µg/l of trichlorfon took place after the mussels were incubated in the clean ASW for seven days (Figure 1). The ChE activity of that mussel's hemolymph showed a mean value of 73.17 ± 19.16 nmol/min/mg protein.

Gill

Parametric one-way ANOVA on the transformed data of the ChE activity from gill of mussel showed that significant differences in the ChE activity among the treatments were observed. Further calculation using Bonferroni's multiple comparison revealed that trichlorfon inhibited ChE activity significantly at 50 µg/l (3.44 ± 1.17 nmol/min/mg protein) (Figure 1). At this concentration, the pesticide reduced 29% of the ChE activity from the control level (4.83 ± 1.36 nmol/min/mg protein). The inhibition of the pesticide on the ChE activity persisted significantly at all higher concentrations.

Decreasing activities of the ChE in all treatments were observed when mussels were transferred to the clean condition compared to the control of the 96 h exposure experiment (Figure 1). Furthermore, the differences of the ChE activities of the control and the treatments in the recovery experiment were not confirmed when the data were calculated using Kruskal-Wallis test.

Foot

The ChE activity from foot of mussel was induced significantly by trichlorofon at 200 µg/l (3.10 ± 0.56 nmol/min/mg protein) (Dunn's Multiple Comparation test) (Figure 1). The reduction of the ChE activity at this concentration was 35% compared to the control (4.77 ± 1.19 nmol/min/mg protein). The inhibition of the pesticide persisted significantly when mussels were exposed to other higher concentrations 500 (2.89 ± 0.64 nmol/min/mg protein) and 1000 µg/l (2.10 ± 0.42 nmol/min/mg protein) (Dunn's multiple comparation test).

Transferring the pesticide-exposed mussels to clean condition resulted in relatively slight reduction of the ChE activities for all treatments. However, when the mussels of the control was compared (3.12 ± 0.79 nmol/min/mg protein) to other treatments at clean condition, there were slight increase in the ChE activities of the mussels at 50 (3.40 ± 0.84 nmol/min/mg protein) and 100 µg/l (3.48 ± 1.10 nmol/min/mg protein). Furthermore, insignificant inhibitions of the ChE activities occurred at 200 (2.94 ± 0.63 nmol/min/mg protein) and 500 µg/l (3.11 ± 0.33 nmol/min/mg protein). The inhibition of the ChE activity appeared significantly at 1000 µg/l (2.11 ± 0.188 nmol/min/mg protein) (Dunn's Multiple Comparison test) (Figure 1).

Mantle

Kruskal-Wallis, followed by Dunn's multiple comparison demonstrated that a significant inhibition of ChE activity from mantle by the pesticide occurred at 200 µg/l (Figure 1). Compared to the control (3.82 nmol/min/mg protein), the ChE activity of mussels which were exposed to 200 µg/l of trichlorfon reduced by 26%. Subsequently at 500 µg/l, the inhibition remained (26%) and seemed more distinct at 1000 µg/l (34%).

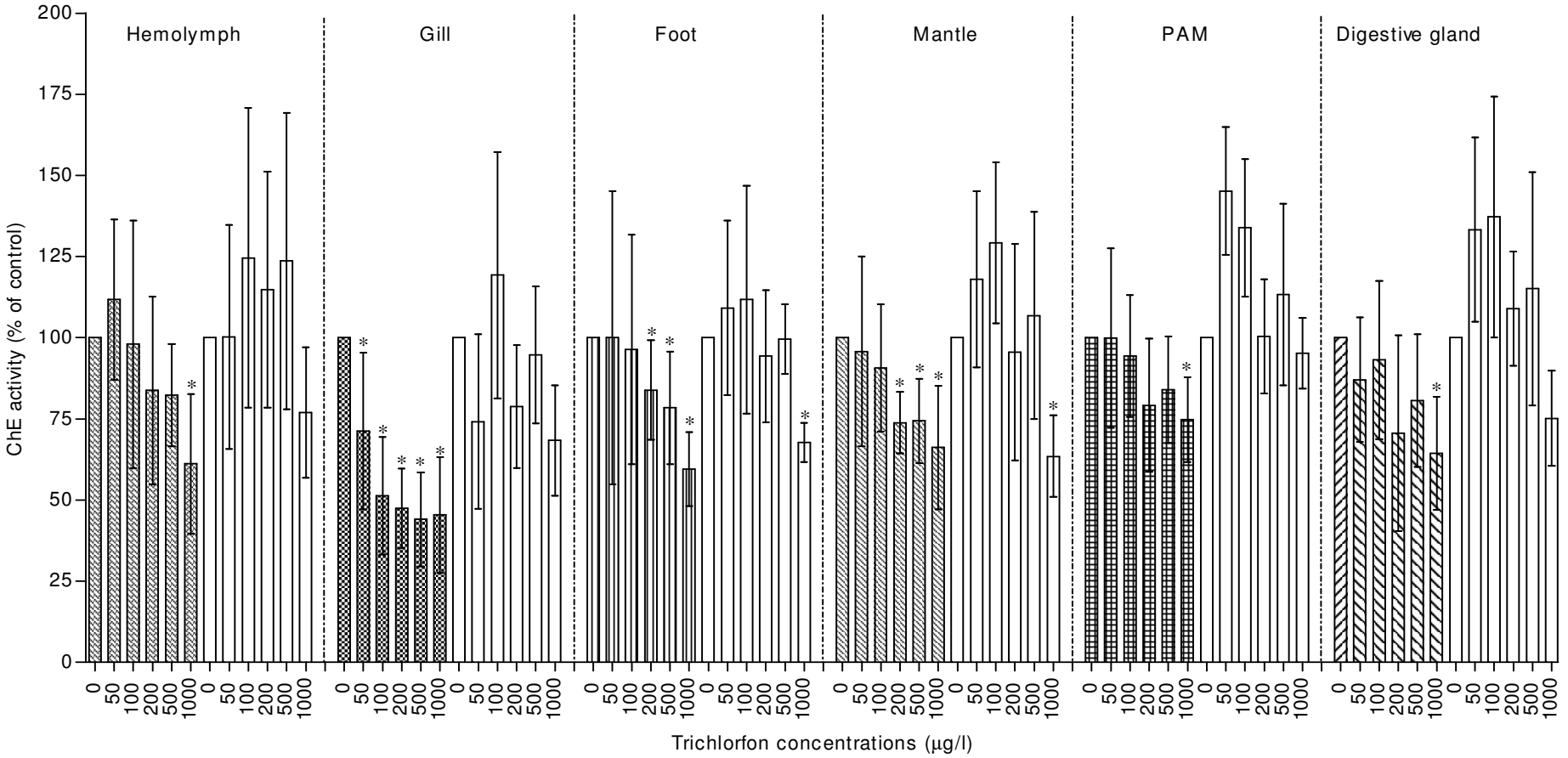

Figure 1. Comparison between pre- and post-incubated mussels *M. edulis* after exposed to trichlorfon concentrations in term of the ChE activity from the six organs. The ChE activity in the six organs of mussels exposed to trichlorfon for 96-h (striated area with ± standard deviation). The ChE activity in the six organs of post-trichlorfon exposed mussels which were incubated in clean ASW for 7 days (empty area with ± standard deviation). * indicate significant difference from control (0 µg/l) ($P < 0.05$).

After the mussels were replaced to the clean ASW media, the stimulation of the ChE activities occurred seemingly at 50 (3.28 nmol/min/mg protein) and 100 µg/l (3.59 nmol/min/mg protein). Significantly, the level of the ChE activities returned to the level of the control at 200 (2.65 nmol/min/mg protein) and 500 µg/l (2.96 nmol/min/mg protein). In contrast, the effect of the pesticide on the ChE activity of mantle (1.76 nmol/min/mg protein) still appeared significantly (Dunn's Multiple Comparison test) at 1000 µg/l when compared to the control (2.78 nmol/min /mg protein) (Figure 1).

Posterior adductor muscle (PAM)

The data set from the ChE activity of PAM were

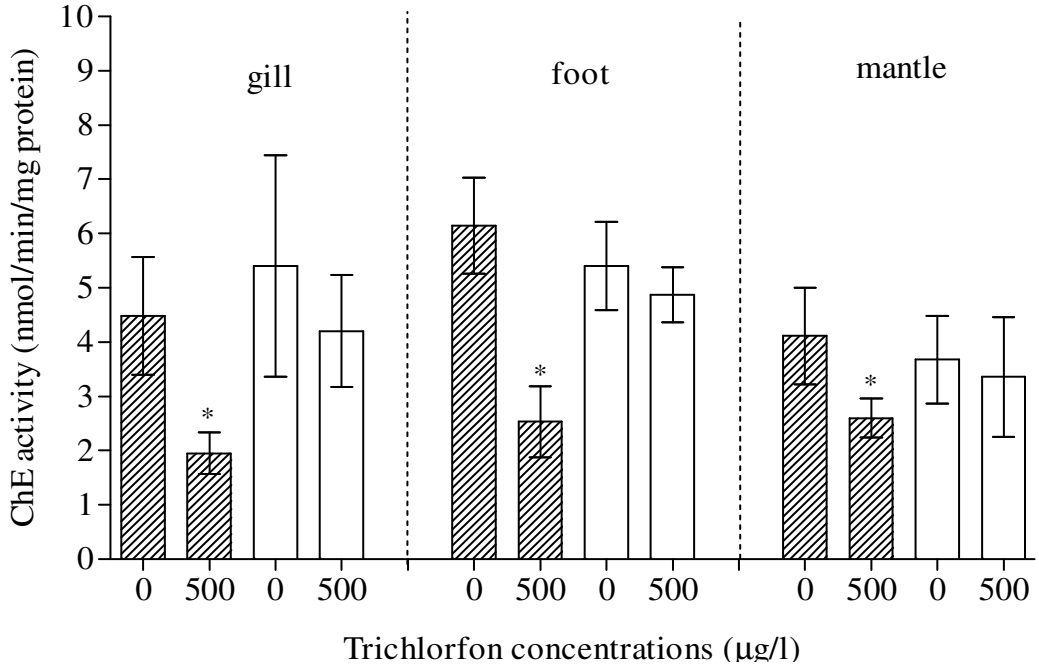

Figure 2. Effects of trichlorfon on the ChE activity from three organs of *M. edulis*. The ChE activity in three organs of mussels exposed to trichlorfon for 96-h (striated area with ± standard deviation). The ChE activity in three organs of post-trichlorfon exposed mussels which were incubated in clean water of original habitat of mussels for 7 days (empty area with ± standard deviation). * indicate significant different from control (0 µg/l) ($P < 0.05$).

homogenous and normally distributed. Parametric ANOVA showed that the pesticide induced significantly the ChE activity only at 1000 µg/l which produced the lesser ChE activity (1.82 nmol/min/mg protein) from PAM (Figure 2). The percentage of inhibition was 25% compared to the control (2.43 nmol/min/mg protein).

The ChE activity of PAM from the control of the clean incubated-mussels showed a relatively lower level compared to the control of the 96 h pesticide-exposure. Since the data also were distributed normally and the variant was homogenous, parametric ANOVA was used to distinguish the differences among the treatments. The results revealed that there were no differences in ChE activities among the treatments.

Digestive gland

The ChE activity in the digestive gland of mussels in the control showed mean value of 2.57 ± 0.55 nmol/min/mg protein. The ChE activity was only inhibited significantly (Bonferroni's Multiple Comparison test) when mussels were exposed to the highest concentration (1000 µg/l), resulting in 35 % inhibition (Figure 1).

The persistence of the pesticide inhibition on the ChE activity of digestive gland after incubation in the clean ASW media was not observed statistically (Dunn's Multiple Comparison test). However, stimulations of the ChE activities in the control (1.95 ± 0.48 nmol/min/mg protein) appeared insignificantly at 50 (2.30 ± 0.49 nmol/min/mg protein) and 100 µg/l (2.33 ± 0.37 nmol/min/mg protein). Eventually, the level of the ChE activities returned to the control level at 200 and 500 µg/l and reduced slightly at 1000 µg/l (Figure 1).

In situ recovery experiment

In situ recovery experiment was conducted only by exposing mussels to 500 µg/l of trichlorfon and control. Consequently, data analysis was performed on the organs of mussels which showed inhibition of the ChE activity at concentrations ≤500 µg/l only. The organs were gill, foot and mantle. Repeated *in vivo* experiment for 96 h showed that the ChE activities of the three organs were significantly inhibited by 500 µg/l trichlorfon. After transplanting the mussels to the original habitat in the coastal area of the Island of Sylt, the ChE activities of the organs returned significantly to the activity level of the control (Figure 2).

DISCUSSION

Cholinesterase (ChE) activity of different organs

Although, there are considerable diversity of biochemical properties of ChEs in aquatic organisms (Escartin and

Porte, 1997), ChEs in mussels are generally divided into two main classes; acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BuChE; EC 3.1.1.8). The distribution and the properties of ChEs in mussels are organ-dependent (Bocquene et al., 1990; Brown et al., 2004). This study referred to the enzyme as ChEs since they employed acetylthiocholine as non-specific substrate of the enzyme. Therefore, the term of ChE that was used in this study referred to the sum of AChE and BuChE as frequently used as a diagnostic tool for ecotoxicological risk assessment studies in aquatic ecosystem (Torre et al., 2002).

The alteration of ChE activity is a well-known cellular response of marine mussels which is particularly induced by OP and carbamate pesticides. Subsequently, this enzyme activity from the mussels was considered as a respectable tool for detecting the effects of the pesticides (Herbert et al., 1995). Mussel organs such as hemolymph, gill, mantle, PAM, digestive gland and gonad have been employed as target organs for measuring the alteration of ChE activity induced by contaminants. The preference of the used organs usually depends on some factors such as the goal of the study and the availability of the organs that are used for analysis. The ChE activity from hemolymph of mussel is considered to be rapid, inexpensive and a reliable means for measuring the biological impact of pesticide mainly if sacrificing the animals is avoided (Moreira et al., 2001; Galloway et al., 2002). Nevertheless, the link effect of the alteration of the ChE activity from hemolymph induced by contaminants to higher biological organization such as feeding behavior is less relevant compared to the innervated organs like gill, mantle and PAM.

This study evaluated the effects of trichlorfon on ChE activity from various organs from *M. edulis*, viz., hemolymph, gill, foot, mantle, PAM and digestive gland. The highest ChE activity was found in hemolymph and was more than 20 fold when compared to the other organs. These activities from different organs of *M. edulis* were comparable to those which were observed by Herbert et al. (1995).

The results displayed that different organs of *M. edulis* had different sensitivity to trichlorfon. The ChEs from gill was the most sensitive to the effects of trichlorfon followed by foot, mantle, the hemolymph, digestive gland and PAM. The lowest observable effect concentration (LOEC) of gill took place at 50 µg/l. Although, the LOEC of the ChE activity from foot and mantle were similar, the inhibition level of the ChE activity from foot (35%) at 200 µg/l was higher than that from mantle (26%). The significant inhibition of trichlorfon on the ChE activities from the hemolymph, digestive gland and PAM were initiated at 1000 µg/l, even though the ChE activity from the hemolymph experienced greater inhibition (39%) than those from digestive gland (36%) and PAM (25%). These results demonstrated that the threshold of trichlorfon to inhibit the ChE activity from *M. edulis* was organ dependent. Accordingly, the sensitivity of *M. edulis* organs to trichlorfon in terms of the ChE activity could be divided into three clusters, which were high (gill), moderate (foot and mantle) and low sensitivities (the hemolymph, digestive gland and PAM).

Most studies on ChE activity from mussels were oriented to identify the sensitivity level of ChE activity to pesticides by using single organ. There were some ChEs studies which used multiple organs of mussels in the laboratory scale. Herbert et al. (1995) conducted *in vitro* test to evaluate the sensitivity of ChEs from different organs of *M. edulis* which were hemolymph, gill, PAM, digestive gland and gonad. The experiment revealed that gill and the hemolymph were the most sensitive organs which were followed by digestive gland, PAM and the gonad. Comparison between solid tissue homogenates which were gill, mantle, PAM and whole body showed that gill was the most sensitive organ when the tissue homogenates were exposed to aldicarb. Escartin and Porte (1997) also found that the ChE activity of *M. galloprovincialis* gill tissue homogenate was more susceptible to inhibition by fenitrothion, fenitrooxon and carbofuran compared to that from digestive gland. In addition, a recent study conducted by Canty et al. (2007) recorded that the ChE activity from gill of *M. edulis* was more sensitive than that from hemolymph when exposed to azamethiphos.

Comparison of the sensitivity level of the ChE activities from *M. edulis* organs to the pesticides from different studies was carried out carefully. Different sensitivities of the studies did not merely reflect the sensitivities of the organ *per se*, but mostly they resulted from different methodology and the tested pesticides (Brown et al., 2004).

Recovery of ChE activity from different organs

Recovery mechanisms in sentinel organisms after exposed to environmental stress are point of interest in the use of biomarker in biomonitoring campaigns. To be useful tool in biomonitoring, biomarker must reveal the environmental stress over time so that the knowledge about induction, adaptation and recovery of the stress are required and considered prior to monitoring (Wu et al., 2005).

This study applied two strategies to investigate the recovery of the ChE activity from *M. edulis* after been inhibited by trichlorfon in artificial and natural conditions. The results demonstrated that before transferring to the clean media, the percentages of inhibitions of the ChE activities in gill, foot, and mantle from the trichlorfon exposed mussels, which were used in laboratory scale recovery were 55.94, 39.31 and 25.65%, respectively. Furthermore, for the mussels that were used in the recovery experiment and transplanted in natural sea water, the percentages of inhibitions induced by

trichlorfon on the ChE activity in gill, foot, and mantle were 56.51, 58.84 and 36.89% compared to the control respectively. After incubation for seven days, in both laboratory and natural conditions, the ChE activities from the three organs returned to the level of control statistically. This indicated that both artificial and natural conditions served as a suitable media for mussels to recover from ChE activity inhibition. Besides, the result also indicated that the artificial media used in the laboratory scale was sufficient to mimic natural sea water for the mussels to recover from the induction caused by 500 µg/l of trichlorfon for 96 h.

In the laboratory scale, mussels were exposed to serial concentrations of trichlorfon. After been replaced and incubated in clean media for seven days, the ChE activities of the tested organs increased to the level of control. This recovery occurred in mussels which were exposed to the pesticide in concentrations from 50 to 500 µg/l. Nonetheless, the exposed mussels to 1000 µg/l of trichlorfon did not show increase in the ChE activities from all studied organs.

After transferring the animals to the clean media only the ChE activities from gill, hemolymph, digestive gland and PAM that returned to the level of the control, but the evidence were not observed in foot and mantle. These evidences suggested that recovery mechanisms on trichlorfon inhibited ChE activity from mussels were organs specific. In other words, the complete recovery of the ChE activities in the whole organs of mussels after been exposed to 1000 µg/l of trichlorfon was not confirmed. Gill illustrated a sensitive and quick recovery organ regarding the pesticide effects. Mc.Henery et al. (1997) also observed that fast induction and recovery of the ChE activity from gill of *M. edulis* occurred when the animals were exposed to serial concentrations of dichlorvos (10, 100 and 1000 µg/l) and replaced in clean media for seven days. In contrast, this study demonstrated foot and mantle as moderate sensitive organs, which could retain pesticides effects for such periods. The lowest sensitive organs, that is, the hemolymph, digestive gland and PAM elucidated fast recovery responses. On that account, it is suggested that the use of foot and mantle from *M. edulis* in studies on induction of neurotoxic contaminants on the ChE activity and the recovery mechanism is recommended.

The application of these organs viz. foot and mantle as target organs for measuring the ChE activity from mussels could be considered as counterparts of common employment organ such as gill to elucidate more comprehensive understanding on neurotoxic xenobiotic effects in mussels when the ChE activity would be applied as biomarker. As demonstrated in the study the sensitivity and the recovery response of the ChE activity in mussels differed from organ to organ. Combining all types of inductions and recoveries of target organs is needed to reduce potential false positive or negative on assessing the impacts of the pesticides on mussels.

Conclusion

The study depicted that the sensitivity of ChE activity from *M. edulis* to trichlorfon is organ dependent. The sensitivity of ChE activity in *M. edulis* organs to trichlorfon can be discriminated into three clusters, which are high (gill), moderate (foot and mantle) and low sensitivities (hemolymph, digestive gland and PAM). Moreover, the recovery of the ChE activity induction from different organs is also organ specific which can be divided to three clusters as well. The most sensitive (gill) and insensitive organs, (hemolymph, digestive gland and PAM) delineated the recovery from pesticide induction, while the moderate sensitive organs (foot and mantle) still retained the effect of the pesticide during the experiment. The results of the study demonstrated that the use of battery of target organs in screening test using ChE activity as a biomarker in laboratory scale is recommended.

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