Acetylcholinesterase Activity in Nile Tilapia (Oreochromis niloticus Linn.) Following Exposure to Carbamate Insecticide

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ABSTRACT

Acetylcholinesterase (AChE) activities were determined as affected by exposure to sublethal doses of fenobucarb (BPMC) on Nile tilapia. Fish were exposed for different periods, namely: 0, 7, 14, 21, and 28 days. AChE activities of brain and muscle tissues were measured spectrophotometrically. The sublethal doses of the insecticide did not induce treatment-related poisoning but were enough to significantly induce effects on the hepatosomatic index. The concentration used was also enough to significantly induce AChE inhibition on brain and muscle. AChE inhibitions were significantly different between unexposed and exposed fish in both brain (KW=14.02, P<0.05) and muscle (KW=6.87, P<0.05) tissues. The inhibition on brain AChE was highest on the 14 day-exposed fish. The inhibition on muscle AChE was highest on the 21-day-exposed fish which could be due to the direct exposure of muscle tissues to toxicants. The sensitivity of the relative liver weights and AChE activity of Nile tilapia could therefore be used as potential bioindicator of carbamate insecticide contaminated waters.

Keywords: fenobucarb (BPMC), acetylcholinesterase (AChE) inhibition, bioindicator, Nile tilapia, Oreochromis niloticus Linn.

INTRODUCTION

Pesticides are agents that are synthetically produced to rid humans of anything considered as “pests” such as mosquitoes, midge larva, pests of various agricultural crops, undesirable fishes, aquatic weeds, and aquatic snails (Que Hee 1993, Aragon 2000). Synthetic pesticides are well known as a cost-effective method of pest control, but these chemicals are toxic to aquatic species, particularly fish. The indiscriminate use of such chemicals is a growing concern worldwide which results in environmental pollution and toxicity risk to non-targeted organisms (Coppage & Bradeich 1976, Venkateswara Rao 2004).

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In the Philippines, the use of pesticides is an essential part of crop production, especially vegetables and rice, which increases crop yields by 28%. Insecticides are the most commonly used pesticide in the Philippines and represent 39% of the Php 6 billion pesticide market for 2005 (Rondon 2005).

Presently, organophosphorus pesticides (Ops), carbamates, pyrethroids, and triazines have largely replaced the organochlorine compounds in agricultural practices. They have the advantage of being less persistent in the environment (Varo et al 2000). Although they have such advantage, Gruber and Munn (1998) suggested that these substances could still affect non-target aquatic biota since they are applied for extended periods of the year, especially during growing season, when most usage occurs.

In determining chemical characteristics of pollutants and their concentrations, organisms and their biomolecules represent a useful choice as biomarkers, since they employ both the chemical and the biological approaches in environmental biomonitoring. Moreover, they also allow estimation of the impact of these pollutants to such species that provide the target molecules (Wijesuriya & Rechnitz 1993, Watson & Mutti 2004). Among these compounds, enzymes play an important role due to their degree of specificity and fast response to relevant changes in the surrounding medium. The use of enzymes as bioindicators is based on the inhibition or negative interference in catalytic activity triggered by analytes (Marco & Barceló 1996). Cholinesterase inhibition has been used as biomarker of carbamate exposure. AChE is one of the oldest environmental biomarkers (Payne et al 1996).

This study focused on carbamate, a cholinesterase inhibitor. It acts by carbamoylating the serine residue at the active site of the ChEs. Their structures present either similarities to the substrates or their hydrolytic intermediates and interact very slowly with the enzyme by forming stable conjugates (Töügu 2001). This mechanism hinders the normal functioning of the enzyme, which cannot prevent the accumulation of the neurotransmitter in the synaptic cleft. The overstimulation caused by acetylcholine continuously firing its receptors generates a range of signs and symptoms. Because of their low environmental persistence and high toxicity, particularly to aquatic organisms, water must be continuously monitored (Beauvais et al 2002).

Carbamate pesticides are presently used in the Philippines, particularly in farms around Laguna de Bay (Calumpang et al 1997, Varca 2012) which could find its way to the lake and could have detrimental effects on non-target species. The findings of this study would provide a tool for evaluating the efficiency of using AChE activity in the central nervous system (brain) and peripheral NS (skeletal muscle) as potential biomarkers for the presence of carbamate pesticides in freshwater ecosystems such as rivers and lakes.

**MATERIALS AND METHODS**

*Procurement and Selection of Tilapia Samples*

A total of 50 fry of Nile Tilapia (*Oreochromis niloticus* L.) was used in this study. The test animals were obtained from the Demonstration Farm of the Bureau of Fisheries and Aquatic Resources (BFAR) in Bay, Laguna. The fish averaged 3cm in length and 7g in weight. From the farm, they were brought to the laboratory
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inside plastic bags half-filled with water and adequately provided with oxygen. The fishes were then transferred to a 40-liter (25 x 50 x 30cm) aquarium filled with 35 liters of aged tap water. The tanks were fully aerated using a compressor. The fishes were allowed to acclimate for two weeks and were given fish commercial starter crumble pellets regularly. Water in the aquaria was changed daily.

Description of Test Chemical

The carbamate insecticide used was fenobucarb or BPMC (Hopcin®). Hopcin 50 EC (Wuhan Chemwisch Technology Company) is a formulation with 500g BPMC/kg. This test material was chosen among a number of insecticides because this pesticide is being used in rice fields and farms in Lumban and Pangsanjan, Laguna and was detected in waters of Laguna de Bay and tributary rivers (Bajet et al 2012, Varca 2012).

Time-course pattern experiment

All 50 fishes were randomly distributed into 25 x 50 x 30cm tanks (labeled A to E) filled with 40L aged tap water. The test concentration of BPMC used was 0.08mg/L. This concentration was reported by Calumpang et al (1997) as the detectable amount of residues of BPMC in irrigation areas. At the start of the experiment, all the fish, except those in Tank A, were exposed to the treatment dose. Fish in Tank A served as the control and were sacrificed at the start of the treatment. The remaining fish were sacrificed at the end of the 7th, 14th, 21st and 28th day exposure periods. Brain and muscle samples were obtained for AChE evaluation. The number of dead test fish and any abnormalities in gross morphology and/or behavior, which may be due to insecticide exposure, were also noted. The general management and feeding of the fish were the same as already described above during the acclimatization period. Tanks were cleaned and replaced with new aged tap water daily, and fresh doses were provided.

Post-mortem Analysis

Test fishes were sacrificed at the end of every exposure period by decapitation. Individual fish were weighed using a top loading balance and were dissected. The liver and spleen were excised and weighed. Using the following formula, the relative organ weight (ROW) of each organ was calculated:

\[
ROW = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of fish on the day of sacrifice (g)}} \times 100
\]

Brain and muscle samples were obtained to be used for AChE activity evaluation.

Evaluation for AChE Activity

AChE activities of the brain and muscle samples were determined spectrophotometrically following the procedure described by Ellman et al (1961). A
20-mg tissue sample of muscle and brain was mixed with 1 mL of phosphate buffer (pH 8, 0.1) and homogenized using a homogenizer. A 0.4mL aliquot of the homogenate was added to a cuvette containing 2.6mL of 0.1M phosphate buffer (pH 8). 100μl of dithio-bis-nitrobenzoic acid (DTNB) was then added to the photocell. The mixture was mixed by bubbling for about 30 seconds and absorbance was measured at 412nm every minute for a period of six minutes. A 20 μL of acetylthiocholine iodide (0.075M) was then added to the initial mixture, and absorbance was again measured for another six minutes. Changes in absorbance were recorded and the change per minute was calculated. The rates were calculated as follows:

\[ R = 5.74 \times 10^{-4} \times \frac{\Delta A}{C_o} \]

Where: \( R \) = rate, in moles substrate hydrolyzed per minute per gram of tissue;

\( \Delta A \) = change in absorbance per minute;

\( C_o \) = original concentration of tissue (mg/ml)

Inhibition rates were computed using the following formula:

\[
\text{Inhibition rate} = \frac{R \text{ (control fish)} - R \text{ (treated fish)}}{R \text{ (control fish)}} \times 100
\]

**Statistical Analysis**

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS 16.0 for Windows). Differences in relative organ weights, and AChE activities of the brain and muscle tissues among test groups were analyzed using the Kruskall-Wallis H Test Analysis of Variance.

**RESULTS AND DISCUSSION**

**Treatment-related Symptoms of Toxicity**

There were no treatment-related symptoms observed among individuals. This may be due to the very low concentration level of BPMC. Nile tilapia has an LC₅₀ value of 9 mg/L for BPMC exposure as reported by De Silva and Ranasinghe (1989) which is -112 higher than the concentration used in this study (0.08 mg/L). This suggests that the concentrations used in this study were not enough to induce symptoms of toxicity.

Although there were no treatment-related symptoms observed among individuals, there were still minimal mortalities observed. Mortalities were observed in both treated and control group. This may be due to the presence of dominant and subordinate fish in the tanks. Evans et al (2008) observed behavior such as biting, ramming, and mouth-fighting among tilapia raised in aquaria.
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These behaviors were also observed among the fishes in the tanks in this study. These behaviors lead to stress or trauma on the subordinate fish, which resulted in breaks in the fins or scales (Figure 1). Scales and skin function as a physical barrier that protects the fish against injury. When these are damaged, a window for both biotic and chemical toxins starts to invade the system of the fish. Breaks in fins left the fish unable to swim and were incapable of moving towards the food. The subordinate fish that were traumatized by these aggressive behaviors eventually died.

![Figure 1. Subordinate fish suffered breaks in the fins (black arrows) due to presence of a dominant fish in tanks.](image)

Influence of Test Pesticide on Relative Organ Weights

Figure 2 shows the relative mean weights of liver of Nile tilapia exposed to BPMC at different lengths of exposure. Analysis shows significant difference of relative weights of the fish (KW =21.88, P<0.05). There is a decreasing trend of relative weights of the liver observed from 0-days exposure until 14-day exposure and increased afterwards. This decrease in relative liver weights were also observed by Monteiro et al (2006), Chung et al (2002) and Gawish et al (2011) as a response to pesticide poisoning. This decrease in relative liver weights could have been the result of depletion of protein fraction as observed by Bose et al (2011), Tripathi et al (2003) and Singh et al (1996) on fish exposed to pesticides. The higher relative liver weights of 21- and 28-day exposed fish may have been an adaptive process and also suggests recovery as stated by Bose et al (2011). There were no definite patterns observed in the relative weights of the spleen. This could suggest that the test pesticides did not induce effects on the spleen but histological analysis could be recommended to further test the effects of the test pesticides on both spleen and liver.

Time-course pattern for brain AChE activity

Analysis revealed that there were significant inhibitions of brain AChE among fish exposed to BPMC (KW=14.02, P<0.05). Figure 3 shows the pattern of brain AChE inhibition. The peak of brain AChE inhibition was observed on the 14-day-exposed fish which was 40.7% inhibition rate. The AChE inhibition on the 21
- and 28-day-exposed fish was lower compared to the 14-day-exposed fish. Liwag et al (2009) observed the same trend in which AChE activity of *O. niloticus* exposed to carbaryl and chlorpyrifos insecticides, which are both cholinesterase inhibitors, improved after longer exposure to the constant concentration level of the insecticides. Morgan et al (1990) explained that recovery of AChE activity was a function of the degree of initial inhibition. It was explained that recovery was a result of de novo synthesis of the enzyme protein. This suggests that the concentration used in the present study was quite low and that the initial inhibition was not great, thereby enabling the fish to recuperate to the continued exposure.

![Figure 2](image2.png)

**Figure 2.** Relative liver weight of *Oreochromis niloticus* juveniles at different length of exposure to constant dose of BPMC (0.08 mg/L). N=10 fishes per group. (KW=14.02, P<0.05)

![Figure 3](image3.png)

**Figure 3.** Inhibition rate of brain acetylcholine (AChE) activity in *Oreochromis niloticus* juveniles at different length of exposure to constant dose of BPMC (0.08 mg/L). N=10 fishes per group. (KW=14.02, P<0.05)
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**Time-course pattern for muscle AChE activity**

Muscle AChE activity was also significantly inhibited by the exposure to BPMC (KW=6.87, P<0.05). The highest inhibition rate observed was on the 21-day-exposed fish (Figure 4). John and Prakash (2003) observed that cholinesterase inhibitors accumulate more in muscle than other tissues and explained that it may be due to their direct contact to the insecticides. The direct exposure of muscle tissues to the test compound could also explain why muscle tissues recuperated late compared to brain tissues.

The brain is the most sensitive and appropriate tissue to be analyzed for AChE activity. de Almeida et al (2005) explained that the degree of tissue enervation is related to the amount of AChE. Meaning, the higher the AChE concentration, the higher the inhibition susceptibility is.

![Graph showing inhibition rate of muscle acetylcholine (AChE) activity in Oreochromis niloticus juveniles at different length of exposure to constant dose of BPMC (0.08 mg/L). N=10 fishes per group. (KW=6.87, P<0.05)](image)

**CONCLUSION**

Fish exposed to the residual concentrations of BPMC did not show any overt treatment-related symptoms of poisoning. There was a significant influence of test insecticide on the relative weights of the liver and it is generally lower than that of the control fish. These could have been a direct effect of the pesticide or an adaptive process to cope with constant exposure to the toxicant.

AChE inhibitions were found to be significantly different among control and treated groups. BPMC-exposed fish had the highest enzyme brain AChE inhibition rate of 40.7% on the 14th day of exposure and lower in the 21- and 28-day exposed fish. The same pattern was also observed in muscle AChE inhibition wherein inhibition rate decreased as exposure continued.
This study shows that Nile tilapia is sensitive enough to be affected by environmentally detected levels of the insecticide BPMC. Effects on relative organ weights, as well as the brain and muscle AChE activity, were observed. The apparent decrease of AChE inhibition rate could provide insights as to how frequent should sampling of fishes be in the wild if Nile tilapia will be used as biomarkers for carbamate exposure. It is suggested to add parameters such as histological examination to further strengthen the potential of Nile tilapia as a bioindicator to monitor the impact of carbamate insecticides.

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Disclaimer: The use of trade names in this study does not imply endorsement or criticism of the mentioned products.

REFERENCES

Aragon MR. 2000. Subchronic toxicity effects of the residual levels of chlorpyrifos on cytochrome P₄₅₀ induction, selected hematological and histological parameters of Nile tilapia (Oreochromis niloticus Linn., MS thesis), University of the Philippines at Los Baños, Philippines.


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