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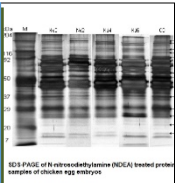

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Acetylcholinesterase as an In Vitro Assay for Insecticides: A Mini Review

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ABSTRACT

Acetylcholinesterase (AChE) is recognised as the key enzyme in the nerve signal system. It terminates nerve impulses by catalysing the hydrolysis of the neurotransmitter acetylcholine. Acetylcholinesterase is a unique molecular aim of a target for insecticides such as organophosphate (OP) and carbamate (CB) pesticides. Recent studies have recognized acetylcholinesterase activity and its inhibition as a useful human's and animal's biological indicator of toxicant poisoning. Fish may not be directly exposed by insecticide since this insecticide is created to kill pest which eventually affecting the crops. But when insecticide applied to crops or directly to the soil, it can be absorbed by the soil and affect nearest water bodies through the rain. This is how insecticide can contaminate the water resources thus affecting aquatic organisms especially the fish. Many insecticides such as organophosphates are highly toxic to aquatic organisms. Measurement of toxicant by using AChE inhibition has been widely used in toxicity studies, a biosensor or as a bioindicator having an effect on the nervous system of aquatic animals following exposure to pesticides or any other toxicants present in the environment especially in water bodies. The aim of this review is to discuss the latest study of *in vitro* assay of acetylcholinesterase for the detection of insecticide, the relation of insecticide contamination to the aquatic environment including its effect on aquatic life especially the fish.

INTRODUCTION

AChE inhibition occurs after complex exposure to toxicant such as pesticide, where it normally happens in the nervous system one of the early biomarkers characterized by human environmental exposure [1]. AChE is grouped as Cholinesterase family where it specifically catalyses the breakdown or hydrolysis of choline esters in the presence of water [1]. AChE is very sensitive to toxicants including insecticides such as organophosphates and carbamates. Contamination of toxicants like insecticides in the water bodies is due to the use and application of insecticide in the plantation crop industries. Whether the plant is grown for food or textile, the development of crop plantation has led to the rising in insecticides consumption.

These insecticides are spread to the plant or directly to the soil will then be transported to the nearest water bodies through the action of rain. Prolonged use of insecticides such as OP and CB are dangerous due to these compounds' neurotoxicity effect. Acetylcholinesterase of aquatic organisms especially fish has been extensively used as biosensor or biomarker to detect this toxicant in water bodies, as it can inhibit cholinesterase activity. In Malaysia, based on a study conducted along the shore indicates a positive detection of pesticides in fish [2].

Pesticide

In agriculture, pesticides are used to control the activity of the pests by an external contact on the surface reaction or by internal contact which is though consuming the pesticides [3–12]. The crisis of pesticides happens when improper applications occur which contaminate rivers and water sources thus endangered the aquatic organism, birds and human. The impact of pesticides toxicity is one of the major public health concerns globally [13–23].

Beside its generic term, pesticide can also be known as herbicide, insecticide, nematicide, bactericide, rodenticide, avicide, piscicide, molluscicide, termiticide, antimicrobial, fungicide, disinfectant, and sanitizer based on their targeted pest [4,12,24–26]. In pest control, the most effective chemical application to kill pest for agriculture is pesticide [27]. Apart from its efficiency to kill the pest, a pesticide also can give side effect to a non-targeted organism such as human or organism with a beneficial relationship to the crops such as bees and butterflies.

Examples of common insecticides in agriculture include organochlorines (OC), Organophosphates (OP) and carbamates (CB). Since most of the OC is banned, only OP and CB are commonly used [14,28–33]. Organophosphorus compounds and CB are also called cholinesterase inhibitors. Implementation of OPs and CBs for pest control in modern agriculture was due to their low persistence and high insecticidal activity.

Many studies highlighted OP and CB insecticides as one of the most widely used type of pesticide in agriculture due to their toxic properties to the neural system [34]. Although AChE activity is said to be more sensitive towards OP and CB pesticides than other toxicants, other types of toxicant have also been used to inhibit the activity of AChE and study the effect of its exposure to fishes. [35]. There are also AChE inhibitors which are chemicals and has toxic effects where they block the normal breakdown of neurotransmitter, acetylcholine (ACh).

A compound which restrain the cholinesterase enzyme from hydrolysing ACh, are AChE inhibitors or anti-cholinesterases compound which also can affect in raising both the level and period of the neurotransmitter action. There are two groups of AChE inhibitors; irreversible and reversible. The one that mostly has therapeutic applications is reversible inhibitors, competitive or non-competitive, such as CB, while the one that has toxic effects are irreversible AChE activity modulators such as OP [29]. CB has severe toxicity to humans which may cause sneezing and cough due to irritation of skin, eyes and throat [14,36–39].

Mostly, AChE is located at the cholinergic synapses and myoneural junction in the central nervous system, where it is involved in the normal synaptic communication activity. The present of OP and CB pesticides can inhibit the activity of AChE in both the central and peripheral nervous system of pests [28]. Beside its function to detect present of toxicant in fish, in a situation where OP and CB are accidently exposed to human, AChE is very useful as a main biosensor by using blood cholinesterase measurement [40]. In occupational and environmental medicine, there are other two types of ChE; Erythrocyte AChE and plasma or serum BChE which measured in blood for commonly used as a biomarker.

AChE and BChE potential of inhibition varies widely among different organophosphorus compounds and give variable AChE and BChE potential of inhibition, for example, some pesticides is stronger in inhibiting BChE than AChE such as OP [1]. BChE activity is inhibited or stopped of its function, which is highly relied on high intensity and period of exposure to the massive amount of OP and CB pesticides. The function of the normal nervous system is disrupted by these insecticides [14,36–39].

Nowadays, production and use of OP and CB pesticides have become rapidly increased which lead to concern when pesticides from the plantation area have started to contaminate the waterways and urban run-off whether after direct application or by flow through soil into the water system, thus increasing the possibility to cause damage to human health and population of non-target wildlife [14,36–39]. Aquatic organisms like fish is one of the common and widely used aquatic organisms as a biosensor or biomarker for cholinesterase inhibition to detect the presence of various pesticides [14,36–39].

Acetylcholinesterase

For the past few years, the use of biological assessments have recorded that the environmental impact by chemical substances such as pesticides and heavy metals have increased in detection in soils and aquatic bodies. AChE is one of the widely used enzymes for this purpose [14,36–39].

AChE is a group of the cholinesterases (ChEs) family, which are known as specialized carboxylic ester hydrolases that break down choline esters. AChE can mostly be found located at the centre of the nervous system at the neuromuscular junctions and cholinergic synapses. The product of the breakdown of acetylcholine by AChE into choline and acetate in the nervous system generate the activation of the acetylcholine receptors at the postsynaptic membrane. This is very important for a normal central and peripheral nervous system function (**Fig. 1**). Besides the neural junction, AChE can also be found on the erythrocytes membranes, where it constitutes the Yt blood group antigen [41].

There are two different protein domains which comprise the AChE molecule as the main large catalytic domain with 500 residues, and the small one is a C-terminal peptide which is composed of not more than 50 residues. Only a single AChE gene encoded AChE which is then transformed into the various types of tissues and splicing forms [42].

In the 3′ terminus of AChE pre-mRNA, alternative splicing forms produces three variants; “Synaptic” AChE-S which is the primary (also called as “tailed,” AChE-T) [43], the soluble AChE-R variant, stress-induced and the erythrocyte AChE-E [44]. They differ in their C-terminal domain, although these isoforms share a similar catalytic domain which will affect their molecular form, localization gives their distinct and specific features. In the brain and muscle, “Synaptic” AChE-S forms the principal multiple polypeptide chains of enzyme. Erythrocytic AChE-E is a glycophosphatidylinositol- (GPI-) linked dimer targeted to the plasma membrane of erythrocyte and lymphocytes [45]. AChE-S and AChE-R have also been described in the peripheral blood cells [46].

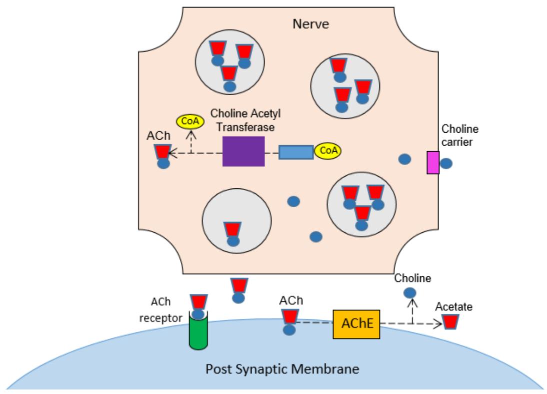

Fig. 1 Depiction of the mechanism of action of the enzyme AChE in synaptic transmission.

There are two subsites on the active site of AChE: the anionic site and the esteratic subsite. The binding site for the positive quaternary amine of acetylcholine is called the anionic site.

While the site where acetylcholine is hydrolysed into acetate and choline is called as the esteratic subsite, the carboxyl ester undergoes hydrolysis and form an acyl-enzyme and release free choline. Then, the water molecule attacks the acyl-enzyme regenerating the free enzyme and release acetic acid [43].

The major disadvantages in pest control from the use of OP and CR chemicals is that it can cause chronic toxicities to mammals. AChE is known as an enzyme that acts fast. It participates in cleavage of the neurotransmitter, acetylcholine (ACh) by the addition of water which then settled at the synaptic cleft. AChE inhibition cause AChE to accumulate which then leads to hyper excitation, convulsions of the muscles, and death [47].

A quasi-irreversible form from the binding of OP and AChE which cause the enzyme to inactivate and disturb the normal function of the nervous system thus lead to pests and mammals death [48]. The splitting of the neurotransmitter acetylcholine in cholinergic synapsis into choline and acetic acid is done by AChE [40]. Because of AChE sensitivity towards neurotoxic compounds such as pesticides, some drugs and nerve agents, therefore, its measurement is very useful to detect the amount of toxicant which may affect other organisms [40]. The method which has been commonly using in pesticide detection is based on the enzymatic hydrolysis reaction of an artificial substrate; acetylthiocholine, which release thiocholine and by mixing with the Ellman's reagent, producing a measurable 5-thiol-2-nitrobenzoate, which is yellow and can be measured through spectrophotometry at 412 nm [40]. The lower the concentration of the thiocholine produced, the yellow colour of the compound will reduce meaning the higher inhibition of the acetylcholinesterase activity.

Fish as targeted biomarkers and biosensors

For many years, inhibition of ChE-based assay has been used as a tool to monitor the effects of toxicants and also as multiple detection markers to living organism and environment. Many toxicants or xenobiotics including pesticides, industrial chemicals waste, pathogens and heavy metals which release to the land may be concentrated inside the bodies of marine organisms thus cause the fish to become toxic to handlers and farmers, and finally to mankind as the major consumer [49–57].

Fish is the type of aquatic organisms which have been widely studied and traditionally chosen as one of the organisms classically use as a biomarker and biosensor for various pesticides and toxicants detection, by inhibition of cholinesterase activities as an *in vivo* assay. The levels of the enzymes after the fish is exposed to the toxicant is a marker for that particular toxicant toxicity. Example of toxicants used for this bioassay include detergents, pesticides, textile dyes and heavy metals. This indicates the sensitivity of fish to toxicants [58–63]**.** Another important use of AChE is as *in* vitro assay, where AChE from healthy fish are partially purified and exposed *in vitro* to toxicants especially pesticides and heavy metals (**Tables 1** and **2**) and the level of decrease of AChE activity in comparison to the control indicates toxicity.

Table 1. Different types of organs dissected form various type of fish for in vitro detection of acetylcholinesterase activity to different pesticides from various studies.

Fish species	Organs	Pesticides detected	Ref.
Clarius batricus	Brain, muscle	Chlorphyrifos (CPF) and monochrotophos (MCF)	[67]
Oreochromis mossambicus	Brain	Carbaryl, carbofuran, eserin ect.	[65]
Hemibagrus nemurus	Brain	Acephate, bendiocarb, carbaryl, carbofuran, chlorpyrifos, diazinon, dimethoate, malathion, methomyl, parathion, and trichlorfon	[66]
Oreochromis mossambicus	Brain	Organophosphate and carbamate	[65]
Tor tambroides	Brain	Carbamate	[68]
Puntius javanicus	Liver	Malaoxon and carbofuran	[69]
Puntius schwanenfeldii	Brain	Carbamate (bendiocarb carbaryl, propoxur, carbofuran and methomyl)	[70]
Channa micropeltes	Brain	Carbofuran Carbaryl Methomyl Propoxur Bendiocarb Parathion-oxon Malathion-oxon Diazinon-oxon Chlorpyrifos oxon	[71]
Periophtalmodon schlosseri	Brain	Carbofuran, methomyl, carbaryl, parathion, malathion, diazinon, bendiocarb, chlorpyrifos, acephate, dimethoate and trichlorfon	[72]
Oreochromis mossambicus	Brain	Organophosphate azinphos-methyl	[73]
Osteochilus hasselti	Brain	Carbamate; carbaryl, bendiocarb, carbofuran, methomyl and propoxur; oxonate organophosphate, chlorpyrifos, diazinon, malathion and parathion	[74]
Clarias batrachus	Brain	Bendiocarb, carbaryl, carbofuran, methomyl, acephate, chlorpyrifos, diazinon, dimethoate, malathion, parathion	[40]

Tham et al. [40] had chosen *Clarius batrachus* as AChE from the fish source for *in vitro* insecticide detection research. The AChE from this species of fish has been reported to be very sensitive towards carbamate; an organic compound derived from carbamic acid such as carbaryl [64].Fish is very sensitive insecticides and other environmental contamination, and hence is a good biomarker or bioassay for toxicants [3]. Example of sources of fish AChE for pesticide bioassay and biosensor technology are *Drosophila melanogaster* and *Electrophorus electricus*. Other fish types that originate from Malaysian waters, such as tiger grouper; *Epinephelus fuscoguttatus* [63], Javanese carp; *Puntius gonionotu* and grass carp; *Ctenopharyngodon Idella* are useful fish sources which can be used as biomarker and biosensor agents for insecticides or any other toxicants. Current studies use *Channa micropeltes* or Toman, *Oreochromis mossambicus* [65] and *Hemibagrus nemurus* or Baung [66] (**Table 1**).

Table 2. Current studies result of IC_{50} and Ki from different species of fish.

Species	IC_{50} (μmol/L)	Ki (mM^{-1} min^{-1})	Source	Ref.
Methyl-paraoxon				
Mugil liza	2.87883	10.77	Brain	[58]
Genidens genidens	1.0312	35.28	Brain	[58]
Lagocephalus leavigatus	2.8425	9.31	Brain	[58]
Paralonchurus brasiliensis	0.455	-	Brain	
Genidens genidens	0.468	-	Brain	[75]
Haemulon steindachneri	1.035	-	Brain	[75]
Pagrus pagrus	1.087	-	Brain	[75]
Menticirrhus americanus	1.579	-	Brain	[75]
Cynoscion striatus	1.595	-	Brain	[75]
Dules auriga	1.624	-	Brain	[75]
Merluccius hubbsi	3.339	-	Brain	[75]
Percophis brasiliensis	3.259	-	Brain	[75]
Chlorpyrifos (CPF)				
Odontesthes bonariensis	0.030	-	Head	[76]
Eserine				
Colossoma macropomum	0.158	-	Brain	[77]
Odontesthes bonaeriensis	0.0743	-	Brain	[78]
Cyprinus carpio	0.5	-	Brain	[41]
Cnesterodon decemmaculatus	1.43	-	Brain	[41]
Cathorops spixii	0.0770	-	Brain	[79]
Micropogonias furnieri	4.47	-	Brain	[79]

CONCLUSION

It can be concluded that acetylcholinesterase has been recognized as important in vivo and currently in vitro assays for toxicants. Emerging use of *in vitro* assay is in biosensor development to detect the present of pesticides by determining the concentration of acetylcholine after exposure. There have been a lot of studies done on acetylcholinesterase as an *in vitro* assay for insecticide from various source, mostly fishes. However, the variation in sensitivity to different pesticides means that the use of multiple AChEs from various sources are needed to cover a wide range of pesticides. More fish species need to be screened and their sensitivity to various pesticides determined to have a wider range or spectrum of insecticides detection ability.

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