

# SENSITIVITY OF ACETYLCHOLINESTERASE TO ENVIRONMENTAL POLLUTANTS

Moe Pwint Phyu, Jitbanjong Tangpong\*

Biomedical Science, School of Allied Health sciences and Public health, Walailak University,  
Nakhon Si Thammarat 80160, Thailand

**ABSTRACT:** Acetylcholinesterase (AChE) is the human biomarker of environmental and occupational exposure to toxicants. Toxic materials are disposed or used incorrectly in developing countries, where they lead to long term exposure and high risks of unintentional health problems. In addition to poor urban planning, the establishment of settlements in industrial areas has led to generation of and exposure to more hazardous wastes as a consequence of poor disposal systems. The health problems of human are associated with environmental and occupational exposure to organophosphate and carbamate pesticides and heavy metals including lead, mercury, arsenic and cadmium. Even low level exposures of pesticides and heavy metals can be responsible for behavioral abnormalities and cognitive deficit related with acetylcholinesterase (AChE) dysfunction. AChE is the key enzyme which terminates nerve impulses by hydrolyzing neurotransmitter acetylcholine (ACh). Its activity is inhibited in a dose-dependent manner on exposure to environmental pollutants. Dysfunction of AChE activity can be attributed to cognitive dysfunction and memory loss. The aim of this study is to review and discuss the recent finding about the effects of environmental pollutants, pesticides and heavy metals exposure induced AChE dysfunction and the possible mechanisms of AChE alteration related to neurodegeneration.

**Keywords:** Acetylcholinesterase (AChE), Environmental pollutants, Heavy metals, Pesticides

## INTRODUCTION

The pollutant emissions are increasing worldwide and inducing enormous human health and environmental problems, particularly in urban areas. The problem has adverse effects from long-term exposure to low or sub-lethal concentrations of single chemicals or to mixtures of chemicals including heavy metals, polycyclic aromatic hydrocarbons and pesticides [1-3]. Heavy metals such as mercury (Hg), cadmium (Cd), lead (Pb) and arsenic (As) are toxic metals and their accumulation can result in central nervous system disorders, low energy production and damage to blood composition, liver, kidney, lungs and other vital organs [4]. Long-term pollutants exposure results in slowly progressing physical and mental problems such as neurodegenerative process of Alzheimer's disease and Parkinson's disease [5]. Heavy metals and organophosphates are effective

inhibitor of the enzyme acetylcholinesterase (AChE) and can alter this enzyme effectively [6-8].

Acetylcholinesterase (AChE), or acetylhydrolase, is a serine protease enzyme that hydrolyzes the neurotransmitter acetylcholine (ACh). Acetylcholinesterase is found mainly in neuromuscular junctions and is the key enzyme in central nervous system of cholinergic brain synapses, where its activity serves to terminate synaptic transmission. Irreversible inhibition of AChE may lead to muscle paralysis, convulsion, bronchial obstruction and death by asphyxiation [9]. Organophosphate and carbamate pesticides are known as inhibitors of AChE catalytic activity [8]. There is an interaction between heavy metals such as mercury/lead and the etiology of neurodegenerative diseases. These metals can accumulate in the brain by crossing blood brain barrier and can promote the oxidative stress and induce the neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease [4]. Metal toxicity such as lead (Pb) especially in

\* Correspondence to: Jitbanjong Tangpong  
E-mail: njibjoy@yahoo.com

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hippocampus and cerebellum showed significantly AChE activity decrease in rat [10]. Since cholinergic system is responsible for the behavioral manifestations in animals, Reddy et al. [11] suggested that Pb induced impairments in AChE system can be attributed to cognitive dysfunction in Pb-exposed animals.

Inhibition activity of the enzyme AChE is one of the early biomarkers characterized in human environmental exposure to toxins, and it exhibits a link to health adverse effects on nervous system following exposure to heavy metal and organophosphate poisoning [12]. Measurement of AChE inhibition as a human biomonitoring test is a precious tool in estimation of toxicants exposure. The basic concept of monitoring and applying AChE inhibition was used as a biomarker for surveillance tool providing real time detection for exposure to hazardous substances in the workplace and in occupational and environmental medicine [13]. The cholinergic system plays a pivotal role in the central nervous system functioning. Impairment of this system is associated with the behavioral disturbances, learning deficits and memory loss usually observed in humans and animals.

Therefore, considering the increase of the pollution, incorrect disposal of heavy metal as industrial waste products, the immersion of organisms in this impaired environment and the possible adverse effects of exposure, the present study aims to review and discuss about the effects of environmental pollutants and alteration AChE activity in relation to health problems and neurodegeneration leading to learning deficit and memory loss.

#### **ACETYLCHOLINESTERASE (AChE)**

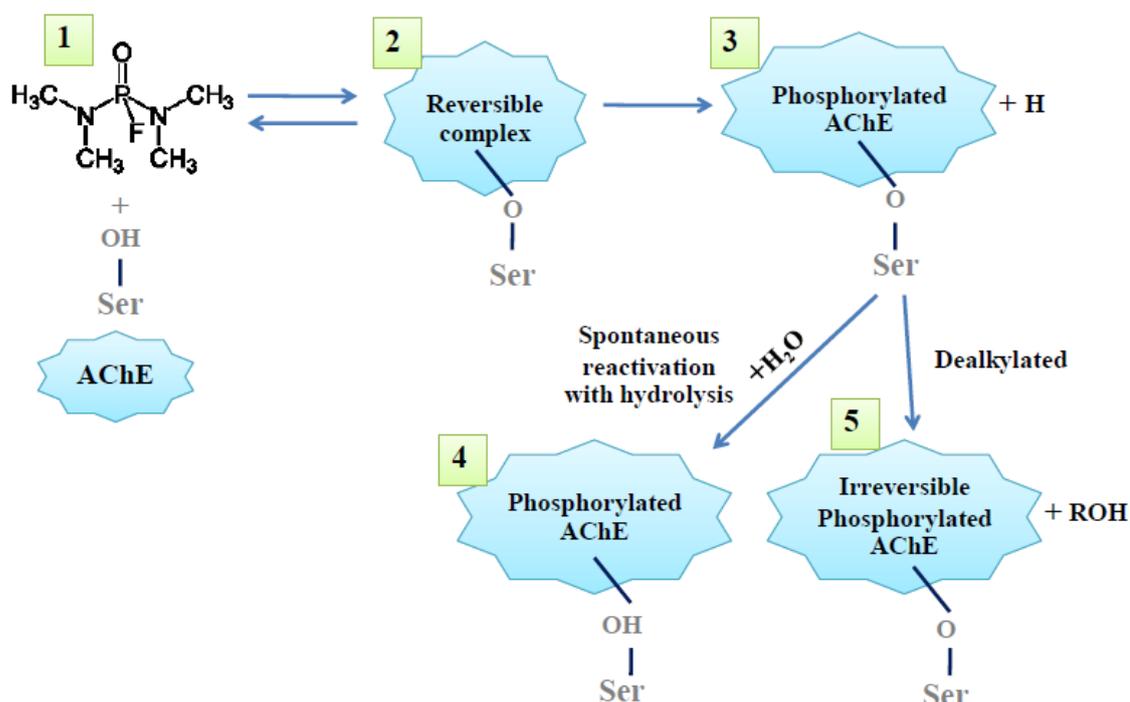
Acetylcholinesterase (AChE) is the key enzyme released by rapid hydrolysis of the neurotransmitter, acetylcholine (ACh). AChE belongs to cholinesterase class that hydrolyzes neurotransmitter acetylcholine (ACh), and butyrylcholinesterase or pseudocholinesterase (BChE) utilize butylcholine as a substrate. AChE is mainly found at neuromuscular junctions and cholinergic synapses in the central nervous system [14]. AChE locates on post synaptic membrane, terminates the neurotransmission by hydrolysis of acetylcholine into choline and acetic acid after activation of acetylcholine receptor at the post cholinergic synaptic membrane in central nervous system. In the peripheral nervous system, acetylcholine is located at the neuromuscular junction where it acts to control muscular contraction. The liberated choline is taken up again

by the pre-synaptic nerve and ACh is synthesized by combining with acetyl CoA through the action of choline acetyltransferase. AChE activity does to prevent continuous nerve firings at nerve endings, which is essential for the normal functioning of the central and peripheral nervous system [15, 16]. AChE is found in red blood cell membrane and BChE is found in plasma. However, their physiological function on erythrocyte membrane and in blood is to date unknown [17, 18].

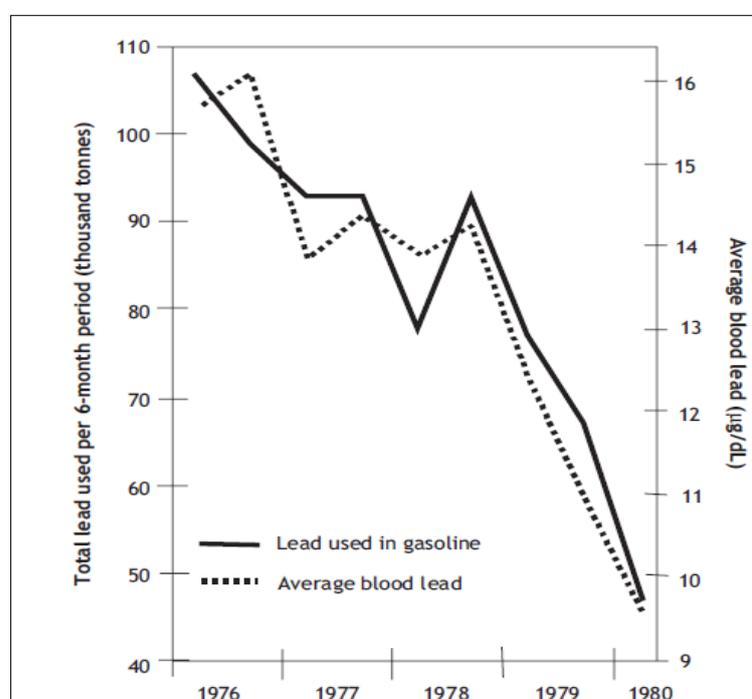
Acetylcholinesterase is a product of a single gene which is expressed in different tissues. AChE molecule has two different protein domains: a large catalytic domain of about 500 residues and a small C-terminal peptide of less than 50 residues. The active catalytic site of AChE composed of two subsites: the anion subsite and esteratic subsite. The anion subsite accommodates the positive quaternary amine of acetylcholine. The esteratic subsite is the site where acetylcholine is hydrolyzed to acetate and choline. The carboxyl ester is hydrolyzed and leads to form an acyl-enzyme and free choline. Then, acyl enzyme undergoes nucleophilic attack by a water molecule, liberating acetic acid and regenerating free enzyme [19].

#### **AChE'S ACTIVITY IS ALTERED BY ORGANOPHOSPHATE (OP) AND CARBAMATE INSECTICIDES**

Organophosphate (OP) compounds are diverse group of chemicals used in domestic, industrial and agricultural settings. Examples of organophosphates are insecticides, nerve gases, ophthalmic agents and anti-helminthics. OP and carbamate insecticides have increased in production and are widely used around the world replacing the more persistent organochlorine pesticides which effects bird populations and was removed from use in agricultural activities [20]. Inhibition of AChE is the primary mechanism of toxicity of OP leading to inactivation of the enzyme which has an important role in neurotransmission. OP cholinesterase inhibitor blocks the function of AChE, causing the accumulation of excessive acetylcholine in the synaptic cleft. Therefore, consequences of organophosphates are neurotoxic effects and continuous muscle over stimulation throughout the whole body. OP inhibits acetylcholinesterase by phosphorylation serine hydroxyl residues on AChE (Figure 1). Spontaneous reactivation of inhibited AChE occurs very slowly for most OP and very rapidly for carbamates. Phosphorylated AChE when dealkylated lead to a stable irreversible of phosphorylated AChE [21]. Exposure to OP compound can be fatal; death is usually caused by



**Figure 1** Acetylcholinesterase (AChE) interact with organophosphorus (OP) and carbamates. Reaction 1 shows interaction of organophosphate molecule with the serine hydroxyl group at the active site of AChE leading to phosphorylated enzyme (Reaction 2 and 3). Reaction 4 is a spontaneous reactivation of inhibited AChE which occurs very slowly for most OP and very rapidly for carbamates. Reaction 5 represents non-enzymatic time-dependent loss of one alkyl group (ROH) bound to the phosphorus. The irreversible phosphorylated AChE reaction depends on the chemical structure of the inhibitor and leads to a stable non-reactivable form of phosphorylated AChE



**Figure 2** Lead (Pb) concentrations in petrol and the blood Pb levels in children's in United State (USA)

respiratory failure resulting from paralysis of the diaphragm and intercostal muscles, depression of the brain respiratory center, bronchospasm, and excessive bronchial secretions [22]. Presently, a combination of an anti-muscarinic agent, e.g. atropine, AChE reactivator such as one of the recommended pyridiniumoximes (pralidoxime, trimedoxime, obidoxime and HI-6) and diazepam are used for the treatment of OP poisoning in humans. Administration of oral activated charcoal, in conventional doses, may be considered for reducing further absorption of some OP pesticides. OPs are highly toxic chemicals that are well controlled in developed countries but the amount of exposure and usage of OPs are continuously increasing in developing countries. Therefore, protective measures and regulatory restrictions are necessary.

#### **LEAD (Pb) INDUCED ACETYLCHOLINESTERASE (AChE) DYSFUNCTION**

Lead (Pb) is a ubiquitous environmental and industrial pollutant that mainly conveyed to humans through water, food and occupational sources. During past centuries, 50% of Pb emission originated from petrol to the air. Lead concentrations in petrol showed correlation to children's blood Pb levels in United State of America (USA) [23] (Figure 2). Many countries have reduced the concentration of Pb in gasoline since 1990, including United State, European nations, South Africa and Thailand. Pb levels in blood have decreased significantly in developed countries over the last few decades. However, Pb emission still remained health problem in developing countries. Pb can also be transmitted through maternal milk [24, 25] and low level of Pb (0.3%) exposure can cause long-lasting cognitive deficits [26]. Nervous system is the primary target for the Pb exposure and the developing brain appears to be especially vulnerable to Pb neurotoxicity. Pb's binding can alter enzymes and protein's function [27]. Pb exposure remarkably decreases the RBCs and plasma AChE activity with a negative correlation to blood Pb levels. The decrease in both RBC and plasma AChE is indicative of disruption of the cholinergic function, leading to neurotoxicity in Pb-exposed workers [28]. Recent studies indicated that the activity of AChE is decreased on exposure to free radicals and inhibitory effect of Pb on AChE might be due to free radicals produced by Pb [28, 29]. Pb also has a high affinity for free -SH groups present in enzymes and proteins and its binding can alter their function, including AChE activity and consequent increase in Ach level at synaptic

regions lead to desensitization of the cholinergic receptor on the post-synaptic membrane and ineffective to the transmitter action [30, 31]. There is another effect of ACh and AChE alteration during early postnatal development because Pb can cross blood brain barrier readily [32] and Pb exposure effects directly on AChE activity in developing cerebellum that leads to alteration of motor coordination [33]. According to Reddy et al. [33], the alteration of ACh and AChE activity can continue even after the Pb exposure is withdrawn. Obviously, Pb inhibits synaptosomal AChE in cortex, hippocampus and cerebellum brain regions. However, susceptibilities of brain region to Pb exposure depend on the local differences in their formation and maturation [33]. Pb exposure at the physiological relevant dose cause moderate inhibition of midbrain AChE activity leading to neurobehavioral deficits and region specific alteration in AChE and acetylcholine receptors [34]. Although Pb is a certainly serious problem in central and peripheral nervous system, the mechanisms of inhibition of AChE activity by lead exposure is still further investigation.

#### **EFFECTS OF ARSENIC (As) ON ACETYLCHOLINESTERASE(AChE)DYSFUNCTION**

Arsenic (As) is a chemical element, a naturally occurring metalloid is widely distributed in the environment through natural, geographical and anthropogenic sources and the gray form has important use in industry [35, 36]. As and its compounds are used in the production of pesticides, treated wood products, herbicides, and insecticides [37]. Incidences of As toxicity are occupational, environmental or accidental, major source to arsenic exposure in humans is through consumption of contaminated drinking water or food materials [38-40]. Chronic As exposure has been found to have adverse effects on human reproductive health outcomes, pregnancy associated with abortion, increased risk of infant mortality, preterm and stillbirth [41-44]. Chronic As exposure in adults is common result of arsenicosis, skin lesions, cardiovascular diseases, reproductive problems, carcinogenic effects, and neurologic, psychological and mental disturbances [44-48]. AChE activity was inhibited in the brain regions of animals exposed to As and took longer to acquire the learned behavior [49-53]. Long-term As exposure is related with cognitive and learning deficit in humans [54]. Many researchers showed that exposure to As may lead to neurobehaviour abnormalities [55]. Numerous studies have also

shown that As is rapidly absorbed and accumulated in the body organs such as liver, kidney, heart, lungs [54]. As can easily cross blood brain barrier and accumulate in the brain that is associated with decreased activity of brain AChE activity and impairment of memory that leads to learning deficit and cholinergic dysfunctions [49, 50, 56]. Besides, As affects dopaminergic functions and alters the brain biogenic and nitric oxide level. As induced neurotoxicity has been reported in rats through oxidative stress in brain [39]. Multiple mechanisms about As neurotoxicity have been suggested through different experimental studies but the exact mechanism of As inhibit AChE is still unclear.

#### EFFECTS OF OTHER HEAVY METALS EXPOSURES ON AChE ACTIVITY

Mercury (Hg) compounds are used to coat metals to prevent rust or inhibit foliage growth (marine paints). Under the intense heat of the arc or gas flame Hg vapor is produced. Exposure of these vapors may produce stomach pain, diarrhea, kidney damage and respiratory failure. Long-term exposure may produce tremors, emotional instability, hearing damage and development of cancer. It is classified as carcinogenic to humans by the International Agency for Research on Cancer (IRAC) (Group 2B) [57].

Cadmium (Cd) is used as stabilizer for coloring pigments and also used for producing of re-chargeable nickel Cd batteries. Cd is disposed in the environment incorrectly and it has an anthropogenic effect [58-60]. Cd is responsible for kidney damage (tubular dysfunction), bone effects (low mineral density of bone) and many types of cancers. It is classified as carcinogenic to human by the IRAC (Group 1) [61].

Hg, Cd and Pb have been reported inhibit AChE activity in the central nervous system of the red swamp crayfish at sub-lethal concentrations exposures [62]. Therefore, it is possible to suggest that oxidative brain damage of zebrafish caused by Hg and Pb promoted a significant decrease in AChE activity whereas they did not alter the gene expression pattern. High concentration of Hg can directly inhibit AChE activity *in vitro* experiments whereas lower doses of this metal cannot alter the enzyme activity [7]. Cd in a short-term exposure inhibited brain AChE activity and induced oxidative stress in adult rats brain [63]. Hence, the identification of AChE alteration related to cholinergic system during metal exposure may render some important insight about learning and memory targets in neurotoxicity promoted by heavy metal.

#### CONCLUSION

Acetylcholinesterase (AChE) activity in blood is a recently identified biomarker useful for assessing the organophosphate (OP) or carbamate (CB) pesticide exposure [64]. The recent reports indicate that inhibition of AChE activity is due to heavy metal exposure, including Pb, As, Hg, and Cd showing adverse effects to this enzyme activity [7, 62, 63]. All these findings on inhibition of AChE activity show sensitivity to several heavy metal and contaminants other than organophosphate and carbamate pesticides. Moreover, the combinations of difference pollutant compounds may exert additive or synergistic effects inhibition of AChE activity. The application of AChE activity in biomonitoring and assessment are not only specific for organophosphate and carbamate pesticides exposure but it may be reconsidered for heavy metal exposure. It is a useful biomarker to monitor the neurotoxicant contaminants in the environmental and occupational exposure. Taken together, the selection of a correct biomarker and potent prevention should be taken to reduce these heavy metal and pesticides exposures in order to minimize the risk of adverse neurotoxic effects and neurodegeneration disease.

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