CHAPTER 1

INTRODUCTION

1.1 Pesticide History

In the 1600s, pesticides were largely highly toxic compounds, such as arsenic and hydrogen cyanide (EPA, 2002). Their use was largely abandoned because they were either too ineffective or too toxic. In the late nineteenth century, U.S. farmers used copper acetoarsenite, calcium arsenate, nicotine sulfate and sulfur to control pests in crop fields, results were often unsatisfactory.

After World War II, organochlorine pesticides such as dichlorodiphenyl-trichloro-ethane (DDT), aldrin, dieldrin, endrin and 2,4-Dichlorophenoxyacetic acid were present to use in agriculture. These chemicals were inexpensive, effective and enormously popular. DDT was especially favored for its broad spectrum activity against insect pests of agriculture and human health. In 1962, Racel Carson published the book “Silent Spring” which pointed out that DDT was not safe and discovered DDT was harmful to birds and fish. Recently, most of organochlorine pesticides were banned for use in several countries and the new chemical pesticides such as organophosphate, carbamate and pyrethroid were discovered and widely used to control pests, weeds and others (Keith, 2000).

Organophosphate and carbamate pesticides affect the nervous system by inhibiting the cholinesterase enzyme activity that regulates acetylcholine, a neurotransmitter. They were developed during the early 19^{th} century they had effects on insects and then showed similar effects on human health. Some of them are very poisonous chemicals which were used in World War II as nerve agents such as sarin gas. However, most of them are not persistent in the environment (Bate, 2002).

A natural pesticide group such as pyrethroids group has been developed to be non-persistent and not be harmful to human and animals. However, some synthetic pyrethroids are toxic to the human nervous system (Bert, 1995).

1.2 Pesticide Classification and Usage in Thailand

Pesticides are substances used throughout the world to prevent, repel and destroy pests, insects, plant diseases and others that compete for food and agricultural produce. Pesticides are classed into four major categories based on their chemical structure, organophosphate pesticides (OPs), organochlorine pesticide (OCPs), carbamate pesticide (Carb) and synthetic pyrethorid (Pyr). (Dana, 2002 and Weiss, 2004) Pesticide turns out to be the factor with the most significant economic, environmental and public health impact. In 1997, Thailand imported 29,189 tons of pesticides, increasing to 65,074 tons in 2003. (Department of Agricultural, 1997-2003)

Pesticide usage helps the quality of crops productivity, improves the physical appearance, gives longer storage life and lowers costs of food. Pesticides also reduce the labor cost and assist in other pest control. But pesticides are toxic substances and mostly released and accumulated in crops, vegetables and foods. (Thomas, 2004) The most commonly applied pesticides in agricultural field are in organophosphate and carbamate pesticide groups.

1.3 Pesticide poisoning in Thailand

Pesticide poisoning data in Thailand showed that only 2.4% of workers with poisoning incidents led consulted a hospital, and there were many poisoning cases never reported to a doctor or hospital. Farmers are the most exposed and the highest risk group for pesticide poisoning. According to the Ministry of Public Health report, most of the acute poisoning cases were associated with the organophosphate and carbamate pesticides (The Ministry of Public Health, 2003).

Other studies report that most Thai farmers were not aware or concerned about the potential hazards of pesticides. Most pesticide poisoning among farmers was the result of their behavior of spraying, mixing by bare hand and pesticide handling without proper knowledge (Jungbluth, 1996). Several assumptions have been made to relate symptoms or illness of cholinesterase enzyme inhibition to pesticide exposure level. First, poisoning cases are mainly due to potentially exposure to highly hazardous pesticides. Second, susceptibility on genetic control is attributed specifically to the inhibition of cholinesterase enzyme and the pesticide hydrolysis mechanism depends on the paraoxonase enzyme.

1.4 Molecular biology of enzymes

Paraoxonase and cholinesterase have been proposed as biomarkers of susceptibility to organophosphate toxicity. The human paraoxonase is determined by the PON1 gene, which is located on chromosome 7q21.3. Paraoxonae is a common substrate used to measure enzyme activity. The PON1 activity polymorphism has been shown to be an amino acid substitution at position 192 and 55 (Mackness, 1998).

The polymorphism at amino acid 192, A isoenzyme has glutamine, but B isoenzyme has arginine. This polymorphism could affect the hydrolysis of organophosphate compounds, which is a major detoxification route of pesticides. Another polymorphism is found at amino acid 55 where a Leucine is substituted by methionine. However, amino substitution at the 55 position is believed not to affect paraoxonase activity.

Paraoxonase enzyme activities have been shown to have a wide range in the healthy population and to be under genetic control. Inter-individual differences in the activity of these enzymes may contribute to variations in susceptibility of man to toxic effects of organophosphate compounds. Exposure to organophosphates has acute effects on health but evidence of chronic effect is unclear. The reasons for susceptibility to organophosphate pesticides were explained through PON-1 genetic polymorphism (Mackness, 1998; Allebrantdt, 2002).

Accordingly, the paraoxonase enzyme is a major detoxifier of organophosphate pesticide, which is determined by PON-1 gene. On the other hand, the cholinesterase enzyme is a biomarker of pesticide exposure and indicate an effect on neurological function. Cholinesterase monitoring has been used worldwide and is recommended by the World Healt Organization (WHO) as a means of monitoring for illness prevention among workers exposed to organophosphate pesticides (WHO, 1972).

Although cholinesterase is a good indicator for pesticide exposure, variants of cholinesterase activity are frequently misdiagnosed. The genetic variants of cholinesterase are two loci. E1 is a cholinesterase synthesis gene and E2 is associated with isoenzyme synthesis of genetic variant. E1 locus is located on chromosome 3 in the region 3q 21-25 and at least 4 alleles are usually found on locus E1. The best known alleles are usual (U), the atypical (A), the fluoride resistant (F) and the silent (S). All combinations of heterozygotes have been found and serum cholinesterase activity depends on their genotype. Enzyme activity might reduce their affinity to the substrate or decrease their activity. However, delineation of cholinesterase phenotype requires the use of at least inhibitors. Dibucaine and Fluoride were developed as cholinesterase inhibitors and stratified percentage of cholinesterase inhibitor to delineated U, A, S and F phenotypes (Kalow 1957; 1982; Pinto, 1996).

The heterozygotes of UU, UA and AA cholinesterase show Dibucaine number (DN) between 71-90, less than 20 and 40-70, respectively. Sodium fluoride is another inhibitor used to delineate of FF phenotype, the resistant homozygous gene; cholinesterase activity is as low as zero (Loockridge, 1990). Both paraoxonase and cholinesterase enzymes are included in esterase classification. The paraoxonase enzyme is involved in the detoxification mechanism through the hydrolysis reaction. Cholinesterase could be inhibited by pesticides and thus can play a role as an exposure monitoring enzyme. Paraoxonase and cholinesterase phenotypes will be determined in this study for assessment of the health impact among people who have been exposed to pesticides.

Another important of health risk for farmers is the lack of awareness and knowledge of proper use and handling while using potentially highly hazardous pesticides. However, the health impact assessment is not enough for the farmers to reconsider their exposure and practical behaviors. The study of farmers' susceptibility to pesticides toxicity is another strong support that can enable the farmers to decrease pesticide exposure in their farms.

Cholinesterase monitoring has been used as an indicator of illness in workers exposed to organophosphate pesticides. But the enzyme activity might be reduced due to genetic variants of cholinesterase. Low level of paraoxonase enzyme could be involved in detoxification of pesticide and it might be related to cholinesterase level.

1.5 Objectives of the present study

a. To determine the cholinesterase genetic variants in Thai farmers.

b. To determine the PON1 gene polymorphism in Thai farmers.

c. To determine the relationship between genetic polymorphism and activities of cholinesterase and paraoxonase in Thai farmers.

d. To determine the relationship between genetic polymorphism and enzyme activity of cholinesterase and paraoxonase enzyme in pesticide exposed farm workers.

1.6 Education and Advantage applications

a. Gaining knowledge of relationships among cholinesterase enzyme activity, their genetic variants, and the health impact in the Thai population.

b. Understanding the relationship among paraoxonase enzyme activity, polymorphism of PON-1 gene, and pesticide susceptibility.

c. Gaining more knowledge of genetic variants in the Thai population.

d. Obtaining the optimized method for PON-1 gene determination by using PCR technique.