CHAPTER I

INTRODUCTION

1. Statement and significance of the problem

The mosquito *Aedes aegypti* is the major vector of dengue and dengue hemorrhagic fever, which are serious public health problems worldwide. In Thailand, there were 57,948 reported cases of dengue in 2010, which were increased about 130% as compared to 2009 (WHO, 2010). So, there is a need to strengthen dengue surveillance, prevention and control systems. Currently, a dengue vaccine is not available, therefore the vector control is still of importance to reduce transmission risk of the disease. Vector control is implemented using environmental management methods through preventing or reducing vector propagation and chemical-based control methods (WHO, 1997).

For decades several insecticides such as DDT, organophosphate and carbamate compounds have been heavily used for vector control. However, the removal of DDT was replaced by pyrethroids in 1990s for using in agriculture and public health because of reported vector resistance and perceived adverse impact on the environment (Neely, 1964; Charoenviriyaphap *et al.,* 1999). At present, pyrethroid insecticides are widely used for controlling *Ae. aegypti* at household and community levels because of their less toxicity to vertebrate. Consequently, long-term and heavy using of insecticides for mosquito vector control has caused the resistance of *Aedes* mosquito due to selection pressure. The first report of pyrethroid resistance in *Ae.*

aegypti in northern Thailand was published by Somboon *et al.* (2003). The results revealed that this species has been highly resistant to DDT. In some areas, it has been also resistant to permethrin and deltamethrin, likewise in other research groups showing the moderate levels of permethrin resistance in *Ae. aegypti* in Thailand (Ponlawat *et al.,* 2005; Jirakanjanakit *et al.*, 2007a, 2007b).

There are two major mechanisms of insecticide resistance, i.e. target-site resistance and detoxification enzyme-based resistance. The target-site resistance, known as knockdown resistance (*kdr*), is the mechanism that occurs when the insecticide no longer binds to its target (Soderlund, 2008). The latter mechanism occurs when enhanced levels or modified activities of detoxification enzymes, e.g. esterase, glutathione s-transferase and monooxygenase, prevent the insecticide from reaching its site of action (Brogdon and McAllister, 1998; Hemingway and Ranson, 2000).

Cytochrome P450 monooxygenases are one of the important enzyme systems associated with insecticide detoxification (Hodgson, 1985; Berge *et al.,* 1998; Scott *et al.,* 1998; Feyereisen, 1999; 2006; Prapanthadara *et al.,* 2002; Strode *et al.,* 2008). Many studies have shown the elevated cytochrome P450 monooxygenase activity in many kinds of mosquitoes associated with pyrethroid resistance such as *Anopheles gambiae*, *An. stephensi*, *Culex quinquefasciatus*, and *Ae. aegypti* (Hemingway and Ranson, 2000; Prapanthadara *et al.,* 2002; Nikou *et al.,* 2003; Hardstone *et al.,* 2007; Strode *et al.,* 2008). There were at least two studies suggested that cytochrome P450 may be involved in permethrin resistance in *Ae. aegypti*. Prapanthadara *et al.* (2002) using biochemical assay to investigate the mechanisms of DDT and permethrin resistance reported a significantly increased amount of cytochrome P450 in the

resistant strain. Strode *et al.* (2008) using microarray study has shown over expression of many cytochrome P450 genes in resistant *Aedes* mosquitoes, in particular the CYP6, CYP9 and alpha esterase families.

Although the elevation of cytochrome P450 in insecticide resistance is well recognized, little is known about the metabolic pathway of this enzyme in insect vector. The aim of this study is to define enzymatic involvement in permethrin metabolism pathway in *Ae. aegypti* resistant strain. The knowledge of the resistance mechanism by the function of cytochrome P450 (in the sense of metabolic profile) and/or other enzymes could help to develop and provide some possible management of vector control strategies.

2. Literature Review

2.1 *Aedes aegypti* and insecticide resistance

Aedes aegypti is the major vector of urban yellow fever in the African and American tropics and is also the primary vector of dengue virus throughout most of the tropical world. In Thailand, this species acts as a major vector of dengue and dengue hemorrhagic fever, which are serious public health problems. People suffer from dengue attacks annually, and the number of reported cases continues to increase with 57,948 cases reported in 2010 (Figure 1) (WHO, 2010). Although some vector-transmitting diseases such as Japanese encephalitis and yellow fever have been reasonably brought under control by vaccination, no effective vaccine is available for dengue (Mustafa and Agrawal, 2008). Therefore, the vector control is still important to reduce transmission risk of the disease. The use of insecticides to kill vectors often has been the only feasible method of disease control. There are many kinds of insecticides that are or were used to control *Aedes* mosquito vectors, including organochlorines (DDT), organophosphates (temephos, fenitrothion, malathion and chlopyrifos), carbamates (propoxur, pirimiphodmethyl and bendiocarb), pyrethroids (permethrin, deltamethrin, lamda-cyhalothrin and etofenpox) and biologicals control agents (*Bacillus thuringiensis israelensis* and *Bacillus sphaericus*) (Bang *et al.,* 1969; Charoenviriyaphap *et al.,* 1999).

For years, apart from malaria control, DDT was widely used to control *Aedes* mosquitoes with great success in interrupting dengue vector. DDT has been withdrawn since 1995 in Thailand and its use in other countries has been decreasing over time until recently. The reasons for the removal of DDT were because of reported vector resistance and perceived adverse impact on the environment

(Charoenviriyaphap *et al.* 1999). Eventually, several new and promising insecticides such as organophosphorus insecticides, carbamates and pyrethroids have been recommended for use against agricultural pests and disease vectors. At present, synthetic pyrethroids (e.g., permethrin, deltamethrin) are the current insecticides of choice used in controlling vector-borne diseases throughout worldwide due to their low toxicity in humans, and rapid killing effect on the insect. These insecticides have been widely used to control numerous insects such as house fly (*Musca domestica*), fruit fly (*Drosophila melanogaster*) and mosquito vectors (*An. gambiae*, *An. stephensi*, *Cx. quinquefasciatus*, and *Ae. aegypti*) (Kasai and Scott, 2000; Nikou *et al.*, 2003; Hardstone *et al.*, 2007).

The first report of pyrethroid-resistance in *Ae. aegypti* in Thailand was published by Somboon *et al.* (2003). The results revealed that *Ae. aegypti* has been highly resistant to DDT and in some areas, it has been also resistant to permethrin and deltamethrin; the latter two are currently used for controlling dengue vectors. Later, many studies have revealed the occurrence of pyrethroid resistance in *Ae. aegypti* from many provinces throughout Thailand (Paeporn *et al.*, 2004; Ponlawat *et al.*, 2005; Pethuan *et al.*, 2007).

2.2 Biology of insecticide resistance and resistance management

Resistance is defined by the World Health Organization as "the development of an ability in a strain of some organism to tolerate doses of a toxicant that would prove lethal to a majority of individuals in a normal population of the same species". This ability is brought a result of the selective effects to withstand the insecticides (WHO, 1975). The insecticide resistance might be produced as a result of selective

survival after use of insecticides in the population. Selection increases the frequency of resistance in the population generation by generation and the survival could develop and produce a progeny which represent a larger proportion of resistance.

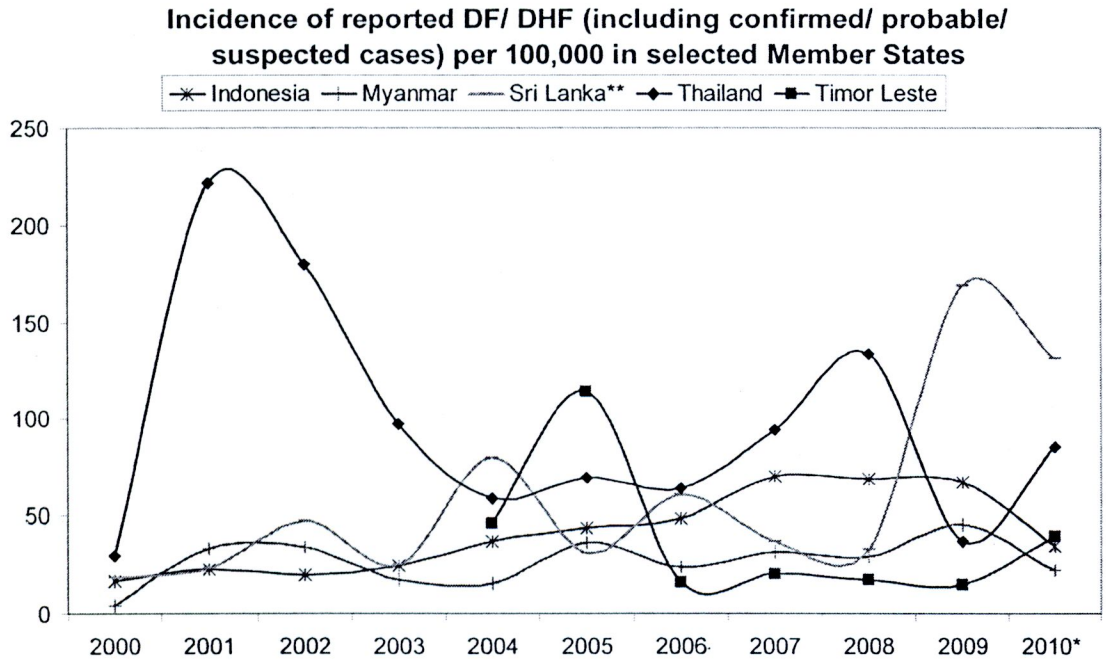

Figure 1 Dengue and dengue hemorrhagic fever cases in Thailand, 2000-2010 (WHO, 2010).

One explanation for the high resistance levels is the fact that the increasing on the volume and frequency of applications of insecticides used against them. In the past, resistant populations were controlled by increasing the amounts of insecticide applied or replacing older chemicals with new, more effective compounds. The mixtures of insecticides with different modes of action, rotations in time, or spatial patterns of applications have been used in management resistance.

These strategies are of limited value today. These considerations lend urgency to the need for other measures to prevent or reduce the impact of resistance. These measures have been called "resistance management". Today the major emphasis in resistance research is on the basis of molecular characterization of resistance genes and their biochemical products which are important to develop effective resistance management strategies.

2.3 Permethrin and permethrin metabolism

Permethrin, a pyrethroid insecticide, is a synthetic structure derivative of insecticidal pyrethrins. The basic structure (Figure 2) is esters, with an alcohol and an acid moiety. It is an ester of the dichloro analogue of chrysanthemic acid, chemically identified as (3-phenoxyphenyl)methyl-(±)-*cis-trans*-3(2,2-dichloroethyenyl)-2,2 dimethylcyclopropanecarboxylate.

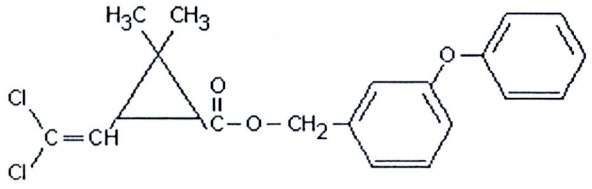

Figure 2 Permethrin structure (Miyamoto *et al.,* 1981).

Modes of action of permethrin are a functional toxin as acts on the axons in the peripheral and central nervous systems of insects, resulting a prolonged opening of sodium channels that causes a complete deporalization of the nervous membrane with blockage of the neuronal excitability. The symptoms poisoning of permethrin on insects are restlessness, incoordination, hyperactivity and paralysis (Aldridge, 1990;

Miyamoto *et al.,* 1995; McGregor, 1999; Sogorb and Vilanova, 2002). Permethrin is unstable when exposed to UV light. This insecticide is considered to be safe because it is easily converted to putatively non-toxic derivatives by hydrolysis in mammalian species (Sogorb and Vilanova, 2002; Ross *et al.,* 2006). In insects, however, the hydrolytic activity is low, accounting for the selective toxicity of the pesticides (Ahmad and Forgash, 1976).

Permethrin degradations have been found to be metabolized mainly through cleavage at the ester linkage by esterases to give the corresponding alcohols which are subsequently oxidized to the carboxylic acids. They are mainly found in liver microsomal fraction of various mammalian species (Miyamoto *et al.*, 1976). Nakamura *et al.* (2007) reported the postulated metabolic pathway of permethrin in rat liver; permethrin was metabolized to 3-phenoxybenzyl alcohol (PBOH), 3-phenoxybenzylaldehyde (PBCHO) and 3-phenoxybenzoic acid (PBCOOH) (Figure 3). The former two metabolized products are more toxic than the parent compound (or permethrin).

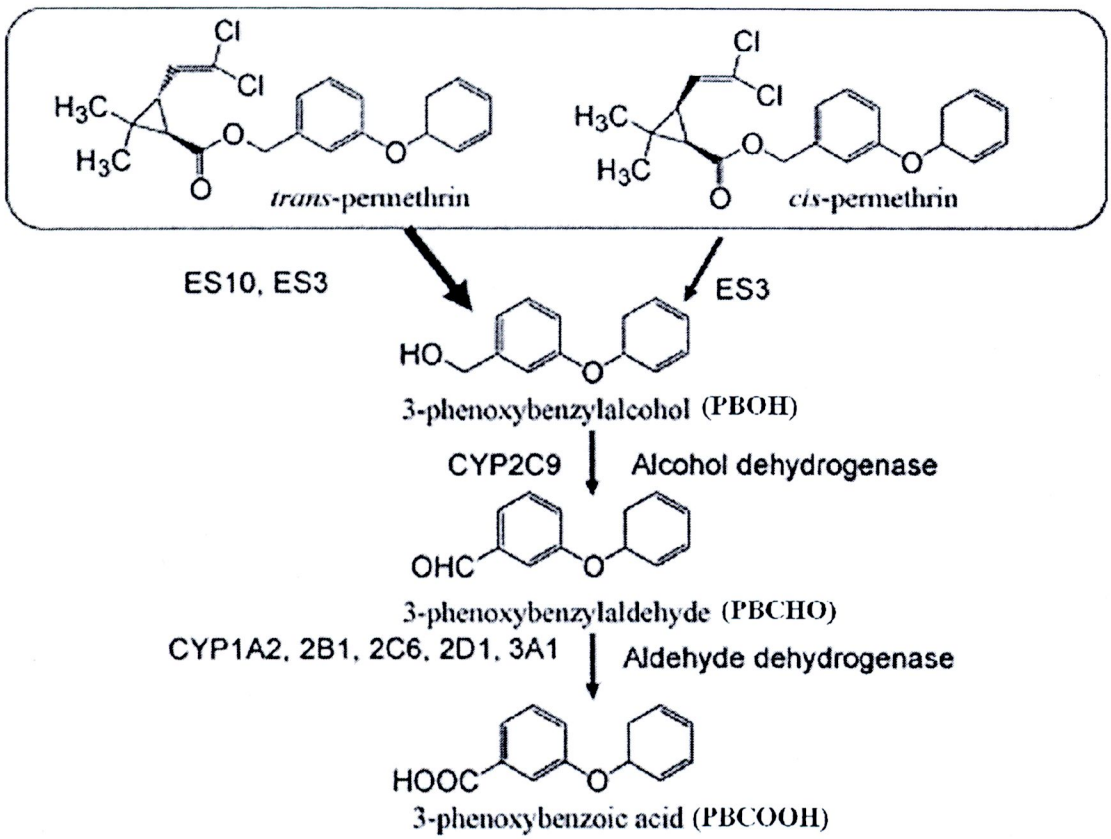

Figure 3 Postulated metabolic pathways of *cis*- and *trans*- permethrins by rat liver preparations (modified from Nakamura *et al.*, 2007).

2.4 Permethrin resistance

Permethrin resistance in *Ae. aegypti* has been reported in many studies. In northern Thailand, populations of *Ae. aegypti* resistant to DDT and permethrin have been isolated (Somboon *et al.*, 2003). Preliminary biochemical characterization of the DDT and permethrin resistance showed 10 fold higher levels of DDTase activity, 4 fold higher levels of cytochrome P450 content and slightly increased GST activities compared to the susceptible strain (Prapanthadara *et al.*, 2002). The over expression of some cytochrome P450 genes in resistant strains were reported (Strode *et al.*, 2008). Subsequently, the mutation in the sodium channel protein, which is the target site for both DDT and pyrethroid insecticides producing a *kdr* phenotype, may play an important role for permethrin resistance in *Ae. aegypti* (Yanola *et al.*, 2010; 2011). According to the above, resistance to permethrin may cause by many mechanisms (Figure 4).

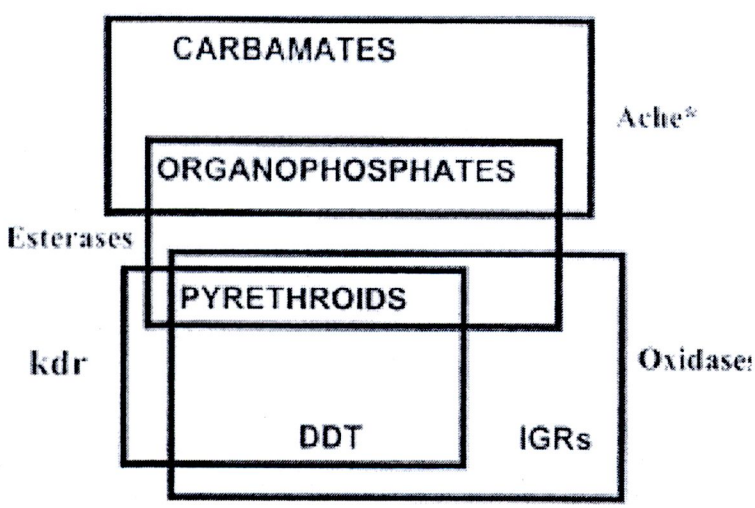

Figure 4 Cross-resistance relationships of commonly used classes of insecticides (Brogdon and McAllister, 1998). Ache* = Acetylcholinesterases, IGRs = Insect growth regulators.

2.5 Insecticide resistance mechanisms

2.5.1 Target site mechanism

Site-insensitivity is an alteration of amino acids of the insecticide's target site which occurs when the insecticides no longer binds to its target that causes the insecticide to be less effective or even ineffective. For example, the target site of organophosphorus insecticides (OPs) (e.g., malathion, fenitrothion) and carbamate (e.g., propoxur) is acethylcholinesterase in nerve synapses. The target site of organocholines (DDT) and synthetic pyrethroids are the sodium channels of the nerve sheath, and the target site of cyclodiene is the γ-aminobutyric acid (GABA) receptor (Brogdon and McAllister, 1998). DDT-pyrethroid cross resistance may be produced by changing single amino acid in the axonal sodium channel insecticide-binding site, known as knockdown resistance (*kdr*) (Brengues *et al.*, 2003).

2.5.2 Metabolic detoxification mechanism

Regarding detoxification mechanisms, they occur when there are enhanced levels or modified activities to prevent the insecticide from reaching its site of action. There are three major enzyme groups being involved for detoxification of xenobiotics in living organisms. Those include members of large multigene families of hydrolase (including esterases), mono-oxygenase (mixed-function oxidases, microsomal oxidases or cytochrome P450 dependent oxidases) and Glutathione S-transferases (GST). Esterases are the most common enzymes and extremely important in resistance to organophosphate, carbamate, and to a lesser extent in pyrethroid resistance (Hemingway and Ranson, 2000; Hemingway *et al.*, 2004). The elevated esterases act effectively as an insecticide sink, rapidly binding and slowly

metabolizing the insecticides. GSTs are primarily involved in DDT, organophosphate and pyrethroid resistance. Monooxygenases, the cytochrome P450-dependent monooxygenases (MFOs), are a large group of oxidative enzymes. These enzymes confer resistance primarily to pyrethroids and carbamates, and to a lesser extent organophosphates and organochlorines (Marquardt, 2005).

Enzymes involved in the detoxification of insecticides

2.5.2.1 Esterase-based resistance

Esterase-based resistance is one of the common resistance mechanisms in insects that metabolizes a wide range of insecticides. Esterase enzymes metabolize many lipophilic xenobiotics by hydrolyzing to the alcohol and carboxylic acid. These metabolites are much more water soluble compounds that are easy to excretion (Brogdon and McAllister, 1998; Hemingway, 2000). In mosquitoes, this mechanism has extensively studied in *Culex*, especially involved broad spectrum in organophosphate resistance.

Study by Pethuan *et al.* (2007) revealed that nonspecific esterase activities were increased in *Ae. aegypti* resistant pyrethroid from Thailand. According to metabolic study on DDT and permethrin resistance in *Ae. aegypti* from northern Thailand, there was a slightly increase of esterase activity compared to the susceptible strain (Prapanthadara *et al.*, 2002).

2.5.2.2 Glutathione S-transferase (GST-based resistance)

Glutathione S-transferases (GST) are major phase II detoxification enzymes. GST can produce resistance to a range of insecticides by conjugating reduced

glutathione (GSH) to the insecticide which is converted to soluble compound, thereby aiding excretion (Hemingway, 2000; Lumjuan *et al.*, 2007). GST-based resistance has been reported for both organophosphate and DDT in many insects such as mosquitoes (*An. gambiae*, *Ae. aegypti*) or flies (*D. melanogaster*) (Hemingway and Ranson, 2000).

In *Ae. aegypti*, there are 26 GST genes which are identified to 6 classes : Delta, Epsilon, Omega, Sigma, Theta and Zeta. The Delta and Epsilon classes are both insect specific whereas the remaining classes are also found in other organisms. In the Epsilon class GST, GSTE2 has been involved in DDT resistance in both *An. gambiae* and *Ae. aegypti*. The recombinant GSTE2 protein is very effective to metabolize DDT and expression of this protein is elevated in DDT resistant *An. gambiae* from East Africa and *Ae. aegypti* from Thailand (Lumjuan *et al.*, 2005; 2007).

2.5.2.3 Oxidases-based resistance

1. Dehydrogenases

Dehydrogenases are enzymes that oxidize a substrate by transferring one or more hydrides (H^-) to an acceptor, usually NAD^+/$NADP^+$ as a coenzyme.

1.1 Alcohol dehydrogenase

Alcohol dehydrogenase (ADH) enzyme catalyses the oxidation of many alcohols to the corresponding aldehydes (Figure 5). This enzyme uses NAD^+ as co-factor.

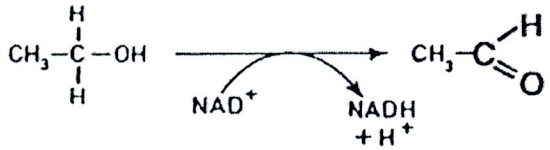

Figure 5 The oxidation of ethanol by alcohol dehydrogenase (Gibson and Skett, 1994).

1.2 Aldehyde dehydrogenase

Aldehyde dehydrogenase (ALDH) enzyme catalyses the oxidation of aldehydes to the carboxylic acid (Figure 6).

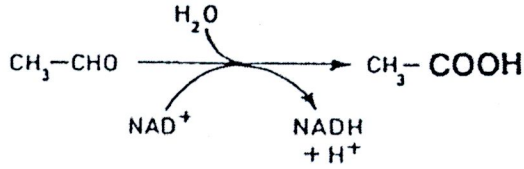

Figure 6 The oxidation of acetaldehyde (Gibson and Skett, 1994).

Alcohol and aldehyde dehydrogenases have been reported that they are involved in mammalian permethrin metabolism. Permethrin is first hydrolyzed to phenoxybenzyl alcohol (PBOH) and further oxidized to phenoxybenzylaldehyde (PBCHO) and phenoxybenzoic acid (PBCOOH) by alcohol and aldehyde dehydrogenases, respectively (Choi *et al.*, 2002; Nakamura *et al.*, 2007). However, little is known about the involvement of these enzymes with permethrin metabolism in *Ae. aegypti*.

2. Oxidations involved the mixed-function oxidase (Cytochrome P450)

The cytochrome P450 monooxygenases are ubiquitous family of enzymes, presenting from bacteria to mammals. They are involved in endogenous metabolism such as hormones, fatty acids, as well as in the metabolism of xenobiotics such as drugs, pesticides and plant toxins. Cytochrome P450 is a hemoprotein which acts as the terminal oxidase in monooxygenase systems. There is so-called "cytochrome P450" because the unique absorbance spectrum that is produced when CO is bound to the reduced, ferrous form of the heme. The spectrum exhibits a peak at approximately 450 nm (Omura and Sato, 1964). In the reaction cycle of cytochrome P450, it binds molecular oxygen and receives electrons from nicotinamide adenine dinucleotide phosphate (NADPH) to introduce a single oxygen atom into the substrate and to form water with the other oxygen atom according to the reaction:

$$\text{Substrate (S)} + \text{NADPH} + H^+ + O_2 \rightarrow \text{S(O)} + \text{NADP}^+ + H_2O$$

The electrons necessary for this reaction are transferred from NADPH on the substrate-P450 complex by an NADPH cytochrome P450 reductase, but this reaction can also be stimulated by cytochrome b_5 (Devlin, 2006). The overall reaction cycle of cytochrome P450 is shown in Figure 7.

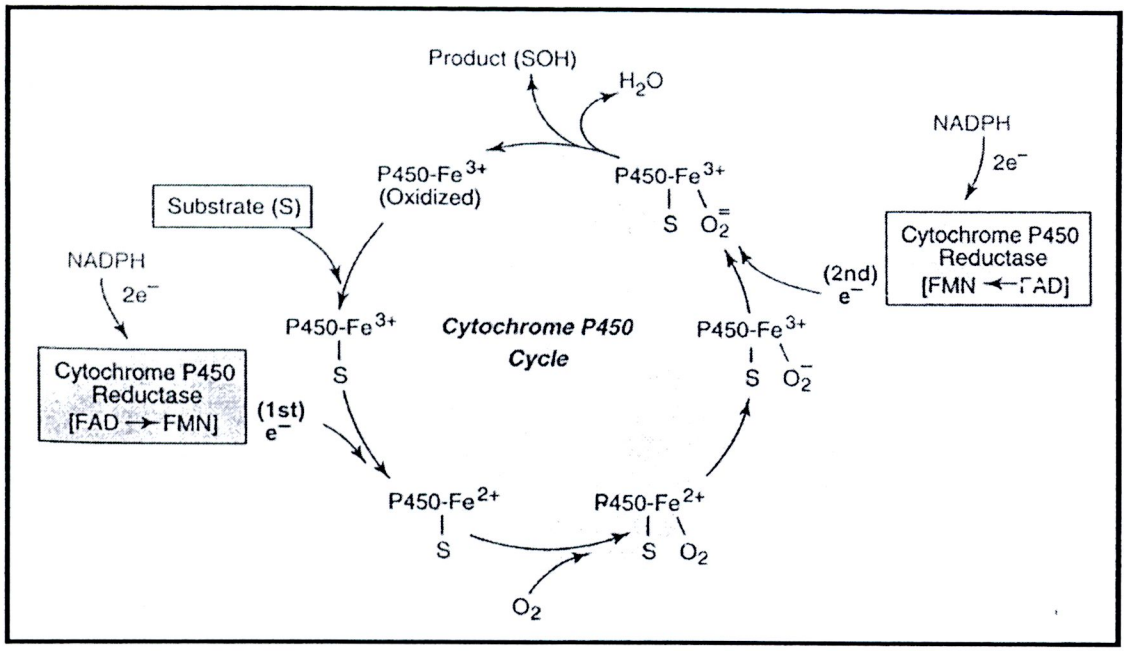

Figure 7 Reaction cycle of cytochrome P450. Diagram shows the binding of substrate, transfer of the first and second electrons from NADPH-cytochrome P450 reductase, and binding of O_2 (Devlin, 2006).

Cytochromes P450 can metabolize a variety of lipophilic compounds of endogenous or exogenous compounds by hydroxylation, epoxidation, O^-, N^- and S^- dealkylations, N^- and S^- oxidation reactions (Table 1). They oxidize the primarily lipophilic (hydrophobic) compounds to make them more water soluble (hydrophilic) for excretion.

Table 1 Reactions catalyzed by cytochromes P450 (Sono *et al.*, 1996; Bernhardt *et al.*, 2006)

Reactions performed by cytochromes P450
Hydrocarbon hydroxylation
Alkene epoxidation
Alkyne oxygenation
Arene epoxidation
Aromatic hydroxylation
N-Dealkylation
S- Dealkylation
O- Dealkylation
N-Hydroxylation
N-Oxidation
S-Oxidation
Oxidative deamination
Oxidative dehalogenation
Alcohol and aldehyde oxidations
Dehydrogenation
Dehydratations
Reductive dehalogenation
N-Oxide reduction
Epoxide reduction
Reductive β-scission of alkyl peroxides
NO reduction
Isomerizations
Oxidative C-C bond cleavage

The nomenclature of P450 has been designated for all gene members of the P450 superfamily having a CYP prefix, for cytochrome P450, followed by an Arabic

numeral (e.g., CYP1, CYP2, CYP3). The subfamily is identified by an additional Arabic, capital letter (e.g., CYP1A1, CYP1B, CYP1C) and the individual members of each subfamily are then numbered in the order in which they are identified (e.g., CYP1A1, CYP1A2, CYP1A3). All members of a family share more than 40% identity at the amino acid sequence level, and members of a subfamily share more than 55% identity (Nelson, 1996; Berge *et al.,* 1998; Feyereisen, 1999; Scott, 1999).

Insect P450 enzymes

In insects, P450 enzymes have several functional roles such as in the biosynthetic pathways of ecdysteroids and juvenile hormones, which are at the center stage of insect growth, development, feeding and reproduction and moreover found in the metabolism of insecticides, resulting either in bioactivation or in detoxification, the latter process being enhanced in resistance to insecticides (Hodgson, 1985; Feyereisen, 1999). The structure and function of P450 enzymes have indeed been conserved from bacteria to *D. melanogaster* and the catalytic cycle is as mentioned above (Berge *et al.,* 1998; Feyereisen, 1999; Scott, 1999).

Expression of insect P450 genes are highly found in many tissues such as the digestive tract, fat body and malpighian tubules being a rich source of P450 (Hodgson, 1983). In general, total P450 levels are undetectable in eggs, rise and fall in each larval stage, undetectable in pupae and expressed at high levels in adults (Agosin, 1985). The patterns of expression of individual P450s can vary within and/or between life stages. Currently, a large number of P450s were purified and characterized. Insect P450s have been assigned to six CYP families: five are insect specific (CYP6, 9, 12, 18 and 28), and another one is CYP4 that shares with

sequences from vertebrates (Berge *et al.*, 1998). A summary of the number of insect P450s is shown in Table 2 (Scott, 2008).

There are two reasons for demonstrating that "why there are so many P450s in some insect species". First, insect P450s are considered in xenobiotic detoxification as the primary purpose. Second, the majority of insect P450s may not be involved in xenobiotic detoxification, but rather in the metabolism of other compounds. The estimation of the relative percentage of P450s involved in various biological processes of a generalized insect is shown in Table 3 (Scott, 2008). However, it should be pointed out that P450s may be able to metabolize both xenobiotics and endogenous compounds.

Table 2 Total number of P450s (excluding pseudogenes) determined from the genomes of six insect species, relative to four mammals (Scott, 2008)

Species	**P450s**
Aedes aegypti	164
Tribolium castaneum	134
Anopheles gambiae	105
Drosophila melanogaster	84
Drosophila pseudoobscura	79
Apis mellifera	48
Mus musculus	102
Ratus norvegicus	89
Homo sapiens	57
Canis familiaris	54

Table 3 A highly speculative estimation of the relative percentages of P450s involved in various biological processes of a generalized insect (Scott, 2008)

% of P450s involved	Process
65	Biosynthetic processes Hormone and pheromone biosynthesis (35-50%) Reproduction (5-15%) Pigmentation (5-10%) Other (10%)
30	Xenobiotic metabolism
5	Sensory physiology

Insect P450 and insecticide resistance

The most common types of resistance found in insects are increased enzymatic detoxification and target site insensitivity. Many reports revealed the metabolic resistance of insects to insecticides due to the results of enhanced P450 activities (Scott *et al.,* 1998; Feyereisen, 1999; Scott, 1999). For some insects, this enzyme is so active that the insecticide does not reach its molecular target before being metabolized and degraded by this enzyme that leads to insecticide resistance.

Monooxygenase-based resistance is most commonly due to increased detoxification by changing in the catalytic activity of the P450 and/or changing in the level of expression of the P450. Scott *et al.* (1998) has proposed two criteria for demonstrating that a P450 is involved in resistance: (1) the P450 must be shown to detoxify (or sequester) the compound to which the strain has monooxygenase-mediated resistance and (2) the resistant strain should have a greater amount of this

P450, or the protein coded for by the resistant strain allele should be shown to have a greater catalytic activity compared to the protein coded for by the susceptible strain allele.

There are several methods that can be used to determine the involvement of P450 enzymes in resistance. For example, the CYP6A1 was the first cloning P450 cDNA obtained from the house fly (Rutgers) resistance strain. Several PCR methods have been used to show that the mRNA of P450s overproduced in resistant strains (Scott *et al.,* 1999). In biochemical analysis, the application of P450 inhibitors such as the synergist piperonyl butoxide (PBO) is most commonly used as a diagnostic for P450 involvement. Treatment of resistant insects by PBO can result in a complete loss of resistance. However, resistance may be due to several mechanisms and the treatment with P450 synergist may not restore complete susceptibility.

***Aedes aegypti* P450s**

Ae. aegypti contains a total of 160 full length, putatively catalytically active P450 genes. Several large clusters of P450s are found in the *Ae. aegypti* genome, the largest being a cluster of 18 CYP6 and a cluster of 16 CYP9 genes. This represents an expansion of approximately 55% compared to *An. gambiae*, 86% compared to flies, *D. melanogaster* and ~ 3 fold higher than the number of P450s found in the honeybee, *Apis mellifera* (Table 4) (Ranson *et al.,* 2002; Claudianos *et al.,* 2006; Strode *et al.,* 2008).

Table 4 Classification of detoxification genes in *Drosophila melanogaster*, *Anopheles gambiae* and *Aedes aegypti* (Strode *et al.*, 2008)

	D. melanogaster	*An. gambiae*	*Ae. aegypti*
Glutathione transferases			
Delta	11	12	8
Epsilon	14	8	8
Omega	5	1	1
Sigma	1	1	1
Theta	4	2	4
Zeta	2	1	1
Others	0	3	3
Total	37	28	26
Cytochrome P450s			
CYP4 clade	32	46	57
CYP3 clade			
CYP6	22	30	44
CYP9	5	9	37
Others	9	1	1
CYP2 clade	7	10	12
Mitochondrial clade	11	9	9
Total	86	105	160
Carboxy/cholinesterases			
Alpha esterases	13	16	22
Hormone processsing			
Beta esterases	3	5	2
Juvenile hormone esterases	2	4	6
Others	3	4	7
Glutactin	4	9	10
Acetylcholinesterases	1	2	2
Total	26	40	49

As in other Diptera, the majority of the P450s are represented by the CYP3 and CYP4 clades, which contain the insect specific families, CYP4, CYP6, CYP9 and CYP325 and include the majority of enzyme implicated in xenobiotic metabolism and insecticide resistance (Tomita and Scott, 1995; Pittendrigh *et al.*, 1997; Nikou *et al.*, 2003; Strode *et al.*, 2008).

Prapanthadara *et al.* (2002) studied biochemical characterization of mechanisms of DDT and permethrin resistance in *Ae. aegypti* by measuring the

activities of DDTase, esterase and cytochrome P450. The results revealed 4-folds increasing activity of cytochrome P450 observed for both DDT and DDT/permethrin resistance comparing with the susceptible strain. This led to the conclusion that cytochrome P450 may also play some part in permethrin resistance in this mosquito. Currently, the analysis of detoxification genes in *Ae. aegypti* by Strode *et al.* (2008) has revealed the cytochrome P450 gene families which are associated with resistance to insecticides, in particular the CYP6 and CYP9 in *Ae. aegypti*.

3. Purposes of this study

3.1 To compare the amount and the activity of cytochrome P450 monooxygenase on permethrin metabolism between two strains of *Ae. aegypti*; permethrin susceptible (PMD) and permethrin resistance (PMD-R).

3.2 To determine the role of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) in detoxifying permethrin in vitro.

4. Significance of this research

This study will extend the knowledge about the role of cytochrome P450 monooxygenase in the permethrin resistant mechanism in *Ae. aegypti*. This will greatly facilitate the monitoring and management of insecticide-based *Aedes* control and may enhance the ability to control this major disease vector in the future.