We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

12

Metabolism of Pyrethroids by Mosquito Cytochrome P450 Enzymes: Impact on Vector Control

Pornpimol Rongnoparut[1], Sirikun Pethuan[1], Songklod Sarapusit[2] and Panida Lertkiatmongkol[1]
[1]*Department of Biochemistry, Faculty of Science, Mahidol University,*
[2]*Department of Biochemistry, Faculty of Science, Burapha University, Thailand*

1. Introduction

Cytochrome P450 enzymes (P450s) are heme-containing monooxygenases that catalyze metabolisms of various endogenous and exogenous compounds. These P450s constitute a superfamily of enzymes present in various organisms including mammals, plants, bacteria, and insects. P450 enzymes are diverse and metabolize a wide variety of substrates, but their structures are largely conserved. A universal nomenclature has been assigned to P450 superfamily based on their amino acid sequence homology (Nelson et al., 1996). In eukaryotes, P450 is membrane-bound and in general functions to insert one molecule of oxygen into its substrate, with its heme prosthetic group playing a role in substrate oxidation. This catalytic reaction requires a pair of electrons shuttled from NADPH via the NADPH-cytochrome P450 reductase (CYPOR) enzyme, a P450 redox partner, to target P450s (Ortiz de Montellano, 2005). In contrast in bacteria and mitochondria, ferredoxin reductase and iron-sulfur ferredoxin proteins act as a bridge to transfer reducing equivalent from NAD(P)H to target P450s. In insects, P450s are membrane-bound enzymes that play key roles in endogenous metabolisms (i.e. metabolisms of steroid molting and juvenile hormones, and pheromones) and xenobiotic metabolisms, as well as detoxification of insecticides (Feyereisen, 1999). It becomes evident that P450s are implicated in pyrethroid resistance in insects.

Insecticides form a mainstay for vector control programs of vector-borne diseases. However intensive uses of insecticides have led to development of insecticide resistance in many insects thus compromising success of insect vector control. In particular pyrethroid resistance has been found widespread in many insects such as house flies, cockroaches, and mosquitoes (Acevedo et al., 2009; Awolola et al., 2002; Cochran, 1989; Hargreaves et al., 2000; Jirakanjanakit et al., 2007). Two major mechanisms have been recorded responsible for insecticide resistance, which are alteration of target sites and metabolic resistance (Hemingway et al., 2004). Metabolic resistance is conferred by increased activities of detoxification enzymes such as P450s, non-specific esterases (Hemingway et al., 2004; Price, 1991). Initial approaches to detect involvement of detoxification mechanisms in metabolic resistance are to compare activities of detoxification enzymes between resistant and

susceptible insect strains, and by identification of corresponding genes that display higher expression level in resistant insects (Bautista et al., 2007; Chareonviriyaphap et al., 2003; Tomita et al., 1995; Yaicharoen et al., 2005). Examinations in various insects such as house fly, cotton ballworm, and mosquito have implicated involvement of up-regulation of different P450 genes in pyrethroid resistance (Liu & Scott, 1998; Müller et al., 2007; Ranasinghe & Hobbs, 1998; Rodpradit et al., 2005; Tomita et al., 1995). Such P450 overexpression has been assumed constituting a defense mechanism against insecticides and responsible for insecticide resistance, presumably by virtue of enhanced insecticide detoxification.

Recent advanced methods employing microarray-based approach, when genomic sequence information for insects is available, have identified multiple genes involved in pyrethroid resistance in mosquitoes. Genes in CYP6 family, in particular, are reported to have an implication in insecticide resistance. In *Anopheles gambiae* malaria vector, microarray analyses reveal that several CYP6 P450 genes could contribute to pyrethroid resistance, these include CYP6M2, CYP6Z2 and CYP6P3 (Djouaka et al., 2008; Müller et al., 2007). These genes were observed up-regulated in pyrethroid resistant mosquitoes (Müller et al., 2008; Stevenson et al., 2011). CYP6M2 and CYP6P3 have shown ability to bind and metabolize pyrethroids, on the other hand CYP6Z2 is able to bind pyrethroids but does not degrade pyrethroids (Mclauglin et al., 2008). Genetic mapping of genes conferring pyrethroid resistance in *An. gambiae* also supports involvement of CYP6P3 in pyrethroid resistance (Wondji et al., 2007). Up-regulation of CYP6 genes has also been found in other resistant insects, for instance CYP6BQ9 in pyrethroid resistant *Tribolium castaneum* (Zhu et al., 2010), CYP6D1 in *Musca domestica* that is able to metabolize pyrethroids (Zhang & Scott, 1996), and CYP6BG1 in pyrethroid resistant *Plutella xylostella* (Bautista et al., 2007). In *T. castaneum* knockdown of CYP6BQ9 by dsRNA resulted in decreased resistance to deltamethrin (Zhu et al., 2010). Similar finding has been observed for CYP6BG1 in permethrin resistant *P. xylostella*, supporting the role of overexpression of these CYP6 genes in pyrethroid resistance (Bautista et al., 2009). In *An. minimus* mosquito, CYP6AA3 and CYP6P7 are upregulated and possess activities toward pyrethroid degradation (Duangkaew et al., 2011b; Rongnoparut et al., 2003).

2. Cytochrome P450 monooxygenase (P450) and NADPH-cytochrome P450 reductase (CYPOR) enzymes isolated from *An. minimus*

In this chapter, we focus on investigation of the P450s that have been shown overexpressed in a laboratory-selected pyrethroid resistant *An. minimus* mosquito. We describe heterologous expression of the overexpressed P450s in baculovirus-mediated insect cell expression system and characterization of their catalytic roles toward pyrethroid insecticides. Tools utilized in functional investigation of *An. minimus* P450s have been developed and described. In parallel the *An. minimus* CYPOR has been cloned and protein expressed via bacterial expression system. Amino acid sequence of *An. minimus* CYPOR is intrigue in that several important residues that might play role in its functioning as P450 redox partner are different from those of previously reported enzymes from mammals and house fly. The *An. minimus* CYPOR is different in enzymatic properties and kinetic mechanisms from other CYPORs. In this context we speculate that *An. minimus* CYPOR could influence electron delivery to target mosquito P450 enzymes, and could act as a rate-limiting step in P450-mediated metabolisms. These results together could thus gain an

insight into pyrethroid metabolisms in this mosquito species and knowledge obtained could contribute to strategies in control of mosquito vectors.

An. minimus is one of malaria vectors in Southeast Asia, including Thailand, Loas, Cambodia and Vietnam. We previously established a deltamethrin-selected mosquito strain of *An. minimus* species A, by exposure of subsequent mosquito generations to LD_{50} and LT_{50} values of deltamethrin (Chareonviriyaphap et al., 2002). Biochemical assays suggested that deltamethrin-resistant *An. minimus* predominantly employ P450s to detoxify pyrethroids (Chareonviriyaphap et al., 2003). We next set out on isolation of P450 genes that have a causal linkage in conferring deltamethrin resistance in this mosquito species. Using reverse-transcribed-polymerase chain reaction (RT-PCR) in combination with degenerate PCR primers whose sequences were based on CYP6 conserved amino acids, we have isolated CYP6AA3, CYP6P7, and CYP6P8 complete cDNAs from deltamethrin-resistant *An. minimus* (Rongnoparut et al., 2003). The three genes showed elevated transcription level in deltamethrin resistant populations compared to the parent susceptible strain. We found that fold of mRNA increase of CYP6AA3 and CYP6P7 is correlated with increase of resistance during deltamethrin selection. However, this correlation was not observed for CYP6P8 (Rodpradit et al., 2005). The three mosquito P450s could thus be used as model enzymes for characterization of their metabolic activities toward insecticides and possibly for future development of tools for mosquito vector control. This can be accomplished by determining whether they possess catalytic activities toward pyrethroid insecticides, thus assuming a causal linkage of overexpression and increased pyrethroid detoxification leading to pyrethroid resistance. Equally important, elucidating properties of the *An. minimus* CYPOR and its influential role in P450 system is beneficial for understanding of P450 metabolisms of this mosquito species.

2.1 *In vitro* insecticide metabolisms

We have heterologously expressed CYP6AA3, CYP6P7, and CYP6P8 in *Spodoptera frugiperda* (*Sf*9) insect cells via baculovirus-mediated expression system. The expression procedure employed full-length CYP6AA3, CYP6P7, and CYP6P8 cDNAs as templates to produce recombinant baculoviruses, and subsequently infected *Sf*9 cells for production of P450 proteins. RT-PCR amplification and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis were performed to verify expression of P450 mRNAs and proteins in the infected *Sf*9 cells. Expression of CYP6AA3, CYP6P7, and CYP6P8, each is predominantly detected in membrane fractions of infected cells after 72 hours of infection, with expected molecular size of approximately 59 kDa detected on SDS-PAGE (Kaewpa et al., 2007; Duangkaew et al., 2011b). The expressed proteins display CO-reduced difference spectrum of a characteristic peak at 450 nm (Omura & Sato, 1964). Total P450 content obtained from baculovirus-mediated expression of CYP6AA3, CYP6P7, and CYP6P8 ranges from 200 to 360 pmol/mg membrane protein. The expressed CYP6AA3, CYP6P7, and CYP6P8 proteins were used in enzymatic reaction assays testing against pyrethroids and other insecticide groups. Knowledge of the metabolic profile of these P450s could give us insight into functioning of these P450s within mosquitoes towards insecticide metabolisms, i.e. how mosquitoes detoxify against a spectrum of insecticide classes through P450-mediated metabolisms.

In enzymatic assay, each P450 in the reaction was performed in the presence of NADPH-regenerating system and was reconstituted with *An.minimus* CYPOR (Kaewpa et al., 2007), as CYPOR is required to supply electrons to P450 in the reaction cycle. Insecticide

metabolism was determined by detection of disappearance of insecticide substrate at different times compared with that present at time zero as previously described (Booseupsakul et al., 2008). This time course degradation was detected through HPLC analysis. Table 1 summarizes enzyme activities of CYP6AA3 and CYP6P7 toward insecticides and metabolites detected. Insecticides that were tested by enzyme assays were type I pyrethroids (permethrin and bioallethrin), type II pyrethroids (deltamethrin, cypermethrin, and λ-cyhalothrin), organophosphate (chlorpyrifos), and carbamate (propoxur). Additional insecticides (bifenthrin, dichlorvos, fenitrothion, temephos, and thiodicarb) belonging to these four insecticide classes were tested by cytotoxicity assays (see Section 2.3). Chemical structures of these insecticides are shown in Fig. 1.

Insecticides	CYP6AA3 Activity (metabolites)	CYP6P7 Activity
Type I pyrethroids		
Bioallethrin	-	-
Permethrin	+ (1 major unknown product)	+, ND
Type II pyrethroids		
Cypermethrin	+ (3-phenoxybenzaldehyde and 2 unknown products)	+, ND
Deltamethrin	+ (3-phenoxybenzaldehyde and 2 unknown products)	+, ND
λ - Cyhalothrin	+, ND	-
Organophosphate		
Chlorpyrifos	-	-
Carbamate	-	-
Propoxur	-	-

Table 1. Presence (+) and absence (-) of P450 activities in insecticide degradation and metabolites obtained. ND, products not determined

The results shown in Table 1 demonstrate that CYP6AA3 and CYP6P7 share overlapping metabolic profile against both type I and II pyrethroids, while no detectable activity was observed toward chlorpyrifos and propoxur (Duangkaew et al., 2011b), nor in the presence of piperonyl butoxide (a P450 inhibitor). Differences in activities of both enzymes could be noted, for CPY6AA3 could metabolize λ-cyhalothrin while CYP6P7 did not display activity against λ-cyhalothrin. For CYP6P8 we initially detected absence of pyrethroid degradation activity, further tests using cytotoxicity assays described in Section 2.3 suggest that CYP6P8 is not capable of degradation of pyrethroids, organophosphates and carbamates.

Determination of products obtained from CYP6AA3-mediated pyrethroid degradations using GC-MS analysis reveal multiple products for type II pyrethroid cypermethrin degradation and for earlier described deltamethrin metabolism (Boonseupsakul et al., 2008). These products were 3-phenoxybenzyaldehyde and two unknown products with chloride and bromide isotope distribution derived from cypermethrin and deltamethrin metabolisms, respectively. In contrast there was only one unknown product that was predominantly detected from CYP6AA3-mediated permethrin (type I pyrethroid) degradation, with mass spectrum profile showing characteristic chloride isotope distribution of permethrin-derived compound. Unlike cypermethrin and deltamethrin metabolisms, we did not obtain 3-phenoxybenzaldehyde from permethrin degradation (Boonseupsakul, 2008).

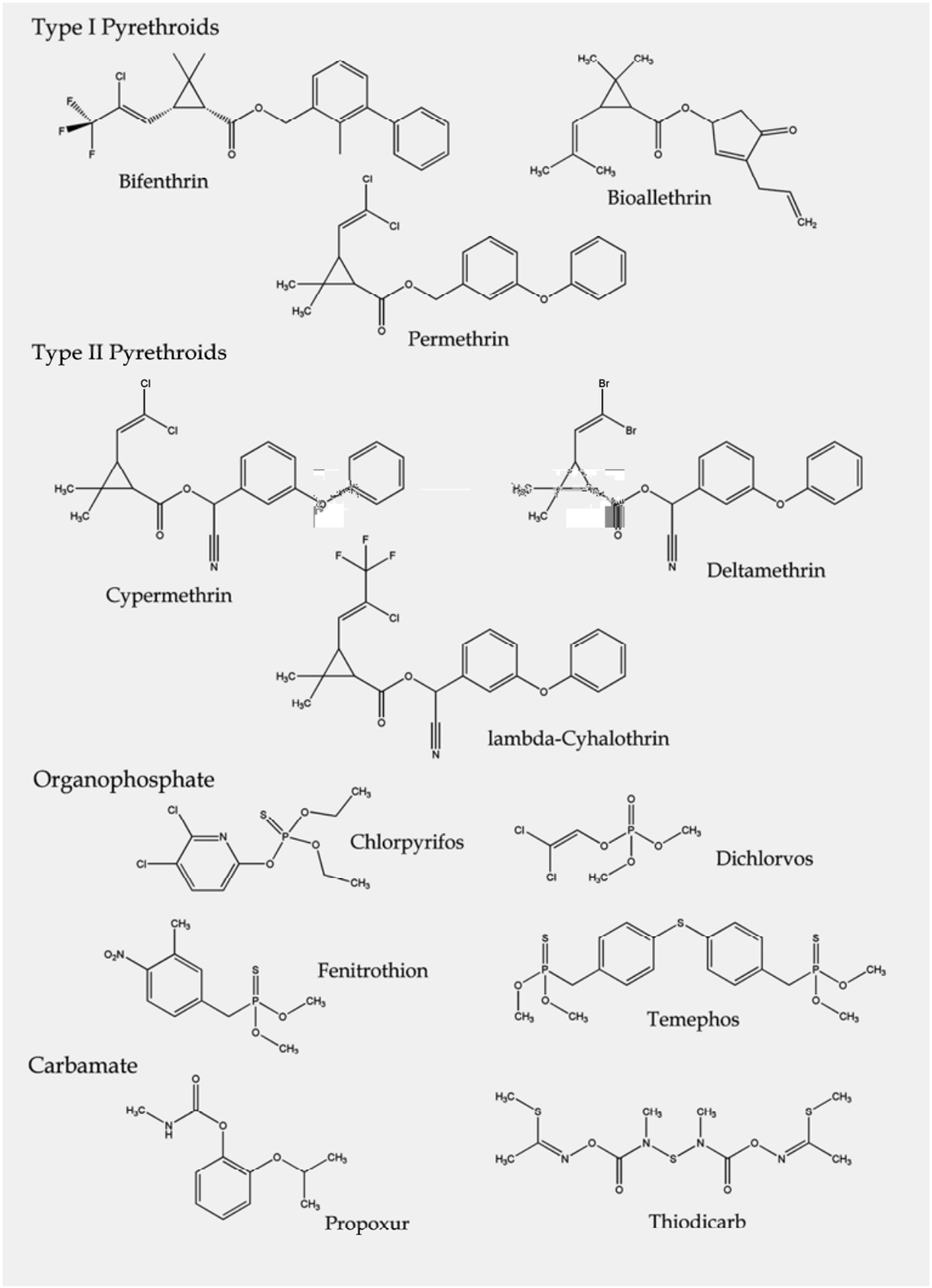


Fig. 1. Chemical structures of insecticides used in the study.

Type I and type II pyrethroids are different by the presence of cyano group (see Fig. 1). Thus our results implicate that presence of cyano group may play role in CYP6AA3-mediated pyrethroid degradations resulting in detection of 3-phenoxybenzaldehyde, possibly through oxidative cleavage reaction. In *An. gambiae* CYP6M2-mediated deltamethrin metabolism and house fly CYP6D1-mediated cypermethrin metabolism, 4′-hydroxylation of deltamethrin and cypermethrin is the major route of their metabolisms since 4′-hydroxylation products were predominantly detected (Stevenson et al., 2011; Zhang & Scott, 1996). The 4′-hydroxylation and 3-phenoxybenzaldehyde products have been observed in *in vitro* pyrethroid metabolisms mediated by mammalian microsomal enzymes (Shono et al., 1979). The absence of detection of 3-phenoxybenzaldehyde in CYP6AA3-mediated permethrin degradation could be predicted that the reaction underwent monooxygenation of permethrin.

2.2 Characterization of CYP6AA3 and CYP6P7 enzymes

As described, both CYP6AA3 and CYP6P7 enzymes have enzymatic activities against pyrethroid insecticides and their metabolic profiles are different. Kinetics and inhibition studies further support their abilities to metabolize pyrethroids, however with different enzyme and kinetic properties that influence substrate and inhibitor selectivity. Such knowledge could have an implication in pyrethroid detoxification in *An. minimus* mosquito, for example how the two P450s redundantly metabolize overlapping sets of pyrethroids. Alongside investigation of pyrethroid metabolisms, we examined their activities toward fluorescent compounds for development of rapid enzymatic assays. Finally we performed cell-based MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) cytotoxicity assays for further determination of substrates and inhibitors of both P450 enzymes as reported herein.

2.2.1 Determination of enzyme kinetics of CYP6AA3 and CYP6P7 enzymes

We recently reported kenetic paremeters for CYP6AA3 and CYP6P7 enzymes (Duangkaew et al., 2011b). Kinetic results reveal that CYP6AA3 has preference in binding to and has higher rate in degradation of permethrin type I pyrethroid than type II pyrethroids (K_m values toward permethrin, cypermethrin, deltamethrin, and λ-cyhalothrin of 41.0 ± 8.5, 70.0 ± 7.1, 80.2 ± 2.0, and 78.3 ± 7.0 μM, respectively and V_{max} values of 124.2 ± 1.2, 40.0 ± 7.1, 60.2 ± 3.6, and 60.7 ± 1.1 pmol/min/pmol P450, respectively). In contradictory CYP6P7 does not have preference for type of pyrethroids (K_m values toward permethrin, cypermethrin, and deltamethrin of 69.7 ± 10.5 , 97.3 ± 6.4, and 73.3 ± 2.9, respectively and V_{max} values of 65.7 ± 1.6, 83.3 ± 7.6, and 55.3 ± 5.7 pmol/min/pmol P450, respectively) and does not metabolize λ-cyhalothrin. Thus although both enzymes are comparable in terms of capability to metabolize pyrethroids *in vitro*, their kinetic values are different. Enzyme structure could account for their differences in kinetic properties and substrate preference.

Since there has been no known crystal structure available for insect P450s, we initially constructed homology models for CYP6AA3, CYP6P7, and CYP6P8 in an attempt to increase our understanding of molecular mechanisms underlying their binding sites toward insecticide substrates and inhibitors. The three enzyme models are different in geometry of their active-site cavities and substrate access channels. Upon docking with various insecticide groups, results of its active site could predict and explain metabolic behavior toward pyrethroid, organophosphate, and carbamate insecticides (Lertkiatmongkol et al., 2011). These results suggest that differences in metabolic activities among P450 enzymes in

insects could be attributed to structural differences resulting in selectivity and different enzymatic activities against insecticides.

In human, CYP2C8, CYP2C9, CYP2C19, and CYP3A4 have been reported abilities to metabolize both type I and II pyrethroids (Godin et al., 2007; Scollon et al., 2009). The preference for type I pyrethroid in CYP6AA3 is similar to human CYP2C9 and CYP2C19, while similar metabolic activity toward both types of pyrethroids found for CYP6P7 is similar to that of human CYP2C8 enzyme (Scollon et al., 2009). Nevertheless efficiency of CYP6AA3 and CYP6P7 in deltamethrin degradation is 5- to 10-fold less effective than human CYP2C8 and CYP2C19. It is noteworthy that more than one P450s residing within an organism can metabolize pyrethroids as described for human and mosquito, multiple rat P450s are also found capable of pyrethroid metabolisms (Scollon et al., 2009). When comparing to *An. gambiae* CYP6P3, both CYP6AA3 and CYP6P7 possess at least 10 fold higher K_m than CYP6P3, but V_{max} values of both *An. minimus* CYP6AA3 and CYP6P7 are at least 20 fold higher (Müller et al., 2008). Higher values of K_m and V_{max} of CYP6AA3 and CYP6P7 than those values of *An. gambiae* CYP6M2 (Stevenson et al., 2011) are also observed.

2.2.2 CYP6AA3 and CYP6P7 are inhibited differently by different compounds

To obtain a potential fluorogenic substrate probe for fluorescent-based assays of CYP6AA3 and CYP6P7, we previously screened four resorufin fluorogenic substrates containing different alkyl groups (Duangkaew et al., 2011b) and results in Table 2 suggest that among test compounds, benzyloxyresorufin could be used as a fluorescent substrate probe since both CYP6P7 and CYP6AA3 could bind and metabolize benzyloxyresorufin with lowest K_m (values of 1.92 for CYP6AA3 and 0.49 for CYP6P7) and with highest specific activities (Duangkaew et al., 2011b). The assays of benzyloxyresorufin-*O*-debenzylation activity were further used for inhibition studies of both mosquito enzymes.

Compounds	Specific activity (pmole resorufin/min/pmole P450) CYP6AA3	CYP6P7
Benzyloxyresorufin	6.81 ± 0.65	4.99 ± 0.74
Ethyloxyresorufin	2.88 ± 0.21	3.61 ± 0.17
Methyloxyresorufin	0.02 ± 0.01	-
Penthyloxyresorufin	0.01 ± 0.01	-

Table 2. Specific activities of CYP6AA3 and CYP6P7 toward resorufin derivatives.

Using fluorescence-based assays, we could initially determine what compound types that give mechanism-based inhibition pattern by pre-incubation of enzyme with various concentrations of test inhibitors in the presence or absence of NADPH for 30 min before addition of substrates and IC_{50} values have been determined as described (Duangkaew et al., 2011b). As known, mechanism-based inactivation inhibits enzyme irreversibly, rendering this mechanism of inhibition more efficient than reversible inhibition. Nevertheless information on mode of inhibition for inhibitors is potential for understanding of catalytic nature of enzymes. We thus determined mode of inhibition for all compounds tested. As shown in Table 2, the compounds we have tested are phenolic compounds and their chemical structures are shown in Fig. 2.

It is apparent that none of test flavonoids and furanocoumarins shows mechanism-based inhibition pattern, but piperonyl butoxide (PBO) and piperine that are methylenedioxyphenyl compounds show NADPH-dependent mechanism-based inhibition activities against both

enzymes. Piperine has been commonly found in *Piper sp.* plant extracts, it possesses acute toxicity to mammals (Daware et al., 2000). Inhibition results shown in Table 3 also elucidate that α-naphthoflavone displayed strongest inhibitory effect. Its inhibition pattern suggests that α-naphthoflavone uncompetitively inhibit both enzymes by binding to CYP6AA3– and CYP6P7-benzyloxyresorufin complex. Moreover, a difference was noted for xanthotoxin as it uncompetitively inhibits CYP6AA3 but mixed-type inhibited CYP6P7. Thus inhibition results together with different metabolic profiles thus confirm that CYP6AA3 and CYP6P7 have different enzyme properties. We thus also tested crude extracts of two plants (*Citrus reticulate* and *Stemona spp.*) that were reported containing phenolic compounds (Kaltenegger et al., 2003; Jayaprakasha et al., 1997) and are found in Thailand. Initial results suggest that compounds within both plants may not possess mechanism-based activities against CYP6AA3 and CYP6P7, and both extracts did not inhibit both enzymes as efficient as flavonoids and methylenedioxyphenyl compounds.

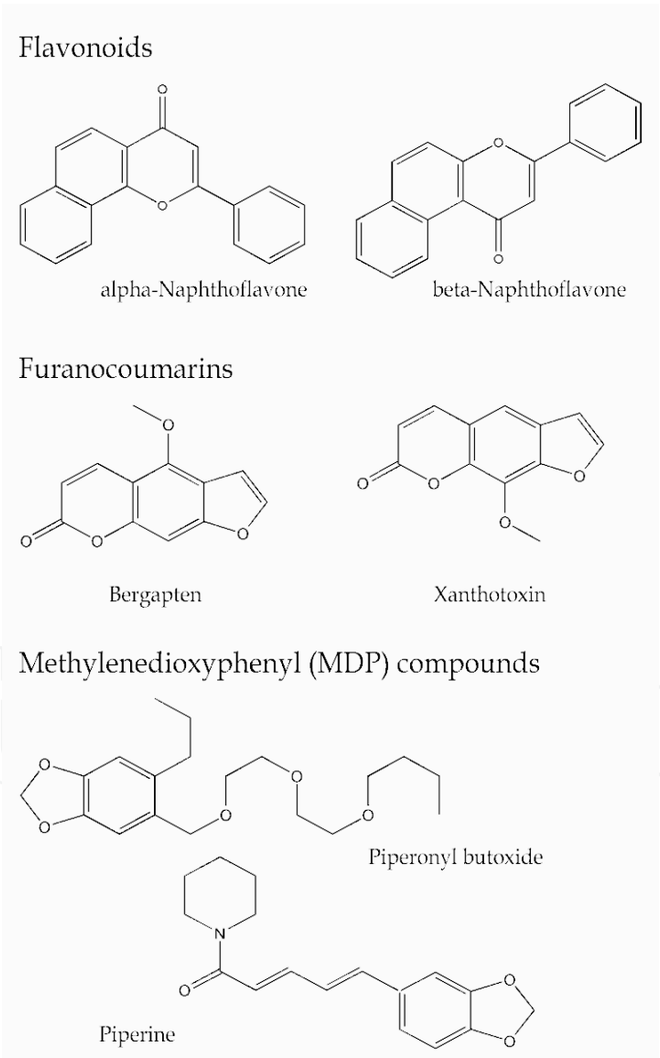


Fig. 2. Chemical structures of different compound types used for inhibition assays of mosquito P450s.

Inhibitor	Inhibition type (K_i, μM)		IC_{50} (M)			
	CYP6AA3	CYP6P7	CYP6AA3 pre-incubation		CYP6P7 pre-incubation	
			w/o NADPH	w/ NADPH	w/o NADPH	w/ NADPH
Flavonoids						
α-Naphthoflavone	Uncompetitive (0.84)	Uncompetitive (2.02)	0.37 ± 0.06	0.38 ± 0.06	2.90 ± 0.27	3.03 ± 0.45
-Naphthoflavone	ND	ND	19.22 ± 3.13[a]	34.44 ± 5.95[a]	17.25 ± 3.67	33.35 ± 9.90
Furanocoumarins						
Xanthotoxin	Uncompetitive (52.45)	Mixed-type (47.14)	51.04 ± 2.15	52.17 ± 2.86	33.77 ± 3.54[a]	78.93 ± 10.04[a]
Bergapten	Mixed-type (93.27)	Mixed-type (65.59)	93.77±10.87[a]	170.3 ± 16.88[a]	52.76 ± 6.77[a]	114.0 ± 11.81[a]
Methylenedioxyphenyl (MDP) compounds						
Piperine	Mechanism-based (ND)	Mechanism-based (ND)	15.26± 1.21[a]	4.86 ± 0.79[a]	52.86 ± 6.92[a]	3.48 ± 0.36[a]
Piperonyl butoxide (PBO)	Mechanism-based (ND)	Mechanism-based (ND)	9.91 ± 0.81[a]	4.04 ± 0.31[a]	31.77 ± 3.21[a]	16.22 ± 1.81[a]
Crude Extracts						
Citrus reticulata	ND	ND	236.1± 32.6	234.9 ± 9.54	116.4 ± 16.54	141.1 ± 15.1
Stemona spp.	ND	ND	56.11 ± 7.05	63.91 ± 5.2	71.77 ± 5.73[a]	105.7 ±10.18 [a]

Table 3. Mode of inhibition and inhibition constants of CYP6P7- or CYP6AA3-benzyloxyresorufin-*O*-debenzylation activities of flavonoids, furanocoumarins, and MDP compounds (Duangkaew et al., 2011b). Crude plant extracts reported herein are ethanolic extracts. Values marked with 'a' are significantly different between reactions with (w/) and without (w/o) NADPH. ND, not determined.

2.3 Use of cell-based MTT cytotoxicity assays to determine insecticide substrates and inhibitiors of *An. minimus* P450 enzymes

Since *in vitro* reconstitution system demonstrated CYP6AA3 and CYP6P7 enzymatic activities against pyrethroids, further investigation of the ability of CYP6AA3 and CYP6P7 enzymes to eliminate pyrethroid toxicity from cells was assessed in P450-infected *Sf*9 cells. This can be accomplished because other than targeting on sodium channels of nervous system, pyrethroids possess toxic effects on cells such as inhibition of cell mitochondrial complex I or causing DNA damage and cell death (Gassner et al., 1997; Patel et al., 2007; Naravaneni & Jamil, 2005). Similar cell death and cytotoxic to cells caused by organophosphates and carbamate insecticides have also been reported (Maran et al., 2010; Schmuck & Mihail, 2004). This is supported by that we previously observed cytotoxic effects of deltamthrin on insect *Sf*9 cells. When using *Sf*9 cells that express CYP6AA3 in MTT assays, cell mortality was drastically decreased in the presence of insecticides due to degradation of deltamethrin by CYP6AA3 and thus posing cytoprotective role on *Sf*9 cells (Boonseupsakul et al., 2008). Use of insect cells to test for toxicity effects of compounds such as fungal metabolites (Fornelli et al., 2004) and pyridalyl insecticide (Saito et al., 2005) has been previously reported. Moreover, insect cells expressing P450 have also been successfully used to test detoxification capability of enzyme against cytotoxic xenochemicals (Grant et al., 1996; Greene et al., 2000). In this context, we used MTT assays to determine insecticide detoxification by P450 expressed in *Sf*9 cells. Insecticides tested were pyrethroids (deltamethrin, permethrin, cypermethrin, bifenthrin, bioallethrin and λ-cyhalothrin), organophosphates (chlorpyrifos, dichlorvos, fenitrothion and temephos), carbamates (thiodicarb and propoxur). Various concentrations (1-500 μM) of insecticides were used for determination of cytotoxic effect of insecticides toward CYP6AA3-, CYP6P7-, and CYP6P8-expressing cells and compared to the control parent *Sf*9 cells. Cell viability of insecticide treated cells was measured by MTT assay as previously described (Boonseupsakul et al., 2008) and plotted against insecticide concentrations. The LC_{50} value of each insecticide was subsequently evaluated and obtained from each plot. Table 4 summarizes LC_{50} values of insecticides against *Sf*9 cells and cells with expression of P450s.

We observed that pyrethroids, organophosphates and carbamates are toxic to *Sf*9 parent cells. Since LC_{50} values of permethrin, bifenthrin, cypermethrin, and deltamethrin against CYP6AA3- and CYP6P7-expressing cells were approximately 4- to 19-folds significantly greater than those from parent *Sf*9 cells, these values imply that CYP6AA3 and CYP6P7 enzymes could cytoprotect *Sf*9 cells from pyrethroid toxicity. Conversely there was no significant difference of IC_{50} values between cells treated with organophosphate (chlorpyrifos, fenitrothion and temephos), carbamates (thiodicarb and propoxur) and bioallethrin pyrethroid insecticide, suggesting that expression of P450s did not cytoprotect cells from these insecticides. In addition CYP6P8 did not cytoprotect *Sf*9 cells against insecticides tested. It should be noted that LC_{50} value of λ-cyhalothrin in CYP6AA3-expressing cells was significantly greater than *Sf*9 parent cells, but not in CYP6P7-expressing cells. These results from MTT cytotoxicity assays are thus in agreement with *in vitro* enzymatic assays as described in Section 2.1. Thus abilities to cytoprotect against insecticide toxicity in infected *Sf*9 cells are due to P450-mediated enzymatic activity toward insecticides of CYP6AA3 and CYP6P7 (Duangkaew et al., 2011a). Together with *in vitro* enzymatic and cytotoxicity assays, we can conclude that both CYP6AA3 and CYP6P7 share metabolic activities against pyrethroids, but both enzymes play no role in degradations of organophosphates and carbamates. The results suggest that CYP6P8 plays no role in

degradation of insecticides tested in this report. Moreover, such cytotoxicity results implicate that the method could also be applied for primary screening of compounds that have an inhibitory effect towards CYP6AA3 and CYP6P7, as well as P450 enzymes that possess enzymatic activities against these insecticides.

Insecticides	LC_{50} (μM)			
	*Sf*9	CYP6AA3	CYP6P7	CYP6P8
Pyrethroids				
Bioallethrin[b]	30.6 ± 2.1	32.7 ± 2.4	23.3 ± 3.9	29
Permethrin[b]	42.7 ± 1.8	406.7 ± 21.5[a]	214.7 ± 48.8[a]	78
Bifenthrin	45 ± 7.6	210 ± 12.4[a]	135 ± 51[a]	45
Cypermethrin[b]	21.8 ± 0.5	192.7 ± 30.4[a]	216.7 ± 21.4[a]	25
Deltamethrin[b]	27.5 ± 9.2	285.0 ± 27.8[a]	379.5 ± 21.9[a]	10
λ-Cyhalothrin[b]	38.4 ± 4.3	133.3 ± 37.5[a]	42.0 ± 1.8	ND
Organophosphates				
Chlorpyrifos[b]	40.3 ± 6.5	56.3 ± 8.5	41.7 ± 2.8	60
Fenitrothion	25.0 ± 5.3	30.0 ± 6.4	ND	25
Temephos	11.0 ± 3.9	19.0 ± 7.5	ND	ND
Dichlorvos	32.0 ± 8.9	39.0 ± 6.4	ND	ND
Carbamates				
Propoxur[b]	4.0 ± 6.6	4.7 ± 0.3	3.6 ± 0.2	ND
Thiodicarb	28.6 ± 2.3	29.2 ± 4.7	ND	ND

Table 4. Cytotoxicity effects by insecticides on P450-infected cells and the parent SF9 cells using MTT assays. Values reported for CYP6P8 were average obtained from experiments performed in duplicate. Those marked with 'a' were significantly different from parent *Sf*9 cells and those marked with 'b' were reported in Duangkaew et al, 2011a. ND, not determined.

To test whether inhibitors can be screened, MTT assays were performed with P450-expressing cells treated with 100 μM deltamethin in the presence or absence of each test inhibitor. Concentrations of test inhibitory compounds were those of approximately LC_{20} values pre-determined by MTT assays on *Sf*9 cells. In cell-based inhibition assays, cell viability was determined upon co-incubation of test compound and deltamethrin, and normalized with viability of cells treated with test compound without deltamethrin. Inhibition experiments were performed with control *Sf*9 cells in the same manner as CYP6AA3-expressing cells and percent cell viability was plotted against test inhibitor concentrations, and results demonstrated that cell viability of parent *Sf*9 cells was not affected by test compounds (data not shown).

The results shown in Fig. 3 indicate that cell viability of CYP6AA3-expressing cells was decreased upon increasing concentration of test inhibitors. Piperine, piperonyl butoxide, and α-naphthoflavone could inhibit cytoprotective activity of CYP6AA3 more than xanthotoxin. This is thus in compliance with *in vitro* enzymatic inhibition assays, although piperonyl butoxide showed more potential than α-naphthoflavone in inhibiting cytoprotective activity of CYP6AA3. Cell permeability of test compounds could be accounted for differences of cell-based MTT and *in vitro* enzymatic assays. The results however indicate usefulness of cells expressing P450 enzymes to primarily screen for P450 substrates and inhibitors. Our results indicated that PBO and piperine could inhibit P450s

and possess synergistic actions against deltamethrin cytotoxicity in Sf9 cells expressing P450. PBO has been used as pyrethroid synergist to enhance pyrethroid toxicity, as it can bind to P450s thereby inhibiting P450 activity (Fakoorziba et al., 2009; Kumar et al., 2002; Vijayan et al., 2007). Unfortunately PBO has been reported acutely toxic to mammals (Cox, 2002; Okamiya et al., 1998).

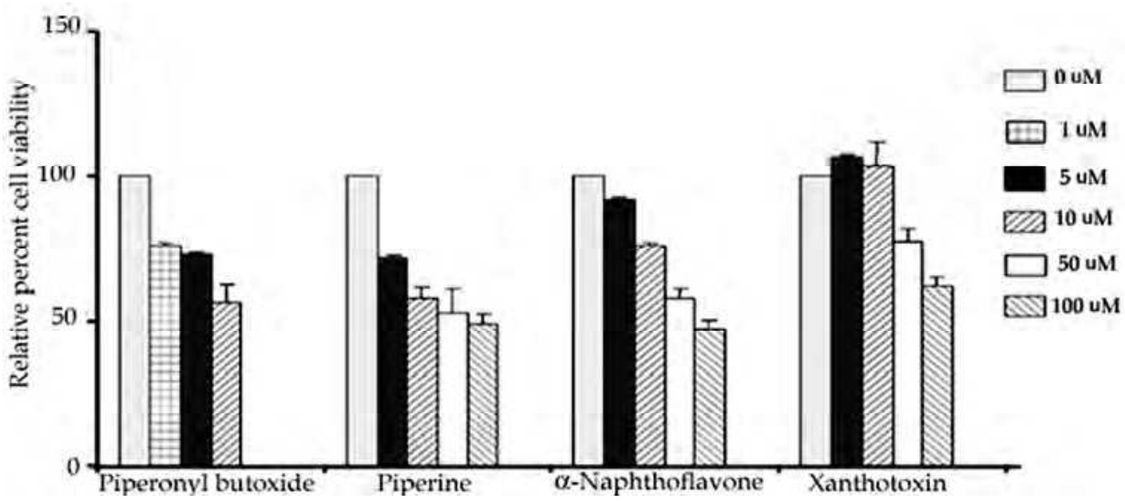


Fig. 3. Inhibition effect of test compounds against cell-based CYP6AA3-mediated deltamethrin detoxification measured by MTT assays.

2.4 *An. minimus* CYPOR and its possible role in regulation of P450-reaction cycle

The NADPH-Cytochrome P450 oxidoreductase (CYPOR) enzyme is a member of di-flavin enzymes that transfers electrons, one by one, from NADPH through FAD and FMN to target enzymes to fulfill functioning of various cytochrome P450 enzymes as well as other enzymes (Murataliev et al., 2004). Other members of this class are those containing a flavoprotein subunit, such as nitric oxide synthase, sulfite reductase, methionine synthase reductase and protein NR 1. Detailed biochemical and structural studies of rat CYPOR reveal several conserved structural domains existed in this enzyme class, these are membrane-bound, FMN-binding, connecting, and FAD/NADPH binding domains (Wang et al., 1997).

The *An. minimus* CYPOR has been cloned and expressed in *E. coli*, and CYPOR could support CYP6AA3- and CYP6P7-mediated pyrethroid metabolisms *in vitro* (Duangkaew et al., 2011b; Kaewpa et al., 2007). However its expression has been of poor yield as a result of inclusion bodies formation. An attempt to obtain soluble protein by deletion of the first 55 amino acid residues comprising of membrane binding region (*Δ55AnCYPOR*) has been successful (Sarapusit et al., 2008). However the protein could not be purified by 2'5'-ADP affinity column, indicating that NADPH binding capacity of mosquito CYPOR is low and this is different from CYPORs of other organisms such as rat and human (Sarapusit, 2009). Low binding affinity to 2'5'-ADP affinity column has also been recently reported in *An. gambiae* CYPOR (Lian et al., 2011). Only under specific condition was *Δ55AnCYPOR* successfully expressed and purified to homogeneity by a combination of Ni^{2+}NTA-affinity chromatography and G200-gel filtration chromatography (Sarapusit et al., 2008). Moreover both purified full-length (*flAnCYPOR*) and membrane-deleted *Δ55AnCYPOR* proteins readily lose FAD and FMN cofactors, they undergo

aggregation and are unstable compared to rat and human CYPORs (Sarapusit et al., 2008, 2010). While supplementation of FAD could increase activity of both full-length and membrane-deleted forms, FMN supplementation could increase activity of full-length form only (Sarapusit et al., 2008, 2010). This behavior is different from membrane-deleted soluble CYPORs of rat and human in which exogenous FMN is readily incorporated into its FMN-binding site (Döhr et al., 2001; Shen et al., 1989). Due to loss of flavin cofactors and instability of *An. minimus* CYPOR, we have identified two key amino acids (Leu86 and Leu219 in FMN binding domain) by amino sequence alignment and shown that mutations of the two leucine residues into conserved phenylalanine residues that are found conserved among other CYPORs could rescue loss of FAD cofactor and increase protein stability of mosquito CYPOR (Sarapusit et al., 2008, 2010). These mutations do not affect kinetic mechanism and constants of enzyme. Double mutations of leucine to the conserved phenylalanine (L86F/L219F) in full-length *flAnCYPOR*, but not in *Δ55AnCYPOR*, could increase binding of FMN and increase CYP6AA3-mediated pyrethroid metabolism (Sarapusit et al., 2010), indicating that membrane-bound region of *An. minimus* CYPOR could influence both structural folding of FMN domain and mediation of P450 catalysis (Murataliev et al., 2004; Wang et al., 1997).

The enzyme activity and kinetic mechanism of *flAnCYPOR* using cytochrome c as substrate are ionic strength dependent, with its mechanism following random Bi-Bi mechanism at low ionic strength and non-classical two-side Ping-Pong at high ionic strength. These mechanisms are different from rat, human, and house fly CYPORs (Murataliev et al., 2004; Sem & Kasper, 1994, 1995). In addition, *flAnCYPOR* could use extra flavins as additional substrates in which FAD binds at FAD/NADPH domain and FMN binds at FMN domain (as depicted in Fig. 4), resulting in an increase in its rate of electron transfer in CYP6AA3-mediated pyrethroid degradation (Sarapusit et al., 2010).

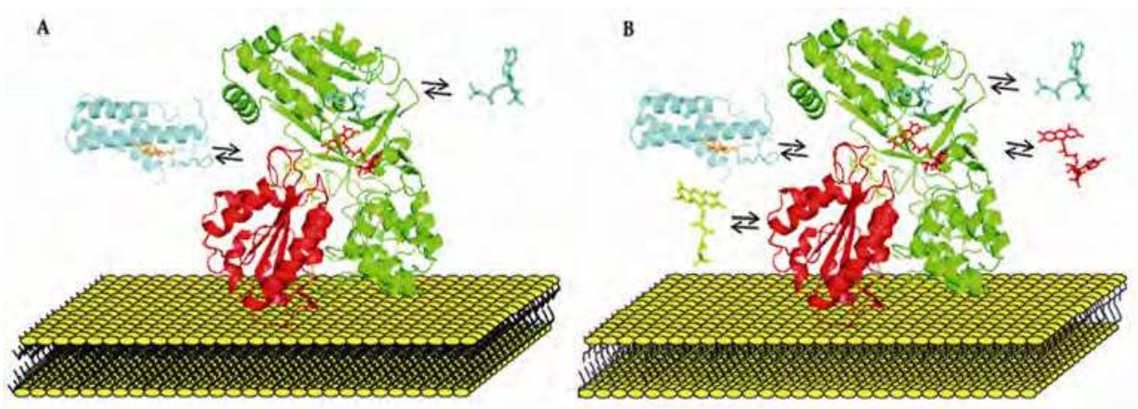


Fig. 4. Schematic representation of enzymatic reactions of CYPOR enzymes. CYPOR enzymes are represented in cartoon model of which FMN domain is in red color and FAD/NADPH domain is in green. Cofactors are represented in the stick mode; FMN is yellow colored, FAD is red, and $NADP^+$ is cyan (rat CYOR: pdb code 1AMO). The cytochrome *c* substrate (cytychrome *c*: pdb code 1BBH) is in cyan cartoon model with an orange heme group residing at the center.

In Figure 4, panel A illustrates common CYPOR (such as rat, human CYPORs) to which NADPH and cytochrome *c* substrate separately binds FAD/NADPH and FMN domains, while in panel B, *flAnCYPOR* could use extra flavins as additional substrates to which FAD

cofactor binds FAD/NADPH domain and FMN cofactor binds FMN domain. We thus speculate that *An. minimus* mosquito uses CYPOR in regulation of P450-mediated metabolisms, since it supplies electrons to a collection of P450s within the cell. Although structural basis for loose binding of flavin cofactors in *An. minimus* CYPOR is not known, it is conceivable that its distinct property that adopt extra flavins as substrates may render the enzyme ability to regulate electron transfer to target mosquito enzymes.

3. Conclusion

The results of this study on CYP6AA3 and CYP6P7 could lay groundwork into an understanding of the mechanisms that control substrates and reaction selectivity of both P450 enzymes, thereby increase an understanding of P450-mediated resistance mechanisms to various pesticides. The kinetic values, metabolic profile of pyrethroid insecticide metabolisms and inhibition patterns by different inhibitors of CYP6AA3 are different from CYP6P7. Future approach could aim at the strategy involving finding a collection of substrates together with structural models and mutation analyses of CYP6AA3 and CYP6P7 that affect specific P450 catalysis. Moreover, characterizing inhibitors and inhibition mechanisms of large collection of compounds with known chemical structures against CYP6AA3 and CYP6P7 enzymes could give insight into an understanding of mechanisms of cytochrome P450s that metabolize pyrethroids. It is conceivable that CYP6P8 does not play role in detoxification of pyrethroid, organophosphate, and carbamate insecticides. Further substrate search for CYP6P8 may help to learn about its overexpression in pyrethroid-resistant mosquito. Together with knowledge obtained from enzymatic properties of *An. minimus* CYPOR, this could improve our understanding of P450-mediated detoxification of insecticides, as well as provide a foundation for rational design of P450 synergists specific for P450-mediated pesticide resistance and thus resistant management in mosquito vector control program.

4. Acknowledgment

This work is supported by Grant BRG5380002 from Thailand Research Fund and Mahidol University; grant BT-B01-XG-14-4803 from BIOTEC, National Science and Technology Development Agency.

5. References

Acevedo, G.R.; Zapater, M. & Toloza, A.C. (2009). Insecticide resistance of house fly, *Musca domestica* (L.) from Argentina. *Parasitology Research*, Vol. 105, No. 2, pp. 489-493, ISSN 0932-0113

Awolola, T.S.; Brooke, B.D.; Hunt, R.H. & Coetzee, M. (2002). Resistance of the malaria vector *Anopheles gambiae s.s.* to pyrethroid insecticides, in south-western Nigeria. *Annals of Tropical Medicine and Parasitology,* Vol. 96, No. 8, pp. 849-852, ISSN 003-4983

Bautista, Ma.A.M.; Miyata, T.; Miura, K. & Tanaka, T. (2009). RNA interference-mediated knockdown of a cytochrome P450, CYP6BG1, from the diamondback moth *Plutella xylostella*, reduces larval resistance to permethrin. *Insect Biochemistry and Molecular Biology*, Vol. 39, No. 1, pp. 38-46, ISSN 0965-1748

Bautista, Ma.A.M; Tanaka, T. & Miyata, T. (2007). Identification of permethrin-inducible cytochrome P450s from the diamondback moth, *Plutella xylostella* (L.) and the possibility of involvement in permethrin resistance. *Pesticide Biochemistry and Physiology,* Vol. 87, No. 1, pp. 85-93, ISSN 0048-3575

Boonsuepsakul, S. (2008). Characterization of CYP6AA3 in baculovirus expression system. Ph.D. Thesis, Mahidol University, Bangkok, Thailand

Boonsuepsakul, S.; Luepromchai, E. & Rongnoparut, P. (2008). Characterization of *Anopheles minimus* CYP6AA3 expressed in a recombinant baculovirus system. *Archives of Insect Biochemistry and Physiology*, Vol. 63, No. 1, pp. 13-21, ISSN 0739-4462

Chareonviriyaphap, T.; Rongnoparut, P. & Juntarumporn, P. (2002). Selection for pyrethroid resistance in a colony of *Anopheles minimus* species A, a malaria vector in Thailand. *Journal of Vector Ecology,* Vol. 27, No. 2, pp. 222-229, ISSN 1081-1710

Chareonviriyaphap, T.; Rongnoparut, P.; Chantarumporn, P. & Bangs, M.J. (2003). Biochemical detection of pyrethroid resistance mechanisms in *Anopheles minimus* in Thailand. *Journal of Vector Ecology,* Vol. 28, No. 1, pp. 108-116, ISSN 1081-1710

Cochran, D.G. (1989). Monitoring for insecticide resistance in field-collected strains of the German cockroach (Dictyoptera: Blattellidae). *Journal of Economic Entomology,* Vol. 82, No. 2, pp. 336-341, ISSN 0022-0493

Cox, C. (2002). Piperonyl butoxide. *Journal of Pesticide Reform,* Vol. 22, pp. 12–20, ISSN 0893-357X

Daware, M.B.; Mujumdar, A.M. & Ghaskadbi, S. (2000). Reproductive toxicity of piperine in Swiss Albino mice. *Planta Medica,* Vol. 66, No. 3, pp. 231-236, ISSN 0032-0943

Djouaka, R.F.; Bakare, A.A.; Coulibaly, O.N.; Akogbeto, M.C.; Ranson, H.; Hemingway, J. & Strode, C. (2008). Expression of the cytochrome P450s, CYP6P3 and CYP6M2 are significantly elevated in multiple pyrethroid resistant populations of *Anopheles gambiae s.s.* from Southern Benin and Nigeria. *BMC genomics,* Vol. 9, p. 538, ISSN 1471-2164

Döhr, O.; Paine, M.J., Friedberg, T.; Robert, G.C.K. & Wolf, R. (2001). Engineering of a functional human NADH-dependent cytochrome P450 system *Proceedings of the National Academy of Sciences of the United States of America,* Vol. 98, No.1, pp. 81-86, ISSN 0027-8424

Duangkaew, P.; Kaewpa, D. & Rongnoparut, P. (2011a). Protective efficacy of *Anopheles minimus* CYP6P7 and CYP6AA3 against cytotoxicity of pyrethroid insecticides in *Spodoptera frugiperda* (*Sf*9) insect cells. *Tropical Biomedicine*, Vol. 28, No. 2, pp. 293-301, ISSN 0127-5720

Duangkaew, P.; Pethuan, S.; Kaewpa, D.; Boonseupsakul, S.; Sarapusit, S. & Rongnoparut, P. (2011b). Characterization of mosquito CYP6P7 and CYP6AA3: Differences in substrate preferences and kinetic properties. *Archives of Insect Biochemistry and Physiology*, Vol. 76, No. 4, pp. 236-248, ISSN 0739-4462

Fakoorziba, M.R.; Eghbal, F. & Vijayan, V.A. (2009). Synergist efficacy of piperonyl butoxide with deltamethrin as pyrethroid insecticide on *Culex tritaeniorhynchus* (Diptera: Culicidae) and other mosquitoe species. *Environmental Toxicology,* Vol. 24, No. 1, pp. 19-24, ISSN 1520-4081

Feyereisen, R. (1999). Insect P450 enzymes. *Annual Review of Entomology,* Vol. 44, pp. 507-533, ISSN 0066-4170

Fornelli, F.; Minervini, F. & Logrieco, A. (2004). Cytotoxicity of fungal metabolites to lepidopteran (*Spodoptera frugiperda*) cell line (*Sf*-9). *Journal of Invertebrate Pathology,* Vol. 85, No. 2, pp. 74-79, ISSN 0022-2011

Gassner, B., Wüthrich, A., Scholtysik, G. & Solioz, M. (1997). The pyrethroids permethrin and cyhalothrin are potent inhibitors of the mitochondrial complex I. *Journal of Pharmacology and Experimental Therapeutics,* Vol. 281, No. 2, pp. 855–860, ISSN 0022-3565

Godin, S.J.; Crow, J.A.; Scollon, E.J.; Hughes, M.F.; DeVito, M.J. & Ross, M.K. (2007). Identification of rat and human cytochrome P450 isoforms and a rat serum esterase that metabolize the pyrethroid insecticides deltamethrin and esfenvalerate. *Drug Metabolism and Disposition,* Vol. 35, No. 9, pp. 1664–1671, ISSN 0090-9556

Grant, D.F.; Greene, J.F.; Pinot, F.; Borhan, B.; Moghaddam, M.F.; Hummock, B.D.; McCutchen, B.; Ohkawa, H.; Luo, G. & Guenthner, T.M. (1996). Development of an *in situ* toxicity assay system using recombinant baculoviruses. *Biochemical Pharmacology,* Vol. 51, No. 4, pp. 503–515, ISSN 0006-2952

Greene, J.F.; Zheng, J.; Grant, D.F. & Hammock, B.D. (2000). Cytotoxicity of 1,2-epoxynaphthalene is correlated with protein binding and *in situ* glutathione depletion in cytochrome P4501A1 expressing *Sf*-21 cells. *Toxicological Sciences,* Vol. 53, No. 2, pp. 352-360, ISSN 1096-6080

Hargreaves, K.; Koekemoer, L.L.; Brooke, B.D.; Hunt, R.H.; Mthembu, J. & Coetzee, M. (2000). *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. *Medical and Veterinary Entomology,* Vol. 14, No. 2, pp. 181-189, ISSN 0269-283X

Hemingway, J.; Hawkes, N.J.; McCarroll, L. & Ranson, H. (2004). The molecular basis of insecticide resistance in mosquitoes. *Insect Biochemistry and Molecular Biology*, Vol. 34, No. 7, pp. 653-665, ISSN 0965-1748

Kaltenegger, E.; Brema, B.; Mereiter, K.; Kalchhauser, H.; Kählig, H.; Hofer, O.; Vajrodaya, S. & Greger, H. (2003). Insecticidal pyrido[1,2-a]azepine alkaloids and related derivatives from *Stemona* species. *Phytochemistry,* Vol. 63, No. 7, pp. 803-816, ISSN 0031-9422

Jayaprakasha, G.K.; Singh, R.P.; Pereira, J. & Sakariah, K.K. (1997). Limonoids from *Citrus reticulata* and their moult inhibiting activity in mosquito *Culex quinquefasciatus* larvae. *Phytochemistry,* Vol. 44, No. 5, pp. 843-846, ISSN 0031-9422

Jirakanjanakit, N.; Rongnoparut, P.; Saengtharatip, S.; Chareonviriyaphap, T.; Duchon, S.; Bellec, C. & Yoksan, S. (2007). Insecticide susceptible/resistance status in *Aedes (Stegomyia) aegypti and Aedes (Stegomyia) albopictus* (Diptera: Culicidae) in Thailand during 2003-2005. *Journal of Economic Entomology,* Vol. 100, No. 2, pp. 545-550, ISSN 0022-0493

Kaewpa, D.; Boonsuepsakul, S. & Rongnoparut, P. (2007). Functional expression of mosquito NADPH-cytochrome P450 reductase in *Escherichia coli*. *Journal of Economic Entomology,* Vol. 100, No. 3, pp. 946-953, ISSN 0022-0493

Kumar, S.; Thomas, A.; Sahgal, A.; Verma, A.; Samuel, T. & Pillai, M.K.K. (2002). Effect of the synergist, piperonyl butoxide, on the development of deltamethrin resistance in yellow fever mosquito, *Aedes aegypti L.* (Diptera: Culicidae). *Archives of Insect Biochemistry and Physiology,* Vol. 50, No. 1, pp. 1-8, ISSN 0739-4462

Lertkiatmongkol, P.; Jenwitheesuk, E. & Rongnoparut, P. (2011). Homology modeling of mosquito cytochrome P450 enzymes involved in pyrethroid metabolism: insights into differences in substrate selectivity. *BMC Research Notes*, Vol. 4, p. 321, ISSN 1756-0500

Lian, L.Y.; Widdowson, P.; McLaughlin, L.A. & Paine, M.J. (2011). Biochemical comparison of *Anopheles gambiae* and Human NADPH P450 reductases reveals different 2′-5′-ADP and FMN binding traits. *Plos one,* Vol. 6, No. 5, pp. e20574, ISSN 1932-6203

Liu, N. & Scott, J.G. (1998). Increased transcription of CYP6D1 causes cytochrome P450-mediated insecticide resistance in house fly. *Insect Biochemistry and Molecular Biology,* Vol. 28, No.8, pp. 531-535, ISSN 0965-1748

Maran, E.; Fernández-Franźon, M.; Font, G. & Ruiz, M.J. (2010). Effects of aldicarb and propoxur on cytotoxicity and lipid peroxidation in CHO-K1 cells. *Food and Chemical Toxicology,* Vol. 48, No. 6, pp. 1592–1596, ISSN 0278-6915

Mclaughlin, L.A.; Niazi, U.; Bibby, J.; David, J.-P.; Vontas, J.; Hemingway, J.; Ranson, H.; Sutcliffe, M.J. & Paine M.J.I. (2008). Characterization of inhibitors and substrates of *Anopheles gambiae* CYP6Z2. *Insect Molecular Biology,* Vol. 17, No. 2, pp. 125-135, ISSN 0962-1075

Müller, P.; Donnelly, M.J. & Ranson H. (2007). Transcription profiling of a recently colonized pyrethroid resistant *Anopheles gambiae* strain from Ghana. *BMC Genomics,* Vol. 8, p. 36, ISSN 1471-2164

Müller, P.; Warr, E.; Stevenson, B.J.; Pignatelli, P.M.; Morgan, J.C.; Steven, A.; Yawson, A.E.; Mitchell, S.N.; Ranson, H.; Hemingway, J.; Paine, M.J.I. & Donnelly, M.J. (2008). Field-caught permethrin-resistant *Anopheles gambiae* overexpress CYP6P3, a P450 that metabolises pyrethroids. *PLoS Genetics,* Vol. 4, No. 11, pp. e1000286, ISSN 1553-7390

Murataliev, M..B.; Feyereisen, R. & Walker, F.A. (2004). Electron transfer by diflavin reductases. *Biochimica et Biophysica Acta*, Vol. 1698, No. 1, pp. 1 – 26, ISSN 0006-3002

Naravaneni, R. & Jamil, K. (2005). Evaluation of cytogenetic effects of lambda-cyhalothrin on human lymphocytes. *Journal of Biochemical and Molecular Toxicology,* Vol. 19, No. 5, pp. 304-310, ISSN 1095-6670

Nelson, D.R.; Koymans, L.; Kamataki, T.; Stegeman, J.J.; Feyereisen, R.; Waxman, D.J.; Waterman, M.R.; Gotoh, O.; Coon, M.J; Estabrook, R.W.; Gunsalus, I.C. & Nebert, D.W. (1996). P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics,* Vol. 6, No. 1, pp. 1-42, ISSN 0960-314X

Okamiya, H.; Mitsumori, K.; Onodera, H.; Ito, S.; Imazawa, T.; Yasuhara, K. & Takahashi, M. (1998). Mechanistic study on liver tumor promoting effects of piperonyl

butoxide in rats. *Archives of Toxicology,* Vol. 72, No. 11, pp. 744–750, ISSN 0340-5761

Omura, T. & Sato, R. (1964). The carbon monoxide-biding pigment of liver microsome I. Evidence for its hemoprotein nature. *The Journal of Biological Chemistry,* Vol. 239, pp. 2370-2378, ISSN 0021-9258

Ortiz de Montellano, P.R. (2005). *Cytochrome P450: structure, mechanism, and biochemistry*, Kluwer Academic/Plenum Piblishers, ISBN 0-306-48324-6, New York, USA.

Patel, S.; Bajpayee, M.; Pandey, A.K.; Parmar, D. & Dhawan, A. (2007). *In vitro* induction of cytotoxicity and DNA strand breaks in CHO cells exposed to cypermethrin, pendimethalin and dichlorvos. *Toxicology In Vitro,* Vol. 21, No. 8, pp. 1409–1418, ISSN 0887-2333

Price, N.R. (1991). Insect resistance to insecticides: mechanisms and diagnosis. *Comparative Biochemistry and Physiology - C Pharmacology Toxicology and Endocrinology,* Vol. 100, No. 3, pp. 319-326, ISSN 0742-8413

Ranasinghe, C. & Hobbs, A.A. (1998). Isolation and characterization of two cytochrome P450 cDNA clones for CYP6B6 and CYP6B7 from *Helicoverpa armigera* (Hubner): possible involvement of CYP6B7 in pyrethroid resistance. *Insect Biochemistry and Molecular Biology,* Vol. 28, No. 8, pp. 571-580, ISSN 0965-1748

Rodpradit, P.; Boonsuepsakul, S.; Chareonviriyaphap, T.; Bangs, M.J. & Rongnoparut, P. (2005). Cytochrome P450 genes: molecular cloning and overexpression in a pyrethroid-resistant strain of *Anopheles minimus* mosquito. *Journal of the American Mosquito Control Association,* Vol. 21, No. 1, pp. 71-79, ISSN 8756-971X

Rongnoparut, P.; Boonsuepsakul, S.; Chareonviriyaphap, T. & Thanomsing, N. (2003). Cloning of cytochrome P450, CYP6P5, and CYP6AA2 from *Anopheles minimus* resistant to deltamethrin. *Journal of Vector Ecology,* Vol. 28, No. 2, pp. 150-158, ISSN 1081-1710

Saito, S.; Sakamoto, N. & Umeda, K. (2005). Effects of pyridalyl, a novel insecticidal agent, on cultured *Sf*9 cells. *Journal of Pesticide Science,* Vol. 30, No. 1, pp. 17-21, ISSN 0385-1559

Sarapusit, S. (2009). Study of NADPH-cytochrome P450 oxidoreductase from *Anopheles minimus* mosquito. Ph.D Thesis, Mahidol University, Bangkok, Thailand

Sarapusit, S.; Xia, C.; Misra, I.; Rongnoparut, P. & Kim, J-JP. (2008). NADPH-Cytochrome P450 oxidoreductase from *Anopheles minimus* mosquito: Kinetic studies and the influence of Leu86 and Leu219 for cofactors binding and protein stability. *Archives of Biochemistry and Biophysics,* Vol. 477, No. 1, pp. 53-59, ISSN 0739-4462

Sarapusit, S.; Pethuan, S. & Rongnoparut, P. (2010). Mosquito NADPH-cytochrome P450 oxidoreductase: kinetics and role of phenylalanine amino acid substitutions at Leu86 and Leu219 in CYP6AA3-mediated deltamethrin metabolism. *Archives of Insect Biochemistry and Physiology*, Vol. 73, No. 4, pp. 232-244, ISSN 0739-4462

Schmuck, G. & Mihail, F. (2004). Effects of the carbamates fenoxycarb, propamocarb and propoxur on energy supply, glucose utilization and SH-groups in neurons. *Archives*

of Toxicology, Vol. 78, No. 6, pp. 330–337, Epub 2004 Feb 19, ISSN 0340-5761

Scollon, E.J.; Starr, J.M.; Godin, S.J.; DeVito, M.J. & Hughes, M.F. (2009). *In vitro* metabolism of pyrethroid pesticides by rat and human hepatic microsomes and cytochrome P450 isoforms. *Drug Metabolism and Disposition,* Vol. 37, No. 1, pp. 221–228, ISSN 0090-9556

Sem, D.S & Kasper, C.B. (1994). Kinetic mechanism for the model reaction of NADPH-cytochrome P450 oxidoreductase with cytochrome c. *Biochemistry*, Vol. 33, No. 4, pp. 12012–12021, ISSN 0006-2960

Sem, D.S. & Kasper, C.B. (1995). Effect of ionic strength on the kinetic mechanism and relative rate limitation of steps in the model NADPH-cytochrome P450 oxidoreductase reaction with cytochrome c. *Biochemistry*, Vol. 34, No. 39, pp.12768–12774, ISSN 0006-2960

Shen, A.L., Porter, T.D., Wilson, T.E., Kasper, C.B. (1989) Structural analysis of the FMN binding domain of NADPH-cytochrome P-450 oxidoreductase by site-directed mutagenesis. *Journal of Biological Chemistry*, Vol. 254, No. 13, pp. 7584–7589, ISSN 0021-9258

Shono, T.; Ohsawa, K. & Casida, J.E. (1979). Metabolism of *trans*- and *cis*-permethrin, *trans*- and *cis*-cypermethrin and decamethrin by microsomal enzymes. *Journal of Agriculture and Food Chemistry,* Vol. 27, No. 2, pp. 316-325, ISSN 0021-8561

Stevenson, B.J.; Bibby, J.; Pignatelli, P.; Muangnoicharoen, S.; O'Neill, P.M.; Lian, L-Y; Müller, P.; Nikou, D.; Steven, A.; Hemingway, J.; Sutcliffe, M.J. & Paine, M.J.I. (2011). Cytochrome P450 6M2 from the malaria vector *Anopheles gambiae* metabolizes pyrethroids: Sequential metabolism of deltamethrin revealed. *Insect Biochemistry and Molecular Biology,* Vol. 41, No. 7, pp. 492-502, ISSN 0965-1748

Tomita, T.; Liu, N.; Smith, F.F.; Sridhar, P. & Scott, J.G. (1995). Molecular mechanisms involved in increased expression of a cytochrome P450 responsible for pyrethroid resistance in the housefly, *Musca domestica*. *Insect Molocular Biology,* Vol. 4, No. 3, pp. 135-140, ISSN 0962-1075

Vijayan, V.A.; Sathish Kumar, B.Y.; Ganesh, K.N.; Urmila, J.; Fakoorziba, M.R. & Makkapati, A.K. (2007). Efficacy of piperonyl butoxide (PBO) as a synergist with deltamethrin on five species of mosquitoes. *The Journal of Communicable Diseases,* Vol. 39, No. 3, pp. 159-163, ISSN 0019-5638

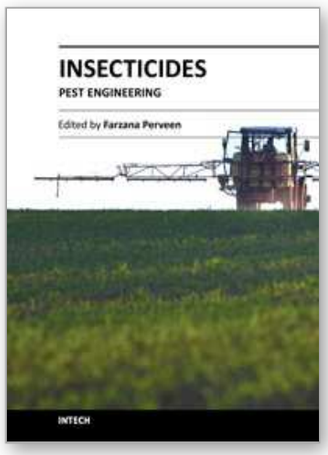
Wang, M.; Roberts, D.L.; Paschke, R.; Shea, T.M.; Masters, B.S. & Kim, J.J. (1997). Three-dimensional structure of NADPH-cytochrome P450 reductase: prototype for FMN- and FAD-containing enzymes. *Proceedings of the National Academy of Sciences of the United States of America,* Vol. 94, No. 16, pp. 8411-8416, ISSN 0027-8424

Wondji, C.S.; Morgan, J.; Coetzee, M.; Hunt, R.H.; Steen, K.; Black IV, W.C.; Hemingway, J. & Ranson, H. (2007). Mapping a Quantitative Trait Locus (QTL) conferring pyrethroid resistance in the African malaria vector *Anopheles funetus*. *BMC Genomics*, Vol. 8, p. 34, ISSN 1471-2164

Yaicharoen, R.; Kiatfuengfoo, R; Chareonviriyaphap, T. & Rongnoparut, P. (2005). Characterization of deltamethrin resistance in field populations of *Aedes aegypti* in Thailand. *Journal of Vector Ecology,* Vol. 30, No. 1, pp. 144-150, ISSN 1081-1710

Zhang, M. & Scott, J.G. (1996). Cytochrome b5 is essential for cytochrome P450 6D1-mediated cypermethrin resistance in LPR house flies. *Pesticide Biochemistry and Physiology,* Vol. 55, No. 2, pp. 150-156, ISSN 0048-3575

Zhu, F.; Parthasarathy, R.; Bai, H.; Woithe, K.; Kaussmann, M.; Nauen, R.; Harrison, D.A. & Palli S.R. (2010). A brain-specific cytochrome P450 responsible for the majority of deltamethrin resistance in the QTC279 strain of *Tribolium castaneum*. *Proceedings of the National Academy of Sciences of the United States of America,* Vol. 107, No. 19, pp. 8557-8562, ISSN 0027-8424



Insecticides - Pest Engineering

Edited by Dr. Farzana Perveen

ISBN 978-953-307-895-3
Hard cover, 538 pages
Publisher InTech
Published online 15, February, 2012
Published in print edition February, 2012

This book is compiled of 24 Chapters divided into 4 Sections. Section A focuses on toxicity of organic and inorganic insecticides, organophosphorus insecticides, toxicity of fenitrothion and permethrin, and dichlorodiphenyltrichloroethane (DDT). Section B is dedicated to vector control using insecticides, biological control of mosquito larvae by Bacillus thuringiensis, metabolism of pyrethroids by mosquito cytochrome P40 susceptibility status of Aedes aegypti, etc. Section C describes bioactive natural products from sapindacea, management of potato pests, flower thrips, mango mealy bug, pear psylla, grapes pests, small fruit production, boll weevil and tsetse fly using insecticides. Section D provides information on insecticide resistance in natural population of malaria vector, role of Anopheles gambiae P450 cytochrome, genetic toxicological profile of carbofuran and pirimicarp carbamic insecticides, etc. The subject matter in this book should attract the reader's concern to support rational decisions regarding the use of pesticides.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Pornpimol Rongnoparut, Sirikun Pethuan, Songklod Sarapusit and Panida Lertkiatmongkol (2012). Metabolism of Pyrethroids by Mosquito Cytochrome P450 Enzymes: Impact on Vector Control, Insecticides - Pest Engineering, Dr. Farzana Perveen (Ed.), ISBN: 978-953-307-895-3, InTech, Available from: http://www.intechopen.com/books/insecticides-pest-engineering/metabolism-of-pyrethroids-by-mosquito-cytochrome-p450-enzymes-impact-on-vector-control

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen