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5

CARBOXYLESTERASES IN THE METABOLISM AND TOXICITY OF PESTICIDES

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5.1 INTRODUCTION

Carboxylesterases (CarbEs) play an important role in xenobiotic metabolism in many organisms, from humans to blowflies. The introduction of pesticides such as organophosphates (OPs), carbamates, and synthetic pyrethroids (SPs) during the twentieth century has led to extensive research into the role of CarbEs in pesticide metabolism and detoxification. In the case of the OPs and carbamates, their pesticidal activity is a consequence of their inhibition of acetylcholinesterase (AChE) at the nerve synapse. Due to the structural and mechanistic similarities between AChE and CarbEs, hydrolysis of these pesticides by CarbEs is a prominent pathway for their metabolism. In the case of the SPs, although their primary pesticidal activity is not via inhibition of AChE, their prominent carboxylester bond renders them labile to metabolism by the CarbEs. This chapter describes the chemico-biological interactions between CarbEs and various pesticides (OPs, carbamates, SPs) in vertebrates and invertebrates in detail, with

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particular emphasis on the role of CarbEs in metabolism and toxicity.

5.2 ORGANOPHOSPHATES

5.2.1 Structural and Chemical Basis of the Carboxylesterase-Organophosphate Interaction

Organophosphate pesticides (OPs) are structurally diverse, as over 30 different commercially available OPs are actives listed by the U.S. Environmental Protection Agency (www.epa.gov). They generally share a number of characteristics, being triesters of phosphoric acid with two alkyl side chains, a variable leaving group, and a terminal oxygen or sulfur moiety (Fig. 5.1; O'Brien 1960). The sulfur atom of phosphothionate OPs is replaced by an oxygen through the action of cytochrome P450 enzymes during metabolism (Sultatos and Murphy 1983). This desulfuration step markedly increases the toxicity of OP insecticides because the oxon form is considerably more effective as an inhibitor of acetylcholinesterase (AChE) (Feyereisen 1999).

The acute toxicity of OPs derives from their inhibition of AChE, which is an essential enzyme that functions by terminating signal transduction at the nerve synapse through the hydrolysis of acetylcholine (Casida and Quistad 2004). Inhibition of AChE results in interminable signal transduction at the nerve synapse, leading to paralysis and death. Acetylcholine is rapidly hydrolysed via a catalytic mechanism in which nucleophilic attack from a serine side chain, activated by a catalytic triad also involving a histidine and a glutamic acid, occurs at the electrophilic carbon of acetylcholine. This results in hydrolysis of the ester bond to the choline moiety, departure of the leaving group, and formation of an acyl-enzyme intermediate (Fig. 5.2). The acyl-enzyme intermediate is then hydrolysed via nucleophilic attack from a solvent molecule activated by the catalytic histidine, thereby regenerating the active site (Sussman et al. 1991). The initial steps in the inhibition of AChE by OPs are very similar to the mechanism of acetylcholine hydrolysis (Fig. 5.2): after hydrolytic attack at the electrophilic phosphorus of the OP and departure of the leaving group, a phospho-enzyme intermediate is formed. However, unlike the planar acyl-enzyme intermediate that is produced during the hydrolysis of acetylcholine, the phospho-enzyme intermediate is tetrahedral. Steric constraint then prevents rapid regeneration of the nucleophilic serine via attack from a solvent molecule activated by the catalytic histidine in an analogous manner to the regeneration of the acyl-intermediate (Millard et al. 1999). Instead, the enzyme has two possible fates: it can be very slowly hydrolytically reactivated via free (unactivated) solvent molecules, or undergo a subsequent aging reaction. The aging reaction involves dealkylation of one of the side chains and has been proposed to occur either through hydrolysis or carbocation bond scission (Shafferman et al. 1996; Nachon et al. 2005). Most importantly, aging results in the irreversible inhibition of AChE.

Carboxylesterases belong to the same serine hydrolase superfamily as AChE (E.C.: 3.1.1.1) and have an essentially identical catalytic mechanism. As an example of their similarity, human carboxylesterase 1 (hCE1) has 34% sequence identity with AChE and the two structures have only 1.2 Å r.m.s.d. over the main-chain atoms when superimposed (Fleming et al. 2007). However, the interaction between CarbEs and OPs appears to be slightly different from the AChE:OP interaction. Most importantly, many CarbEs appear to be resistant to the aging reaction (Maxwell and Brecht 2001). This phenomenon has been investigated in structural studies (Fleming et al. 2007) showing that the resistance to aging may be a consequence of a differently oriented phospho-serine adduct in the active site. Specifically, the catalytic histidine, which has been implicated in activating the alkyl side chain in aging (Nachon et al. 2005) is positioned in close proximity to the phosphorylated serine in

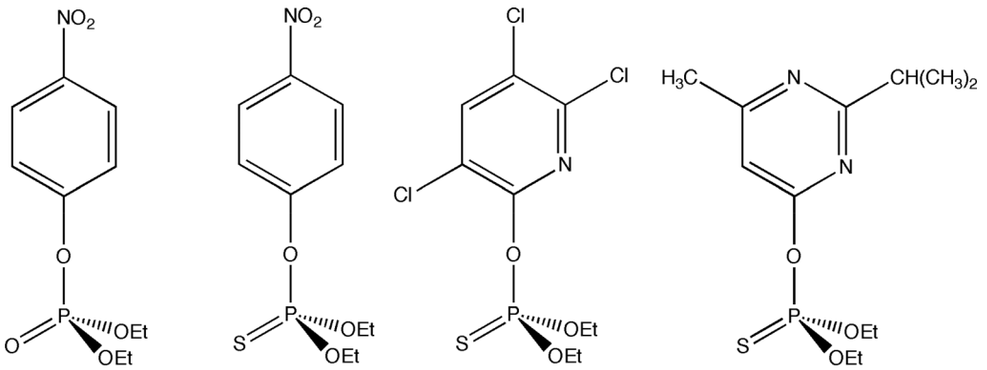

Figure 5.1 The chemical structure of some commonly used phosphotriester and phosphothionate organophosphorus insecticides. From left, paraoxon, parathion, chlorpyrifos, and diazinon.

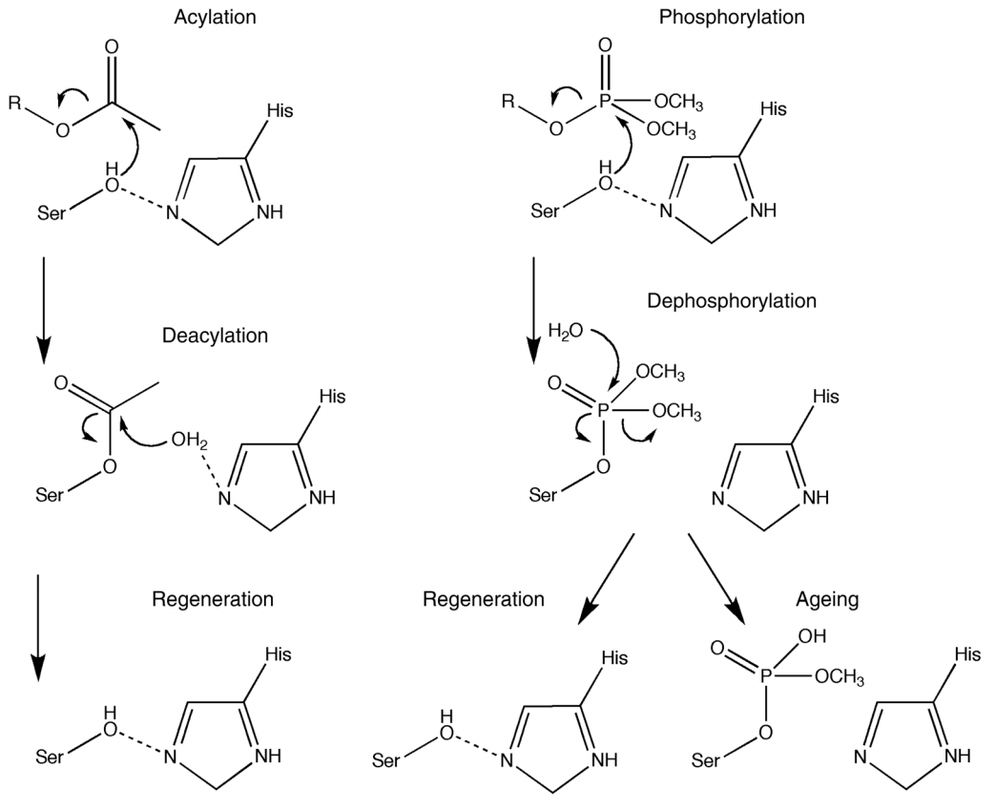

Figure 5.2 The mechanism of acetylcholine and organophosphate hydrolysis by AChE. Acetylcholine hydrolysis proceeds via a planar acyl intermediate, enabling rapid regeneration by an activated solvent molecule. Organophosphate hydrolysis proceeds via a tetrahedral phosphoenzyme intermediate that must be hydrolyzed by free solvent.

AChE, but is further away from the intermediate in the hCE1 structure. Additionally, two other regions implicated in promoting the aging reaction, namely, the helix containing tryptophan 86 (Shafferman et al. 1997), and the acyl loop (Hornberg et al. 2007; Millard et al. 1999) are also missing, or different, in CarbEs (Fig. 5.3). In summary, owing to the close similarity between their catalytic mechanisms and substrates, CarbEs also have high affinity for OPs and are, initially, inhibited in the same manner as AChEs. However, subtle differences between the structures appear to prevent CarbEs from becoming irreversibly inhibited by the aging reaction.

5.2.2 The Role of CarbEs in Vertebrate Organophosphate Metabolism

There is evidence that CarbEs may be involved in conferring low-level metabolic resistance to OP toxicity to mammals. Early studies exploited the induction of liver CarbEs by phenobarbitol to test the effects of increased amounts of CarbEs on OP toxicity (Chambers and Chambers 1990; Clement 1984; Jokanovic 1989). All studies showed that CarbEs contribute to increased resistance against OP poisoning *in vivo*, suggesting that they function either by sequestering OPs, and thereby acting as a sink, or through catalytic detoxification. The inverse approach, in which CarbEs were selectively inhibited by addition of 2-(*O*-cresyl)-4H-1,3,2-benzodioxaphosphorin-2-oxide (Maxwell 1992), a metabolite of the phosphotriester tri-*o*-cresylphosphate that differentially inhibits CarbEs versus ChEs (Jimmerson et al. 1989), further supported this interpretation.

It has also been noted that animals show differential susceptibility to OP poisoning at different life stages (Benke and Murphy 1975; Pope et al. 1991). It was found that although the sensitivity of cholinesterases to OPs, and hepatic activation of OPs, did not change throughout the life cycle, the levels of protection conferred by CarbE sequestration and/or cytochrome P450-mediated dearylation did vary (Atterberry et al. 1997). This observation has been supported by later studies showing a clear correlation across life stages between the level of CarbE activity and protection against OP poisoning (Karanth and Pope 2000; Moser et al. 1998), and it appears that plasma CarbE activity may be of more importance than liver CarbE activity. Studies have

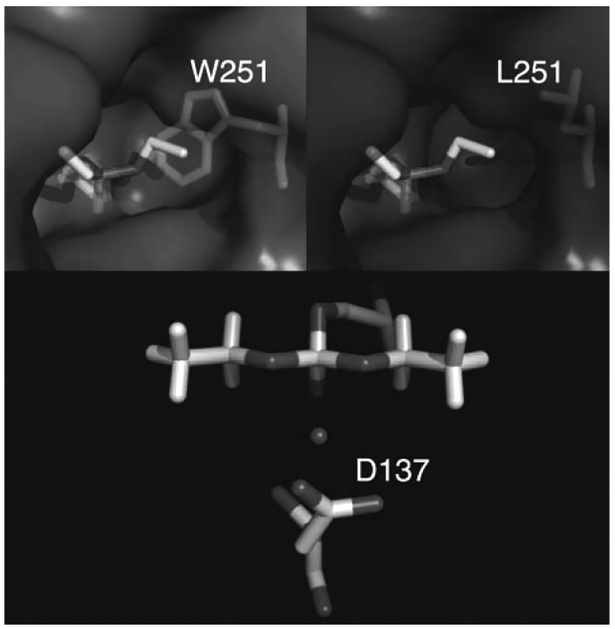

Figure 5.3 The positions of G137D and W251L in the active site of the αE7 CBE. The G137D is positioned to orient a water molecule to hydrolyze the phospho-enzyme intermediate. The W251L mutation will reduce the stabilizing contact between the enzyme and the phospho-adduct.

also been performed to address the question of whether the affinity of CarbEs for OPs, or the number of CarbE molecules, is the most important factor in conferring protection against OPs (Chanda et al. 1997). These studies have shown that, although the affinity of CarbEs did not always correlate with the level of OP sequestration, the number of CarbE molecules did appear to correlate with the level of metabolic resistance.

The studies described above were typically carried out with rodent models. There is comparatively little known about the role of OP metabolism by CarbEs in humans. Recent work has shown that there is little difference in either the levels of CarbE activity or sensitivity to OPs at different stages of postnatal maturation (Pope et al. 2005). Furthermore, unlike the levels of paraoxonase, butyrylcholinesterase and paraoxonase activity in plasma, which vary significantly throughout different life stages, there is little CarbE activity in human plasma at any life stage (Ecobichon and Stephens 1973). In fact, it appears that the major enzyme involved in OP metabolism in humans is not a CarbE, but the serum paraoxonase, or PON1 (Costa et al. 2005).

5.2.3 Physiological Effects of CarbE Inhibition in Vertebrates

The work described up until this point has focused on the role of CarbEs in preventing acute neurotoxicity through minimizing the interaction between the OP and AChE. However, chronic low-level exposure to OPs, such that AChE-mediated neurotoxicity is not lethal, can lead to harmful effects via the inhibition of CarbEs. This is particularly important in the case of interactions with other xenobiotics; affected organisms often show significantly altered metabolism of other xenobiotics (Cohen 1984), which can predispose affected organisms to adverse reaction to medications or even other OPs. Other studies have recently shown that inhibition of CarbEs by OPs can lead to increased cholesterol retention in macrophages, suggesting that other important physiological roles of CarbEs, such as hydrolysis of cholesterol esters in the prevention of atherosclerosis, might also be inhibited (Crow et al. 2008).

5.2.4 The Role of CarbEs in OP Metabolism in Insects

Although CarbEs may only play a relatively minor role in the metabolism of OPs in mammals, a number of insect CarbEs have been found to be directly involved in OP metabolism and resistance (Oakeshott et al. 2005). These typically fall into two categories: (1) overexpression of one or multiple CarbEs that have naturally low levels of catalytic activity, or (2) changes in the activity of the CarbEs leading to an increased rate of OP hydrolysis. The sequestration mechanism works by virtue of the fact that the close similarity between CarbEs and AChEs means that they also bind OPs with very low dissociation constants (Chanda et al. 1997). Thus, if overexpressed in the target organism, they can serve as an OP sink, thereby minimizing inhibition of AChE function in nerve signal transduction. This strategy also works because the native function of many CarbEs is less essential than that of AChE and their inhibition is not lethal. For example, many CarbEs are involved in xenobiotic degradation already (Mikhailov and Torrado 1999). The catalytic improvement mechanism is successful because the high affinity of CarbEs for OPs means they can bind to and hydrolyze, thereby detoxifying, the OP to prevent the interaction between the OP and the target (AChE). Over 20 different examples of CarbE-mediated OP resistance have been documented in insects (Oakeshott et al. 2005). The majority of these examples fall into the first category, that is, overexpression of CarbEs. Of these, the E4/FE4 example in the peach-potato aphid (*Myzus persicae*) and the *estα* and *estβ* genes in mosquitoes (*C. pipiens*) are the best characterized. A well-characterized instance of catalytic improvement leading to metabolic resistance is seen in the αE7 CarbEs from *L. cuprina* and *M. domestica*. These three examples are discussed in detail below.

***5.2.4.1 The E4/FE4 CBEs in* Myzus persicae** Early work on the OP-resistant peach-potato aphid *M. persicae* identified a correlation between CarbE activity and metabolic

resistance (Needham and Sawicki 1971). It was later discovered that the metabolic resistance observed was a result of the increased expression of two β-esterase isozymes, E4 and FE4 (Devonshire and Sawicki 1979; Field et al. 1993; Field and Devonshire 1998). The E4 and FE4 genes are almost identical (98% identity) and genetically linked, suggesting that one of these genes has recently been duplicated (Field et al. 1988). As mentioned, the mechanism of resistance via E4 and FE4 overexpression appears to be through sequestration of OPs, thereby preventing significant interaction between the OP and AChE (Devonshire and Moores 1989). Indeed, the steady-state rate constant for the hydrolysis of dimethyl OPs by these enzymes is on the order of $3\,h^{-1}$, and is even less for diethyl-substituted OPs (Devonshire and Moores 1982). It is interesting that these CarbEs are secreted; salivary secretions typically contain large amounts of digestive hydrolases, which could provide a plausible explanation for an ancestral function for these enzymes (Oakeshott et al. 2005).

It appears as though the major mechanism by which the expression of these enzymes is increased via gene amplification (Field et al. 1999). Either gene can be amplified up to 80 times within a highly resistant strain. This remarkable level of amplification is reflected in the levels of increased CarbE expression, which can reach levels up to approximately 1% of the total soluble protein (Devonshire 1989). It is rare that both isozymes are amplified; most commonly only one of the isozymes is amplified within large (approximately 24 kb for E4 and 20 kb for FE4) amplicons in which the E4 or FE4 gene is the only open reading frame (Blackman et al. 1995, 1999). Genetic analysis of the E4 and FE4 genes from various resistant strains has indicated that the amplification of E4 and FE4 originated from a single event in each instance (Field et al. 1994).

***5.2.4.2 Estα and Estβ in* Culex pipiens** The *estα* and *estβ* genes encode two CarbEs that have higher activities for α- and β-naphthyl acetate as substrates, respectively (Hemingway et al. 2000). The *estα* and *estβ* genes share approximately 50% identity and, like the E4/FE4 genes, are also thought to have derived from a duplication event (Ranson et al. 2002; Vaughan and Hemingway 1995; Vaughan et al. 1997). As is seen in the E4/FE4 esterases, the resistance mechanism mediated by the amplified *C. pipiens* esterases appears to derive from sequestration. Again, the steady-state rate constant for OP hydrolysis is effectively negligible (Ketterman et al. 1992). Whereas E4/FE4 are secreted, *estα* and *estβ* are associated with the microsome, which has long been known to be involved in xenobiotic metabolism (Terriere 1984).

Susceptible *C. pipiens* strains have significantly lower levels of CarbE activity than resistant strains, which generally express much higher levels of these genes as a consequence of gene amplification (Karunaratne et al. 1995). Indeed, at least nine distinct *estα* and/or *estβ* amplifications have been described in *C. pipiens*, involving either each gene in isolation, or both in combination (Guillemaud et al. 1998; Hemingway et al. 1998, 2000; Pasteur et al. 1981). Many mosquito populations are polymorphic for several amplicons; however, two amplicons ($est\beta1^2$ and $est\alpha2^1/est\beta2^1$) are the most common (Qiao and Raymond 1995; Raymond et al. 1987). As in the E4/FE4 esterases, the level of amplification can be remarkable, with amplifications of up to 250-fold corresponding to increases in CarbE activity of up to 500-fold (up to 0.4% of the total soluble protein of the organism) and resistance factors of 800-fold (Karunaratne et al. 1993; Mouches et al. 1990).

5.2.4.3 Catalytic Improvement of the αE7 CarbEs from L. cuprina and M. domestica Unlike the sequestration mechanism used by the E4/FE4 CarbEs in *M. persicae*, or the *estα/estβ* CarbEs in *C. pipiens*, the *αE7* genes from *L. cuprina* (*LcαE7*) and *M. domestica* (*McαE7*) utilize an altered catalytic mechanism to confer metabolic resistance to OPs on their host. The kinetic characteristics of the wild-type enzymes make them good candidates to evolve towards metabolic resistance. Most importantly, the affinity of these CarbEs for their target is higher than that of AChE (k_i of LcαE7 with paraoxon $= 6.3 \times 10^7\,M^{-1}\,min^{-1}$ compared with $5.1 \times 10^6\,M^{-1}\,min^{-1}$ for *L. cuprina* AChE; Newcomb et al. 1997; Chen et al. 2001). Additionally, these microsomal esterases are expressed in relatively high abundance across the larvae and adult life stages during which the insects will be exposed to insecticides (Parker et al. 1991, 1996). Metabolic resistance to OPs in *L. cuprina* and *M. domestica* can be conferred by either of two amino acid polymorphisms in αE7; namely, G137D and W251L. The G137D substitution confers broad spectrum resistance against a range of OPs. In contrast, the W251L substitution confers a different class of resistance, which is particularly effective against malathion (Campbell et al. 1998a, 1998b).

The G137D substitution now dominates in contemporary populations of both *L. cuprina* and *M. domestica* (Claudianos et al. 1999; Newcomb et al. 1997, 2005; Scott and Zhang 2003; Smyth et al. 2000). Although the mutation appears to have occurred multiple times in different allelic backgrounds, a single allele is now relatively common in each species. The G137D variant displays significantly faster steady-state rate improvements for the hydrolysis of diethyl (about 55-fold) over dimethyl (about 33-fold) OPs (Devonshire et al. 2003). These improvements can result in up to 20-fold greater OP resistance in adult *L. cuprina* (Campbell et al. 1998b).

Although a crystal structure of an αE7 CarbE is not available, the structural effects of this mutation can be inferred from the structures of related enzymes, such as other CarbEs and the cholinesterases (Fleming et al. 2007; Sussman et al. 1991). In the wild-type enzyme, G137 is a highly conserved residue that forms part of the oxyanion hole via its main

chain nitrogen. As seen in Figure 5.3, this residue is directly opposite the catalytic serine; mutation to D137 results in the introduction of a potential general base in a position to activate a water molecule to hydrolyze the phosphorylated serine and regenerate the active site (Newcomb et al. 1997). By activating a solvent molecule, the catalytic mechanism of E3 has similarities to the deacylation step in the native carboxylester hydrolysis activity (Fig. 5.2). Interestingly, these structural changes, which result in an increase in the promiscuous OP hydrolase activity, trade off with the original carboxylesterase function, which decreases significantly. This decrease in the native activity is most likely as a consequence of altering the otherwise highly conserved oxyanion hole (Claudianos et al. 1999; Newcomb et al. 1997). This coincident gain of OP hydrolytic activity and loss of aliesterase activity defines the so-called mutant aliesterase mechanism (Oppenoorthand van Asperen 1960).

As a consequence of the loss of the native CarbE activity, *L. cuprina* expressing the variant G137D form of αE7 display signs of reduced fitness in the absence of the insecticide. Specifically, measurable fluctuating (bilateral) asymmetry is seen in resistant strains of the insects in the absence of the insecticide (McKenzie et al. 1982). Indeed, it was not until this fitness cost was relieved by an epistatic *modifier* mutation at a second gene that the polymorphism became widespread (A. G. Davies et al. 1996).

The second polymorphism in *αE7* genes that is associated with insecticide resistance in *L. cuprina* and *M. domestica*, W251L, is considerably less common (Claudianos et al. 1999; Smyth et al. 2000). Significantly, unlike the G137D variant, the W251L variant retains significant, albeit different to wild-type, CarbE activity (Campbell et al. 1998a). It is interesting that in a recent PCR study of museum collections, polymorphism at position 251 was found to exist at high frequency (up to approximately 20%) in *L. cuprina* populations prior to the introduction of pesticides (Hartley et al. 2006). Thus, this mutation might be effectively neutral where the native CarbE activity is concerned. The level of improvement to the OP hydrolase activity is also significantly less; 34-fold and 10-fold increases in the turnover of dimethyl- and diethyl-substituted OPs, compared with the wild-type enzyme from *L. cuprina*, respectively (Devonshire et al. 2003). These *in vitro* rates are reflected in the *in vivo* resistance of the insects; 5- to 27-fold increased resistance in adult *L. cuprina* for dimethyl OPs versus 2- to 6-fold increases in the resistance to diethyl OPs (Campbell et al. 1998b).

Like the G137D mutation, the structural effects of the W251L mutation can be inferred from the structures of close homologues. In fact, W251 is quite well conserved throughout this class of enzymes. As can be seen in Figure 5.3, there is no significant change in the catalytic machinery; rather, the acyl binding pocket is widened. The improved OP hydrolase activity is proposed to result from a loss of stabilizing interactions between the protein and the phosphorylated serine, leading to an increased rate of active site regeneration. However, the loss of size of this residue also results in losses of stabilizing hydrophobic interactions with the remainder of the enzyme. Indeed, the W251L variant of *L. cuprina* αE7 appears to be less stable than the wild-type or G137D variant *in vitro* (C.J.J, J.G.O, unpublished data), **Q2** which agrees with the results of another study that has shown mutations at the equivalent residue in the αE7 gene in *Anisopteromalus calandrae* also resulted in unstable protein (Baker et al. 1998).

An interesting variation on these forms of catalytic metabolic resistance is the resistance to malathion in insects expressing W251L αE7, which occurs through OP hydrolase as well as CarbE activity and can result in up to 130-fold increased resistance to malathion (Campbell et al. 1998a). Malathion is unusual among the OP insecticides in that it contains a carboxylester bond within its leaving group. Thus, the enzyme displays two activities in its presence: (1) OP hydrolase activity at the phosphoester bond to the leaving group and (2) CarbE activity at the carboxylester bond in the leaving group. It appears that the exceptional resistance to malathion shown by insects expressing the W251L αE7 enzyme is a result of increases in both the malathion CarbE activity and the OP hydrolase activity. However, unlike the G137D mutation, the *in vitro* activity does not correlate with the levels of *in vivo* resistance (Smyth et al. 2000). First, the wild-type enzymes have comparable malathion carboxylesterase activities to the W251L variant, yet almost no native resistance (Devonshire et al. 2003). Second, the G137D variant has very low malathion CarbE activity *in vitro* yet greater resistance levels than wild-type αE7 *in vivo* (Campbell et al. 1998b). These observations suggest that the malathion CarbE activity is of secondary importance to the OP hydrolase activity. However, the primary metabolite found in resistant flies is the product of the CarbE activity. This may be explained by the fact that although wild-type αE7 can efficiently hydrolyze the carboxylester bond in the leaving group, it is still rapidly inhibited by the OP. Thus, the W251L mutation likely confers high resistance because it allows regeneration of the active site through the OP hydrolase activity, allowing the enzyme to perform as a CarbE with significantly less inhibition.

In addition to these point mutations, there appears to have been a recent duplication event in *L. cuprina* that has resulted in the duplication of the chromosomal region containing *αE7*, with two copies of this gene and others from the α-esterase cluster being carried on one chromosome (Newcomb et al. 2005; Smyth et al. 2000). Interestingly, to date these duplications have always resulted in the combination of a high CarbE variety or the gene (wild-type or W251L) and a low CarbE/high OP hydrolase variety (G137D). Some of these strains have broader spectrum resistance profiles than those containing either variety in isolation (Campbell et al. 1998b).

5.3 CARBAMATES

5.3.1 Structural and Chemical Basis of the Carboxylesterase-Carbamate Interaction

Carbamate pesticides are ester derivatives from particular carbamic acids and have a wide range of biocidal activities. When the hydrogen atoms associated with the nitrogen are substituted by methyl groups, the compounds generated (methyl or dimethyl carbamates) present insecticidal activity (Fig. 5.4). However, herbicidal or fungicidal activity is acquired when one of the hydrogen atoms associated with the nitrogen atom is replaced by an aromatic or benzimidazol group, respectively. Sulfur can substitute for one or two oxygen atoms in the carbamic acid moiety to form thio- or dithiocarbamate compounds. The majority of carbamates registered in the world market are fungicides and herbicides. Among the carbamate insecticides, carbofuran, carbaryl, aldicarb, methomyl, and propoxur are the most frequently applied in agriculture.

The mechanism of acute toxicity of carbamate insecticides is similar to that of OP insecticides, that is, inhibition of AChE activity at the cholinergic synapses (Fukuto 1990). The mechanism of inhibition is similar to that proposed for hydrolysis and involves carbamylation of the nucleophilic serine, followed by release of an alcohol leaving group (Sogorb and Vilanova 2002). Subsequently, the AChE can be reactivated by attack of a water molecule, and the carbamic acid is released and rapidly decomposed into CO_2 and methylamine (Fig. 5.5). However, the onset and recovery of carbamylated AChE activity is faster than that for equipotent exposure to OP insecticides. This is due to two factors: first, unlike OP insecticides, the *in vivo* inhibition of AChE activity by carbamates does not require prior metabolic activation of the carbamate for maximum anti-ChE potency; second, carbamylated AChE does not undergo the previously described aging reaction with OPs and can be reactivated at a significantly quicker rate because the adduct is planar and not tetrahedral.

The high diversity of the molecular structures of insecticidal carbamates accounts for their complex metabolism, which can involve oxidation, hydroxylation, dealkylation, hydrolysis, and conjugation reactions (Fig. 5.5). As with other lipophilic xenobiotics, carbamates are converted to more soluble metabolites by the introduction of a polar group into the parent compound by a group of enzymes referred to as phase I enzymes. These enzymes include the cytochrome P450-dependent monooxygenases (CYP), flavin-containing monooxygenases (FMO), and CarbEs (Kulkarni and Hodgson 1980; Tang et al. 2006). The phase I metabolites can be conjugated with an endogenous molecule (gluthathione, glucuronic acid, sulfate, or amino acids) to

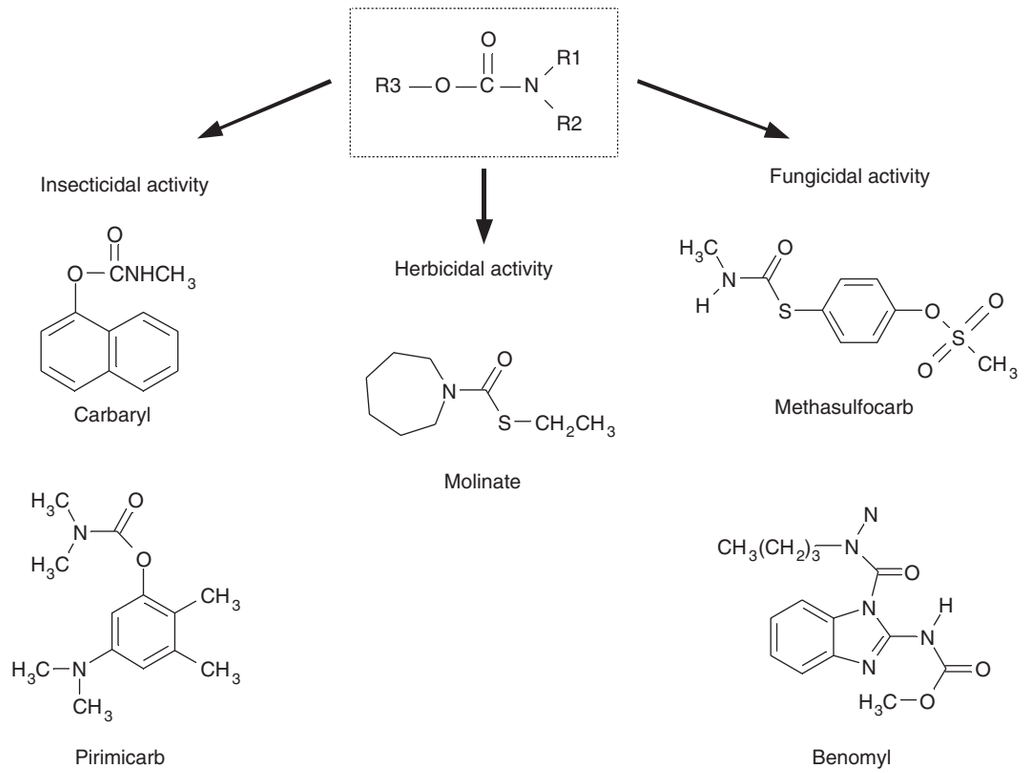

Figure 5.4 General chemical structure of carbamate compounds.

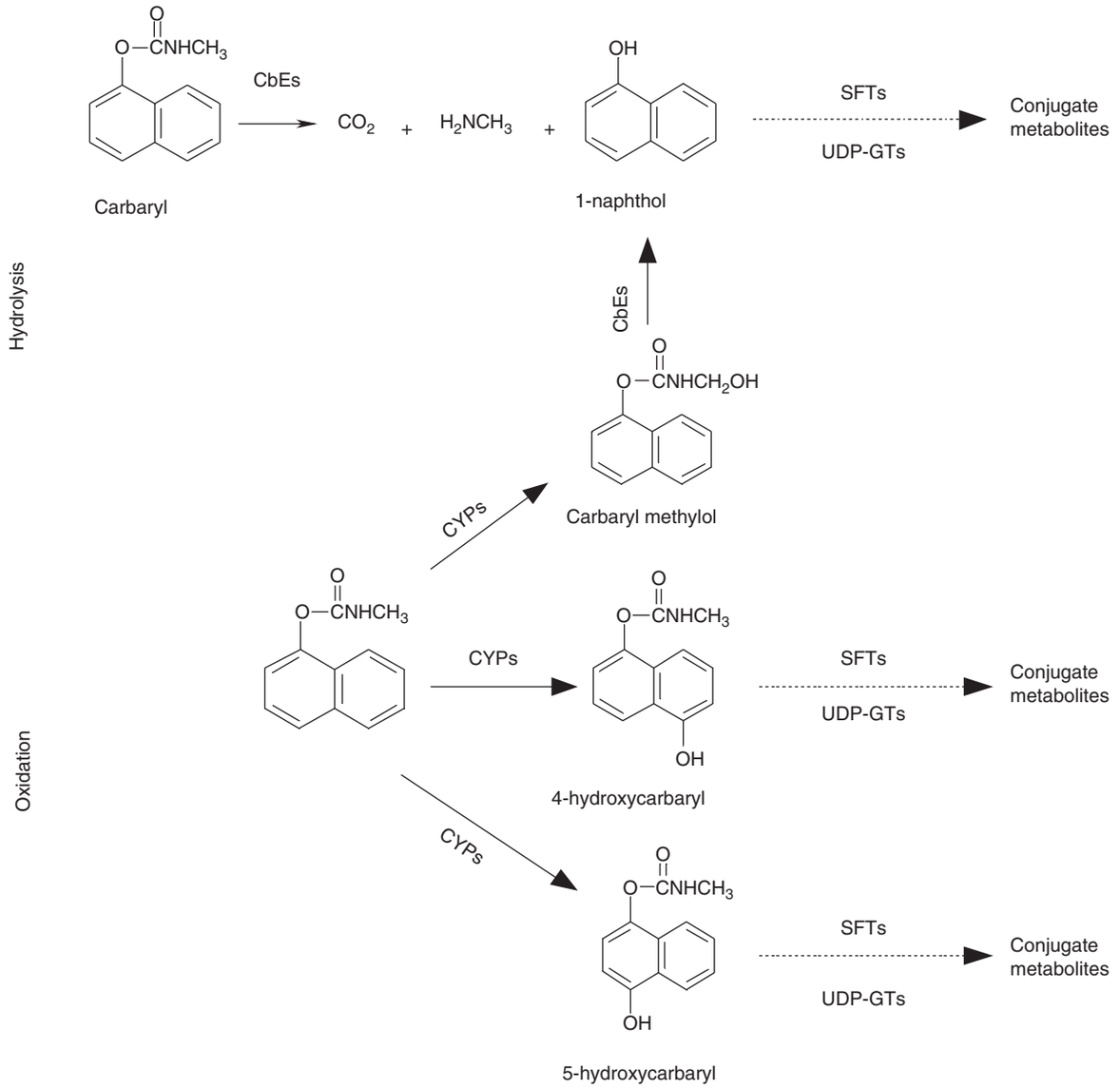

Figure 5.5 Main pathways of hydrolytic and oxidative metabolism of carbaryl. CbEs = carboxylesterases, CYPs = cytochrome-dependent monooxygenase isozymes, SFTs = sulfotransferases, UDP-GTs = UDP-glucuronosyltransferases. (Adapted from Kulkarni and Hodgson 1980 and Tang et al. 2002.)

yield a more polar secondary metabolite. Conjugation is catalyzed by a group of enzymes known as phase II enzymes which include glutahione *S*-transferase, sulfotransferases or UDP-glucuronosil transferase. Figure 5.5 summarizes the wide variety of metabolic reactions to metabolize carbaryl. CarbEs can hydrolyze either the parent compounds or the phase I metabolites, provided the carbamic ester bond remains intact. For example, the oxidative metabolites of carbaryl (5-hydroxycarbaryl) or carbofuran (3-hydroxycarbofuran) can undergo further hydrolysis by CarbEs (Dorough 1968). Herein, the role of CarbEs can be crucial to reducing the *in vivo* toxicity of the carbamate because occasionally the oxidative metabolite can evidence greater toxicity than the parent compound. This is the case for 5-hydroxycarbaryl, which shows an acute oral LD_{50} of 297 mg/kg to rats, whereas the LD_{50} of carbaryl is 430 mg/kg (Dorough 1970).

5.3.2 Vertebrate Metabolism of Carbamates via CarbE Activity

Most of the studies on the metabolism of carbamate compounds in vertebrates have been performed using either tissue homogenates or isolated whole organs from animal

models. The majority of the data imply that a CarbE is at least partly involved in carbamate metabolism. For example, isolated perfused rat lung was used by Pillai et al. (1993) to investigate the metabolism of carbofuran. They found that 11% of the administered dose of carbofuran was metabolized by the lung after 1 hour of exposure and that the main metabolites detected in the perfusate were 3-hydroxycarbofuran and carbofuran phenol. These oxidative and hydrolytic metabolites were also the most abundant in rat liver microsomes incubated with this carbamate (Dorough 1968). Indeed, CarbE-catalyzed hydrolysis of carbofuran *in vivo* seems to be an important route of carbofuran detoxification; in an experimental study using reverted sacs of rat small intestine, Pekas (1972) found that carbamate hydrolysis catalyzed by CarbEs was the rate-limiting step of intestinal detoxification of methyl carbamates. The importance of hydrolysis as a detoxification route of carbamates was also evidenced by Ferguson et al. (1984), who found that the metabolic rate constant for *in vivo* hydrolysis of carbofuran was higher than that for oxidative metabolism in rats.

The importance of CarbE-mediated metabolism of carbamates in providing some toxicological resistance to these compounds in vertebrates has been evidenced by studies using rat plasma and liver CarbEs, which show that the CarbEs play an important role as carbamate scavengers by reducing the impact of carbamates on brain AChE activity (Gupta and Dettbarn 1993). However, recent studies with liver microsomes from other mammalian species have shown that oxidative metabolism by CYP isozymes is the primary pathway for carbamate metabolism, whereas the contribution of esterases is minimal (Tang et al. 2002; Usmani et al. 2004). Thus, although the literature is inconclusive regarding the extent to which CarbEs are responsible for carbamate metabolism, they are clearly involved to some degree.

Beside carbamate insecticides, CarbE activity can also participate in the metabolic detoxification of other carbamate compounds such as ethyl carbamate, a potent carcinogen also known as urethane (Zimmerli and Schlatter 1991). Ethyl carbamate is actively metabolized by both the CYP2E1 isozyme and the CarbE isozyme hydrolase A (Forkert and Lee 1997; Nomeir et al. 1989; Yamamoto et al. 1990). Although, some investigations support the proposition that CarbE-catalyzed ethyl carbamate hydrolysis is physiologically important (Nomeir et al. 1989; Yamamoto et al. 1990), others have demonstrated that this chemical inhibits liver and lung CarbEs, consequently decreasing the concentration of ethyl carbamate available to oxidation by CYP2E1 (Lee et al. 1998; Lee and Forkert 1999). Likewise, the main oxidative metabolite of ethyl carbamate, vinyl carbamate, also inhibits CarbE activity in human and mice lung microsomes (Forkert et al. 2001).

In contrast to the effects of OPs, which often show greater inhibition of CarbEs than AChE, carbamates generally inhibit AChE activity more strongly. For example, plasma CarbE activity was also more sensitive to the OP diazinon than AChE activity in the nestling European starling *Sturnus vulgaris*, whereas aldicarb resulted in higher inhibition of plasma AChE activity (Parker and Goldstein 2000).

5.3.3 Physiological Effects of CarbE Inhibition by Carbamates

Inhibition of CarbE activity by carbamates is not a generalized phenomenon as with OPs; it depends mainly on the organism, the CarbE isozyme and the variety of carbamate. Interactions of CarbE activity with anti-cholinesterase pesticides have been widely investigated in the liver and plasma of mammals, or in the microsomal fraction of metabolically active organs such as liver, lung, and intestine. However, some studies have reported high levels of CarbE activity in the reproductive organs, with a possible role in the differentiation and maturation of spermatozoids (Mikhailov and Torrado 1999). A CarbE activity named hydrolase-A has been described in rat testicular microsomes (Yan et al. 1995). This esterase seems to be implicated in the testicular toxicity caused by the thiocarbamate herbicide molinate (Jewell and Miller 1998). Molinate and its sulfoxide and sulfone metabolites are able to inhibit CarbE activity in the Leydig cells, leading to a depletion of steroidogenesis. It was postulated that the inhibition of CarbE activity by molinate accounts for the inhibition of the mobilization of cholesterol esters and consequently the synthesis of testosterone (Jewell and Miller 1998). Some insecticidal carbamates such as carbofuran or carbaryl affect the function and morphology of reproductive organs and estrogen serum levels in rats and fish, and inhibition of CarbE activity could be involved in the manifestation of these adverse reproductive effects (Kitamura et al. 2006).

5.3.4 Invertebrate Metabolism of Carbamates by CarbEs

Unlike OPs, there are relatively little data regarding the role of CarbEs in conferring metabolic carbamate resistance to insects. This is surprising considering that carbamates are considerably better substrates for CarbEs than OPs. Indeed, structurally related enzymes have evolved in bacteria that catalyze their hydrolysis at relatively fast rates (Pohlenz et al. 1992). There is, however, a notable instance in which metabolic carbamate (thiodicarb) resistance has been clearly linked to an overexpressed CarbE in the moth *Heliothis virescens* (Goh et al. 1995). This enzyme was also shown to be immunoreactive to specific antibodies to, and share sequence similarity to, the E4 esterase from *M. persicae*, suggesting it is likely to be related.

Carbamate metabolism by aquatic invertebrates has been studied in some detail. As seen in aquatic vertebrates, carbamates often more strongly inhibit AChE activity than CarbE

activity in aquatic invertebrates. For example, in their study of the Mediterranean mussell *Mytilus galloprovincialis*, Galloway et al. (2002) reported that CarbE activity was more strongly inhibited by the organophosphates paraoxon ($IC_{50} = 0.4$ mM) and chlorpyrifos ($IC_{50} = 10.1$ mM) than was its AChE activity ($IC_{50} = 1.83$ mM for paraoxon and $IC_{50} = 15.0$ mM for chlorpyrifos), whereas the carbamate eserine hemisulfate strongly inhibited AChE activity and had no significant effect on CarbE activity. However, despite differences in CarbE sensitivity to carbamates, sequestration of carbamates by CarbEs is thought to be an effective stoichiometric mechanism of detoxification. For example, Barata et al. (2004) investigated the inhibition of ChE and CarbE activities in the water flea *Daphnia magna* exposed to carbofuran. A mean IC_{50} value of 900 nM of carbofuran was reported for CarbE activity, suggesting a tight association between the CarbE and the carbamate. Similarly, when organisms were previously exposed to the known CarbE inhibitors triphenyl phosphate or 2-(*O*-cresyl)-4H-1,3,2-benzodioxaphosphorin-2-oxide, the LC_{50}s for carbofuran decreased from 760 nM (carbofuran alone) to 160–110 nM if the daphnids were previously exposed to the CarbE inhibitors. This significant increase in the acute toxicity of carbofuran was explained by a higher availability of the carbamate to inhibit the AChE activity.

The insensitivity of CarbEs to carbamate inhibition has been reported recently in an *in vitro* study evaluating the potential use of CarbE inhibition as a biomarker of pesticide exposure in the terrestrial snail *Xeropicta derbentina* (Laguerre et al. 2009). It was found that *in vitro* inhibition of CarbE activity using three different substrates, α-naphthyl acetate (α-NA), 4-nitrophenyl acetate (4-NPA), and 4-nitrophenyl valerate, followed a sigmoidal concentration-effect model when the inhibitors were OP pesticides (dichlorvos and chlopryrifos-oxon), but esterase inhibition was very low or absent when the homogenate was incubated with carbamates such as carbofuran or carbaryl. Conversely, carbaryl and other carbamates caused an inhibition of some CarbE isozymes in the hepatopancreas of the mussel *Mytilus galloprovincialis* (Ozretic and Krajnovic-Ozretic 1992). Carbaryl also strongly inhibits the hepatopancreas CarbE activity of the crayfish *Procambarus clarkia*, showing an IC_{50} value of 7×10^{-7} M, whereas the esterase was resistant to serine hemisulfate (Vioque-Fernandez et al. 2007).

This latter result suggests that a possible explanation for some of the wide variation in the sensitivities of CarbEs to carbamates found in the literature concerns the substrate used for enzyme measurement (usually α-NA or 4-NPV). Indeed, Sanchez-Hernandez and Wheelock (2009) found that the inhibitory potency of chlorpyrifos-oxon on earthworm CarbE activity was highly dependent on the substrate used for esterase assay. In a similar study, substrate-specific differences in CarbE activity in *Xeropicta derbentina* were observed with several OP and carbamate insecticides (Laguerre et al. 2009). Further work is needed both on the sensitivities of CarbEs to specific carbamates and on the relationships between their sensitivities to inhibition and abilities to hydrolyze the pesticides.

5.4 SYNTHETIC PYRETHROIDS

5.4.1 Structural and Chemical Basis of the Synthetic Pyrethroid Carboxylesterase Interaction

Pyrethroid insecticides are synthetic analogs of the naturally occurring pyrethrum flower (*Chrysanthemum cinerariaefolium*; Casida 1973; Elliott 1976; J. H. Davies 1985). Pyrethrum extract contains six different ester-containing compounds that demonstrate insecticidal activity, with varying degrees of non-target-organism toxicity. In the 1960s, significant advances in pyrethroid research resulted in the replacement of pyrethrum extracts with synthetic pyrethroids (Katsuda 1999). These new synthetic pyrethroids (Fig. 5.6) were developed to overcome the photolability of pythrethrin as well as to increase the selectivity for insects. The first major modifications to the original pyrethrum structure were made by researchers at Sumitomo Chemical Co. and Michael Elliott's group at the Rothamsted Research Center in the United Kingdom (Elliott et al. 1965, 1973a, 1973b, 1974; Ohno et al. 1976).

The structures of pyrethroids can be most easily described in terms of their alcohol and acid moieties, which are synthesized via condensation to form an ester moiety. The initial synthetic efforts to improve on pyrethrum involved multiple structures, which were iteratively standardized to optimize the insecticidal and physiochemical properties. The initial acid moiety was standardized as chrysanthemic acid (Elliott et al. 1965) and was incorporated into allethrin, the first commercial synthetic pyrethroid. A range of alcohol moieties was then incorporated into the pyrethroid structure to optimize both the insecticidal activity and the chemical stability. The next product was resmethrin, which included a phenyl ring into the alcohol moiety (Elliott et al. 1967). However, due to chemical instability, the 5-benzyl-3-furylmethyl alcohol moiety was replaced with 3-phenoxybenzyl alcohol (Elliott et al. 1973b). To further increase the stability, chrysanthemic acid was modified by substituting the methyl groups in the isobutenyl side chain with chlorine atoms to form permethrin (or with bromine to form deltamethrin; Elliott et al. 1973b, 1974). Further modifications involved replacing the cyclopropane carboxylate moiety with α-isopropyl 4-chlorophenylacetate (fenvalerate; Ohno et al. 1976).

The next major advance was the synthesis of type II pyrethroids, which use 3-phenozybenzaldehyde cyanohydrin instead of 3-phenoxybenzyl alcohol as the alcohol moiety to give an α-cyano group-substituted ester (Elliott 1976). This substitution converted the ester linkage from a primary

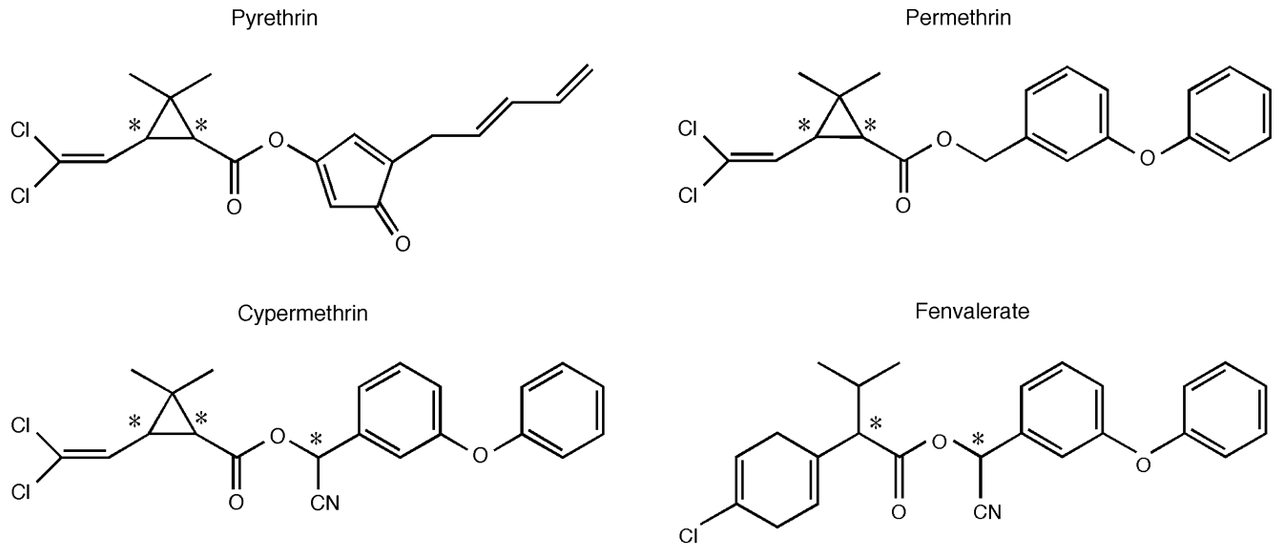

Figure 5.6 A variety of natural (pyrethrum) and type I (permethrin and fenvalerate) and type II (cypermethrin) synthetic pyrethroids. Chiral centers are indicated (*).

to a secondary ester, which is the distinguishing feature between type I and type II pyrethroids (i.e., permethrin vs. cypermethrin). This developmental process has continued and there are numerous additional pyrethroids available, often with niche applications. For example, the sensitivity of many fish to pyrethroid application has led to the development of so-called "fish-safe" derivatives, including cycloprothrin, etofenprox, flufenprox, and silafluofen (Pap 2003).

Pyrethroids are primarily sodium channel toxins that prolong excitation, but they exhibit little or no direct cytotoxic effects (Casida 1973). The major site of action of all pyrethroids is the voltage-dependent sodium channel; however, a number of other potential interaction sites exist, including the voltage-gated chloride channels (Farag et al. 2007), GABA-gated chloride channels (Spencer and O'Malley 2006), and protein phosphorylation (Bradberry et al. 2005). The degree of sodium channel excitability is dose related, but the nature of the excitability is structure dependent (Kulkarni and Hodgson 1980). Pyrethroids exhibit ~2250 times greater toxicity to insects relative to mammals because insects have increased sodium channel sensitivity, lower body temperature, and smaller body size (Tang et al. 2002). In addition, mammals are protected by poor dermal absorption of pyrethroids and rapid metabolism to nontoxic metabolites.

Pyrethroids contain a variable number of chiral centers and accordingly have a variety of optical isomers, often with variable biological activity. For example, there are eight different optical isomers of cypermethrin. The *S,S*-isomer of fenvalerate has greater insecticidal activity relative to the *R,R*-isomer or the racemic mixture, leading to the selective manufacture and sale of the *S,S*-isomer (esfenvalerate vs. fenvalerate). Pyrethroid toxicity varies greatly with species and is also life stage-dependent; for example, the oral LD_{50} for deltamethrin in adult rats is 81 mg/kg vs. 5.1 mg/kg in weanling rats.

Pyrethroid metabolism involves CarbE-mediated hydrolysis of the esters to the corresponding alcohol and acid, with organism-specific levels of CarbE activity often negatively correlated with pyrethroid-associated toxicity (Abernathy and Casida 1973; Wheelock et al. 2005a, 2005b, 2008). The general catalytic mechanism for CarbE-mediated hydrolysis involves the previously discussed serine-histidine-glutamate catalytic triad (Bencharit et al. 2003a, 2003b). ~~This process is shown in Figure 5.7 for the pyrethroid permethrin.~~

Studies on allethrin (Abernathy and Casida 1973), resmethrin (Miyamoto et al. 1971; Ueda et al. 1975), phenothrin, permethrin, and cypermethrin (Shono et al. 1979), have demonstrated that the *trans*-isomers of the pyrethroids are more rapidly hydrolyzed than the corresponding *cis*-isomers in both mammals and insects. These findings support the generally observed lower toxicity of the *trans*-isomers (Miyamoto 1976; Soderlund and Casida 1977). A recent study with a recombinant pyrethroid-hydrolyzing CarbE originally isolated from mouse liver found that *trans*-permethrin and *trans*-cypermethrin were hydrolyzed 22 and 4 times faster than their *cis*-isomers, respectively (Stok et al. 2004). **Q3** In addition, assays conducted with four fenvalerate isomers showed that the two less toxic enantiomers *(αR)(2R)*- and *(αS)(2R)*-fenvalerate were hydrolyzed ~50 and 5 times faster than the *(αR)(2R)* enantiomer. The most toxic fenvalerate enantiomer *(αS)(2S)*-fenvalerate (esfenvalerate) exhibited essentially no CarbE-mediated hydrolysis, which

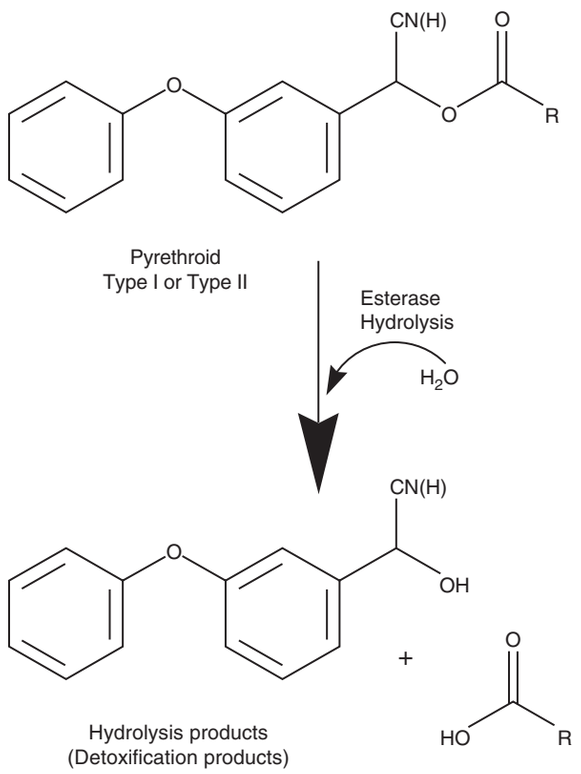

Figure 5.7 Esterase-mediated hydrolysis of pyrethroids. Esterases hydrolyze an ester via the addition of water to form the corresponding alcohol and acid, which are generally detoxification products.

supports the observations from previous studies with crude mouse liver microsomes (Kaneko 1988; Takamatsu et al. 1987). Further testing on recombinantly expressed mammalian pyrethroid-specific esterases (BAC36707 and NM_133960) using pyrethroid-surrogate substrates found that the esterase-specific stereospecificity varied significantly among the eight cypermethrin enantiomers; the least toxic *1S trans, αS*-stereoisomer was hydrolyzed ~300 times faster than the two most potent isomers *1R cis, αR*- and *1R cis,* Q3 *αS*-cypermethrin (Huang et al. 2005; Stok et al. 2004). These findings indicate that the study of esterase stereospecificity is important for understanding the efficacy, toxicity, and metabolism of pyrethroids.

5.4.2 The Role of CarbEs in SP Metabolism in Vertebrates

Pyrethroids are rapidly hydrolyzed in the liver, thereby preventing the nervous system effects that are lethal to insects (Aldridge 1990). It was recognized as early as 1977 that the main routes of SP metabolism in vertebrates involve the function of esterases (Abernathy and Casida 1973), but more recent work has shown that this esterase activity can be attributed to specific CarbEs. There are two main human CarbE isozymes: hCE-1, which is primarily located in the liver, and hCE-2 (or hiCE), which is primarily located in the intestines (Redinbo and Potter 2005). Both isozymes have been demonstrated to hydrolyze pyrethroids, but with very different activity profiles (Ross and Crow 2007). For example, hCE-1 and hCE-2 were shown to hydrolyse *trans*-permethrin 8- and 28-fold more efficiently than *cis*-permethrin, respectively, whereas hydrolysis of bioresmethrin was catalyzed efficiently by hCE-1, but not by hCE-2 (Ross et al. 2006). These results were supported by Nishi et al. (2006) who examined a range of authentic pyrethroids as well as fluorescent surrogates. Additional work by Ross and coworkers demonstrated that *trans*-permethrin was effectively hydrolyzed by a sample of pooled human intestinal microsomes (containing predominantly hCE-2 activity), while deltamethrin and bioresmethrin were not.

Studies by Godin et al. (2006, 2007) demonstrated significant species differences in the hydrolysis of deltamethrin in rat and human liver microsomes, which were in part due to differences in the intrinsic activities of rat and human CarbEs. Humans lack serum CarbE activity, but a serum CarbE isozyme has been identified in rats. This purified isozyme was demonstrated to hydrolyze both bioresmethrin and *trans*-permethrin effectively, but to only very slowly hydrolyze deltamethrin, esfenvalerate, alpha-cypermethrin, and *cis*-permethrin (Crow et al. 2007).

5.4.3 Physiological Effects of CarbE Inhibition by SPs

Pyrethroids generally exhibit low mammalian toxicity (Vijverberg and van den Bercken 1990; Ray and Forshaw 2000). Despite their extensive worldwide use, there are relatively few reports of human pyrethroid poisoning, with as few as 10 deaths having been reported from ingestion or following occupational exposure (Bradberry et al. 2005). Nevertheless, SP toxicity does present some hazard to human health, either through deliberate ingestion (Yang et al. 2002) or as a consequence of pesticide application (Perry et al. 2007). There is an expanding body of research data suggesting that SPs have toxic effects in vertebrates, such as impaired testosterone production (Zhang et al. 2007), impaired offspring development (Farag et al. 2007), and adverse effects on the developing nervous system (Sinha et al. 2006). Indeed, over 300 cases of human illness associated with pyrethroid exposure have been documented in California alone between 1996 and 2002 (Spencer and O'Malley 2006).

5.4.4 The Role of CarbEs in SP Metabolism in Invertebrates

Although the major form of insect resistance to SPs appears to result from mutations to the target site (kdr) (Bradberry et al. 2005), over a dozen cases of SP resistance associated

with increased expression of esterase activity have now been reported (Anstead et al. 2007; Guerrero and Barros 2006; McAbee et al. 2004; Oakeshott et al. 2005). In some cases the increased activity is due to specific CarbE isozymes, but in other cases several isozymes show more intense staining. Increases of over 100-fold have been reported in the staining intensities of particular isozymes. None of these cases have been elucidated as yet at a molecular level, although the early literature at least (see Oakeshott et al. 2005 for a review) suggested that at least some of the cases may be due to overexpression of CarbEs rather than structural changes in the enzymes affecting their catalytic activities. The increased activities appear to be stably expressed and not inducible by the SPs. Interestingly, a few of the cases involve cross-resistance to the unusual carboxylester OP malathion (Oakeshott et al. 2005).

While there are no direct data as yet on the molecular basis of esterase-mediated SP resistance, several studies have shown that many insect CarbEs, even from SP-susceptible strains, can hydrolyze SPs (Oakeshott et al. 2005). The most detailed study in this respect involves the αE7 esterase from the sheep blowfly *Lucilia cuprina* (Mouches et al. 1990), the same enzyme that has mutated to confer OP resistance on this species (see above). The common, OP-susceptible form of this enzyme has high SP hydrolytic activity (up to 90,000 M^{-1} sec^{-1}) for SPs and, like its mammalian counterparts, shows strong preferences for type I over type II SPs, dichloro- over dibromo- substituents, and *trans*- over *cis*- isomers across the cyclopropane ring. Significantly, the general trend in the activities of the enzyme across different SPs and their isomers is negatively correlated with the relative efficacies of the different compounds and isomers as insecticides. Also important, however, is the finding that some naturally occurring variants of αE7 (including the W251L change associated with resistance to dimethyl OPs and, in particular, malathion) differ substantially in isomer preference, and in particular their *cis*/*trans* preference across the cyclopropane ring and *R*/*S* preference with respect to the orientation of the α cyano group on type 2 SPs. SPs are not in fact used in the field to control *L. cuprina*, but this result nevertheless suggests that structural mutations in ~~CBEs~~ could play a part in esterase-based resistance to SPs. The most recent work on this enzyme by Devonshire et al. (2007) has strongly supported this latter idea. These authors showed that two variants of αE7 (again including W251L) have more than 10- to 100-fold higher activities than wild-type for fluorescent analogs of the most insecticidal isomers of cypermethrin and fenvalerate, respectively.

5.5 CONCLUSIONS

The proliferation of synthetic pesticides in the twentieth century has brought the role of ~~CBEs~~ in xenobiotic metabolism into sharp focus. Their role in metabolizing pesticides in human and other vertebrates appears to be at least partly responsible for ameliorating their toxic effects. Similarly, the function of CarbEs in insects, and the changes in their expression and activity have provided target insects with the means to detoxify pesticides and thereby survive and reproduce, providing a fascinating system for the study of evolutionary processes. The discoveries to date regarding the role of CarbEs in pesticide detoxification justify further investigations into this enzyme class. There is significant research that remains to be done to fully elucidate the substrate specificity, function, and distribution of these enzymes. In particular, studies should focus on isozyme identification and characterization, with an emphasis on substrate specificity. These data would be useful in predicting CarbE-mediated metabolism of pesticides as well as understanding the potential for resistance development in target pests.

REFERENCES

Abdel-Aal Y. A. I., Soderlund D. M. (1980) Pyrethroid-hydrolyzing esterases in southern armyworm larvae: Tissue distribution, kinetic properties and selective inhibition. *Pest Biochem Physiol* 14: 232–289.

Abernathy C. O., Casida J. E. (1973) Pyrethroid insecticides: Esterase cleavage in relation to selective toxicity. *Science* 179: 1235–1236.

Aldridge W. N. (1990) An assessment of the toxicological properties of pyrethroids and their neurotoxicity. *Crit Rev Toxicol* 21: 89–104.

~~Amweg E. L., Weston D. P., You J., Lydy M. J. (2006) Pyrethroid insecticides and sediment toxicity in urban creeks from California and Tennessee. *Environ Sci Technol* 40: 1700–1706.~~ **Q4**

Anstead J. A., Mallet J., Denholm I. (2007) Temporal and spatial incidence of alleles conferring knockdown resistance to pyrethroids in the peach-potato aphid, *Myzus persicae (Hemiptera: Aphididae)*, and their association with other insecticide resistance mechanisms. *Bull Entomol Res* 97: 243–252.

Atterberry T. T., Burnett W. T., Chambers J. E. (1997) Age-related differences in parathion and chlorpyrifos toxicity in male rats: Target and nontarget esterase sensitivity and cytochrome P450-mediated metabolism. *Toxicol Appl Pharmacol* 147: 411–418.

Baker J. E., Fabrick J. A., Zhu K. Y. (1998) Characterization of esterases in malathion-resistant and susceptible strains of the pteromalid parasitoid *Anisopteromalus calandrae*. *Insect BiochemMol Biol* 28: 1039–1050.

Barata C., Solayan A., Porte C. (2004) Role of B-esterases in assessing toxicity of organophosphorus (chlorpyrifos, malathion) and carbamate (carbofuran) pesticides to *Daphnia magna*. *Aquat Toxicol* 66: 125–139.

Bencharit S., Morton C. L., Hyatt J. L., Kuhn P., Danks M. K., Potter P. M., Redinbo M. R. (2003) Crystal structure of human carboxylesterase 1 complexed with the Alzheimer's drug tacrine: From **Q27**

binding promiscuity to selective inhibition. *Chem Biol* 10: 341–349.

Q27 Bencharit S., Morton C. L., Xue Y., Potter P. M., Redinbo M. R. (2003) Structural basis of heroin and cocaine metabolism by a promiscuous human drug-processing enzyme. *Nat Struct Biol* 10: 349–356.

Benke G. M., Murphy S. D. (1975) The influence of age on the toxicity and metabolism of methyl parathion and parathion in male and female rats. *Toxicol Appl Pharmacol* 31: 254–269.

Blackman R. L., Spence J. M., Field L. M., Devonshire A. L. (1995) Chromosomal location of the amplified esterase genes conferring resistance to insecticides in *Myzus persicae (Hemiptera: Aphididae)*. *Heredity* 75: 297–302.

Blackman R. L., Spence J. M., Field L. M., Devonshire A. L. (1999) Variation in the chromosomal distribution of amplified esterase (FE4) genes in Greek field populations of *Myzus persicae* (Sulzer). *Heredity* 82: 180–186.

Bloomquist J. R., Adams P. M., Soderlund D. M. (1986) Inhibition of gamma-aminobutyric acid-stimulated chloride flux in mouse brain vesicles by polychlorocycloalkane and pyrethroid insecti-
Q5 cides. *Neurotoxicology* 7: 11–20.

Bradberry S. M., Cage S. A., Proudfoot A. T., Vale J. A. (2005) Poisoning due to pyrethroids. *Toxicol Rev* 24: 93–106.

Bradbury S. P., Coats J. R. (1989) Comparative toxicology of the pyrethroid insecticides. *Rev Environ Contam Toxicol* 108:
Q6 133–177.

Campbell P. M., Trott J. F., Claudianos C., Smyth K. A., Russell R. J., Oakeshott J. G. (1997) Biochemistry of esterases associated with organophosphate resistance in *Lucilia cuprina* with comparisons to putative orthologues in other *Diptera*. *Biochem Genet* 35: 17–40.

Campbell P. M., Newcomb R. D., Russell R. J., Oakeshott J. G. (1998a) Two different amino acid substitutions in the aliesterase, E3, confer alternative types of organophosphorus insecticide resistance in the sheep blowfly, *Lucilia cuprina*. *Insect Biochem Mol Biol* 28: 139–150.

Campbell P. M., Yen J. L., Masoumi A., Russell R. J., Batterham P., McKenzie J. A., Oakeshott J. G. (1998b) Cross-resistance patterns among *Lucilia cuprina* (Diptera: Calliphoridae) resistant to organophosphorus insecticides. *J Econ Entomol* 91: 367–375.

Casida J. E. (1973) *Pyrethrum, the Natural Insecticide*. New York: Academic Press.

Casida J. E., Quistad G. B. (2004) Organophosphate toxicology: Safety aspects of nonacetylcholinesterase secondary targets. *Chem Res Toxicol* 17: 983–998.

Casida J. E.,. Gammon D. W., Glickman A. H., Lawrence L. J. (1983) Mechanisms of selective action of pyrethroid insecticides.
Q7 *Annu Rev Pharmacol Toxicol* 23: 413–438.

Chambers J. E., Chambers H. W. (1990) Time course of inhibition of acetylcholinesterase and aliesterases following parathion and paraoxon exposures in rats. *Toxicol Appl Pharmacol* 103: 420–429.

Chanda S. M., Mortensen S. R., Moser V. C., Padilla S. (1997) Tissue-specific effects of chlorpyrifos on carboxylesterase and cholinesterase activity in adult rats: An in vitro and in vivo comparison. *Fundam Appl Toxicol* 38: 148–157.

Chen Z., Newcomb R., Forbes E., McKenzie J., Batterham P. (2001) The acetylcholinesterase gene and organophosphorus resistance in the Australian sheep blowfly, *Lucilia cuprina. Insect Biochem Mol Biol* 31: 805–816.

Claudianos C., Russell R. J., Oakeshott J. G. (1999) The same amino acid substitution in orthologous esterases confers organophosphate resistance on the house fly and a blowfly. *Insect Biochem Mol Biol* 29: 675–686.

Clement J. G. (1984) Role of aliesterase in organophosphate poisoning. *Fundam Appl Toxicol* 4: S96–S105.

~~Coats J. R. (1990) Mechanisms of toxic action and structure-activity relationships for organochlorine and synthetic pyrethroid insecti-~~
~~cides. *Environ Health Perspect* 87: 255–262.~~ Q8

Cohen S. D. (1984) Mechanisms of toxicological interactions involving organophosphate insecticides. *Fundam Appl Toxicol* 4: 315–324.

Costa L. G., Cole T. B., Vitalone A., Furlong C. E. (2005) Measurement of paraoxonase (PON1) status as a potential biomarker of susceptibility to organophosphate toxicity. *Clin Chim Acta* 352: 37–47.

Crow J. A., Borazjani A., Potter P. M., Ross M. K. (2007) Hydrolysis of pyrethroids by human and rat tissues: Examination of intestinal, liver and serum carboxylesterases. *Toxicol Appl Pharmacol* 221: 1–12.

Crow J. A., Middleton B. L., Borazjani A., Hatfield M. J., Potter P. M., Ross M. K. (2008) Inhibition of carboxylesterase 1 is associated with cholesteryl ester retention in human THP-1 monocyte/macrophages. *Biochim Biophys Acta* 1781: 643–654.

Davies J. H. (1985) The pyrethroids: An historical introduction. In: Leahey J. P., editor. *The Pyrethroid Insecticides*. Philadelphia: Taylor & Francis, pp. 1–41.

Davies A. G., Game A. Y., Chen Z., Williams T. J., Goodall S., Yen J. L., McKenzie J. A., Batterham P. (1996) Scalloped wings is the *Lucilia cuprina* Notch homologue and a candidate for the modifier of fitness and asymmetry of diazinon resistance. *Genetics* 143: 1321–1337.

Devonshire A. L. (1977) The properties of a carboxylesterase from the peach-potato aphid, *Myzus persicae (Sulz.)*, and its role in conferring insecticide resistance. *Biochem J* 167: 675–683. Q9

Devonshire A. L. (1989) The role of electrophoresis in the biochemical detection of insecticide resistance. In: Loxdale H. D., Hollander J. D., editors. *Electrophoresis Studies on Agricultural Pests*. Oxford: Clarendon Press, pp. 363–377.

Devonshire A. L., Sawicki R. M. (1979) Insecticide-resistant *Myzus persicae* as an example of evolution by gene duplication. *Nature* 280: 140–141.

Devonshire A. L., Moores G. D. (1982) A carboxylesterase with broad substrate specificity causes organophosphorus, carbamate and pyrethroid resistance in peach-potato aphids *(Myzus persicae). Pestic Biochem Physiol* 18: 235–246.

Devonshire A. L., Moores G. D. (1989) Detoxification of insecticides by esterases from *Myzus persicae*: Is hydrolysis important? In: Reiner E., Aldridge W. N., Hoskin F. C. G., editors. *Esterases Hydrolysing Organophosphorus Compounds*. Chichester, U.K.: Ellis Horwood, pp. 180–192.

Devonshire A. L., Field L. M. (1991) Gene amplification and insecticide resistance. *Annu Rev Entomol* 36: 1–23.

Devonshire A. L., Field L. M., Foster S. P., Moores G. D., Williamson M. S., Blackman R. L. (1998) The evolution of insecticide resistance in the peach-potato aphid, *Myzus persicae*. *Philos Trans R Soc London B* 353: 1677–1684.

Devonshire A. L., Heidari R., Bell K. L., Campbell P. M., Campbell B. E., Odgers W. A., Oakeshott J. G., Russell R. J. (2003) Kinetic efficiency of mutant carboxylesterases implicated in organophosphate insecticide resistance. *Pestic Biochem Physiol* 76: 1–13.

Devonshire A. L., Heidari R., Huang H. Z., Hammock B. D., Russell R. J., Oakeshott J. G. (2007) Hydrolysis of individual isomers of fluorogenic pyrethroid analogs by mutant carboxylesterases from *Lucilia cuprina*. *Insect Biochem Mol Biol* 37: 891–902.

Dorough H. W. (1968) Metabolism of furadan (NIA-10242) in rats and houseflies. *J Agric Food Chem* 16: 319–325.

Dorough H. W. (1970) Metabolism of insecticidal methylcarbamates in animals. *J Agric Food Chem* 18: 1015–1022.

Ecobichon D. J., Stephens D. S. (1973) Perinatal development of human blood esterases. *Clin Pharmacol Ther* 14: 41–47.

Elliott M. (1976) Properties and applications of pyrethroids. *Environ Health Perspect* 14: 1–2.

Elliott M., Janes N. F., Jeffs K. A., Needham P. H., Sawicki R. M. (1965) New pyrethrin-like esters with high insecticidal activity. *Nature* 207: 938–940.

Elliott M., Farnham A. W., Janes N. F., Needham P. H., Pearson B. C. (1967) 5-Benzyl-3-furylmethyl chrysanthemate: A new potent insecticide. *Nature* 213: 493–494.

Elliott M., Farnham A. W., Janes N. F., Needham P. H., Pulman D. A. (1973a) Potent pyrethroid insecticides from modified cyclopropane acids. *Nature* 244: 456–457.

Elliott M., Farnham A. W., Janes N. F., Needham P. H., Pulman D. A., Stevenson J. H. (1973b) A photostable pyrethroid. *Nature* 246: 169–170.

Elliott M., Farnham A. W., Janes N. F., Needham P. H., Pulman D. A. (1974) Synthetic insecticide with a new order of activity. *Nature* 248: 710–711.

~~Enan E., Matsumura F. (1993) Activation of phosphoinositide/protein kinase C pathway in rat brain tissue by pyrethroids. *Biochem Pharmacol* 45: 703–710.~~

Escartin E., Porte C. (1997) The use of cholinesterase and carboxylesterase activities from *Mytilus galloprovinciallis* in pollution monitoring. *Environ Toxicol Chem* 16: 2090–2095.

Farag A. T., Goda N. F., Shaaban N. A., Mansee A. H. (2007) Effects of oral exposure of synthetic pyrethroid, cypermethrin on the behavior of F1-progeny in mice. *Reprod Toxicol* 23: 560–567.

Ferguson P. W., Dey M. S., Jewell S. A., Krieger R. I. (1984) Carbofuran metabolism and toxicity in the rat. *Fundam Appl Toxicol* 4: 14–21.

Feyereisen R. (1999) Insect P450 enzymes. *Annu Rev Entomol* 44: 507–533.

Field L. M., Devonshire A. L., Forde B. G. (1988) Molecular evidence that insecticide resistance in peach-potato aphids *(Myzus persicae Sulz.)* results from amplification of an esterase gene. *Biochem J* 251: 309–312.

Field L. M., Williamson M. S., Moores G. D., Devonshire A. L. (1993) Cloning and analysis of the esterase genes conferring insecticide resistance in the peach-potato aphid, *Myzus persicae (Sulzer)*. *Biochem J* 294 (Pt 2): 569–574.

Field L. M., Javed N., Stribley M. F., Devonshire A. L. (1994) The peach-potato aphid *Myzus persicae* and the tobacco aphid *Myzus nicotianae* have the same esterase-based mechanisms of insecticide resistance. *Insect Mol Biol* 3: 143–148.

Field L. M., Devonshire A. L. (1998) Evidence that the E4 and FE4 esterase genes responsible for insecticide resistance in the aphid *Myzus persicae (Sulzer)* are part of a gene family. *Biochem J* 330 (Pt 1): 169–173.

Field L. M., Blackman R. L., Tyler-Smith C., Devonshire A. L. (1999) Relationship between amount of esterase and gene copy number in insecticide-resistant *Myzus persicae (Sulzer)*. *Biochem J* 339 (Pt 3): 737–742.

Fleming C. D., Edwards C. C., Kirby S. D., Maxwell D. M., Potter P. M., Cerasoli D. M., Redinbo M. R. (2007) Crystal structures of human carboxylesterase 1 in covalent complexes with the chemical warfare agents soman and tabun. *Biochemistry* 46: 5063–5071.

Forkert P. G., Lee R. P. (1997) Metabolism of ethyl carbamate by pulmonary cytochrome P450 and carboxylesterase isozymes: Involvement of CYP2E1 and hydrolase A. *Toxicol Appl Pharmacol* 146: 245–254.

Forkert P. G., Lee R. P., Reid K. (2001) Involvement of CYP2E1 and carboxylesterase enzymes in vinyl carbamate metabolism in human lung microsomes. *Drug Metab Dispos* 29: 258–263.

~~Forshaw P. J., Lister T., Ray D. E. (1993) Inhibition of a neuronal voltage-dependent chloride channel by the type II pyrethroid, deltamethrin. *Neuropharmacology* 32: 105–111.~~

~~Fukunaga K., Fukami J., Shishido T. (1969) The in vitro metabolism of organophosphorus insecticides by tissue homogenates from mammals and insect. *Residue Rev* 25: 233–249.~~

Fukuto T. R. (1990) Mechanism of action of organophosphorus and carbamate insecticides. *Environ Health Perspect* 87: 245–254.

Galloway T. S., Millward N., Browne M. A., Depledge M. H. (2002) Rapid assessment of organophosphorous/carbamate exposure in the bivalve mollusc *Mytilus edulis* using combined esterase activities as biomarkers. *Aquat Toxicol* 61: 169–180.

Godin S. J., Scollon E. J., Hughes M. F., Potter P. M., DeVito M. J., Ross M. K. (2006) Species differences in the in vitro metabolism of deltamethrin and esfenvalerate: Differential oxidative and hydrolytic metabolism by humans and rats. *Drug Metab Dispos* 34: 1764–1771.

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 Metab Dispos* 35: 1664–1671.

Goh D. K. S., Anspaugh D. D., Motoyama N., Rock G. C., Roe R. M. (1995) Isolation and characterisation of an insecticide resistance associated esterase in the tobacco budworm, *Heliothis virescens (F)*. *Pestic Biochem Physiol* 51: 192–198.

Guerrero F. D., Barros A. T. (2006) Role of kdr and esterase-mediated metabolism in pyrethroid-resistant populations of *Haematobia irritans irritans (Diptera: Muscidae)* in Brazil. *J Med Entomol* 43: 896–901.

Guillemaud T., Lenormand T., Bourguet D., Chevillon C., Pasteur N., Raymond M. (1998) Evolution of resistance in *Culex pipiens*: Allele replacement and changing environment. *Evolution* 52: 443–453.

Gupta R. C., Dettbarn W. D. (1993) Role of carboxylesterases in the prevention and potentiation of N-methylcarbamate toxicity. *Chem Biol Interact* 87: 295–303.

Hartley C. J., Newcomb R. D., Russell R. J., Yong C. G., Stevens J. R., Yeates D. K., La Salle J., Oakeshott J. G. (2006) Amplification of DNA from preserved specimens shows blowflies were preadapted for the rapid evolution of insecticide resistance. *Proc Natl Acad Sci USA* 103: 8757–8762.

Heidari R., Devonshire A. L., Campbell B. E., Bell K. L., Dorrian S. J., Oakeshott J. G., Russell R. J. (2004) Hydrolysis of organophosphorus insecticides by in vitro modified carboxylesterase E3 from *Lucilia cuprina*. *Insect Biochem Mol Biol* 34: 353–363.

Heidari R., Devonshire A. L., Campbell B. E., Dorrian S. J., Oakeshott J. G., Russell R. J. (2005) Hydrolysis of pyrethroids by carboxylesterases from *Lucilia cuprina* and *Drosophila melanogaster* with active sites modified by in vitro mutagenesis. *Insect Biochem Mol Biol* 35: 597–609.

Hemingway K. (1998) Mosquito carboxylesterases: A review of the molecular biology and biochemistry of a major insecticide resistance mechanism. *Med Veterinary Entomol* 12: 1–12.

Hemingway J., Hawkes N., Prapanthadara L., Indrananda Jayawardenal K. G., Ranson H. (1998) The role of gene splicing, gene amplification and regulation in mosquito insecticide resistance. *Philos Trans R Soc London B.* 353: 1695–1699.

Hemingway J., Coleman M., Paton M., McCarroll L., Vaughan A., DeSilva D. (2000) Aldehyde oxidase is coamplified with the world's most common *Culex* mosquito insecticide resistance-associated esterases. *Insect Mol Biol* 9: 93–99.

Hemingway J., Hawkes N. J., McCarroll L., Ranson H. (2004) The molecular basis of insecticide resistance in mosquitoes. *Insect Biochem Mol Biol.* 34: 653–665.

Hick C. A., Field L. M., Devonshire A. L. (1996) Changes in the methylation of amplified esterase DNA during loss and reselection of insecticide resistance in peach-potato aphids, *Myzus persicae. Insect Biochem Mol Biol* 26: 41–47.

Hornberg A., Tunemalm A. K., Ekstrom F. (2007) Crystal structures of acetylcholinesterase in complex with organophosphorus compounds suggest that the acyl pocket modulates the aging reaction by precluding the formation of the trigonal bipyramidal transition state. *Biochemistry* 46: 4815–4825.

Huang H., Stok J. E., Stoutamire D. W., Gee S. J., Hammock B. D. (2005) Development of optically pure pyrethroid-like fluorescent substrates for carboxylesterases. *Chem Res Toxicol* 18: 516–527.

Hughes P. B., Raftos D. A. (1985) Genetics of an esterase associated with resistance to organophosphorus insecticides in the sheep blowfly, *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae). *Bull Entomol Res* 75: 535–544.

Jao L. T., Casida J. E. (1974) Insect pyrethroid-hydrolyzing esterases. *Pest Biochem Physiol* 4: 465–472.

Jewell W. T., Miller M. G. (1998) Identification of a carboxylesterase as the major protein bound by molinate. *Toxicol Appl Pharmacol* 149: 226–234.

Jimmerson V. R., Shih T. M., Maxwell D. M., Kaminskis A., Mailman R. B. (1989) The effect of 2-(o-cresyl)-4H-1:3:2-benzodioxaphosphorin-2-oxide on tissue cholinesterase and carboxylesterase activities of the rat. *Fundam Appl Toxicol* 13: 568575.

Jokanovic M. (1989) Role of carboxylesterase in soman, sarin and tabun poisoning in rats. *Pharmacol Toxicol* 65: 181–184.

Kaneko H. (1988) Comparative metabolism of pyrethroid insecticides, especially fenvalerate isomers. *J Pestic Sci* 13: 535–543.

Karanth S., Pope C. (2000) Carboxylesterase and A-esterase activities during maturation and aging: Relationship to the toxicity of chlorpyrifos and parathion in rats. *Toxicol Sci* 58: 282–289.

Karunaratne S. H., Jayawardena K. G., Hemingway J., Ketterman A. J. (1993) Characterization of a B-type esterase involved in insecticide resistance from the mosquito *Culex quinquefasciatus*. *Biochem J* 294 (Pt 2): 575–579.

Karunaratne S. H., Hemingway J., Jayawardena K. G., Dassanayaka V., Vaughan A. (1995) Kinetic and molecular differences in the amplified and non-amplified esterases from insecticide-resistant and susceptible *Culex quinquefasciatus* mosquitoes. *J Biol Chem* 270: 31124–31128.

Katsuda Y. (1999) Development of and future prospects for pyrethroid chemistry. *Pestic Sci* 55: 775–782.

Ketterman A. J., Jayawardena K. G., Hemingway J. (1992) Purification and characterization of a carboxylesterase involved in insecticide resistance from the mosquito *Culex quinquefasciatus*. *Biochem J* 287 (Pt 2): 355–360.

Kitamura S., Sugihara K., Fujimoto N. (2006) Endocrine disruptionby organophosphate and carbamate pesticides. In: Gupta R. C., editor. *Toxicology of Organophosphate and Carbamate Compounds*. Burlington, MA: Elsevier, pp. 481–494.

Kulkarni A. P., Hodgson E. (1980) Metabolism of insecticides by mixed function oxidase systems. *Pharmacol Ther* 8: 379–475.

Laguerre C., Sanchez-Hernandez J. C., Kohler H. R., Triebskorn R., Capowiez Y., Rault M., Mazzia C. (2009) B-type esterases in the snail *Xeropicta derbentina*: An enzymological analysis to evaluate their use as biomarkers of pesticide exposure. *Environ Pollut* 157: 199–207.

Lee R. P., Forkert P. G. (1999) Inactivation of cytochrome P-450 (CYP2E1) and carboxylesterase (hydrolase A) enzymes by vinyl carbamate in murine pulmonary microsomes. *Drug Metab Dispos* 27: 233–239.

Lee R. P., Parkinson A., Forkert P. G. (1998) Isozyme-selective metabolism of ethyl carbamate by cytochrome P450 (CYP2E1) and carboxylesterase (hydrolase A) enzymes in murine liver microsomes. *Drug Metab Dispos* 26: 60–65.

Lockridge O., Blong R. M., Masson P., Froment M. T., Millard C. B., Broomfield C. A. (1997) A single amino acid substitution, Gly117His, confers phosphotriesterase (organophosphorus acid anhydride hydrolase) activity on human butyrylcholinesterase. *Biochemistry* 36: 786–795.

Matsumura F., Hogendijk G. J. (1965) The enzymatic degradation of parathion in organophosphate-susceptible and resistant houseflies. *J Agric Food Chem* 12: 447–452.

Maxwell D. M. (1992) The specificity of carboxylesterase protection against the toxicity of organophosphorus compounds. *Toxicol Appl Pharmacol* 114: 306–312.

Maxwell D. M., Brecht K. M. (2001) Carboxylesterase: Specificity and spontaneous reactivation of an endogenous scavenger for organophosphorus compounds. *J Appl Toxicol* 21 (Suppl 1): S103–S107.

McAbee R. D., Kang K. D., Stanich M. A., Christiansen J. A., Wheelock C. E., Inman A. D., Hammock B. D., Cornel A. J. (2004) Pyrethroid tolerance in *Culex pipiens pipiens var molestus* from Marin County, California. *Pest Manag Sci* 60: 359–368.

McKenzie J. A., Whitten M. J., Adena M. A. (1982) The effect of genetic background on the fitness of diazinon resistance genotypes of the Australian sheep blowfly, *Lucilia cuprina. Heredity* 49: 1–9.

Mikhailov A. T., Torrado M. (1999) Carboxylesterase overexpression in the male reproductive tract: A universal safeguarding mechanism? *Reprod Fertil Dev* 11: 133–145.

Millard C. B., Kryger G., Ordentlich A., Greenblatt H. M., Harel M., Raves M. L., Segall Y., Barak D., Shafferman A., Silman I., Sussman J. L. (1999) Crystal structures of aged phosphonylated acetylcholinesterase: Nerve agent reaction products at the atomic level. *Biochemistry* 38: 7032–7039.

Miyamoto J. (1976) Degradation, metabolism and toxicity of synthetic pyrethroids. *Environ Health Perspect* 14: 15–28.

Miyamoto J., Nishida T., Ueda K. (1971) Metabolic fate of resmethrin, 5-benzyl-3-furylmethyl dl-*trans*-chrysanthemate in the rat. *Pest Biochem Physiol* 1: 293–306.

Miyamoto J., Suzuki T., Nakae C. (1974) Metabolism of phenothrin or 3-phenoxybenzyl d-*trans*-chrysanthemate in mammals. *Pest Biochem Physiol* 4: 438–450.

Moser V. C., Chanda S. M., Mortensen S. R., Padilla S. (1998) Age- and gender-related differences in sensitivity to chlorpyrifos in the rat reflect developmental profiles of esterase activities. *Toxicol Sci* 46: 211–222.

Mouches C., Pasteur N., Berge J. B., Hyrien O., Raymond M., de Saint Vincent B. R., de Silvestri M., Georghiou G. P. (1986) Amplification of an esterase gene is responsible for insecticide resistance in a California *Culex* mosquito. *Science* 233: 778–780.

Mouches C., Pauplin Y., Agarwal M., Lemieux L., Herzog M., Abadon M., Beyssat-Arnaouty V., Hyrien O., de Saint Vincent B. R., Georghiou G. P., et al. (1990) Characterization of amplification core and esterase B1 gene responsible for insecticide resistance in *Culex*. *Proc Natl Acad Sci USA* 87: 2574–2578.

Nachon F., Asojo O. A., Borgstahl G. E., Masson P., Lockridge O. (2005) Role of water in aging of human butyrylcholinesterase inhibited by echothiophate: The crystal structure suggests two alternative mechanisms of aging. *Biochemistry* 44: 1154–1162.

Narahashi T. (1996) Neuronal ion channels as the target sites of insecticides. *Pharmacol Toxicol* 79: 1–14.

Needham P. H., Sawicki R. M. (1971) Diagnosis of resistance to organophosphorus insecticides in *Myzus persicae (Sulz.). Nature* 230: 125–126.

Nevoigt E., Fischer C., Mucha O., Matthaus F., Stahl U., Stephanopoulos G. (2007) Engineering promoter regulation. *Biotechnol Bioeng* 96: 550–558.

Newcomb R. D., Campbell P. M., Ollis D. L., Cheah E., Russell R. J., Oakeshott J. G. (1997) A single amino acid substitution converts a carboxylesterase to an organophosphorus hydrolase and confers insecticide resistance on a blowfly. *Proc Natl Acad Sci USA* 94: 7464–7468.

Newcomb R. D., Campbell P. M., Russell R. J., Oakeshott J. G. (1997) cDNA cloning, baculovirus-expression and kinetic properties of the esterase, E3, involved in organophosphorus resistance in *Lucilia cuprina*. *Insect Biochem Mol Biol* 27: 15–25.

Newcomb R. D., Gleeson D. M., Yong C. G., Russell R. J., Oakeshott J. G. (2005) Multiple mutations and gene duplications conferring organophosphorus insecticide resistance have been selected at the Rop-1 locus of the sheep blowfly, *Lucilia cuprina. J Mol Evol* 60: 207–220.

Nishi K., Huang H., Kamita S. G., Kim I. H., Morisseau C., Hammock B. D. (2006) Characterization of pyrethroid hydrolysis by the human liver carboxylesterases hCE-1 and hCE-2. *Arch Biochem Biophys* 445: 115–123.

Nomeir A. A., Ioannou Y. M., Sanders J. M., Matthews H. B. (1989) Comparative metabolism and disposition of ethyl carbamate (urethane) in male Fischer 344 rats and male B6C3F1 mice. *Toxicol Appl Pharmacol* 97: 203–215.

Oakeshott J. G., Claudianos C., Campbell P. M., Newcomb R. D., Russell R. J., Lawrence I. G., Kostas I., Sarjeet S. G. (2005) Biochemical genetics and genomics of insect esterases. In: *Comprehensive Molecular Insect Science*. Amsterdam: Elsevier, pp. 309–381.

O'Brien R. (1960) *Toxic Phosphorus Esters*. New York: Academic Press.

Ohno N., Fujimoto K., Okuno Y., Mizutani T., Hirano M., Itaya N., Honda T., Yoshioka H. (1976) 2-Arylalkanoates, a new group of synthetic pyrethroid esters not containing cyclopropanecarboxylates. *Pestic Sci* 7: 241–246.

Oppenoorth F. J., van Asperen K. (1960) Allelic genes in the housefly producing modified enzymes that cause organophosphate resistance. *Science* 132: 298–299.

Ozretic B., Krajnovic-Ozretic M. (1992) Esterase heterogeneity in mussel *Mytilus galloprovincialis*: Effects of organophosphate and carbamate pesticides in vitro. *Comp Biochem Physiol C* 103: 221–225.

Pap L. (2003) Pyrethroids. In: Plimmer J. R., Gammon D. W., Ragsdale N. N., editors. *Encyclopedia of Agrochemicals*. New York: Wiley, http://www.knovel.com/knovel2/Toc.jsp?BookID=964&VerticalID=0.

Parker A. G., Campbell P. M., Spackman M. E., Russell R. J., Oakeshott J. G. (1996) Comparison of an esterase associated with organophosphate resistance in *Lucilia cuprina* with an orthologue not associated with resistance in *Drosophila melanogaster*. *Pestic Biochem Physiol* 55: 85–99.

Parker M. L., Goldstein M. I. (2000) Differential toxicities of organophosphate and carbamate insecticides in the nestling European starling *(Sturnus vulgaris)*. *Arch Environ Contam Toxicol* 39: 233–242.

Parker A. G., Russell R. J., Delves A. C., Oakeshott J. G. (1991) Biochemistry and physiology of esterases in organophosphate-susceptible and -resistant strains of the Australian sheep blowfly, *Lucilia cuprina. Pestic Biochem Physiol* 41: 305–318.

Pasteur N., Iseki A., Georghiou G. P. (1981) Genetic and biochemical studies of the highly active esterases A′ and B associated with organophosphate resistance in mosquitoes of the *Culex pipiens* complex. *Biochem. Genet* 19: 909–919.

Pekas J. C. (1972) Intestinal hydrolysis, metabolism and transport of a pesticidal carbamate in pH 6.5 medium. *Toxicol Appl Pharmacol* 23: 62–70.

Perry M. J., Venners S. A., Barr D. B., Xu X. (2007) Environmental pyrethroid and organophosphorus insecticide exposures and sperm concentration. *Reprod Toxicol* 23: 113–118.

Pillai U. A., Ace L. N., Dick R. M., Ferguson P. W. (1993) Carbofuran metabolism and acetylcholinesterase inhibition in the isolated perfused rat lung. *Pestic Biochem Physiol* 45: 141–146.

Pohlenz H. D., Boidol W., Schuttke I., Streber W. R. (1992) Purification and properties of an *Arthrobacter oxydans* P52 carbamate hydrolase specific for the herbicide phenmedipham and nucleotide sequence of the corresponding gene. *J Bacteriol* 174: 6600–6607.

Pope C. N., Chakraborti T. K., Chapman M. L., Farrar J. D., Arthun D. (1991) Comparison of in vivo cholinesterase inhibition in neonatal and adult rats by three organophosphorothioate insecticides. *Toxicology* 68: 51–61.

Pope C. N., Karanth S., Liu J., Yan B. (2005) Comparative carboxylesterase activities in infant and adult liver and their in vitro sensitivity to chlorpyrifos oxon. *Regul Toxicol Pharmacol* 42: 64–69.

Qiao C. L., Raymond M. (1995) The same esterase B1 haplotype is amplified in insecticide-resistant mosquitoes of the *Culex pipiens* complex from the Americas and China. *Heredity* 74 (Pt 4): 339–345.

Ranson H., Claudianos C., Ortelli F., Abgrall C., Hemingway J., Sharakhova M. V., Unger M. F., Collins F. H., Feyereisen R. (2002) Evolution of supergene families associated with insecticide resistance. *Science* 298: 179–181.

Ray D. E., Forshaw P. J. (2000) Pyrethroid insecticides: Poisoning syndromes, synergies, and therapy. *J Toxicol Clin Toxicol* 38: 95–01.

Raymond M., Pasteur N., Georghiou G. P., Mellon R. B., Wirth M. C., Hawley M. K. (1987) Detoxification esterases new to California, USA, in organophosphate-resistant *Culex quinquefasciatus* (Diptera: Culicidae). *J Med Entomol* 24: 24–27.

Redinbo M. R., Potter P. M. (2005) Mammalian carboxylesterases: From drug targets to protein therapeutics. *Drug Discov Today* 10: 313–325.

Ross M. K., Crow J. A. (2007) Human carboxylesterases and their role in xenobiotic and endobiotic metabolism. *J Biochem Mol Toxicol* 21: 187–196.

Ross M. K., Borazjani A., Edwards C. C., Potter P. M. (2006) Hydrolytic metabolism of pyrethroids by human and other mammalian carboxylesterases. *Biochem Pharmacol* 71: 657–669.

Sabourault C., Guzov V. M., Koener J. F., Claudianos C., Plapp F. W., Jr., Feyereisen R. (2001) Overproduction of a P450 that metabolizes diazinon is linked to a loss-of-function in the chromosome 2 ali-esterase (MdalphaE7) gene in resistant house flies. *Insect Mol Biol* 10: 609–618.

Sanchez-Hernandez J. C., Wheelock C. E. (2009) Tissue distribution, isozyme abundance and sensitivity to chlorpyrifos-oxon of carboxylesterases in the earthworm *Lumbricus terrestris*. *Environ Pollut* 157: 264–272.

Satoh T., Hosokawa M. (2006) Structure, function and regulation of carboxylesterases. *Chem Biol Interact* 162: 195–211.

Scott J. G., Zhang L. (2003) The house fly aliesterase gene (Md[alpha]E7) is not associated with insecticide resistance or P450 expression in three strains of house fly. *Insect Biochem Mol Biol* 33: 139–144.

Shafferman A., Ordentlich A., Barak D., Stein D., Ariel N., Velan B. (1996) Aging of phosphylated human acetylcholinesterase: Catalytic processes mediated by aromatic and polar residues of the active centre. *Biochem J.* 318 (Pt 3): 833–840.

Shafferman A., Ordentlich A., Barak D., Stein D., Ariel N., Velan B. (1997) Aging of somanyl-acetylcholinesterase adducts: Facts and models. *Biochem J* 324 (Pt 3): 996–998.

Sheets L. P., Doherty J. D., Law M. W., Reiter L. W., Crofton K. M. (1994) Age-dependent differences in the susceptibility of rats to deltamethrin. *Toxicol Appl Pharmacol* 126: 186–190.

Shono T., Ohsawa K., Casida J. E. (1979) Metabolism of trans- and cis-permethrin, trans- and cis-cypermethrin, and decamethrin by microsomal enzymes. *J Agric Food Chem* 27: 316–325.

Sinha C., Seth K., Islam F., Chaturvedi R. K., Shukla S., Mathur N., Srivastava N., Agrawal A. K. (2006) Behavioral and neurochemical effects induced by pyrethroid-based mosquito repellent exposure in rat offspring during prenatal and early postnatal period. *Neurotoxicol Teratol* 28: 472–481.

Small G. J., Hemingway J. (2000) Molecular characterization of the amplified carboxylesterase gene associated with organophosphorus insecticide resistance in the brown planthopper, *Nilaparvata lugens. Insect Mol Biol* 9: 647–653. **Q23**

Smyth K. A., Boyce T. M., Russell R. J., Oakeshott J. G. (2000) MCE activities and malathion resistances in field populations of the Australian sheep blowfly *(Lucilia cuprina)*. *Heredity* 84 (Pt 1): 63–72.

Soderlund D. M. (1979) Pharmacokinetic behavior of enantiomeric pyrethroid esters in the cockroach, *Periplaneta americana* L. *Pest Biochem Physiol* 12: 38–48. **Q24**

Soderlund D. M. (1997) Molecular mechanisms of insecticide resistance. In: Sjut V., editor. *Molecular Mechanisms of Resistance to Agrochemicals*. Heidelberg: Springer-Verlag, pp. 21–56. **Q24**

Soderlund D. M., Casida J. E. (1977) Stereospecificity of pyrethroid metabolism in mammals. In: Elliot M., editor. *Synthetic Pyrethroids*. Washington, D.C.: American Chemical Society, pp. 173–185.

Sogorb M. A., Vilanova E. (2002) Enzymes involved in the detoxification of organophosphorus, carbamate and pyrethroid insecticides through hydrolysis. *Toxicol Lett* 128: 215–228.

Spencer J., O'Malley M. (2006) Pyrethroid illnesses in California, 1996–2002. *Rev Environ Contam Toxicol* 186: 57–72.

Stok J. E., Goloshchapov A., Song C., Wheelock C. E., Derbel M. B., Morisseau C., Hammock B. D. (2004a) Investigation of the role of a second conserved serine in carboxylesterases via site-directed mutagenesis. *Arch Biochem Biophys* 430: 247–255.

Stok J., Huang H., Jones P. J., Wheelock C. E., Morisseau C., Hammock B. D. (2004b) Identification, expression and purification of a pyrethroid hydrolyzing carboxylesterase from mouse liver microsomes. *J Biol Chem* 279: 29863–29869.

Sultatos L. G., Murphy S. D. (1983) Kinetic analyses of the microsomal biotransformation of the phosphorothioate insecticides chlorpyrifos and parathion. *Fundam Appl Toxicol* 3: 16–21.

Sussman J. L., Harel M., Frolow F., Oefner C., Goldman A., Toker L., Silman I. (1991) Atomic structure of acetylcholinesterase from *Torpedo californica*: A prototypic acetylcholine-binding protein. *Science* 253: 872–879.

Takamatsu Y., Kaneko H., Abiko J., Yoshitake A., Miyamoto J. (1987) *In vivo* and *in vitro* stereoselective hydrolysis of four chiral isomers of fenvalerat. *J Pestic Sci* 12: 397–404.

Tang J., Cao Y., Rose R. L., Hodgson E. (2002) In vitro metabolism of carbaryl by human cytochrome P450 and its inhibition by chlorpyrifos. *Chem Biol Interact* 141: 229–241.

Tang J., Rose R. L., Chambers J. E. (2006) Metabolism of organophosphate and carbamate pesticides. In: Gupta R. C., editor. *Toxicology of Organophosphate and Carbamate Compounds*. Burlington, MA: Elsevier, pp. 127–143.

Terriere L. C. (1984) Induction of detoxication enzymes in insects. *Annu Rev Entomol* 29: 71–88.

Ueda K., Gaughan L. C., Casida J. E. (1975) Metabolism of four resmethrin isomers by liver microsomes. *Pest Biochem Physiol* 5: 280–294.

Usmani K. A., Hodgson E., Rose R. L. (2004) In vitro metabolism of carbofuran by human, mouse, and rat cytochrome P450 and interactions with chlorpyrifos, testosterone, and estradiol. *Chem Biol Interact* 150: 221–232.

Vaughan A., Hemingway J. (1995) Mosquito carboxylesterase Est alpha 2(1) (A2). Cloning and sequence of the full-length cDNA for a major insecticide resistance gene worldwide in the mosquito *Culex quinquefasciatus*. *J Biol Chem* 270: 17044–17049.

Vaughan A., Rodriguez M., Hemingway J. (1995) The independent gene amplification of electrophoretically indistinguishable B esterases from the insecticide-resistant mosquito *Culex quinquefasciatus*. *Biochem J* 305 (Pt 2): 651–658.

Vaughan A., Hawkes N., Hemingway J. (1997) Co-amplification explains linkage disequilibrium of two mosquito esterase genes in insecticide-resistant. *Culex quinquefasciatus. Biochem J* 325 (Pt 2): 359–365.

Vijverberg H. P., van den Bercken J. (1990) Neurotoxicological effects and the mode of action of pyrethroid insecticides. *Crit Rev Toxicol* 21: 105–126.

Vioque-Fernandez A., de Almeida E. A., Lopez-Barea J. (2007) Esterases as pesticide biomarkers in crayfish (*Procambarus clarkii*, Crustacea): Tissue distribution, sensitivity to model compounds and recovery from inactivation. *Comp Biochem Physiol C Toxicol Pharmacol* 145: 404–412.

Voss G., Dauterman W. C., Matsumura F. (1964) Relation between toxicity of malathion analog and organophosphate resistance in the two-spotted spider mite. *J Econ Entomol* 57: 808–811.

Wheelock C. E., Eder K. J., Werner I., Huang H., Jones P. D., Brammell B. F., Elskus A. A., Hammock B. D. (2005a) Individual variability in esterase activity and CYP1A levels in Chinook salmon (*Oncorhynchus tshawytscha*) exposed to esfenvalerate and chlorpyrifos. *Aquat Toxicol* 74: 172–192.

Wheelock C. E., Shan G., Ottea J. A. (2005b) Overview of carboxylesterases and their role in metabolism of insecticides. *J Pestic Sci* 30: 75–83.

Wheelock C. E., Phillips B. M., Anderson B. S., Miller J. L., Miller M. J., Hammock B. D. (2008) Applications of carboxylesterase activity in environmental monitoring and toxicity identification evaluations (TIEs). *Rev Environ Contam Toxicol* 195: 117–178.

Yamamoto T., Pierce W. M., Jr., Hurst H. E., Chen D., Waddell W. J. (1990) Ethyl carbamate metabolism: *in vivo* inhibitors and *in vitro* enzymatic systems. *Drug Metab Dispos* 18: 276–280.

Yan B., Yang D., Brady M., Parkinson A. (1995) Rat testicular carboxylesterase: Cloning, cellular localization, and relationship to liver hydrolase A. *Arch Biochem Biophys* 316: 899–908.

Yang P. Y., Lin J. L., Hall A. H., Tsao T. C., Chern M. S. (2002) Acute ingestion poisoning with insecticide formulations containing the pyrethroid permethrin, xylene, and surfactant: A review of 48 cases. *J Toxicol Clin Toxicol* 40: 107–113.

Zhang S. Y., Ito Y., Yamanoshita O., Yanagiba Y., Kobayashi M., Taya K., Li C., Okamura A., Miyata M., Ueyama J., Lee C. H., Kamijima M., Nakajima T. (2007) Permethrin may disrupt testosterone biosynthesis via mitochondrial membrane damage of Leydig cells in adult male mouse. *Endocrinology* 148: 3941–3949.

Zimmerli B., Schlatter J. (1991) Ethyl carbamate: Analytical methodology, occurrence, formation, biological activity and risk assessment. *Mutat Res* 259: 325–350.

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Thompson C.M., Richardson R.J. (2004) Anticholinesterase insecticides. In: Marrs T.C., Ballantyne B., editors. *Pesticide Toxicology and International Regulation*. West Sussex, England: John Wiley & Sons, pp. 89-127.