

INSECTICIDE RESISTANCE AND ITS UNDERLYING MECHANISMS IN THE GERMAN COCKROACH, *BLATTELLA GERMANICA* (LINN.) (DICTYOPTERA: BLATTELLIDAE)

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Abstrak: Tinjauan ini menyimpulkan kes kerintangan insektisid terpilih pada lipas Jerman yang pernah diterbitkan sejak tahun 1952, dengan menumpu kepada mekanisme kerintangannya (penurunan penembusan kutikel, monooxygenase, esterase, glutathion S-transferase, asetilkolinesterase terubah dan kerintangan jenis *kdr*). Setiap mekanisme kerintangan ini dibincangkan dengan menumpu kepada kes lipas Jerman yang telah dilaporkan. Satu ringkasan pendek mengenai beberapa aspek utama yang memerlukan penyelidikan mendalam juga disertakan.

Abstract: This review summarizes selected published cases of German cockroach resistance to insecticides since 1952, with emphasis on resistance mechanisms (reduced cuticular penetration, monooxygenase, esterase, glutathione S-transferase, altered acetylcholinesterase and *kdr*-type resistance) that had been reported to date. Each resistance mechanisms is discussed with emphasis on cases reported for German cockroaches. A short summary on several priority research areas that warrants further studies is also included.

INTRODUCTION

"Whereas the presence of resistance was a rare phenomenon during the early 1950s, it is the fully susceptible population that is rare at present...." (Georghiou 1986).

Pesticide resistance is found in many living forms ranging from bacteria to plants and mammals (Georghiou & Mellon 1983). However, the worst case of pesticide resistance is insecticide resistance. According to the World Health Organization (1992), resistance is defined as "an inherited characteristic that imparts an increased tolerance to a pesticide, or group pesticides, such that the resistant individuals survive a concentration of the compound(s) that would normally be lethal to the species." The first case of insecticide resistance was observed in San Jose scale on apples in Washington State, USA where the insect became resistant to lime sulphur (Melander 1914). With the discovery of organic insecticides in the 1940s followed by extensive usage in the 1950s, cases of insecticide resistance have increased each

year since then. Today, more than 504 species of insects have been reported as being resistant to insecticides. There has been more cyclodienes and DDT resistance than resistance to any other group of insecticides (organophosphates, carbamates and pyrethroids), probably owing to their long persistence in the environment (Georghiou 1990).

The impact of insecticide resistance can range from increases in cost of control programme to total control failures by chemical tools (Moberg 1990). An example of control cost increase is in the international malaria control programme. Due to the DDT resistance in many anopheline populations around the world, the replacement of DDT by malathion has increased the cost by 6- to 7-folds. Replacement with other organophosphates, carbamates and pyrethroids cost even more! Due to these financial constraints, DDT has not been replaced completely; thus human morbidity and mortality due to ineffective control are still on the increase (World Health Organization 1992).

HISTORY OF INSECTICIDE RESISTANCE IN THE GERMAN COCKROACH

The German cockroach, *Blattella germanica* (L.) is an important urban insect pest in many parts of the world. Control of this pest species relies heavily on the use of neurotoxic insecticides. Extensive usage of insecticides has led to the development of insecticide resistance in the German cockroach. The first case of German cockroach resistance to insecticide was detected in Corpus Christi, Texas, USA in 1952, where a strain was found to be resistant to chlordane at >100-fold by the glass jar method (Heal *et al.* 1953). Subsequently, resistance to other organochlorines such as DDT, dieldrin and lindane were also noted in many strains of the German cockroach in many parts of the world (Cornwell 1976). Resistance to organophosphates and carbamates in the German cockroach was first detected in the 1960s. Failure to achieve satisfactory control with pyrethroids was observed in the 1980s when cypermethrin no longer provided effective control against German cockroaches in apartments (Schal 1988, Zhai & Robinson 1991). A thorough resistance study on 45 strains of the German cockroach from all over the USA against various insecticides was reported by Cochran (1989). Rust & Reiersen (1991) trapped and studied chlorpyrifos resistance level in German cockroaches from 35 restaurants in California, USA. They found that resistance level (at LD₅₀) ranged from 4.2 to 29.7. The Village Green strain, which was collected from Gainesville, Florida, USA, demonstrated high pyrethroid resistance ranging from 29 to 337-fold when tested against 10 pyrethroids (Atkinson *et al.* 1991).

In Malaysia, the German cockroach is the major insect pest in hotels and food preparative industries (Lee *et al.* 1993). Broad spectrum resistance in twelve field collected strains where resistance were low to high for carbamates, low for organophosphates and low to moderate for pyrethroids were reported lately (Lee 1995, Lee *et al.* 1994, Lee *et al.* 1995, Lee *et al.* 1996a). Recently, Lee *et al.* (1997) reported malathion and bendiocarb resistance in two strains of German cockroach collected from food outlets in Kuala Lumpur, Malaysia.

To avoid repetition of citation in this review, a summary of selected cases of insecticide resistance in the German cockroach documented from 1953 to 1997 is presented in Table 1. This summary excludes reports in which field-collected cockroaches have been subjected to laboratory selection pressure (eg. Scott *et al.* 1990, Siegfried & Scott 1991, Bull & Patterson 1993, Hemingway *et al.* 1993a, 1993b).

RESISTANCE MECHANISMS IN THE GERMAN COCKROACH

The mechanism of insecticide resistance in insects can be divided into physiological and behavioural resistance. Physiological resistance occurs when biochemical/physiological-related mechanisms are involved in reducing the efficacy of an insecticide. On the other hand, the ability of insects to avoid lethal insecticide exposures due to the nature of or changes in their behaviour is classified as behavioural resistance. Only physiological resistance mechanisms is reviewed in this paper. Prior to this paper, a good review on the subject had been reported by Siegfried and Scott (1992).

There are three general resistance mechanisms which can be classified as physiological resistance: reduced cuticular penetration; increased metabolic detoxification and target site insensitivity.

Reduced cuticular penetration

Reduced cuticular penetration confers low level of resistance in insects, usually less than three-fold (Scott 1990). This mechanism was first noted by Fine *et al.* (1963). Generally, this mechanism provides protection against a wide variety of insecticides (Plapp & Hoyer 1968). However, it is the least understood and considered as the least significant single resistance mechanism (Soderlund & Bloomquist 1990).

In the German cockroach, Siegfried & Scott (1991) found that penetration of [¹⁴C]-propoxur into the body was reduced when the chemical was applied topically in a propoxur-resistant strain. A highly

pyrethroid-resistant strain, Village Green, was also found to exhibit this mechanism where the penetration rate of [¹⁴C]-permethrin was found to be slower than that of the susceptible strain (Bull & Patterson 1993, Anspaugh *et al.* 1994). In another report, no difference in [¹⁴C]-chlorpyrifos penetration rate was demonstrated in susceptible and chlorpyrifos-resistant cockroaches (Siegfried *et al.* 1990).

Increased metabolic detoxication

Increased metabolic detoxication is the most common resistance mechanism in insects. The most conclusive way to study this type of resistance mechanism is through *in vivo* and *in vitro* metabolism studies using radio-labeled insecticides (Scott 1990). In the German cockroach, several studies utilizing this technique have been reported (Bull *et al.* 1989, Siegfried *et al.* 1990, Siegfried & Scott 1991, Bull & Patterson 1993, Anspaugh *et al.* 1994).

However, if radio-scintillation facilities are not readily available, increased metabolism can be studied by performing enzyme assays. With enzyme assays, a specified centrifugal fraction or purified sample from susceptible and resistant insects is assayed against model substrates and then compared on the basis of product/min/mg protein. This method, however, will not provide direct evidence for the resistance mechanism involved (Scott 1990).

Three groups of metabolic enzymes which are involved in detoxication of insecticides:

Monooxygenases

Monooxygenase or mixed function oxidase (older nomenclature) systems play an important role in detoxication of many groups of insecticides (ie carbamates, pyrethroids and organophosphates) in both mammals and insects (Agosin & Perry 1974, Hodgson 1983). They catalyze a series of oxidative reactions where one atom of an oxygen molecule is reduced to water, while the other atom is incorporated into its substrate (Hodgson 1983). They are located

in the microsomes and can be obtained by centrifuging postmitochondrial homogenates at 100,000g for 60 minutes. Monooxygenases usually require NADPH and O₂ for activity and are inhibited by CO (Agosin & Perry 1974).

The most important component of a monooxygenase system is cytochrome P₄₅₀ (Agosin & Perry 1974). This name is derived from the prominent peak at or about 450 nm in the CO optical difference spectrum (Omura & Sato 1964). In insects, the activity of cytochrome P₄₅₀ has been demonstrated in the midgut, fat body and malpighian tubules with the midgut being the site of greatest activity (Hodgson 1983). Reactions catalyzed by cytochrome P₄₅₀ include epoxidation, aromatic ring hydroxylation, O- and N-dealkylation, N-methyl hydroxylation, oxidation of thioester a-phosphorothioates and hydroxylation of aliphatic hydrocarbons.

Cytochrome P₄₅₀ monooxygenases usually prefer to metabolize lipophilic substances into products with high water solubility, hence promoting excretion (Soderlund & Bloomquist 1990). It has been suggested that foreign compounds will initially form a complex with the oxidised form of cytochrome P₄₅₀; this complex is then reduced by one electron from NADPH. The reduced cytochrome P₄₅₀/ substrate complex then reacts with and activates O₂. The resulting oxygenated complex break and form an end-product and water. Two flavoproteins, (ie NADPH-cytochrome P₄₅₀ reductase and cytochrome b₅) are responsible for the electron transfer (Wilkinson 1983, Soderlund & Bloomquist 1990).

This resistance mechanism can be overcome by the action of methylenedioxyphenyl (MDP) synergists (eg piperonyl butoxide [PBO] and sesamex) which block the monooxygenases. The presence of these synergists can revert the level of resistance in resistant strains to a level approaching that of the susceptible strain (Sun & Johnson 1960, Casida 1970).

Table 1: Selected cases of insecticide resistance in the German cockroach from 1953-1997

Class insecticide method	Assessment	Resistance ratio	Location	Reference
<i>Organochlorines</i>				
chlordane	SC (time)	> 100	Texas, USA	Heal et al. (1953)
	SC (time)	16- 21	U.S.A.	Keller et al. (1956)
	SC (time)	30	U.S.A	Lofgren et al. (1957)
	SC (time)	1.2- 14.4	France & Germany	Webb (1961)
	SC (time)	>25	California, USA	Micks (1960)
	T (dose)	152-322	Hawaii, USA	Ishii & Sherman (1965)
	T (dose)	>42	Japan	Yasutomi et al. (1966)
	T (dose)	117-452	Louisiana, USA	Bennett & Spink (1968)
	T (dose)	16 - >218	Canada	Bath (1977)
	T (time)	8.2	Baltimore, USA	Nelson & Wood (1982)
	T (dose)	4.3	Muncie, IN, USA	Scharf et al. (1996)
lindane	SC (time)	10-12	Texas, USA	Heal et al. (1953)
	SC (time)	5-14	U.S.A	Keller et al. (1956)
	T (dose)	28-39	Hawaii, USA	Ishii & Sherman (1965)
	T (dose)	14-16	Japan	Yasutomi et al. (1966)
dieldrin	SC (time)	9-17	U.S.A	Keller et al. (1956)
	SC (time)	LT50 = >48 h	England	Gradidge (1960)
	SC (time)	35	Canada	Anonymous (1961)
	T (dose)	2000	Denmark	Keiding (1964)
	T (dose)	360- >750	Japan	Yasutomi et al. (1966)
	T (dose)	> 54	London, UK	Cornwell (1968)
DDT	SC (time)	5 - 6	Texas, USA	Heal et al. (1953)
	SC (time)	4 - 12	Germany & France	Webb (1961)
	T (dose)	4 - 7	Hawaii, USA	Ishii & Sherman (1965)
	T (dose)	>6	Malaysia	Lee et al. (1996a)
<i>Organophosphates</i>				
malathion	SC (time)	1.5 - 3.0	Germany & France	Webb (1961)
	SC (time)	5.1 - 11.2 (at LT ₉₀)	U.S.A	Johnston et al. (1964)
	T (dose)	6.8 - 8.5	Hawaii, USA	Ishii & Sherman (1965)
	SC (dose)	2.2 - 12.8	Texas, USA	Grayson (1965)
	T (dose)	7 - 110	Louisiana, USA	Bennett & Spink (1968)
	T (dose)	3 - 4	Canada	Bath (1977)
	SC (time)	>60 (13 of 45 strains)	U.S.A	Cochran (1989)
	SC (time)	6.5	Baltimore, USA	Nelson & Wood (1982)
	SC (time)	1.9 - 41.1	Malaysia	Lee et al. (1997)
diazinon	SC (dose)	3 - 15 (at LC ₉₀)	Kentucky, USA	Grayson (1965)
	SC (time)	1.3 (at LT ₉₀)	U.S.A	Johnston et al. (1964)
	SC (dose)	3 - 8	Texas, USA	Grayson (1965)
	T (dose)	6 - 13	Louisiana, USA	Bennett & Spink (1968)
	T (dose)	3.8	London	Cornwell (1968)
	T (dose)	2 - 4	Canada	Bath (1977)
	SC (time)	3.7	Baltimore, USA	Nelson & Wood (1982)

(Table 1 continued)

Class insecticide method	Assessment	Resistance ratio	Location	Reference
chlorpyrifos	SC (time)	1.9	New Jersey, USA	Schal (1988)
	T (dose)	1 - 2	Denmark	Vagn-Jensen (1993)
	T (dose)	3.4	Muncie, IN, USA	Scharf et al. (1996)
	T (dose)	1.7 - 2.3	Canada	Batth (1977)
	(time)	1.3	New Jersey, USA	Schal (1988)
	SC (time)	3 - 5 (3 of 45 strains)	U.S.A	Cochran (1989)
	T (dose)	4 - 25	California, USA	Rust & Reiersen (1991)
	T (dose)	1 - 4	Denmark	Vagn-Jensen (1993)
	T (dose)	1 - 31	UK, USA	Chapman et al (1993)
	T (dose)	2 - 8	Malaysia	Lee et al. (1996a)
T (dose)	7	USA	Valles & Yu (1996)	
T (dose)	3.7 - 8.3	Muncie, IN, USA	Scharf et al. (1996)	
fenthion	LC (dose)	3 - 8	Texas, USA	Grayson (1965)
	T (dose)	8 - 11	Louisiana, USA	Bennett & Spink (1968)
fenitrothion	T (dose)	1.3 - 3.7	UK and USA	Chapman et al. (1993).
<i>Carbamates</i>				
bendiocarb	SC (time)	> 60 (32 of 45 strains)	U.S.A	Cochran (1989)
	SC (time)	8 - 11	England	Barson & McCheyne (1979)
	SC (time)	94	Baltimore, USA	Nelson & Wood (1982)
	SC (time)	> 100	New Jersey, USA	Schal (1988)
	T (dose)	1.4 - 7.9	UK, USA	Chapman et al. (1993).
	T (dose)	>63	Malaysia	Lee et al. (1996a)
	T (dose)	46	USA	Valles & Yu (1996)
	SC (time)	1.6 - 4.8	Malaysia	Lee et al. (1997)
	T (dose)	10.6	Muncie, IN, USA	Scharf et al. (1996)
SC (time)	5.1 - 13.3	Malaysia	Lee et al. (In press)	
propoxur	T (dose)	2 - 15	Louisiana, USA	Bennett & Spink (1968)
	T (dose)	2 - 8	Canada	Batth (1977)
	SC (time)	13.3	Baltimore, USA	Nelson & Wood (1982)
	SC (time)	> 100	New Jersey, USA	Schal (1988)
	SC (time)	> 60 (2 of 45 strains)	U.S.A	Cochran (1989)
	T (dose)	1.4 - 10	UK, USA	Chapman et al. (1993)
	T (dose)	3 - 90	Malaysia	Lee et al. (1996a)
	T (dose)	17	USA	Valles & Yu (1996)
	T (dose)	4.0	Muncie, IN, USA	Scharf et al. (1996)
SC (time)	5.4 - 11.5	Malaysia	Lee et al. (In press)	
<i>Pyrethroids</i>				
pyrethrin	SC (time)	3 - 31	U.S.A.	Keller et al. (1956)
	SC (time)	> 80 (20 of 45 strains)	U.S.A	Cochran (1989)
	T (dose)	43.4 - 98.8	UK, USA	Chapman et al. (1993)
cypermethrin	SC (time)	4.5	New Jersey, USA	Schal (1988)
	T (dose)	103.6	Florida, USA	Atkinson et al (1991b)

(Table 1 continued)

Class insecticide method	Assessment	Resistance ratio	Place	Reference
	T (dose)	180	Roanoke, VA, USA	Zhai & Robinson (1991)
	T (dose)	11.6 - 29.1	UK, USA	Chapman et al. (1993)
	T (dose)	1 - 22	Malaysia	Lee et al. (1996a)
	T (dose)	28	USA	Valles & Yu (1996)
	T (dose)	3.5 - 4.2	Muncie, IN, USA	Scharf et al. (1996)
	SC (time)	1.5 - 3.6	Malaysia	Lee et al. (In press)
deltamethrin	T (dose)	20	Australia	Horwood et al. (1991)
	T (dose)	2 - 31	Denmark	Vagn-Jensen (1993)
	T (dose)	6 - 24	Malaysia	Lee et al. (1996a)
permethrin	SC (time)	>100 (1 of 45 strains)	USA	Cochran (1989)
	T (dose)	45	Florida, USA	Atkinson et al. (1991b)
	T (dose)	3 - 57	Denmark	Vagn-Jensen (1993)
	T (dose)	1 - 15	Malaysia	Lee et al. (1996a)
	T (dose)	12	USA	Valles & Yu (1996)
	T (dose)	2.2	Muncie, IN, USA	Scharf et al. (1996)
	SC (dose)	1.9 - 3.2	Malaysia	Lee et al. (In press)
allethrin	SC (time)	>100 (7 of 45 strains)	USA	Cochran (1989)
phenothrin	SC (time)	> 80 (3 of 45 strains)	USA	Cochran (1989)
	T (dose)	13 - 52	Malaysia	Lee et al. (1996a)
fenvalerate	SC (time)	> 60 (1 of 45 strains)	USA	Cochran (1989)
	T (dose)	97.7	Florida, USA	Atkinson et al. (1991b)
cyfluthrin	SC (time)	5 - 6 (1 of 45 strains)	USA	Cochran (1989)
	T (dose)	87.5	Florida, USA	Atkinson et al. (1991b)
	T (dose)	3.0	Muncie, IN, USA	Scharf et al. (1996)

¹ SC = surface contact; T = topical application.

Multiple forms of cytochrome P_{450} monooxygenases have been reported. Evidence showed that different inducing agents cause synthesis of different forms of cytochrome P_{450} monooxygenases with varying catalytic and structural properties (Lu *et al.* 1976, Coon *et al.* 1977). They are also not equally susceptible to inhibition by PBO or other enzyme inhibitors. Therefore, lack of synergism when using any MDP compound does not imply an absence of oxidative metabolism (Soderlund & Bloomquist 1990).

Resistance due to cytochrome P_{450} monooxygenase is common in the German cockroach. Propoxur and chlorpyrifos resistance in Baygon-R and Dursban-R strains, respectively were suppressed partially with PBO and confirmed with higher rates of NADPH-dependent microsomal metabolism of [14 C]-propoxur and [14 C]-chlorpyrifos (Siegfried *et al.* 1990, Siegfried & Scott 1991). Further studies showed that the Baygon-R strain possessed 1.6x more total cytochrome P_{450} than a susceptible strain, but no difference was found in cytochrome b_5 and NADPH-cytochrome c-reductase in both strains. This suggested that the latter two components were not involved in the resistance mechanism (Siegfried & Scott 1992). Hemingway *et al.* (1993b) studied 30 pyrethroid-resistant strains from three continents and found elevated levels of total cytochrome P_{450} in 15 strains. Scharf *et al.* (1996, 1997) also reported increased levels of cytochrome P_{450} in two strains of German cockroaches collected from a housing project in Indiana, USA. Valles *et al.* (1996) reported higher monooxygenase activity in late nymphs as compared to adult males which contributed to stage-dependent propoxur tolerance in the German cockroach.

The use of PBO to characterize the possible involvement of increased oxidative metabolism as a resistance mechanism has also been reported in strains of the German cockroach which are carbamate- and organophosphate-resistant (Cochran 1987, Scott *et al.* 1990, Chapman *et al.* 1993, Hemingway *et al.* 1993a, Lee *et al.* 1996) and pyrethroid-resistant (Scott *et al.* 1990, Atkinson *et al.* 1991, Chapman *et al.* 1993, Hemingway *et al.* 1993b, Cochran 1994a,

Lee *et al.* 1996a, Valles & Yu 1996, Valles *et al.* 1996).

Esterases

Esterases hydrolyze carboxylester and phosphotriester bonds, mainly in OP and CARB insecticides. Like monooxygenases, they exist in multiple forms (Soderlund & Bloomquist 1990). Isozymes are separable with electrophoretic techniques and can also be detected by spectrophotometric assays using model substrates (eg naphthyl acetate) (Scott 1990). These isozymes can be further characterized by using selective inhibitors after electrophoresis and prior to visualization, eg eserine, sodium flouride and cupric sulphate (Sudderuddin & Tan 1973, Setakana 1989, Lim & Tan 1993, Prabhakaran & Kamble 1993).

Two groups of esterases involved in the detoxication of insecticides are carboxylesterases and arylesterases. Carboxylesterases are the most important group in organophosphate and carbamate resistance. These enzymes cleave one or both ethyl ester groups in malathion, leaving it as a mono or diacid (Brattsten 1990). This activity is so specific that it does not hydrolyze any other phosphoester bond. Arylesterases catalyze the hydrolysis of OP oxons, but do not hydrolyze the parent phosphorothioate insecticides. This mechanism, however, is quite rare in insects (Brown 1990).

Since many organophosphates inhibit serine hydrolases by phosphorylation, they are potential inhibitors of esterases (Brattsten 1990). Some relatively non-toxic organophosphates [eg S,S,S-tributylphosphorotrithioate (DEF[®]) and S-benzyl O,O-diisopropyl phosphorothionate (IBP)] can act as inhibitors of esterase because they cause irreversible or slowly reversible inhibition (Soderlund & Bloomquist 1990). Another compound, triphenyl phosphate (TPP) can also serve as an inhibitor against malathion-carboxylesterase. Dong & Scott (1992) compared fifteen compounds as synergists for chlorpyrifos and found that two substituted N,N-dimethylcarbamates (SK-37 and SK-102) performed better than DEF[®] in reducing the resistance levels of a chlorpyrifos-resistant strain.

Elevated esterase has been reported as a common resistance mechanism in the German cockroach. Chlorpyrifos and propoxur resistance in the Dursban-R and Baygon-R strains, respectively, were partially suppressed with DEF[®]. This was confirmed with in vitro metabolism studies using [¹⁴C]-chlorpyrifos and [¹⁴C]-propoxur where high levels of hydrolytic activity were also detected in both strains (Siegfried *et al.* 1990, Siegfried & Scott 1991). Further study revealed that esterases in both strains showed greater hydrolytic activity against α -naphthyl propionate and α -naphthyl butyrate when a series of α -naphtholic ester substrates with varying alkyl chain length were assayed (Siegfried & Scott 1992).

Hemingway *et al.* (1993a) had conducted biochemical esterase assays on 15 resistant strains of the German cockroach from 3 continents and found 11 strains had elevated esterase activities. This finding was confirmed with synergism studies using DEF[®]. Scott *et al.* (1990) partially reduced the bendiocarb resistance level in two German cockroach strains (Kenly and Rutgers) with DEF[®]; however, they were not successful in suppressing pyrethrin and cypermethrin resistance with the same synergist in Kenly and Ectiban-R strains, respectively.

Prabhakaran & Kamble (1993) found two resistant strains of the German cockroach with higher hydrolytic activity than the susceptible strains when using p-nitrophenyl acetate (PNPA) as a model substrate. Ten esterase bands (E₁ to E₁₀) separated by electrophoresis and characterized with selective inhibitors suggested that E₁ - E₅ were cholinesterases, E₆ and E₇ were phosphatases and E₈ - E₁₀ were carboxylesterases. Further studies using differential centrifugal fractions showed that soluble enzymes (the cytosolic fraction) are responsible for most of the total esterase activities in the German cockroach, although esterases are also found at microsomal level (Prabhakaran & Kamble 1994). Recently, the authors purified three isoenzymes (E₅, E₆ and E₇) using column chromatography and preparative gel electrophoresis. They suggested that insecticide resistance in the

strain of German cockroach studied is due to overproduction of esterase E6 which sequesters rather than hydrolyzes the insecticide (Prabhakaran & Kamble 1995). A summary of their three earlier papers was reported recently (Prabhakaran & Kamble 1996).

In Malaysia, possible involvement of elevated esterase in ten resistant strains of field collected German cockroaches was documented (Lee *et al.* 1996a). Four strains demonstrating low to high propoxur resistance levels were then chosen and further studied using biochemical enzyme assays and native polyacrylamide gel electrophoresis. Elevated esterase activity in all four resistant strains were confirmed and these activities were also well-correlated with propoxur resistance levels. Seven bands were also detected by native polyacrylamide gel electrophoresis, with bands E₁, E₅ and E₇ more intensely stained. This indicated that these esterase isozymes were overproduced. Inhibition studies with selective inhibitors suggested that E₁ belongs to the cholinesterase group, while E₅ and E₇ were carboxylesterases (Lee 1995, Lee *et al.* 1996b, Lee *et al.* In review). More recently, Lee *et al.* (in press) detected elevated esterase activity in five resistant strains of German cockroach using modified Pasteur-Georghiou's filter paper method which is simple and economical. However, its sensitivity for detecting esterase resistance is lower than that of the microplate technique.

As practically all pyrethroids are esters, esterases have also been shown to cause pyrethroid resistance by hydrolysis of the central carboxylester bond in pyrethroids, although this is very rare (Casida *et al.* 1983, Soderlund *et al.* 1983). In the cattle tick (*Boophilus microplus*), resistance to permethrin and cypermethrin appeared to be partially due to elevated esterase activity (Schnitzerling *et al.* 1982). Riskallah (1983) observed low levels of pyrethroid resistance in *Spodoptera littoris* in Egypt, which were correlated with enhanced hydrolysis of α -naphthyl acetate. Dowd *et al.* (1987) reported complete synergism of trans-permethrin by profenofos (serving as a synergist) in a resistant strain of *H. virescens*. In *M. domestica*, esterase

activity was found to confer low level of resistance to pyrethrin and pyrethroids (Sawicki *et al.* 1984).

In the German cockroach, permethrin resistance level in a strain from Osaka, Japan, did not decline with the addition of PBO, but reverted with NIA 16388, a monooxygenase and esterase inhibitor, suggesting the possible involvement of esterase (Umeda *et al.* 1988). Unselected Village Green German cockroaches showed partial elimination of cypermethrin and permethrin resistance with DEF[®], suggesting the involvement of esterase in the resistance mechanism (Atkinson *et al.* 1991). This was later confirmed by Anspaugh *et al.* (1994) who found elevated esterase activity (1.7 - 2.4x) in this strain.

Glutathione S-transferase (GST)

GST has not been associated with insecticide resistance in as many cases as monooxygenases or esterases (Dauterman 1983). GST catalyzes the nucleophilic attack of the endogenous tripeptide glutathione (Brattsten 1990, Soderlund & Bloomquist 1990). Principally, these enzymes catalyze *o*-dealkylation of OP (both phosphorothioates and oxon analogues). They also catalyze dehydrochlorination of DDT to DDE (Clark & Shamaan 1984). Like monooxygenases and esterases, GST exists in multiple forms (Clark & Dauterman 1982, Clark *et al.* 1984).

Elevated GST is not a common cause of resistance in the German cockroach. Babers & Roan (1953) found that in the Corpus Christi strain, DDT was dehydrochlorinated to DDE and other metabolites at a faster rate when compared to a susceptible strain. Hemingway *et al.* (1993a) found that only 7 out of 15 strains from 3 continents had elevated GST activity. Anspaugh (1994) found a 1.6-fold elevated GST activity in the Village Green strain when compared to that of a susceptible strain. In addition, two resistant strains from Malaysia (Melia II and HangTuah) also demonstrated elevated GST activity, but at low frequencies (Lee 1995, Lee *et al.* 1996b, Lee *et al.* In press).

Target site insensitivity

Besides reduced cuticular penetration and increased metabolism, insecticide resistance may also due to an insensitive target site. Reduced sensitivity to insecticides due to modification of target site can be divided into: (1) altered acetylcholinesterase (AChE) which confers resistance to organophosphates and carbamates, (2) knockdown resistance (*kdr*-type), where insects become insensitive to DDT and pyrethroids.

Altered AChE

Altered AChE, which becomes insensitive to organophosphate and carbamate inhibitions was first reported in spider mites, *Tetranychus urticae* (Smitsaert 1964). Subsequently, altered AChE has been found in several species of insects including green rice leaf hopper (Hama & Iwata 1971, 1978), mosquitoes (Ayad & Georghiou 1975, Raymond *et al.* 1986, Hemingway *et al.* 1986), house flies (Tripathi & O'Brien 1973) and army worm (Yu 1991). Two comprehensive reviews on the subject have since been published (Hama 1983, Fournier & Mutero 1994).

Modification at the active site of AChE will cause carbamylation or phosphorylation of the active site serine to be less effective (Fournier & Mutero 1994). Method of studying AChE activity generally follows that of Ellman *et al.* (1961). Fraction containing AChE is assayed against acetylthiocholine (ATCh) as substrate and the rate of thiocholine production is measured. This is made possible with the reaction between thiocholine and dithio-bis-nitrobenzoic acid (DTNB) which produces yellow-coloured thionitrobenzoic acid.

Like many other metabolic enzymes, altered AChE exists in multiple forms (Tripathi & O'Brien 1973, Devonshire & Moores 1984a, 1984b). Altered AChE has serious implications for resistance management because: (1) there is no suitable synergist available to inhibit the altered enzyme, and (2) cross-resistance to other insecticides with the same mode of action (Brattsten 1990).

This mechanism, however, is not common in the German cockroach. To date, only three publications have reported altered AChE in the German cockroach. Hemingway *et al.* (1993a) found only one of 15 strains studied to possess altered AChE. This strain (Dubai) showed low organophosphate and high carbamate resistance. Lee *et al.* (1997) detected two strains of German cockroaches from food outlets with AChE insensitivity. In four strains of resistant German cockroaches from hotels and restaurants, low frequencies of altered AChE were detected (Lee 1995, Lee *et al.* 1996b, Lee *et al.* In press). Altered AChE was not found in an earlier study (Siegfried & Scott 1990).

Knockdown resistance (*kdr*-type)

Insensitivity to DDT and pyrethroids due to *kdr*-type resistance has been observed in many insects such as German cockroaches (Scott & Matsumura 1981, 1983, Umeda *et al.* 1988, Bull & Patterson 1993, Hemingway *et al.* 1993b, Anspaugh *et al.* 1994), house flies (DeVries & Georghiou 1981, Ahn *et al.* 1986, Scott & Georghiou 1986), mosquitoes (Omer *et al.* 1980, Priester & Georghiou 1980, Bradley *et al.* 1984, Hemingway *et al.* 1989a, Umeda *et al.* 1990), diamond-back moths (Liu *et al.* 1982) and predatory mites *Amblyseius fallacis* (Scott *et al.* 1983). To date, three reviews on *kdr*-type resistance in insects have been published (Miller *et al.* 1983, Shono 1985, Scott & Dong 1994).

Basic characteristics of *kdr*-type resistance include: (1) reduced sensitivity to DDT/pyrethroids in neurophysiological studies (Scott & Matsumura 1981, Umeda *et al.* 1988), (2) resistance to all pyrethroids (Scott & Matsumura 1983), and (3) failure of any synergist to increase toxicity of DDT and pyrethroids. However, it was found recently that formamidine compounds (eg chlormedifom and Amitraz) can act as putative target site synergists for pyrethroids (Liu & Plapp 1992).

Although the incidence of *kdr*-type resistance has been reported extensively, its underlying molecular mechanism is still very much debated. Three hypotheses have been proposed: (1) changes in number of pyrethroid binding site on the voltage-

sensitive sodium channel (Chang & Plapp 1983, Rossignol 1988, Kasbekar & Hall 1988, Bull & Pryor 1990) or altered binding affinity (Salgado *et al.* 1983, Pauron *et al.* 1989, Dong & Scott 1991) or combination of both. (2) changes in lipid components of the neuronal membranes (Chiang & Devonshire 1982), and (3) altered Ca⁺⁺ binding protein (Ca-ATPase) (Ghiasuddin *et al.* 1981).

The altered binding affinity due to structural changes in sodium channel seems to be the most likely mechanism with recent evidence that reduction in binding sites at the sodium channel is associated with metabolic resistance rather than to *kdr*-type resistance (Liu & Plapp 1991) and that modification of *para*-homologous sodium channels is associated with *kdr*-type resistance (Dong & Scott 1994).

In German cockroaches, a DDT-resistant strain (VPIDLS) was first shown to be due to a target site insensitivity to DDT and permethrin. In this strain, the time required to cause repetitive discharges by the two insecticides was longer than that of the susceptible strain (Scott & Matsumura 1981). After 3 generations of DDT selection, VPIDLS showed cross resistance to all pyrethroids tested (Scott & Matsumura 1983). Following that, Umeda *et al.* (1988) collected a strain of German cockroach from Osaka, Japan, which showed resistance to DDT and 7 pyrethroids and exhibited *kdr*-type resistance (based on electrophysiological studies on the central nervous system). The Village Green strain, which demonstrated resistance to 10 pyrethroids (Atkinson *et al.* 1991) was indirectly shown to possess *kdr*-type resistance based on results from metabolism (Bull & Patterson 1993) and dose-knockdown studies (Anspaugh *et al.* 1994). Recently, Hemingway *et al.* (1993b) also demonstrated this resistance mechanism in two US and one Denmark pyrethroid-resistant strains of the German cockroach. Possible involvement of *kdr*-type resistance can also be deduced when resistance level to pyrethroids did not decrease in PBO or DEF-treated cockroaches (Atkinson *et al.* 1991, Lee *et al.* 1996a).

Conclusion and future research priorities

Insecticide resistance status and its underlying mechanisms in the German cockroach is a timely important topic that warrants more studies. Currently there is only a small pool of scientists working in this area. Except for USA, United Kingdom, Malaysia and Japan, there is a serious lack of information on the status of German cockroach resistance in other parts of the world. In addition, many of the studies reported also lack information on its underlying mechanisms, which is essential for constructing resistance management options.

There have been a serious lack of information in methods to overcome the development of insecticide resistance in the German cockroach. Although several laboratory studies have suggested the feasibility of insecticide rotation and mixture as possible solution to the problem (eg Scharf 1997), only a handful of studies actually addressed its possibility under field situation (eg Scharf *et al.* 1997). Possible resistance development models can be constructed to provide a useful tool to pest control operators for proper and correct insecticide usage and rotation. In addition, the prospects of using insect growth regulators and insecticidal baits against insecticide resistant German cockroaches should also be looked into in the near future.

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