INSECTICIDE RESISTANCE PROFILE, SYNERGISM STUDIES AND BAIT EVALUATION AGAINST FIELD-COLLECTED GERMAN COCKROACH, *Blattella germanica* (L.) (BLATTODEA: BLATTELLIDAE) FROM SINGAPORE

by

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'Scientia est lux lucis'

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LIST OF SYMBOLS AND ABBREVIATIONS

%	percentage
°C	temperature in degree Celsius
χ^2	chi-square
ACh	acetylcholine
AChE	acetylcholinesterase
ATPase	adenosine triphosphatase
CNS	central nervous system
DCJW	N-decarbomethoxyllated S-metabolites
d.f.	degrees of freedom
DD	discriminating dose
DDT	dichlorodiphenyltrichloroethane
DEF	*S,S,S*-tributylphosphorotrithioate
DMC	4,4'-dichloro-α-methylbenzhydrol
GABA	gama-aminobutyric acid
GluCl	glutamate-gated chloride channel
GST	glutathione-S-transferase
IGR	insect growth regulator
IPM	integrated pest management
kdr	*knockdown resistance*
LD_{50}	lethal dose that causes 50% mortality in a tested population
LD_{95}	lethal dose that causes 95% mortality in a tested population
LT_{50}	lethal time where mortality of 50% in tested population is observed
n	total of sample
nAChR	nicotinic acetylcholine receptor
PBO	piperonyl butoxide
Rdl	*Resistance to dieldrin*
R.H.	relative humidity
RR_{50}	resistance ratio
WHO	World Health Organization

INSECTICIDE RESISTANCE PROFILES, SYNERGISM STUDIES & BAIT EVALUATION AGAINST FIELD-COLLECTED GERMAN COCKROACH, BLATTELLA GERMANICA (L.) (BLATTODEA: BLATTELLIDAE) FROM SINGAPORE

ABSTRACT

This research project focuses on the resistance profile, possible resistance mechanism and bait evaluation of German cockroaches, *Blattella germanica* (Linnaeus) from Singapore. Twenty-two strains of the German cockroach were collected from various localities in Singapore and their resistance levels against various commercial insecticides were determined using topical bioassay. Results revealed that insecticide resistance is prevalent in these German cockroach populations. When compared against a laboratory susceptible strain, the levels of resistance were low to very high for pyrethroid (deltamethrin and beta-cyfluthrin), low to high for carbamate (propoxur) and organophosphate (chlorpyrifos), low to moderate for phenyl pyrazole (fipronil), no or low for neonicotinoid (imidacloprid) and oxadiazine (indoxacarb). One strain demonstrated broad spectrum resistance to most of the insecticides tested.

Pyrethroid resistance was reduced with the synergists, piperonyl butoxide (PBO) and *S,S,S*-tributylphosphorotrithioate (DEF), implying monooxygenase- and esterase-based metabolism in conferring resistance. Other additional mechanisms (e.g. *kdr*-type resistance) may also be involved in some strains in which the resistance levels were not affected by the synergists. Propoxur resistance was suppressed with PBO and DEF; coadministration of both synergists resulted in complete negation of the resistance, indicating the involvement of both monooxygenase and esterase. In six of the field strains, esterases could have played a major role in chlorpyrifos resistance as greater synergism occurred with DEF than with PBO.

To assess the potential of using cockroach gel bait for control of these insecticide-resistant strains, the laboratory performance of four commercial gel baits formulated with

fipronil (Maxforce ® FC), hydramethylnon (Maxforce®), imidacloprid (Premise®) and indoxacarb (Advion®) was evaluated. Continuous exposure tests were conducted for 14 days in the presence of water, harbourage and alternative food for three life stages of the German cockroach (male, female, mid-instars). With exception to the indoxacarb baits, all tested baits exhibited variable effectiveness against the insecticide-resistant strains. The indoxacarb bait showed excellent performance against all field strains with 100% mortality of all stages of test insects within 9 days post-treatment. The efficacy of other baits against adult males was encouraging (fipronil: 77.5 – 100%, hydramethylnon: 92.5 – 100%, imidacloprid: 82.5 – 100%). When tested against the adult females and mid-instars of the field strains, fipronil caused 35.0–95.0% and 18.8–93.8% mortality, respectively. The effectiveness of hydramethylnon bait was moderate to high against adult females (42.5–100%) and mid-instars (40.0–97.5%), whereas the imidacloprid bait showed poor to high performance against mid-instars (10.0–83.8%) and moderate to high performance against adult females (52.5–100%). The likelihood of glucose or bait aversion in these German cockroach populations cannot be ascertained at this stage. Therefore, further investigations should be conducted.

PROFIL KERINTANGAN INSEKTISID, KAJIAN SINERGISME & UJIAN PENGUMPANAN TERHADAP LIPAS JERMAN, *Blattella germanica* (L.) (BLATTODEA: BLATTELLIDAE) DARI SINGAPURA

ABSTRAK

Kajian ini berfokus kepada profil kerintangan, mekanisma kerintangan yang mungkin dan ujian pengumpanan lipas Jerman, *Blattella germanica* (Linnaeus) dari Singapura. Sebanyak 22 strain lipas Jerman dikutip dari beberapa lokasi di Singapura dan tahap kerintangan terhadap beberapa insektisid komersial ditentukan dengan menggunakan bioesei topikal. Keputusan menunjukkan bahawa kerintangan insektisid adalah berleluasa di kalangan populasi lipas Jerman tersebut. Apabila dibanding dengan strain makmal rentan, tahap kerintangan adalah dari rendah hingga sangat tinggi untuk piretroid (deltamethrin dan beta-cyfluthrin), rendah hingga tinggi bagi karbamat (propoxur) dan organofosfat (chlorpyrifos), rendah hingga sederhana bagi fenil pirazol (fipronil), tiada atau rendah untuk neonikotinoid (imidacloprid) dan oksadiazin (indoxacarb). Terdapat satu strain yang berkerintangan spektrum luas terhadap kebanyakan insektisid yang diuji.

Kerintangan piretroid dapat dikurangkan dengan sinergis, piperonil butoksida (PBO) and *S,S,S*-tributilfosforotritioat (DEF), menunjukkan metabolisma berasaskan monooksigenas dan esteras sebagai mekanisma kerintangan. Mekanisma lain mungkin juga terlibat dalam beberapa strain kerana tahap kerintangan tidak terjejas oleh sinergis. Kerintangan propoxur dapat ditindas dengan PBO dan DEF; aplikasi kedua-dua sinergis mengakibatkan perencatan sepenuh terhadap kerintangan propoxur. Esteras mungkin memainkan peranan utama dalam kerintangan chlorpyrifos dalam enam strain lapangan kerana tahap sinergisme yang lebih tinggi dicapai dengan DEF berbanding dengan PBO.

Untuk menilai potensi penggunaan umpan gel lipas untuk kawalan strain-strain lipas Jerman yang rintang, keberkesanan empat umpan gel komersial yang diformulasi dengan fipronil (Maxforce ® FC), hydramethylnon (Maxforce®), imidacloprid (Premise®) dan indoxacarb (Advion®) diuji dalam eksperimen makmal. Ujian pendedahan selanjar dijalankan selama 14 hari dengan kehadiran air, termpat perlindungan, dan makanan alternatif terhadap ketiga-tiga peringkat hidup lipas Jerman (jantan, betina, nimfa pertengahan). Semua umpan yang diuji, kecuali umpan indoxacab, menunjukkan keberkesanan yang tidak tetap terhadap strain lipas yang rintang. Umpan indoxcarb mempunyai prestasi yang terbaik kerana 100% mortaliti dicapai bagi semua strain lipas Jerman dalam masa 9 hari. Keberkesanan umpan yang lain terhadap lipas jantan adalah menggalakkan (fipronil: 77.5–100%, hydramethylnon: 92.5–100%, imidacloprid: 82.5–100%). Apabila diuji dengan lipas betina dan nimfa pertengahan strain lapangan, fipronil masing-masing menyebabkan mortaliti 35.0–95.0% dan 18.8–93.8%. Keberkesanan umpan hydramethylnon adalah sederhana hingga tinggi untuk lipas betina (42.5–100%) dan nimfa pertengahan (40.0–97.5%), manakala umpan imidacloprid menunjukkan prestasi yang lemah hingga tinggi terhadap nimfa pertengahan (10.0–83.8%) serta prestasi sederhana hingga tinggi terhadap lipas betina (52.5–100%). Kemungkinan kehadiran aversi glukosa atau umpan dalam populasi lipas Jerman ini tidak dapat disahkan pada tahap ini. Oleh itu, kajian lanjutan diperlukan.

CHAPTER ONE: INTRODUCTION

The German cockroach, *Blattella germanica* (L.) is an omnipresent, abominable insect pest, normally associated with deplorable sanitary condition. It is an important urban pest in various parts of the world. There is a possibility of German cockroaches infesting any location with the presence of food. Not only is this insect an aesthetic pest, it can also cause psychological disturbances and stress (Cornwell 1968). They are also known to harbour medically important microorganisms and act as potential vectors or reservoirs of pathogens (Fotedar et al. 1991, Brenner 1995, Gliniewicz et al. 2003).

Insecticide resistance in the German cockroach has been an area of interest for many researchers since Heal et al. (1953) first documented a case of severe chlordane resistance in German cockroaches from Corpus Christi, Texas. With continued heavy reliance on residual chemicals for German cockroach control, resistance soon extended to other groups of chemicals. Insecticide usage dictates the pattern of resistance development; organochlorine resistance occurred in the 1950s due to extensive usage of chemicals such as DDT, chlordane and lindane (Butts and Davidson 1955).

In the 1960s organophosphate and carbamate were widely used due to control failure with chlorinated hydrocarbons. Thus cases of organophosphate and carbamate resistance were then brought to attention (Ishii and Sherman 1965, McDonald and Cochran 1968, Collins 1973). This was followed by pyrethroid resistance by mid 1980s (Scott et al. 1986, Cochran 1989, Holbrook et al. 1999) when pyrethroids were favoured for their rapid action and low mammalian toxicity.

The German cockroach has developed resistance to virtually all classes of insecticides. This is a serious problem as it leads to control failure, affecting many parts of the world, most notably the US (Bennett and Spink 1968, Cochran 1989), Europe (Webb 1961), Australia (Horwood et al. 1991), the Middle East (Ladonni 2001) and South East Asia (Lee et al. 1996b, Choo et al. 2000). Cross resistance among various groups of insecticides (van den Heuvel and Cochran 1965, Nelson and Wood 1982) aggravates the problem further, and with the involvement of multiple

resistance mechanisms (Siegfried and Scott 1992, Hemingway et al. 1993, Scharf et al. 1996), the German cockroach appears to survive all arsenals of chemicals that we have unleashed upon it. Even with the introduction of novel compounds such as imidacloprid and fipronil, resistance is still being studied as there is a potential for resistance development.

In recent years, baiting is gaining popularity as an alternative to chemical control. Baits are favoured for control of resistant German cockroaches as they work as a stomach poison, compared residual chemicals which are neurotoxins. In addition, baits are known to cause secondary transmission, an added advantage as cockroaches that have not physically feed on the bait itself are also subjected to the lethal dose mainly through coprophagy (Durier and Rivault 2000a). However, poor performance of baits has also been reported, due to glucose aversion (Silverman and Bieman 1993), or aversion to the active ingredient itself (Ross 1998).

Insecticide resistance in the German cockroach populations in Singapore has not been well studied except for the report by Choo et al. (2000), which revealed high levels of deltamethrin resistance in German cockroach populations collected from hotels in Singapore. This study aims to provide a more detailed and comprehensive investigation on the current resistance status of German cockroaches from Singapore. In addition to that, several commercial cockroach gel baits will also be tested for their efficacy against the field strains before baiting can be proposed to be incorporated in the control programme.

The objectives of this research are:

(i) To survey the resistance status of field-collected strains of German cockroaches from Singapore;

(ii) To characterize the possible resistance mechanisms in the resistant strains through synergism studies; and

(iii) To evaluate laboratory performance of commercial gel baits against the field-collected German cockroaches from Singapore.

CHAPTER TWO: LITERATURE REVIEW

2.1 German cockroach (*Blattella germanica* [L.]) (Plate 1)

Adult German cockroaches measure 10 – 15 mm long and are pale to dark brown in colour, with two parallel black bands along the pronotum (Cornwell 1968). The female has a rounder body, and carries the ootheca (containing 30 – 40 eggs) until the nymphs are ready to emerge. The nymphs moult 6 – 7 times to become adults in the final moult (Christensen 1989).

German cockroaches congregate in groups, in warm and moist areas as such environment is optimal for their growth and development (Ross and Mullins 1995). They are nocturnal insects, with male German cockroaches being most active (Metzger 1995).

2.2 Economic and medical importance

German cockroaches are found infesting kitchens, restaurants, food outlets, hotels, motels, bathrooms, even within heated structures and hospitals (Christensen 1989, Brenner 1995). They contaminate food and surfaces with their faeces and distinctive odour.

Apart from being mechanical vectors of pathogens such as viruses, bacterias, fungi and parasitic worms (Dow 1955, Ash and Greenberg 1980, Clorec et al. 1992, Gliniewicz et al. 2003, Zurek and Schal 2004, Salehzadeh et al. 2007), the German cockroach also causes psychological stress and delusory parasitosis (Brenner 1995). Several German cockroach allergens have been identified and are believed to cause sensitization, allergic response and respiratory complications in human (Santos et al. 1999, Gore and Schal 2004).

Because of their ability to reproduce rapidly, infestations can be rampant and thus considerable amount of money is needed to eradicate these insects.

2.3 Chemical control of German cockroaches

The usage of insecticides has always been the principal means of German cockroach population control. Insecticides, mostly neurotoxins, are formulated into residual sprays and

applied onto surfaces, cracks, crevices and voids. Cockroaches pick up the lethal dose of insecticide as they come into contact with the treated surfaces. Residual insecticides are usually long lasting, persisting up to several months.

Rust (1995) reviewed that several biotic and abiotic factors affect the performance of residual insecticides for cockroach control, such as type of substrate, ambient temperature, relative humidity, behaviour of cockroach and repellency. The findings of Schal (1988) supported the claim by Gupta et al. (1973) that improved sanitation increased the efficacy of residual insecticides, particularly for cypermethrin. Correct placement of residual insecticide treatment also greatly increased the residual longevity (Braness and Bennett 1990). On the contrary, contamination of cockroach faeces significantly reduced the performance of insecticides (Strong et al. 2000).

2.4 Insecticides

2.4.1 Organic chemicals (of plant or animal origin)

Pyrethrum, extracted from the flowers of *Chrysanthemum cinerariaefolium*, contains about 25% pyrethrin. Pyrethrin shows rapid knockdown with fairly good killing and flushing action. The disadvantage of pyrethrin is that it is not photostable, and recovery is possible at low dosage (Wickham 1995).

Another example of botanical insecticide is nicotine, which is extracted from the stems and leaves of the tobacco plant (Hassall 1982). Other organic insecticides are neem, rotenone, as well as toxins from *Bacillus thuringiensis* and *Streptomyces avermilitis*, but these are of minor importance for German cockroach control (Wickham 1995).

2.4.2 Synthetic organic chemicals

(i) Chlorinated hydrocarbon compounds

Chlorinated hydrocarbons contain chlorine, hydrogen, carbon and sometimes oxygen or sulphur (Hassall 1982). Examples of such compounds are DDT, lindane, chlordane and heptachlor.

They are generally slow-acting but long lasting as residual insecticide (Wickham 1995). The exact mode of action of chlorinated hydrocarbons still remains unclear and debatable, although it is generally accepted that chlorinated hydrocarbons interfere with axonal nerve impulse transmission through ionic channels. Various reviews of the theories of its toxic action have been published (Metcalf 1955, Coats 1982, Cutkomp 1985, Matsumura 1985).

DDT alters the ionic balance in neurons, causing repetitive discharges and death by hyperexcitation (Narahashi 1979). On the other hand, lindane and cyclodiene insecticides (e.g. chlordane, aldrin, dieldrin) have been proposed to be antagonists of insect gama-aminobutyric acid (GABA) (Matsumura 1985). Chlorinated hydrocarbon insecticides have also been reported to inhibit ATPase (Koch 1969). DDT remains in the environment for a very long time due to its stable nature. Being liphophilic, DDT accumulates in animal fats, which leads to biological magnification (Metcalf 1955) and is banned in most countries (Stetter 1983).

(ii) Organophosphates

Representatives of this group of chemicals are chlopryrifos, diazinon, malathion, fenitrothion and pirimiphos-methyl. These compounds contain carbon, hydrogen, phosphorus, ethyl or methyl groups, with a sulphur or an oxygen atom. These insecticides must undergo activation by converting into their oxygen analogue before they can be insecticidal (O'Brien 1967), where the new compound is more toxic than the original insecticide compound (Matsumura 1985).

The molecules of organophosphate compounds mimic acetylcholine (ACh), thus binding to cholinesterase, which is the enzyme responsible for breaking down ACh after transmitting impulse at the synapse (Matsumura 1985). This interrupts inter-cellular nerve impulse transmission, as the concentration of ACh remains high at the postsynaptic membrane and stimulation of muscle continues (Fest and Schmidt 1982).

(iii) Carbamates

The first commercially-developed carbamate was carbaryl, but it was not popular for German cockroach control (Wickham 1995). Carbamates are less persistent in the environment than

organochlorine insecticides (Kuhr and Dorough 1976). These compounds are esters of carbamic acid (Draber 1983).

Propoxur, with its fast knockdown action, is the major compound used to control German cockroach in many parts of the world (Wickham 1995). As with organophosphates, carbamates inhibit acetylcholinesterase (AChE) (O'Brien 1967), causing a build-up of ACh in the nerve synapse and results in prolonged stimulation of nerves, which eventually leads to death (Kuhr and Dorough 1976).

(iv) Pyrethroids

Pyrethroids are essentially synthetic pyrethrins, but are more stable than natural pyrethrins (Matsumura 1985). Pyrethroids are fast-acting knockdown or flushing agent and are toxic to insects at very low doses (Wickham 1995). Though pyrethroids do not persist for a long time in the environment, there is a risk of water contamination, with some of the materials being toxic to aquatic organisms (Wickham 1995). The first pyrethroid to be commercially used for German cockroach control is cypermethrin (Atkinson et al. 1991).

Based on the different mode of action, pyrethroid compounds can be classified into Type I pyrethorids (e.g. tetramethrin, resmethrin, bioallethrin) and Type II pyrethroids (e.g. cypermethrin, deltamethrin, cyhalothrin, esfenvalerate). Type I pyrethroids induce repetitive dischargers in the nervous system following a single electrical stimulation, a mechanism that closely resembles that of DDT (Vijverberg et al. 1982), while Type II pyrethroids interfere with impulse transmission along nerve cells by blocking action potentials in axons (van den Bercken et al. 1979). Both Type I and Type II pyrethroids prolong Na^+ current in insect nerves, but Type II pyrethroid do not cause repetitive discharges (Eldefrawi and Eldefrawi 1990). Different pyrethroids induce repetitive firing in a specific nerve at different temperature ranges (Gammon 1979).

(v) Novel compounds

(a) Fipronil

A phenylpyrazole insecticide, fipronil is a potent insect control agent; its neurotoxicity is hardly affected by exposure to sunlight (Hainzl and Casida 1996). Fipronil and its sulfonite metabolite are both toxic to insects (Durham et al. 2001). Fipronil disrupts central nervous system (CNS) activity in insects via the gama-aminobutyric acid (GABA) regulated chloride channels, nerve hyperexcitation occurs when fipronil inhibits GABA, which serves to attenuate nerve impulse (Gant et al. 1998).

Fipronil shows good selective toxicity against insects, due to the higher sensitivity of insect GABA receptors (Zhao et al. 2003). Although it is generally accepted that fipronil primarily blocks the insect GABA receptors, the glutamate-gated chloride channels (GluCls) has also been confirmed as another target site of fipronil (Zhao et al. 2004). Fipronil has been shown to be highly toxic to German cockroaches, both as contact and stomach poison (Kaakeh et al. 1997b, Valles et al. 1997, Buczwoski and Schal 2001b).

(b) Hydramethylnon

Hydramethylnon is a compound from a series of amidinohydrazones introduced in the early 1980s, marketed for use in the control of red imported fire ants (*Solenopsis invicta*) (Williams et al. 1980) and cockroaches (Hollingshaus and Little 1984). It inhibits mitochondrial electron transport, thereby interfering with cellular respiration (Hollingshaus 1987). Unlike neurotoxins such as pyrethroids, organophosphates or fipronil in which overstimulation of nerves results in the death of insects, hydramethylnon causes insects to succumb to energy depletion.

Hydramethylnon is highly toxic by ingestion to house fly, German cockroach and Southern armyworm, but it is also toxic by topical application, although it is more active as a stomach poison (Hollingshaus and Little 1984). Hydramethylnon is slow-acting, signs of poisoning are only evident 24 hrs after ingestion, with deaths occurring several days later

(Hollingshaus 1987). The toxicity of hydramethylnon increases with a rise in ambient temperature (Silverman and Shapas 1986).

(c) Imidacloprid

Imidacloprid belongs to a group of insecticides known as chloronicotinyls or second-generation neonicotinoids. Introduced in the early 1990s, imidacloprid and nicotine share some structural similarities, both targeting nicotinic acetylcholine receptors (nAChRs) in insects, which mediate rapid synaptic transmission (Casida and Quistad 2004).

Imidacloprid binds to the postsynaptic nAChRs in insects and interferes with cholinergic transmission, resulting in overexcitement of nervous system (Matsuda et al. 2001), and is able to act on multiple nAChR subtypes (Buckingham et al. 1997). It exhibits selective toxicity for insects, attributable to its higher affinity for insect nAChRs compared to that of mammalians (Liu and Casida 1993) and is frequently employed to control sucking and mining agricultural pests (Matsuda et al. 2001).

Imidacloprid is a fast acting insecticide, with symptoms of poisoning that manifest as early as 2 hours after topical application in the German cockroach, but recovery is possible up to 72 hrs later. Nevertheless, it can be a highly potent insecticide when used together with piperonyl butoxide (PBO), a synergist (Wen and Scott 1997).

Other second-generation neonicotinoids are thiamethoxam, acetamiprid and thiacloprid.

(d) Indoxacarb

Indoxacarb is the first commercially produced pyrazoline-type insecticide from the oxadiazine group of insecticides (Silver and Soderlund 2005). It is a broad spectrum insecticide, toxic via oral or dermal route, and yet demonstrates selective toxicity for mammals and non-target organisms. It has been employed for the control of sucking insects (Homoptera and Hemiptera) and Lepidoptera (Wing et al. 2000).

The novel mode of action, which requires indoxacarb to be bioactivated into a more insecticidally active compound by the target insects, distinguishes it from other commercial

insecticides. Insect esterase or amidase metabolizes the parent compound into N-decarbomethoxyllated S-metabolites (DCJW), which is a highly potent voltage-dependent inhibitor of the sodium channel (Wing et al. 1998, 2000). Mammals are not affected by indoxacarb due to the inability of mammalian species to convert the insecticide to the more toxic metabolites (Silver and Soderlund 2005).

2.4.3 Inorganic chemicals

Inorganic compounds such as borax, boric acid, phosphorus and sodium fluoride are considered old-fashioned remedies for the control of the German cockroach (Reierson, 1995). These chemicals were usually formulated in baits and dusts. Boric acid is gaining popularity again as the issues of insecticide resistance and the adverse effects of neurotoxins worries the public (Wickham 1995).

2.5 Synergists

Synergists are compounds that do not have insecticidal properties, but are able to increase the toxicity of insecticides when used together (Matsumura 1985). Examples of such compounds are sesamin, sesamex, piperonyl butoxide (PBO) and *S,S,S*-tributylphosphorotrithioate (DEF). Synergists are important to enhance the efficacy of an insecticide and to reduce the cost of control (O'Brien 1967). For instance, the addition of PBO to various carbamate compounds significantly reduced the toxicities of these chemicals in the house fly (Georghiou and Metcalf 1962).

2.6 Insecticide resistance

WHO has defined insecticide resistance as 'the inherited ability of a strain of insect to survive a dose of insecticide that would prove lethal to a normal population of the same species'. 'Behaviouristic resistance' describes the ability to avoid a lethal dose.

Insecticide resistance is an inherited trait where genetic mutations in insects confer specific defence mechanism for insecticide tolerance; the mutations however, are not induced

by insecticides (Brown 1958). In most cases, inheritance of resistant gene is not sex-linked, such as DDT and pyrethroid resistance in the German cockroach as described by Cochran and Ross (1962) and Ebbett and Cochran (1997).

Resistance to insecticides represents a case of pure Darwinian selection, as progressive selection of resistant individuals over several generations amplify the numbers of insects that are unaffected by insecticides (O'Brien 1967). Insecticide resistance can be a major hindrance to pest population control not just in public health (Busvine 1956), but also in agriculture (Barbers 1953). The situation is further compounded by the development of cross resistance, where a population of insect that has been subjected to selection pressure with a particular insecticide will also demonstrate some degree of resistance to other insecticides, even though it has never been exposed to the chemicals, owing to the similar mode of action of the insecticides (Brown and Pal 1971).

2.7 Insecticide resistance in the German cockroach

The German cockroach was first documented to show extreme degree of resistance (over 100 fold) to chlordane in Corpus Christi, Texas, by Heal et al. (1953). This strain was also found to possess significant level of resistance to lindane (Heal et al. 1953, Grayson 1954). Further investigations revealed that stocks from this original strain, cultured for several generations, exhibited more than 10-fold resistance to other chlorinated hydrocarbons, such as dieldrin (Fisk and Isert 1953) and heptachlor (Butts and Davidson 1955). Following this, several other reports confirmed that field populations of the German cockroach had developed resistance to this group of chemicals (Stapp 1964, Ishii and Sherman 1965). The severity of insecticide resistance resulted in the discontinuation of chlorinated hydrocarbons for German cockroach control.

Pest control operators then switched to other classes of insecticide for more effective German cockroach control. However, the problem of resistance remained unsolved, and

gradually more reports highlighted that German cockroach populations throughout the United States had also developed resistance to other classes of insecticides, i.e. organophosphates (Grayson 1965, Bennett and Spink 1968, Rust and Reierson 1991), carbamate (McDonald and Cochran 1968, Ross and Bret 1986) as well as pyrethrin (Cochran 1973) and pyrethroid (Cochran 1989, Atkinson et al. 1991, Valles 1999).

Insecticide resistance develops with constant exposure of an insect population to insecticides which subject the population to high selection pressure. Chlordane, a chlorinated hydrocarbon, was the primary choice of insecticide for cockroach control in the late 1940's (Cochran 1995). This explains the earlier occurrence of chlordane resistance in German cockroach populations in Heal et al. (1953).

Resistance to organophosphate material became apparent when this group of chemicals, especially diazinon, malathion and fenthion, was widely employed to control chlorinated hydrocarbon-resistant German cockroaches (Grayson 1965). Stapp (1964) reported slight loss of susceptibility to malathion in 9 field-collected strains of German cockroach when compared with normal colonies, but no marked degree of resistance was found. In a survey by Grayson (1965), 5 field strains from Texas showed low to high degree of resistance to malathion, diazinon and fenthion.

It was then observed that exposure of German cockroaches to a particular organophosphate insecticide leads to cross-resistance to other organophosphates. In a laboratory experiment by van den Heuvel and Cochran (1965), a diazinon-resistant German cockroach showed some degree of tolerance to several other organophosphorus compounds, in contrast, malathion resistance is rather specific and imparted no cross resistance to organophosphates. Due to the resemblance in the mode of action of both organophophate and carbamate insecticides, cross resistance occurred among these two groups of chemical. A bendiocarb-selected strain of German cockroach collected from Baltimore Cross was reported to show cross resistance to malation, diazinon and propoxur (Nelson and Wood 1982). McDonald and

Grayson (1966) had earlier on noted that moderate resistance to diazinon led to slight loss of susceptibility to propoxur in a strain of German cockroach.

Pyrethroid insecticides became available for use against cockroaches in the 1980s. Cypermethrin, with its good residual life, low odour, as well as effectiveness against German cockroaches with resistance to other classes of insecticides became a favourable choice by pest control professionals (Koehler and Patterson 1988). With heavy reliance on pyrethroids, insecticide resistance in the German cockroach soon enough extended to this class of chemical, when field strains of German cockroaches were found to be highly resistant to various types of pyrethroid, such as permethrin (Bull and Patterson 1993, Anspaugh et al. 1994) and newer compounds with alpha-cyano functional group (Atkinson et al. 1991). In Denmark, Spencer et al. (1999) revealed high degree of permethrin and deltamethrin resistance in several field populations. In the early part of the 21st century, Wei et al. (2001) discovered a strain of German cockroach with 480-fold resistance to deltamethrin.

Several researchers have shown that resistance in the German cockroach can be induced under laboratory conditions. Earlier on, Grayson (1951) exposed strains of German cockroach to sublethal dose of DDT and benzene hexachloride over several generations, resulting in development of slight resistance to these chemicals. Subsequently, malathion resistance was detected in a chlordane-resistant and a normal strain after exposing the nymphs to residual malathion (Grayson 1960). Resistance to malathion remained through 15 generations in the presence of continuous selection pressure (Grayson 1963). However, significant degree of resistance to diazinon was not detected even after 15 or 16 generations of selection with this chemical. Burden et al. (1960) noticed that alternating the use of malathion with chlordane or using the two chemicals together in each generation of an experimental laboratory colony failed to prevent the development of resistance to malathion.

Cochran (1987, 1991) demonstrated that pyrethroid resistance can be achieved in a pyrethrins-susceptible and a pyrethrins-resistant strain of German cockroach after selecting with

permethrin and fenvalerate over several generations under laboratory conditions. Using gene frequency (GF) estimates, Cochran (1993) later proved that in the absence of insecticide pressure, resistance to pyrethroids declined substantially in the pyrethrin-resistant strain. The findings of Zhai and Robinson (1996) further proved that cypermethrin resistance in a field population of German cockroach dropped from 180 to 2.5 after 13 generations without pyrethroid exposure. On the contrary, cypermethrin resistance exacerbated more than 9-fold in a resistant strain following intense selection pressure with cypermethrin for four generations (Valles et al. 2003).

The first case of broad spectrum resistance in the German cockroach in South East Asia region was documented by Lee et al. (1996b). Using topical application technique, Lee et al. (1996b) revealed that 12 strains of German cockroach from Peninsular Malaysia exhibited resistance levels of low to high for propoxur and bendiocarb, low for chlorpyrifos, and low to moderate for cypermethrin and permethrin.

Subsequently, Lee et al. (1999) screened 23 field populations using the modified WHO tarsal contact method for resistance to novel and conventional insecticides. Majority of the field strains showed moderate to high resistance to propoxur, which was a popular candidate for German cockroach control in this region since 1980's. Cross resistance with bendiocarb was detected, although the German cockroach populations were never exposed to this chemical. It was also noted that these strains showed only low levels of resistance to organophosphate insecticides. Low to moderate levels of resistance to pyrethroid insecticides was observed as a sign of potential development of pyrethroid resistance, resulting from the reliance on pyrethroids for German cockroach control when propoxur failed to provide satisfactory control.

Using the discriminating dose technique, Lee and Lee (2002) later discovered that more than 50% of German cockroach strains in Peninsular Malaysia demonstrated low to moderate resistance to propoxur and deltamethrin, resistance to chlorpyrifos was still fairly low. A study by Lee and Lee (2004) further confirmed that resistance to propoxur remained prevalent in the

field even though the usage of this chemical has been reduced due to control failure; resistance to chlorpyrifos was not widespread as organophosphates are less favoured by the pest control industry in Malaysia.

Choo et al. (2000) highlighted that deltamethrin resistance was prevalent in field populations of German cockroach in Singapore. Ten strains were screened using topical application and tarsal contact method. Comparing with a susceptible strain, the cockroaches were reported to show 17.7 to 4235 fold deltamethrin resistance for topical application and 2.2 to 22 fold for tarsal contact method.

Recently, it has been disclosed that insecticide resistance in the German cockroach did not merely affect Malaysia and Singapore, but also its neighbouring country, Indonesia. Ahmad et al. (2009) presented the first report on pyrethroid resistance in several strains of *B. germanica* from Bandung, West Java. In their investigation using the WHO glass jar method, all strains showed very low resistance to permethrin, cypermethrin and d-allethrin, except for one particular strain which exhibited > 90 fold resistance to permethrin.

The German cockroach appeared to be able to develop resistance to virtually all classes of insecticides. With the advent of novel insecticide compounds such as fipronil, avermectin and indoxacarb, researchers fear that German cockroaches may also develop resistance to these chemicals.

For instance, fipronil and cyclodiene compounds (e.g. aldrin, dieldrin, chlordane) shared some common mode of action. Hosie et al. (1995) predicted that cyclodiene-resistant *Drosophila melanogaster* flies with mutated *Resistance to dieldrin* (*Rdl*) gene are likely to show cross resistance to fipronil. Incidentally, cross resistance between cyclodiene and fipronil in German cockroaches has been detected at a relatively low level by Scott and Wen (1997). In the United States, five strains of field-collected German cockroaches exhibited a remarkable degree of tolerance towards fipronil, even though fipronil has not been registered for German cockroach control (Valles et al. 1997).

As to date, no reports of extensive imidacloprid resistance have been described in the German cockroach. Nevertheless, Wen and Scott (1997) noted two strains of the German cockroach with > 4 fold levels of cross resistance to imidacloprid which is not suppressible with PBO. Similarly, Wei et al. (2001) reported a 10 fold cross resistance to imidacloprid in a pyrethroid-resistant strain. Resistance to indoxacarb in the German cockroach has also not been detected hitherto.

On the contrary, insecticide resistance has been detected in places with relatively low selection pressure, i.e. where usage of insecticide was minimal (Holbrook et al. 1999). Resistance to pyrethrin in the German cockroach is rather stable and can be maintained even in the absence of insecticide selection pressure (Cochran 1993, 1994). Scharf et al. (1996) also found that moderate resistance to chlorpyrifos and cypermethrin remained in a field population without significant insecticidal pressure.

It is possible that the alleles for resistance inheritance are consistently maintained at a high level, such that individuals with resistant genes continue to multiply without any reduced fitness. For example, although dieldrin has been outdated for German cockroach control, resistance to dieldrin has remained in field German cockroach populations for more than three decades, as documented by Hansen et al. (2005). The presence of a mutated *Resistance to dieldrin* (*Rdl*) gene imputed to the persisting dieldrin resistance, homozygous cockroaches exhibited high level of resistance, while moderate resistance was manifested by heterozygous individuals, with no detectable form of fitness cost.

The German cockroach has become a rather challenging pest to control due to its ability to develop resistance to various insecticides. In cases where considerable level of resistance is detected, one may expect control failure to occur in the field. For instance, Ballard et al. (1984) observed that chlorpyrifos and/or dichlorvos treatment failed to provide adequate control of German cockroach infestation in multifamily apartment buildings in Nebraska. This was attributed to the fact that the cockroaches had been found to show more than 10-fold resistance

to chlorpyrifos. Similarly, high resistance to pyrethroid in field populations of the German cockroach resulted in control failure with cypermethrin (Atkinson et al. 1991) and permethin (Ahmad et al. 2009).

2.8 Resistance mechanisms in the German cockroach

Bret and Ross (1985) noticed that a resistant field strain of the German cockroach did not disperse as much as a susceptible laboratory strain after contact with propoxur vapours. Similarly, this same strain also displayed less irritability, such as antennal and tarsal cleaning, following exposure to propoxur vapour, whereas susceptible cockroaches showed greater responses (Bret and Ross 1986). They hypothesized that such differences in behavioural response of cockroaches was due to reduced sensitivity to insecticide vapours in the resistant strain.

Several explanations can be made to account for such observations. The German cockroach has been proven to be resistant to insecticides by physiological and behavioural modifications. Resistance can occur through enhanced metabolism, decreased cuticular penetration, or target site insensitivity.

2.8.1 Physiological resistance

2.8.1.1 Enhanced metabolism

Enhanced metabolism is perhaps the most common resistance mechanism in insects. Resistant individuals may possess a higher level of detoxication enzyme than susceptible individuals, resulting in rapid metabolism of insecticides. The ability of synergist compounds, such as PBO, DEF and DMC (4,4'-dichloro-α-methylbenzhydrol), to decrease the level of insecticide resistance is generally an indication that increased oxidative or hydrolytic metabolism of insecticides plays a role in the resistance mechanism (Scott 1990).

(i) Monooxygenase

The cytochrome P450 dependent monooxygenases are crucial in the metabolization of various compounds in insects. Comprehensive reviews of the importance of cytochromes P450 and its involvement in numerous insect biochemical reactions have been given by Scott (1999, 2001).

Soderlund and Bloomquist (1990) revealed that the monooxygenase system of resistant insects is different from that of susceptible strains in several ways. Resistant insects may have higher levels of monooxygenase components, modifications in the gene regulation of cytochrome P450, a unique form of cytochrome P450 with higher affinity for insecticide substrate, or an overexpression of cytochrome P450.

Monooxygenases are normally associated with pyrethroid resistance (Scott 2001), although they can also mediate resistance to other types of insecticides. In a study conducted by Scharf et al. (1999), strains of German cockroaches which were more tolerant of cypermethrin indicated higher total cytochrome P450 content, while chlorpyrifos-resistant strains exhibited higher P450 MA (an isoform of cytochrome P450 monooxygenase enzyme) expression as well as increased demethylation activity.

Piperonyl butoxide (PBO), an inhibitor of cytochrome P450 dependent monooxygenases, has been found to reduce the level of resistance to permethrin in house flies (Scott and Georghiou, 1986) as well as resistance to deltamethrin (Pridgeon et al. 2002), propoxur (Scott et al. 1990, Lee et al. 1999, Hemingway et al. 1993), chlorpyrifos (Hemingway et al. 1993), malathion (Scott et al. 1990) and chlordane (Scharf et al. 1996) in the German cockroach. Monooxygenases do not play a major role in the metabolism of fipronil in German cockroaches and house flies, as PBO was not found to affect the susceptibility of several strains tested to this insecticide (Scott and Wen 1997).

(ii) Esterase

Esterases are important for the metabolism of organophosphorus inseciticides (Matsumura 1985). Enhanced esterase activity (Prabhakaran and Kamble 1993) and overproduction of

esterase (Prabhakaran and Kamble 1995) have been linked to insecticide resistance in the German cockroach. A relationship between higher esterase activity and organophosphate (chlorpyrifos) resistance was evident in several resistant strain of the German cockroach (Spencer et al. 1999).

In laboratory studies by Scott et al. (1990), DEF (5,5,5-tributyl phosphorotrithioate), a general esterase inhibitor, managed to reduce resistance to propoxur and bendiocarb in resistant strains of German cockroaches. Likewise, Lee et al. (1999) reported partial or complete suppression of chlorpyrifos and malathion resistance with DEF in 11 field strains of the German cockroaches from Peninsular Malaysia, suggesting the possible involvement of esterase in organophosphate resistance.

(iii) Glutathione-S-transferase (GST)

Glutathione-S-transferases are a group of enzymes involved mainly in resistance to organophosphates (Siegfried and Scharf 2001, Sun et al. 2001) and DDT dehydrochlorination in insects (Matsumura 1985). Scharf et al. (2000) have proven that GST is also involved in fipronil metabolism in western corn rootworm.

Barbers and Roan (1953) found that a chlordane-resistant strain of German cockroach was able to dehydrochlorinate DDT at a greater rate compared to a susceptible strain. Increased GST activity has been associated with resistance to azamethiphos in the house flies (Kristensen 2005) as well as DDT resistance in four of the field-collected strains of German cockroaches from Malaysia (Lee et al. 2000). In addition, GST activity was detected in five field strains from Denmark which showed high permethrin and deltamethrin resistance (Spencer et al. 1999).

2.8.1.2 Reduced cuticular penetration

According to Cochran (1995), this mechanism probably plays a minor role as it only confers very low levels of resistance. Delayed penetration of insecticide through the insect cuticle allows ample time for other defensive mechanisms, such as metabolic detoxication, to take effect and thereby reducing the efficacy of the insecticide.

Collins (1973) suggested that reduced penetration was probably the mechanism for diazinon and DDT resistance in a diazinon-selected strain of the German cockroach, a conclusion drawn from observing the significantly lower resistance values obtained from injection assays compared to values generated using topical application assays. Decreased insecticide penetration has also been documented in the German cockroach for conferring resistance to cabaryl (Ku and Bishop 1967), permethrin (Bull and Patterson 1993) and cypermethrin (Valles et al. 2000).

2.8.1.3 Target site insensitivity

(i) Modified acetylcholinesterase

Acetylcholinesterase (AChE) is the enzyme that hydrolyses acetylcholine (ACh), a neurotransmitter, at the nerve synapse after transmission of impulse. Organophosphate and carbamate insecticides inhibit AChE and prevent such hydrolysis from taking place, causing ACh to accumulate and death by overstimulation of nerves. Mutation of the AChE however, renderd AChE insensitive to inhibition by organophosphates or carbamates. In a review by Gunning and Moores (2001), it is stated that multiple forms of altered AChE can exist in insecticide-resistant insects.

Altered acetylcholinesterase is considered rare in the German cockroach (Siegfried and Scott 1992), but several reports have reflected its significance as a resistance mechanism. Hemingway et al. (1993) attributed propoxur and chlorpyrifos resistance in a Dubai strain of German cockroach to altered acetylcholynesterase, which is likely to confer broad spectrum resistance to various carbamate and organophosphate insecticides. In Denmark, AChE insensitivity presumably contributed to chlorpyrifos resistance in two populations of German cockroach (Spencer et al. 1999). An investigation by Lee et al. (2000) indicated that <10% of Malaysian field populations of German cockroach possess modified AChE. Notwithstanding the low frequency, this mechanism is in reality occurring at a more frequent rate in German cockroach populations in Malaysia compared to other regions.

(ii) *kdr*-type resistance

In insects with *kdr*-type resistance, synergists fail to increase the toxicity of DDT or pyrethroids (Soderlund and Bloomquist 1990). Dong (1997) explained that *kdr* resistance in the German cockroach was due to the substitution of a single amino acid in the *para* sodium channel protein. Such mutation has been found to confer target-site insensitivity related to pyrethroid resistance (Pridgeon et al. 2002). In laboratory assays, an increase in the frequency of *kdr* allele was correlated with an increase in mean knockdown time, cockroaches that possessed homozygous *kdr* allele showed significantly higher mean knockdown time (Valles et al. 2003).

Scott et al. (1990) had earlier reported that cypermethrin resistance in a strain of German cockroach was not affected by the use of PBO or DEF, thus kdr-type resistance was suspected. When synergists partially eliminated resistance to pyrethroid compounds in a field strain, Atkinson et al. (1991) deduced that *kdr*-like insensitivity might be responsible. Bull and Patterson (1993) further verified that permethrin resistance in this very same strain was primarily affected by *kdr*-type insensitivity at neuron sites.

Likewise, Lee et al. (1996b, 1999) suggested the possible involvement of *kdr*-type resistance in several field-collected strains from Malaysia when PBO failed to significantly reduce the low to moderate level of resistance to pyrethroid insecticides. An investigation by Ross (1997a) revealed that a pyrethroid resistant German cockroaches were found to settle on cyfluthrin-treated papers instead of demonstrating avoidance behavior like how susceptible counterparts would have shown, again indicating the possible involvement of *kdr* resistance.

2.8.2 Behavioural Resistance

It is difficult to confirm whether behavioural resistance is responsible for conferring resistance to insecticides in the German cockroach, as such documentations are largely based on observations. It is possible that resistant insects have evolved to the extent that they may be able to detect the presence of an insecticide and thus overcome it by either avoiding it or not being in contact with the surface long enough to pick up a lethal dose (Cochran 1995). For example, Lee

et al. (1996) observed that male German cockroaches in three field-collected strains from Peninsular Malaysia demonstrated reduced amount of walking when exposed to propoxur-treated glass jar surface.

While this may be true, behavioural resistance in the German cockroach is more often associated with baits, such as avoiding toxic bait due to its base (Silverman and Biemann 1993) or its active ingredients (Ross 1997b). Physiological resistance is more predominant in resistant German cockroaches selected with conventional residual insecticides.

As with resistance to residual sprays, behavioural resistance to gel baits also declines in the absence of selection pressure (Wang et al. 2006). Behavioural resistance to bait is also inherited (Ross 1997c, Wang et al. 2006).

2.8.3 Multiple resistance mechanisms

It is possible for a resistant strain of insect to possess different resistance mechanisms, a condition described as multi-resistance (Scott 1990). Broad spectrum insecticide resistance is generally the result of multiple resistance mechanisms. Multiple resistance mechanisms have been documented in several strains of the German cockroach (Siegfried and Scott 1992, Hemingway et al. 1993, Scharf et al. 1996, Valles et al. 1996).

Ku and Bishop (1967) reported that increased excretion of cabaryl, coupled with reduced rate of insecticide penetration was observed in a cabaryl-resistant strain of German cockroach. Atkinson et al. (1991) concluded that increased metabolism and *kdr*-like target site insensitivity both contributed to the high levels of resistance to various pyrethroids in a field-collected strain. The combined effects of enhanced oxidative and hydrolytic metabolism were found to confer resistance to propoxur and chlorpyrifos in two resistant strain of German cockroaches (Siegfried and Scott 1992). In cockroaches with high resistance to fipronil, multiple physiological resistance mechanism against fipronil is suspected (Wang et al. 2006).

2.9 Baits for German cockroach

Cockroach baits have been used for decades to control the German cockroach. Initially, pest control operators prepared their own concoction of cockroach bait, using a variety of food attractants with inorganic insecticides such as boric acid, phosphorous and sodium fluoride, and relying on their experience to prepare the best bait blends (Mallis 1997).

Cockroach baits are essentially a mixture of insecticide (active ingredient) and food attractant, designed to lure cockroaches so that as they feed on the bait, they receive a lethal dose of the active ingredient and subsequently succumb. Cockroach baits come in a variety of formulation: gel (Miller and Peters 1999, Appel and Tanley 2000), paste (Appel 1992), powder (Appel 2003) or dust (Appel 2004), dry (wafer or cube) (Appel 1990) aerosol foam (Milio et al. 1986) and aerosol gel (Appel and Benson 1995).

Gel and paste baits typically contain ≈14 – 80% water content (Appel 1992, Appel and Benson 1995, Appel 2003, Appel and Tanley 2003). Gel bait formulations induced higher bait consumption in the German cockroach compared to drier formulations, such as dust or dry baits and is therefore more toxic (Appel 2004).

Water is crucial for the survival of cockroaches (Willis and Lewis 1957). Hence, moist baits are expected to be more appealing to German cockroaches. However, bait formulation type did not seem to significantly influence bait attractiveness, as proven by Nalyanya et al. (2001) with their olfactometer assays, but the formulation is crucial to maintain the attractiveness of baits. With the aging of baits, some formulation could become less palatable, due to desiccation or chemical change in the bait ingredients (Nalyanya et al. 2001). Depending on the condition of treatment sites, appropriate bait formulation should be selected to enhance bait performance, such as using powder baits for moist environment to minimize the growth of mould (Appel and Benson 1995).

Baiting was once considered a supplemental treatment to residual insecticide sprays, but are gaining more favour in view of its potential as a stand-alone control measure. Previously,

Cochran (1990) evaluated the effects of abamectin-laced dog food in several pyrethroid-resistant strains of German cockroach and commented that abamectin showed considerable potential as an insecticide for cockroach bait.

Ogg and Gold (1993) also assessed the effectiveness of abamectin bait stations in single-family houses and concluded that abamectin (0.05%) bait stations provided the same level of German cockroach population reduction as with chlorpyrifos spray treatment, or a combination of both chlorpyrifos residual spray with hydramethylnon bait stations. Miller and Peters (1999) demonstrated that in long term assessment, significantly higher German cockroach population reduction can be achieved with fipronil baits compared with deltamethrin sprays.

Various intrinsic and extrinsic factors influence the performance of cockroach baits. Detailed review of such factors has been described by Silverman and Bieman (1996) and Reierson (1995).

2.9.1 Fast-acting baits

Cockroach baits formulated with fipronil, (e.g. Maxforce® FC, Goliath®), are popular for use in German cockroach control due to its rapid action. Fipronil is about 100-fold more toxic than commonly used carbamate and organophosphate insecticides (Scott and Wen 1997) and an effective stomach poison with secondary kill.

Fipronil could be effectively translocated to other members of the cockroach aggregation through emetophagy (ingestion of insecticide-induced regurgitate). Adults and nymphs were particularly attracted to the orally regurgitated bait and, to some lesser extent, the anal secretions of fipronil-fed moribund or dead cockroaches (Buczkowski and Schal 2001a). The exudates exerted its greatest effect to other conspecifics in its fresh form (Buczkowski and Schal 2001a), where majority of fipronil was eliminated 4 – 6 hours after bait ingestion along with the display of poisoning symptoms (Buczkowski and Schal 2001b). When fipronil-poisoned *B. germanica* die in close proximity to shelter, other cockroaches have higher chance

of encountering the dying insects, especially the early instars that forage minimally and remain within the harbourage. This facilitates the transfer of fipronil, which in turn causes secondary mortality (Buczkowski and Schal 2001b).

Kaakeh et al. (1997b) reported that higher nymphal mortality was achieved with fipronil bait than with chlorpyrifos bait in continuous exposure assays. Chlorpyrifos baits and fipronil baits are toxic by both contact and ingestion (Gahlhoff et al. 1999). However, baits that are more toxic require less consumption to deliver its lethal dose (Appel 1992), such as in the case of fipronil baits. Durier and Rivault (2000a) observed that through secondary transmission, mortality rates for German cockroaches were higher for fipronil bait than for bait with hydramethylnon, another insecticide used in cockroach bait.

Based on a study by Appel (1990), complete mortality in < 24 hrs can be achieved with chlorpyrifos baits when tested with a laboratory susceptible strain of German cockroach. However baits containing chlopyrifos were also found to be more repellent than bait formulations with hydramethylnon or boric acid.

Imidacloprid, a neonicotinoid insecticide formulated in cockroach bait (e.g. Premise®), is fast-acting and produces symptoms of poisoning as early as 30 minutes, but moribundity is brief and recovery is possible (Kaaekeh et al. 1997a). Nevertheless, imidacloprid bait (2.15%) was found to reduce German cockroach populations in infested apartments by 80% after 4 weeks (Appel and Tanley 2000).

2.9.2 Slow-acting baits

Hydramethylnon (e.g. Combat™, Maxforce™ or Siege®) cockroach bait is relatively slow-acting. The excretion of hydramethylnon from the German cockroach is a slow process, residues of hydramethylnon were detected in the carcass of German cockroach up to 21 days after exposure (Silverman and Shapas 1986).

Baits formulated with hydramethylnon are known to cause secondary kill in the German cockroach through coprophagy. The ingestion of faeces, or coprophagy, is vital to promote the

survival of German cockroach nymphs, especially to the first instars (Kopanic et al. 2001). Silverman et al. (1991) verified that all stages of the German cockroach excreted hydramethylnon in their faeces, with ≈ 50% in its unmetabolized form, after ingesting baits that contained the compound. After feeding, the cockroaches have ample time to return to their harborages and defecate before they succumb. This is made possible by the delayed action of hydramethylnon (Hollingshaus 1987) and its poor metabolism (Silverman and Shapas 1986).

When exposed to hydramethylnon baits, mortality of early instars of the German cockroach increased significantly in the presence of adult cockroaches, as newly emerged nymphs were less likely to forage and fed more on the faeces excreted by other cockroaches within the harbourage (Silverman et al. 1991, Kopanic and Schal 1997). Kopanic and Schal (1999) further reiterated that early instars of *B. germanica* were more vulnerable to horizontally transferred toxicants via contaminated faeces of foragers, especially in the absence of competitive food and when food is further from aggregation site. Mortality of first instars was predominantly contributed by adult-mediated transfer of hydramethylnon through nymphal ingestion of adult faeces, conversely, second and older instars were less affected by such translocation of hydramethylnon, since they forage more actively than first instars, and were thus susceptible to insecticide through both direct bait consumption and horizontal transmission (Kopanic and Schal 1997, 1999).

Sometimes, cockroaches may have ingested only a sublethal dose of hydramethylnon during initial feeding of baits. Mortality ensues if subsequent feeding occurs, as Silverman and Shapas (1986) had demonstrated that cockroaches succumbed to two sublethal doses of hydramethylnon, administered orally, spaced 6 days apart.

Indoxacarb, with its unique mode of action, has also been explored and incorporated into German cockroach bait, marketed as Advion®. Appel (2003) recommended the use of indoxacarb bait for cockroach population control. A 0.25% indoxacarb gel bait successfully yielded > 95% reduction in German cockroach trap catch after 14 days of treatment in infested