THE SUBLETHAL EFFECTS OF MEPERFLUTHRIN AND TRANSFLUTHRIN ON *Aedes albopictus* (Skuse) AND *Aedes aegypti* (Linnaeus)

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by

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LIST OF ABBREVIATIONS

DDT	Dichoro-diphenyl-tricholoethane
LC	Lethal concentration
LD	Lethal dose
RH	Relative humidity
SE	Standard error

KAJIAN KESAN SUBMAUT MEPERFLUTRIN DAN TRANSFLUTHRIN KE ATAS *Aedes albopictus* (Skuse) AND *Aedes aegypti* (Linnaeus)

ABSTRAK

Suatu kajian makmal telah dijalankan mengenai kesan submaut meperfluthrin dan transfluthrin ke atas *Aedes albopictus* dan *Aedes aegypti*. Beberapa parameter biologi *Ae. albopictus* dan *Ae. aegypti* telah dikaji termasuk kesuburan, peratusan penetasan telur, kadar pembentukan pupa dan kemunculan dewasa, nisbah seks, tabiat menghisap darah dan jangka hayat. Nilai LD_{50} meperfluthrin dan transfluthrin yang diperolehi untuk *Ae. albopictus* ialah 0.0061 ng/mg dan 0.0597 ng/mg, masing-masing, Manakala nilai LD_{50} meperfluthrin and transfluthrin yang diperolehi untuk *Ae. aegypti* ialah 0.0090 ng/mg dan 0.0905 ng/mg, masing-masing. Peringkat induk *Ae. aegypti* dan *Ae. albopictus* yang dirawat dengan dos submaut meperfluthrin menunjukkan penurunan yang signifikan dalam kadar penetasan telur. Penurunan yang signifikan telah kelihatan dalam kadar penetasan telur yang dihasilkan oleh induk *Ae. albopictus* yang dirawat dengan dos submaut transfluthrin. Akan tetapi, tiada perbezaan yang signifikan diperhatikan pada bilangan telur yang dihasilkan oleh nyamuk yang dirawat dengan meperfluthrin dan transfluthrin. Peringkat induk *Ae. albopictus* dan *Ae. aegypti* yang dirawat meperfluthrin dan transfluthrin menunjukkan peningkatan dalam tempoh pencarian perumah. Tiada perbezaan signifikan dalam masa kenyang dan jumlah pengambilan darah bagi kedua-dua species nyamuk yang dirawat. Terdapat perbezaan yang signifikan dalam kadar kemunculan nyamuk dewasa generasi F1 *Ae. aegypti*

selepas dirawat dengan meperfluthrin. Walau bagaimanapun, generasi F2 kedua-dua spesies nyamuk yang induknya dirawat dengan meperfluthrin dan transfluthrin tidak menunjukkan perbezaan yang signifikan dalam kadar kemunculan nyamuk dewasa. Nisbah jantina kedua-dua spesis nyamuk yang dirawat juga tidak menunjukkan perbezaan yang signifikan dibanding dengan kawalan. Meperfluthrin dan transfluthrin juga memendekkan jangka hayat peringkat induk kedua-dua spesies nyamuk yang dirawat. Kesimpulannya, LD_{50} meperfluthrin dan transfluthrin menunjukkan kesan signifikan terhadap *Ae. albopictus* dan *Ae. aegypti* dalam kadar penetasan telur, kadar kemuncuran nyamuk dewasa, tempoh percarian perumah dan jangka hayat. Akan tetapi, tiada kesan submaut didapati pada generasi *Ae. albopictus* dan *Ae. aegypti* yang seterusnya.

THE SUBLETHAL EFFECTS OF MEPERFLUTHRIN AND TRANSFLUTHRIN ON *Aedes albopictus* (Skuse) AND *Aedes aegypti* (Linnaeus)

ABSTRACT

A laboratory study was conducted on the sublethal effects of meperfluthrin and transfluthrin on *Aedes albopictus* and *Aedes aegypti*. Several biological parameters of *Ae. albopictus* and *Ae. aegypti* were tested namely fecundity, fertility, pupation and emergence rate, sex ratio, blood feeding behavior and longevity. The LD_{50} value of meperfluthrin and transfluthrin obtained for *Ae. albopictus* was 0.0061 ng/mg and 0.0597 ng/mg, respectively. Whereas the LD_{50} value of meperfluthrin and transfluthrin obtained for *Ae. aegypti* was 0.0090 ng/mg and 0.0905 ng/mg, respectively. Parental batch of *Ae. aegypti* and *Ae. albopictus* treated with a sublethal dose of meperfluthrin showed a significant reduction in egg hatchability. A significant reduction in egg hatchability was also observed in the parental stage of *Ae. albopictus* treated with a sublethal dose of transfluthrin. However, no significant difference in the mean number of eggs deposited was observed in meperfluthrin and transfluthrin treated mosquitoes. Meperfluthrin and transfluthrin treated parental stage *Ae. albopictus* and *Ae. aegypti* also showed increased host seeking time. There were no significant differences in the engorgement time and total amount of blood meal for both the treated mosquito species. There was significant difference in the adult emergence rate of F1 *Ae. aegypti* and F1 *Ae. albopictus* after being treated with meperfluthrin. However, the F2 generation of both species of mosquitoes treated with meperfluthrin and transfluthrin did not show any

significant difference in the adult emergence rate. The sex ratio of both treated mosquitoes also showed no significant difference compared to the control. Meperfluthrin and transfluthrin also shortened the life span of both F1 treated species of mosquitoes. In conclusion, LD_{50} of meperfluthrin and transfluthrin showed significant effect on treated *Ae. aegypti* and *Ae. albopictus* in fertility, emergence rate, host seeking rate and longevity. However, no significant sublethal effects were found in the subsequent generations of treated *Ae. aegypti and Ae. albopictus.*

CHAPTER 1

INTRODUCTION

Mosquito is an insect that can survive well in different conditions and surfaces of the earth. Mosquito can be found not only in tropical and temperate regions, it can also be found in the Arctic region. The high Arctic mosquitoes *Aedes impiger* and *Aedes nigripes*'s eggs are able to resist freezing and dry conditions. Both species lay their eggs around pond margins on the warm moist sites that are sheltered from the wind (Corbet and Danks, 1975).

Mosquito is also very important from the medical aspect. Many species of mosquitoes are important vectors of many dangerous diseases in the world. For example dengue fever, the West Nile virus, malaria, filariasis, Japanese encephalitis and chikungunya are among the diseases that are vectored by mosquitoes. Dengue is spread by two species of the *Aedes* mosquito. The main vector for dengue fever is *Aedes aegypti* (Linnaeus) whereas *Aedes albopictus* (Skuse) is also involved in dengue transmission as a secondary vector (Mulyaningsih, 2004). They are also involved in the transmission of chikungunya (Rozilawati *et al*., 2011). The West Nile virus is transmitted by the *Culex* mosquitoes. High survival of *Cx. pipiens* during overwintering may be important in the maintenance of the West Nile virus in the northeastern United States (Nasci *et al*., 2001). *Anopheles* mosquitoes, on the other hand, are susceptible to the *Plasmodium* parasites which cause malaria, and *Culex* mosquitoes are also responsible in transmitting filariasis. Lymphatic filariasis has infected over 120 million people, 40 million of whom were deformed and incapacitated by the disease (WHO, 2015).

Mosquito is able to breed well in artificial and natural habitat. According to Dom *et al.* (2013) mosquitoes were found abundant in discarded tires, water tanks, flower pots, jars, coconut shells and ant traps. Besides, larvae were also found in leaf axils of giant taro and mango trees. *Aedes* mosquitoes were discovered to breed in artificial and natural containers around housing areas and construction sites. Industrial and economic development of human has caused big infrastructure changes and indirectly created a man-made environment that is suitable for *Aedes* mosquito to breed (Chua *et al*., 2005).

Aedes albopictus and *Aedes aegypti* are important vectors in transmitting dengue in Malaysia. Dengue has emerged as one of the major public health problems in Malaysia. Up to 22 November Malaysia cumulative number of reported Dengue cases was 93402, whereas the cumulative number of death reported were 178 cases (WHO, 2014). A lot of hard work has been put into the control of the transmission of the disease especially control of the vectors. The main method used to control mosquito is by insecticide. Gratz (1999) reported that if the ultra-low-volume (ULV) is properly applied and timed, it could be effective in suppressing dengue vectors at the time of an epidemic. Sulaiman *et al.* (2000) also reported that deltamethrin, s-bioallethrin, piperonyl butoxide and cyfluthrin are used against dengue vectors at high-rise apartment buildings in

Malaysia. Although diseases spread by mosquitoes can be well controlled by insecticides, long term usage can cause mosquitoes to develop resistance to these insecticides.

Although there are many approaches in controlling mosquitoes, insecticide application either directed at the adult or larval stage remains as the main approach in the control of mosquitoes since the advent of DDT in the 1940s. Insecticides have become powerful weapon to overcome major public health issues and to improve agricultural productivity. Chemical control has remained number one until today (Zairi *et al*., 2004; Karunamoorthi *et al*., 2012)

As a result, the understanding of the effects of insecticides on mosquitoes is crucial. According to Lee (2000) there are two types of insecticidal effects on insects, which are direct toxic effects and sublethal effects. Direct toxic effects are the effects which causes mortality of the insects. As for the sublethal effects it is defined as changes in biology and behavior of the surviving insects after coming in contact with a sublethal amount of an insecticide. Research in sublethal effects are still very limited and not well studied.

Synthetic insecticides were reported to induce sublethal effects on *Aedes aegypti.* However, different insecticides even in the same insecticides' group show different sublethal effects. Three pyrethroids including d-phenothrin, d-allethrin and tetramethrin showed reduction in egg production in *Aedes aegypti*. However, only d-phenothrin and d-allethrin showed reduction in blood engorgement (Liu *et al.*, 1986). Another study showed that dieldrin effects feeding and egg-laying capacity in *Aedes aegypti*, but the effects was not carried over to the progeny (Duncan, 1963).

This study was initiated with the following objectives:

1. To evaluate the efficacy of meperfluthrin and transfluthrin on the adult of *Ae. albopictus* and *Ae. aegypti*.
2. To study the blood feeding behavior changes induced by these insecticides in *Ae. albopictus* and *Ae. aegypti*
3. To determine the sublethal effects of these insecticides on the fecundity, fertility and longevity of *Ae. albopictus* and *Ae. aegypti*.

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy

Mosquitoes comprised of about 3450 species and subspecies belonging to 38 genera. They all belong to the family Culicidae which is divided into three subfamilies namely Toxorhynchitinae, Anophelinae (anophelines) and Culicinae (culicines) (Zairi *et al*., 2004)

Sample Classification of *Aedes aegypti* and *Aedes albopictus* Mosquitoe

Class	:	Insecta	(all insects)
Order	:	Diptera	(all the two winged flies)
Family	:	Culicidae	(all the mosquitoes)
Tribe	:	Culicini	
Genus	:	*Aedes*	
Species	:	*Ae. aegypti, Ae. albopictus*	

All mosquitoes undergo complete metamorphosis which includes egg, larval, pupal and adult stages (Loker & Hofkin, 2015). Plate 2.1 showed life cycle of *Aedes* mosquito.

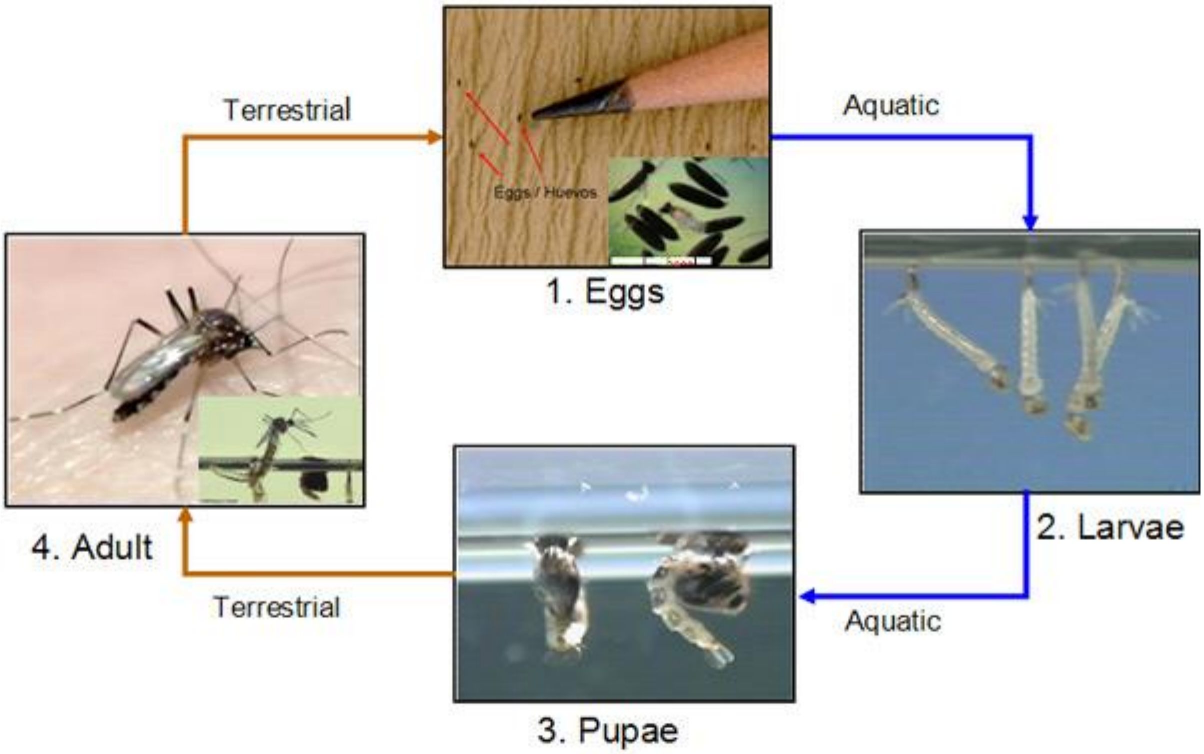

Plate 2.1: ***Aedes*** **mosquito life cycle**

Adapted from:

https://www.cdc.gov/dengue/entomologyecology/m_lifecycle.html

2.2 Morphology and biology of *Aedes* sp

2.2.1 Egg stage

Aedes mosquito eggs are elongated in shape and are always laid singly. It is bound with a thick shell and approximately one millimeter long. The eggs are white in colour when first laid but turn silky black within minutes. The eggs are smooth, long and ovoid shape. They are sharp tapered at the anterior and gradually tapered at the posterior (Clements, 1963; Dengue virus net, 2012; Reinert, 1972). The eggs are capable of resisting and withstanding desiccation for very long periods of time, usually more than one year. The eggs will hatch immediately if immersed in water. However, the hatching process may take quite a long period because the eggs do not hatch at the same time; they hatch in installments (Mattingly, 1969; Dengue virus net, 2012; Service, 1997). *Aedes* likes to lay their eggs separately at the edge of the water surface in tree holes, pools, wet earthen jars and containers which are easily flooded after rain (WHO, 1982; Abu Hassan & Yap, 1999). *Aedes* mosquito usually will not lay all eggs at a single site, they will spread out the eggs over several sites (Foster and Walker 2002).

Aedes mosquito can lay approximately 5 batches of eggs in her lifetime. The productivity of eggs are strongly related with the blood a female *Aedes* mosquito consume (Maricopa, 2006)

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2.2.2 Larval stage

Mosquito larvae are very different from other aquatic insects. They are legless and possess a bulbous thorax that is wider than their head and abdomen. *Aedes* species usually have short barrel-shaped siphon and one pair of subventral tufts. Besides, there are at least 3 pairs of ventral brush on the 9^{th} segment and a few setae on their thorax. Combed teeth can be seen on most *Aedes* larvae, but not in *Anopheles* larvae (Service, 1997; Abu Hassan & Yap, 1999). After hatching, mosquito larvae will feed on detritus and algae for survival. They will undergo four instars and shed their skins three times. During the fourth instar, if the larvae obtain enough energy and reach the minimum size required, metamorphosis will be initiated and transform the larvae to pupae (Norris, 2004; CDC, 2012). They are usually found in clear water. The development of *Aedes* larvae is dependent on the surrounding temperature, competition, water condition and food. However, duration of the larval stage usually lasts around 5 to 12 days (Sulaiman, 1990; Becker *et al.* 2010).

2.2.3 Pupal stage

The pupal stage is very short, around 2 to 3 days. The pupae is coma shape, their body structure can be divided into cephalothorax and abdomen. Cephalothorax possesses a pair of respiratory trumpets and a pair of eyes (Sulaiman, 1990). They can be recognized by looking at the abdominal segments. They do not possess any peg-like spines at abdominal segments 2 to 7 like the anopheline pupae. Although they do not feed, the pupal stage spends most of their time at the water surface taking in air by using their respiratory trumpets. If they are disturbed they will swim up and down in a jerky fashion (Service, 1997).

2.2.4 Adult stage

The life cycle of *Aedes* from eggs to adults can be very fast, lasting around 6 to 8 days. However, it usually takes 10 to 12 days; for some temperate species their life cycle may reach a few months. Most *Aedes* adults have a clear pattern of black and white scales on the thorax and legs. Their abdomen is usually covered with black and white markings. Besides, *Aedes* mosquitoes's wing veins are covered with scales which are narrow and mostly all black. *Ae. aegypti* is recognized by the lyre-shaped silvery scales on the lateral edges of the scutum of the thorax, whereas *Ae. albopictus* is recognized by the broad stripe of white scales extending vertically in the middle of the scutum (Abu Hassan & Yap, 1999; Service, 1997; CDC, 2011).

Adult *Ae. aegypti* and *Ae. albopictus* are active in the day time and at dawn and dusk. Their biting activity is the highest during dawn and dust. Female mosquitoes will start to feed on blood around 1 to 3 days after emergence. *Ae. aegypti* usually bites inside houses during the day time especially during morning and dawn. However, *Ae. albopictus* prefers to bite outside houses and rests on plants such as bushes. The flight distance of both species can reach around 400 meter (Sulaiman, 1990). *Aedes* are able to survive without feeding on blood. A report showed that female *Ae. aegypti* can survive by feeding on sugar solution in the absence of blood meals (Briegel *et al.,* 2001). Xue *et* *al*., (2010) also reported that both sexes of adult *Ae. albopictus* that fed on sucrose solution have a longer life span compared with adults that fed on blood. However, blood meal is very important in mosquito's reproduction. Blood meal is needed to provide protein for egg maturation (Klowden, 1990).

2.3 Blood feeding behavior

Most female mosquitoes need to feed on blood to lay eggs. However, all male mosquitoes only feed on plant's nectar. After feeding on blood, the abdomens of female mosquitoes are enlarged and red in colour. White coloured eggs will start to develop once the blood has been digested. When the anterior part of the abdomen is observed to be white in colour while the posterior part is red, the mosquito is in the half-gravid stage. When the blood is fully digested, the abdomen of the mosquito is enlarged and fully whitish. This shows that the eggs are fully developed and are ready to be laid. After laying the eggs, the gonotrophic cycle is repeated (Abu Hassan & Yap, 1999; Sulaiman, 1990).

Most mosquito species prefer to feed on animal blood than human blood, but some, especially the vector species prefer to feed on human blood. Mosquitoes that prefer to feed on human blood are called anthropophilic; mosquitoes that prefer to feed on animal blood such as mammals, amphibian, reptile and birds are called zoophilic. As for mosquitoes that only blood feed on birds, they are called ornitophilic. Moreover, mosquitoes that frequently bite humans inside the houses are called endophagic, whereas those that bite humans outside houses are called exophagic (Sulaiman, 1990). According to Delatte *et al.,* (2010) *Ae. albopictus* significantly preferred human compare to four other vertebrate hosts which are calf, chicken , goat and dog.

According to Tandon and Ray (2000) *Ae. aegypti* and *Ae. albopictus* are anthropophilic species. However, Rezza (2012) stated that *Ae. albopictus* is less anthropophilic than *Ae. aegypti* and that is one of the reasons *Ae. aegypti* is a better vector of arboviruses. Most female adult start to blood feed after 23 to 24 hours. When they are ready for their first blood meal, they will land with 4 legs on the skin of their target. They will either walk while making directional thrusts or make some light labella taps before settling themselves on the side that they want to puncture or they will instantly insert their fascicle once they land on their target (Jones and Pilitt, 1973).

2.4 Host seeking behavior

Host seeking behavior of mosquitoes can be defined as the flying orientation of the hungry females toward possible blood meal hosts (Bowen, 1991). Factors that affect mosquitoes in locating their potential blood meal host include temperature, olfactory, and visual signals (Hocking, 1971). Human skin emanates chemical that can attract mosquitoes. Mosquitoes respond to carbon dioxide, human skin odour, visual cues and L-(+)-lactic acid. Visual perception of the environment is very important to mosquitoes in their major activities such as searching for sugar sources and potential hosts (Geier *et al*., 1999; Allan *et al*., 1987; Reifenrath, 1996).

Host odour is a crucial factor that affects mosquitoes' orientation in searching for hosts (Smallegange, *et al.,* 2005). According to Geier *et al*. (1999) mosquitoes are able to fly upwind if there is continuous odours stimulation. Many anautogenous mosquitoes have utilized host odours to search for blood meal. They are attracted to almost all higher animals especially mammals and birds (Takken & Knol, 1999). Lactic acid is one of the human odour compound. There is a pair of chemoreceptor neurons located on the antennae of *Aedes aegypti* that is sensitive to lactic acid (Davis & Sokolove, 1976). Besides lactic acid, carbon dioxide, ammonia and short-chain fatty acids also attract mosquitoes (Geier *et al*., 2002).

2.5 Medical importance of mosquitoes

There are 434 species of mosquitoes from 20 different genera in Malaysia (Rahman *et al.,* 1997). *Aedes*, *culex*, *Anopheles* and *Mansonia* are the four major genera that contain medically important species of mosquitoes. Among the four genera, *Aedes* is the main concern because it transmits dengue fever, dengue haemorrhagic fever and chikungunya (Lam, 1993; Lam *et al*., 2001). Besides, there are nine species of *Anopheles* recorded as the main vectors of malaria and filariasis (Rahman *et al.,* 1997; Marzhuki *et al*., 1993), *Mansonia* transmits filariasis (Marzhuki *et al*., 1993) and *Culex* transmits filariasia and Japanese B-encephalitis (Vythilingam *et al*., 2005; Tobias *et al.*, 2009).

Dengue fever was first discovered in Malaysia in 1902, whereas the first dengue hemorrhagic fever was recorded in 1962, both cases were reported in Penang (Skae, 1902; Rudnick *et al*., 1965). *Aedes aegypti* and *Aedes albopictus* are the vectors that transmit dengue in Malaysia. *Aedes aegypti* is an urban species which is easily found in both inside and outside of houses whereas *Aedes albopictus* is a peri-urban species (Lam, 1993). In the last ten years the number of dengue fever in Malaysia increased tremendously. Dengue fever cases in 1973 were only less than 1000, but had increased to 46000 in 2007 (Benitez *et al.* 2009).

Aedes aegypti and *Aedes albopictus* are the vector of chikungunya in Malaysia. Chikungunya (CHIK) virus is an *Aedes* mosquito-borne alphavirus of the Togaviridae family. The first outbreak of chikungunya in Malaysia was in Klang in 1998 and the latest outbreak was in late 2008 in Johore. (Chem *et al.,* 2010; Rozilawati, 2010). In Malaysia there were over 10000 patients affected since April 2008 (Sam *et al*., 2010).

2.6 Chemical control

Insecticides are still the most effective tool in vector control. Insecticides are needed for fast and maximum vector control especially during epidemic situations. Synthetic organic insecticides are the most common type of insecticides that are used today. They can be classified into four classes: pyrethroids, organophosphates, organochlorines and carbamates (Sulaiman, 1990; Lee *et al.*, 2003). Insecticides can also be categorized into larvicides and adulticides. Larvicides include malathion, pirimiphos-methyl (Actellic), fenitrothion (Sumithion), temephos, propoxur (Baygon), permethrin and deltamethrin. Most larvicides are not effective in organically polluted waters and higher dosage rates must be applied (Service, 1997). As for adulticides, they include malathion, fenitrothion, synergized pyrethrins and most of the household insecticide products (Yap *et al.*, 2003). From 2000 to 2009, the average annual usage of insecticides against dengue vectors was 394 tons of organophosphates and 156 tons of pyrethroid (WHO, 2011).

Most control operations used space spray to apply adulticides instead of residual spray. This is because residual spray is far too costly. Space spray includes thermal fog and ultra low volume (ULV) fog, which is also called “cold fog” (Gratz, 1999). According to Service (1997) aerial ULV application of insecticides such as malathion, fenitrothion, pirimiphos-methyl, propoxur and synthetic pyrethroids is the most suitable control strategy in epidemic situations. In some occasions, aero plane ULV has been used to control *Aedes aegypti* during dengue epidemic. A trial of aerial ULV was carried out against *Aedes aegypti* in Nakhon Sawan, Thailand. The result of the trial was impressive. The population of mosquito in the treated area was rapidly reduced after the treatment. However, the aerial application is very expensive due to large aircraft and well trained crews are needed for this method (Gratz, 1999).

During year 2014, The Malaysian Ministry of Health was using the combination of Abate, fogging, ULV spraying and larviciding to control dengue outbreak. Abate with biological control agent was used in stagnant water areas such as construction sites, water containers, drainage and dump sites to control mosquito larvae (BASF, 2014). In Malaysia common insecticides that are used in thermal fogging are Malathion (Malathion), Sumithion (Fenitrothion), Bayatex (Fenthion), Resigen (s-bioallethrin+permethrin+piperonyl butoxide), Pesguard (d-tetramethrin+ cyphenothrin) Gokilat (Cyphenothrin), Actellic (Primiphos-methyl) and Abate (Temephos) (Ong, 2016) Although thermal fogging is widely used, it is applied in the later part of the day instead of early morning. Besides, it is not frequently repeated and therefore no further evaluation to determine when retreatment is necessary. However, thermal fogs should be repeated every four days to maintain adult mosquito populations at low levels (Gratz, 1999).

Larvicides are applied by spraying on potential or actual breeding sites and rest sites of adult females (Scanlon, 1967). Temephos (Abate) is suitable to be placed in water containers to control *Aedes aegypti* due to its low mammalian toxicity and microencapsulated formulations which slowly release insecticides over weeks (Service, 1997). However, during DHF outbreak the application of larvicides are not effective and costly. This is due to the large number of containers in urban areas and low public acceptance of the application of larvicides in household water containers (Gratz, 1999).

2.6.1 Pyrethroids

Pyrethroids are synthetic insecticides that are modified from the chemical pyrethrin, an active ingredient extract of *Chrysanthemum cinerariaefolium*. The extract from the flower that contain pyrethrin is called pyrethrum. Pyrethrum is not toxic to human, but is effective on insects. However, it is not stable in sunlight and air. As a result, it does not possess a residual effect but has a fast knockdown effect instead. In 1950 allethrin, the first synthetic pyrethroid appeared on the market. It is more stable and possesses better insecticidal activity than natural pyrethrum (Büchel, 1983; Sulaiman, 1990).

Pyrethroids are categorized according to their structure and toxicity. Type I pyrethroids lack a α-cyano group on the phenoxybenzyl moiety, whereas those with a α-cyano group on the phenoxybenzyl moiety are type II and another one is the non-ester pyrethroids (Schleier & Peterson, 2011). Structures of type I and II were showed in figure. 2.1. Pyrethroids are common active ingredients used in public health, household insecticide products and repellents for mosquito control (Yap *et al.,* 2003). This is because pyrethroids are safe for humans, low toxicity and possess fast knock-down effects. WHO has recommended quite a number of pyrethroids for indoor residual spraying which include alpha-cypermethrin, bifenthrin, cyfluthrin, deltamethrin, etofenprox and lambda-cyhalothrin (WHO, 2005).

Pyrethroids can be divided into 4 generations. The first generation pyrethroid is allethrin. It is only effective against house flies and mosquitoes. In 1965, tetramethrin the second generation pyrethroid was introduced. It provided better knockdown effect on flying insects than allethrin. Besides, it is able to join action with other compounds to achieve better insecticidal activity. Permethrin and fenvalerate are the third generation pyrethroids. They were the first pyrethroids used in agriculture. They had exceptional insecticidal activity, photostability and residual properties. Last but not least, the fourth generation pyrethroids. The fourth generation pyrethroids include cypermethrin, cyfluthrin, bifenthrin, deltamethrin and esfenvalerate. They are 10 times more active than the third generation pyrethroids, photostable and stable under ultraviolet light (Lee *et al.*, 2003).

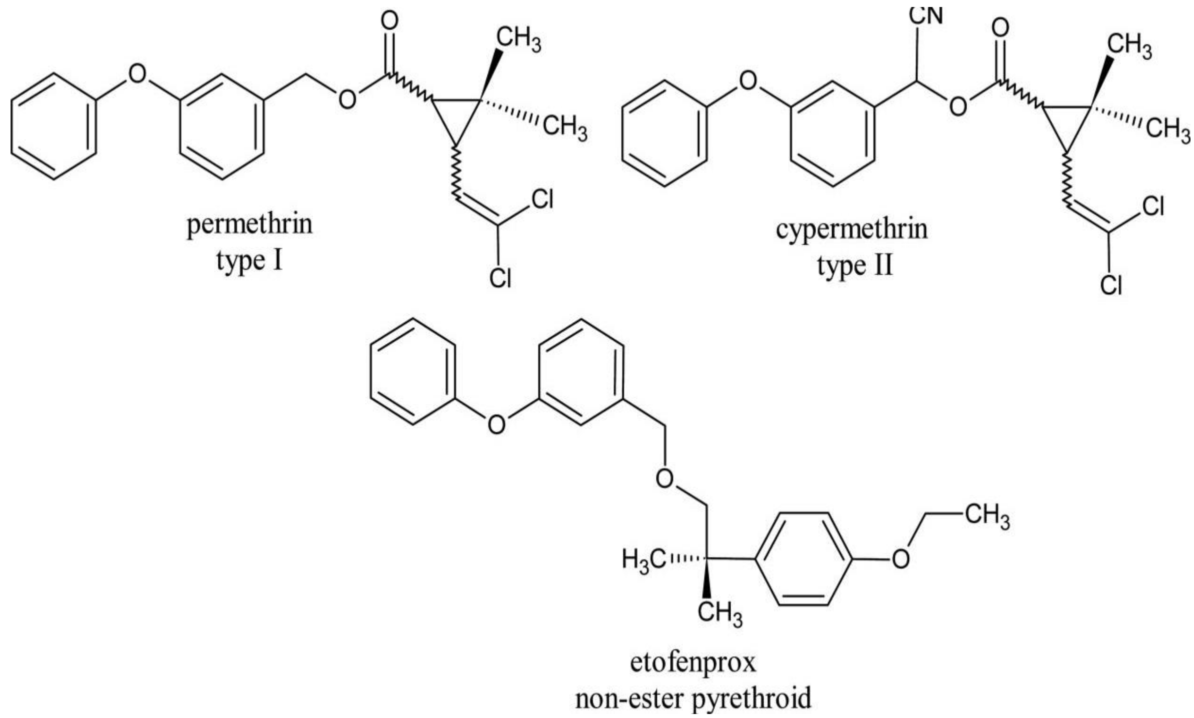

Figure 2.1: Chemical structures of type I, type II, and nonester pyrethroids (Schleier & Peterson, 2011)

2.6.1.1 Mechanism of action

Pyrethroids are a type of neurotoxic that are able to penetrate rapidly into the nervous system of insects through contact. If an insect comes in contact with pyrethroids, it will undergo excitation, disturbance to body orientation, paralysis and finally death (Büchel, 1983). Ion channels are the primary target site for pyrethroids. Type I pyrethroids modify the sodium channels and moderately prolong the channels open time, which induces multiple long action potentials, whereas type II pyrethroids cause a long-lasting prolong of sodium permeability of the nerve membrane during excitation (Schleier & Peterson, 2011).

Pyrethroids cause "knockdown effect", which is defined as symptoms of lost coordination and paralysis. The knockdown effect of sublethal doses of pyrethroids can be prolonged with the present of synergists. This is because synergists inhibit detoxification by the insects. Important synergists for pyrethroids include sesamin, sesame, salfroxan, sesamolin, propyl-isome and so on (Büchel, 1983).

2.6.2 Transfluthrin and meperfluthrin

Transfluthrin and meperfluthrin are synthetic pyrethroids. The molecular formula for transfluthrin is $C_{15}H_{12}Cl_2F_4O_2$ and with a molecular weight of 370.15 g/mol. Structural formula of transfluthrin was shown in figure 2.2. As for meperfluthrin, the molecular formula is $C_{17}H_{16}Cl_2F_4O_3$ and with a molecular weight of 415.22 g/mol.

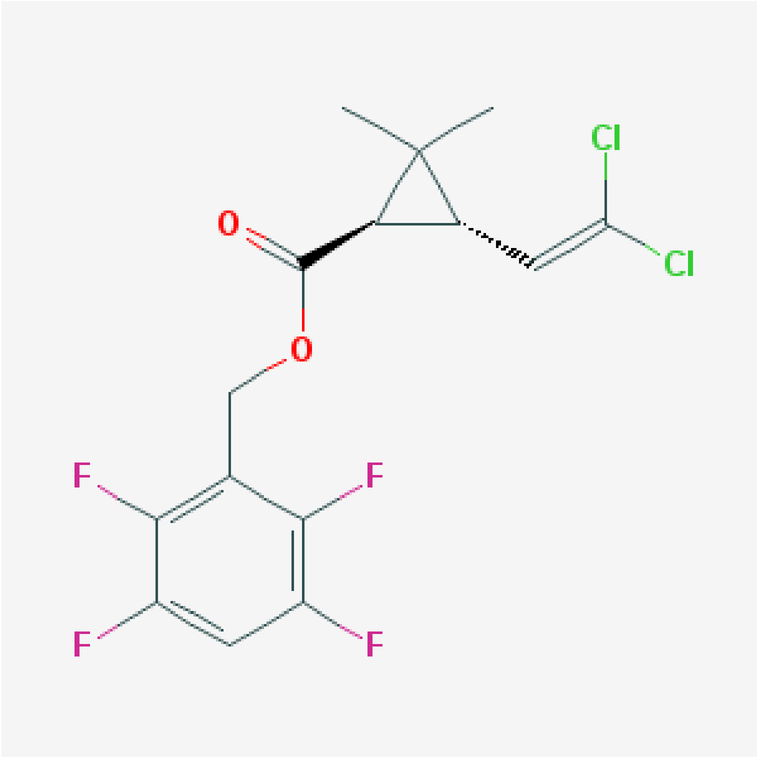

Figure 2.2: Chemical structure of transfluthrin

Adapted from

http://pubchem.ncbi.nlm.nih.gov/compound/656612#section=Top

Structural formula of meperfluthrin was shown in figure 2.3. Both active ingredients can be used in coils, mats, vaporizer and aerosol. Transfluthrin is also suitable for pest control operators (MSR Green Corporation, 2009a; MSR Green Corporation, 2009b).

According to Ogoma *et al.* (2012) strip of hessian sacks treated with transfluthrin showed high protective efficacy against *An. arabiensis.* It reduced mosquito attack rate on human volunteers by more than 99% and the effect lasted for 6 months. Another research of Ogoma *et al.* (2014) had recorded that transfluthrin based coil (0.03% and 0.045%) had reduced *Anopheles gambiae* blood feeding activity for 12 hours. A research also showed that 0.1% of transfluthrin in vegetable oil was able to achieve 50-75% reduction in the biting rate of mosquitoes (Pates *et al.*, 2002). Another research by Yap *et al.* (1996) also showed similar result. Three formulations of mosquito coils that containing transfluthrin (0.018%, 0.027% & 0.046%) able to reduce >90% of landing and biting activities of *Cx. Quinquefasciatus*. Besides, portable battery powered blowers using 0.6% transfluthrin recorded a 2.4 fold lethal effect compared to bioallethrin in electric mats (Lee, 2007).

The research on meperfluthrin is still very limited. It is a relatively new insecticide. Xue *et al.,* (2012) reported that mosquito coils containing 0.08% meperfluthrin showed significantly higher mortality against adult female *An. albimanus, Ae. albopictus,* and *Cx. quinquefasciatus* compared to mosquito coil containing 15% citronella oil. No pest control product using meperfluthrin yet in Malaysia market.

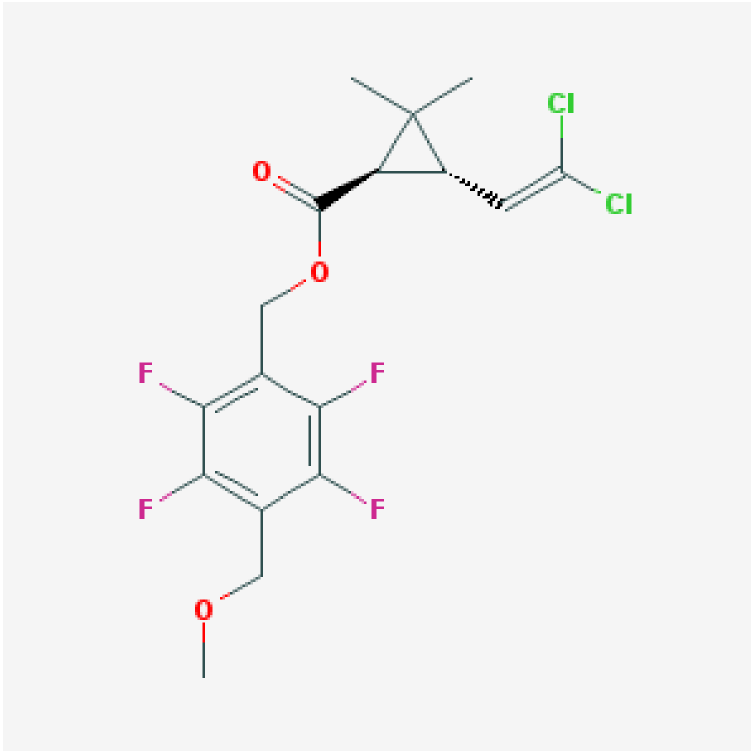

Figure 2.3: Chemical structure of meperfluthrin.

Adapted from

http://pubchem.ncbi.nlm.nih.gov/compound/56841697#section=Top

However, a few commercial mosquito coils with meperfluthrin can be found in China market.

2.7 Sublethal effect

Sublethal effect is defined as the biological and behavioural changes of individual that survived the exposure to a sublethal amount of insecticide. Sublethal effects influence insect survivorship, reproduction and the genetic composition of the following generation (Lee, 2000).

According to Yap *et al.* (1996), *Ae. aegypti* and *Cx. quinquefasciatus* longevity and blood engorgement activity showed significant reduction after exposure to coils containing d-allethrin or d-trans alethrin. Besides, Adanan *et al.* (2005) also reported longevity and blood engorgement activity of *Ae. aegypti* and *Cx. quinquefasciatus* decreased drastically after exposure to coils containing d-allethrin (36 mg/mat) and prallethrin (15 mg/mat). However, fecundity, fertility pupation rate and adult emergence rate were not affected.

Sublethal effects of insecticide influence insects' behavior in many aspects. For example sublethal dose of permethrin caused a significant reduction in the mating activity of the Pink Bollworm Moth (Haynes & Banker, 1985). Besides, Cohnstaedt and Allan (2011) discovered that *Cx. quinquefasciatus* that were exposed to permethrin and deltamethrin flew slower and turned more frequently than control mosquitoes. Boric

Plate 2.2: Sample of meperfluthrin product in China market.

Adapted from

https://www.alibaba.com/product-detail/Eco-friendly-No-smoke-Mosquito

Coil_60422584488.html