REVIEW OF LITERATURE
1. Malaria – a deadly disease

Malaria affects 40% of the world population spread over 92 countries (Martens et al., 1995). Human malaria is widely endemic in tropical and subtropical regions of the world. Despite extensive measures taken since the early fifties in India, both by the central government and state governments to combat the debilitating disease (Sharma et al., 1996), it has become endemic in the central, south eastern and north eastern parts of the country. Indian anopheline fauna comprises 58 species of which nine of them act as vectors of malaria. By managing mosquito vectors; malaria could be kept at check and WHO advocates use of ITN against mosquito vectors.

2. Introduction of ITN and its effect

The World Health Assembly advocated the large-scale use of insecticides for malaria control in 1955, and programmes were carried out to spray as many houses as possible with a residual deposit of insecticide [mostly organochlorine compounds such as dichlorodiphenyltrichloroethane (DDT) and dieldrin]. It was discovered that malaria mosquitoes developed resistance against the insecticides directed against them, and before long insecticidal spraying was abandoned in many countries (Najera, 1989; Georghiou, 1990).

The mechanisms of insecticide resistance are manifold: mostly these involve changes in the enzyme metabolism or neurotransmission of the insects (Soderlund and Bloomquist, 1990; Hemingway and Ranson, 2000), but behavioural changes of mosquitoes have also been reported (Grjebine, 1956; Boreham and Garrett-Jones, 1973). These included changes in biting behaviour, resting behaviour and avoidance of insecticide treated rooms. Indoor spraying with organochlorines has caused
behavioural changes in several malaria vector species (Knols and Takken, 1998), mostly due to the selective pressure placed on the target vector population. In order to overcome the pending crisis in lack of adequate intervention methods, bednets treated with insecticides were re-introduced in the latter part of the 1980s. Bednets were to protect the user(s) against the bites of malaria infectious mosquitoes, and hence contribute to a reduction of transmission risk.

It was soon observed that the use of insecticide-treated bednets (ITNs) provided adequate protection against malaria infections, particularly in children (Lengeler and Snow, 1996).

Based on a series of field studies on the effect of insecticide-treated nets (ITNs) on malaria morbidity and mortality in sub-Saharan Africa (Habluetzel et al., 1999; CuzinQuattara et al., 1999, Lengeler, 2003), promotion of use of ITNs has emerged as a key intervention for malaria control. Large-scale epidemiological field trials involving community use of pyrethroid-impregnated bednets have demonstrated major benefits in reducing malaria morbidity and mortality (Snow et al., 1988; Rashed et al., 2000; Abdulla et al., 2001).

RBM has adopted use of insecticide-treated mosquito nets as a major tool for the achievement of its malaria control objectives. The primary goal of RBM is to achieve a 50% reduction in the global malaria burden by 2010, and the period 2001–2010 has been tagged the "United Nations Decade to Roll Back Malaria" (WHO, 2002). Treatment of mosquito nets with insecticide was probably introduced for the first time during world war II, when nearly half a million American servicemen were stricken with malaria (Hamann, 2001). RBM’s target is to have 60% of the world’s population at risk of malaria sleeping under ITNs by 2005 (WHO, 2002). Realization
of this goal could see tens of millions of doses of pesticides for net impregnation entering thousands of homes in malaria endemic poor countries annually (Hirsch et al., 2002).

3. Insecticide for net treatment

Photo-stable pyrethroids are particularly appropriate for impregnation of bednets, because of their long persistence on materials and their relative safety to humans. Permethrin (200-500 mg a.i./m2), Deltamethrin (15-25 mg a.i./m2), Lambda cyhalothrin (10-30 mg a.i./m2), Cypermethrin (100 mg a.i./m2), Cyfluthrin (100 mg a.i./m2), each one at different dosage, can be used for impregnation of mosquito nets. Results of bioassays may vary depending of the insecticide used and its dosage, mosquito species and the nature of the fibres (Coosemans, 1995).

Most of the pyrethroids will be removed after washing the impregnated nets in cold, soapy water (Miller et al., 1991; Curtis et al., 1991). The deterrent effect, which enhanced personal protection, is produced by the components of the formulation and not by the insecticide itself (Lindsay et al., 1991). Emulsifiable concentrates (EC) are generally used for impregnation of bednets, but Wettable powder (WP) were also successfully used (Le Goff et al., 1992).

A new approach of mosquito net is using a 'two-in-one' combination of pyrethroid and non-pyrethroid insecticides applied to different parts of bednets. Nets are treated on the upper part with residual non-irritant insecticide (carbamate or organophosphate) and with a pyrethroid on the lower part. Sequential exposure to different insecticides with distinct modes of action is equivalent to the use of a mixture as a potential method of managing insecticide resistance (Guillet et al., 2001).
4. Pyrethroids

Pyrethroids (also known as synthetic pyrethroids) are insecticides chemically similar to pyrethrins found in natural pyrethrum extracted from the oleoresin extract of dried flowers of chrysanthemum, known for centuries for their insecticidal activity (CPCN, 2001). First developed in 1973, pyrethroids are more stable to light than natural pyrethrum and possess very good insecticidal activity. At present, the class of pyrethroids includes 42 active ingredients, differing in chemical structure or in relative stereoisomer composition (NPTN, 1998). However, only certain pyrethroids are recommended by WHO for net treatment.

The insecticidal properties are derived from ketoalcoholic esters of chrysanthemic and pyrethroidic acids. These acids are strong lipophilic and rapidly penetrate many insects and paralyse their nervous system. Both pyrethrins and synthetic pyrethroids are sold as commercial pesticides used to control insects. Various formulations of these pesticides are often combined with other chemicals called synergists to increase the potency and persistency in the environment. Insecticides recommended by the WHO Pesticide Evaluation Scheme (WHOPES) for the treatment of mosquito are pyrethroid insecticides, which are presently the only group of insecticides recommended for this use (WHO, 1999).

5. Pyrethroids – Mode of action

Pyrethroids are historically divided into two types, according to their chemical structure: type I pyrethroids, which do not contain an alpha-cyano group in their molecule (for example, Allethrin, Resmethrin, D-phenothrin, and Permethrin) and which cause mainly tremors (T-syndrome); and type II pyrethroids, which do contain an alpha-cyano group (for example, Deltamethrin, Cypermethrin, Cyfluthrin and
Fenvalerate) and which cause choreoathetosis and salivation (CS-syndrome) (Tordoiret al., 1994).

The toxicity of pyrethroids in mammals is caused by similar mechanisms as the insecticidal activity. The marked difference in the toxicity of pyrethroids to insects (target organisms) and mammals is apparently caused by differences mainly in the voltage-sensitive sodium channels (Narahashi, 2000; Soderlundet al., 2002). Pyrethroids impair ion transport through the membrane of nerve axons, causing muscular paralysis in the insect; death seems to follow a nervous system impairment that occurs a few minutes after pesticide absorption (Reigart and Roberts, 1999; Mueller-Beilschmidt, 1990). Pyrethroid insecticides act on the nerves of both insects and higher animals, inducing a transient increase in sodium permeability of the nerve membrane during excitation. This action results in relatively short trains of repetitive nerve impulses in sensory (afferent) nerve fibres (Pollack et al., 1999).

Pyrethroids without an alpha-cyano group (type I pyrethroids) cause a moderate protraction of the sodium channel permeability in the nerve membrane, while alpha-cyano pyrethroids (type II pyrethroids) cause a long-lasting protraction of sodium permeability of the nerve membrane during excitation (EXTOXNET, 1994; EPA, 2000). Invertebrates and some cold-blooded species are more susceptible to the toxic effects of pyrethroids than vertebrates (Narahashi et al., 1998).

Interaction with sodium channels is not the only mechanism of action proposed for pyrethroids in insects and vertebrates (Ray and Forshaw, 2000). The effects of pyrethroids on the central nervous system depend on an antagonism of gamma-aminobutyric acid (GABA)-mediated inhibition, modulation of nicotinic cholinergic transmission, enhancement of nor adrenalin release; or action on calcium
channels. GABA is an important mediator of inhibitory neurotransmission in the mammalian nervous system and a target site for the action of several therapeutic drugs and toxicants. Release of GABA by presynaptic nerve terminals activates a chloride channel on the postsynaptic membrane, leading to hyperpolarization of the postsynaptic nerve terminal and thus enhancing the excitatory threshold of the postsynaptic neuron. This would result in an indirect neuroexcitatory effect. In vitro studies show that the GABA receptor blockade is not observed at concentrations of pyrethroid that disrupt sodium channel function, it is unlikely that GABA inhibition represents the primary mechanism of action, thus explaining the neuroexcitatory effects of pyrethroids (Soderlund et al., 2002). Neurotransmitter release is probably secondary to the increased sodium entry in the neural cell (Ray and Forshaw, 2000).

6. Toxicokinetics of Pyrethroids

The exposure scenario for pesticides used for public health purposes is quite variable, from exposure to larvicides in drinking water, to dermal and inhalation exposure from bednets, or from space spraying and vapour of household insecticides (e.g. mosquito coil). The penetration of pyrethroids into the skin is slow and may cause a typical local paraesthesia (tingling and burning sensations), which may persist for several hours (Bateman, 2000). After absorption, pyrethroids are rapidly distributed throughout the body, mainly in the adipose tissue, stomach, intestine, liver and kidneys and the nervous system. Pyrethroids are rapidly and extensively metabolized, mainly in the liver, by hydrolases and cytochrome P450-dependent monooxygenases (Crawford, 1981). Pyrethroids do not accumulate in the body and their excretion is rather rapid, even after repeated administrations (Aldridge, 1990; Vijverberg and van den Bercken, 1990). Studies carried out on human volunteers have
shown that, after oral administration of Cypermethrin, about 75% of the dose is excreted in 24 hours; the rate of excretion is similar for all dosages. After a 2-day period, no detectable amounts of metabolites were found in urine (Van Sittert et al., 1985). The ratio of excretion between urine and faeces varies with the compound and the route of administration (Soderlund et al., 2002, Vijverberg and van den Bercken, 1990).

7. Toxicity of pyrethroids

Pyrethroids are associated with skin rashes and non-inflammatory irritation of the skin lasting up to 24 hours, and temporary numbness at very high concentrations. Pyrethroids appear to cause immediate irritation to the eye (NIOSH, 1997). Exposure to pyrethroids in the eyes may also result in blurred vision and damage to the cornea of the eye, which can vary in severity depending on exposure levels. Soto et al. (1995) studied effect of the Permethrin-impregnated uniforms.

7.1. Bednet impregnation, use and effects observed

Pyrethroids are recommended as they are safe for domestic handling. Yadav (1995) studied the transient health effects due to the use of bed nets in humans. Satpathy et al. (1997) reported no effect on health or laboratory impairment in the subjects after one month of usage of nets impregnated at the dose of 50 mg/m2 of cyfluthrin. Boman (1995) studied the air concentration in a room where Cyfluthrin (50 mg cyfluthrin/m2) treated net was hung. Barlow et al. (2001) extrapolated the highest concentration observed by Boman (0.055 μg/m3) and estimated the inhalation exposure to humans.

Kolaczinski et al. (2004) opined that, chronic effects presently cannot be excluded with certainty, as relevant toxicological data do not exist in the open
scientific literature. Properly designed neuro-behavioural studies on groups with long-term exposure to low doses of synthetic pyrethroids should be conducted. This will require establishment of a working definition of "case" and "exposure". Meanwhile pyrethroids should continue to be used for public health interventions that contribute substantially to morbidity and mortality reduction, such as ITNs for malaria control.

8. **Insecticide Treated Net**

Insecticide-treated bednets (ITNs) is universally accepted as an efficacious and essential public health service in most parts of sub-Saharan Africa endemic for malaria." (Guyatt and Ochola, 2003).

Deployment of ITN easily wards off people from mosquito bites of endophilic and endophagic mosquitoes and its efficacy enhances when its use coincides with the biting rhythm of the mosquitoes and the sleeping time of the people who use them (NIMR, 2005).

9. **Efficacy of insecticide treated nets**

We consider that the great importance of the pyrethroid deposit on the net arises because the body odour of the sleepers attracts human-seeking (anthropophilic) mosquitoes to make repeated contact with the nets and many are killed (Curtis *et al.*, 2003). The protection of insecticide treated net is due to prevention of physical contact, toxicity due to the presence of insecticide if the insect come in contact which leads to knockdown, mortality, repellency, inhibition of blood feeding etc. The protection offered by the insecticide treated net depends adequacy of surface concentration of the insecticide treated. Bioefficacy serves as a tool to study the adequate presence of insecticide on the net.
Bioassay

Jawara et al. (1998) studied the acceptability and efficacy of bednets treated with three pyrethroid insecticides – Alphacypermethrin, Permethrin and Lambdacyhalothrin in the Gambian village of Saruja, where malaria is transmitted mainly by mosquitoes of the *Anopheles gambiae* complex.

Jana-Kara et al. (1994) compared the efficacy of cotton, nylon or polyester mosquito nets treated with Deltamethrin SC & WP and Lambda-cyhalothrin EC treated conventionally. Ansari et al. (2003) using standard WHO cone, studied the laboratory efficacy and impact of storage of Alpha cypermethrin treated nylon nets against *An. culicifacies* and *Cx. quinquefasciatus* using standard WHO bioassay. Batra et al. (2005) studied the persistence of different formulations of Bifenthrin and Lambda cyhalothrin treated nets against *An. culicifacies* by contact bioassays using cone and ring-net bioassays in field conditions following the method of WHO on day one and thereafter at fortnightly intervals. Curtis et al. (1990 b) studied the effectiveness of nylon net treated with Permethrin EC, Deltamethrin SC and Lambdacyhalothrin EC against *An. gambiae* and *An. funestus* using verandah traps in a Tanzanian village. Darriet et al., 1998 compared the efficacy of SC and WT formulations of Deltamethrin at different doses. A comparative study in experimental huts was done by Todd et al. (1996) to study the persistence Permethrin, Deltamethrin and Etofenprox in relation to storage in light or darkness by chemical assay and bioassay. Curtis et al. (1996) have reported results of bioassays of Etofenprox treated nets, with or without washing. Nguyen et al., (1993) studied the efficacy of EC and EW formulations of Etofenprox in a village scale trial in North Vietnam, where *An. minimus*, is the main malaria vector (susceptible to Etofenprox). Asidi et al. (2005)
evaluated bednets treated with an organophosphate (Chlorpyrifos-methyl) or a pyrethroid (Lambdacyhalothrin) alone and in combination against insecticide-resistant *Anopheles gambiae* and *Culex quinquefasciatus*.

Nguyen *et al.* (1996) studied the efficacy of Olyset Nets when used as screens against *Aedes aegypti* (pyrethroid susceptible). Ikeshoji and Bakotee (1997) carried out the Chemical and biological assays on Olyset Nets and nets conventionally treated with Permethrin at target dose of 500 mg/m². Efficacy of Olyset Nets for malaria vector control was studied in comparison to the use of untreated mosquito nets by the National Malaria Centre, Phnom Penh, where *Anopheles dirus* and *An. minimus* were the main malaria vectors (Chheang and Sandy, 1994).


Curtis *et al.* (1996) compared the bioefficacy of EC formulations of Bofenprox treated bed nets. Lindsay *et al.* (1991) studied the proportional degree of deterrence to the dosage of Permethrin on mosquitoes of the *Anopheles gambiae* complex in Gambia. Njunwa *et al.* (1996) carried out a comprehensive evaluation

Hougard et al. (2002) evaluated the effect on tarsal contact on Bifenthrin treated netting against Anopheles gambiae and Culex quinquefasciatus. Rajendra et al. (2005) evaluated the efficacy of Deltamethrin tablet formulation (K O Tab) against An. culicifacies and Anopheles stephensi using WHO plastic cones. Field trial was conducted by Ansari et al. (2003) to study the bio-efficacy and effect of storage on nets after treatment of Alphacypermethrin in northern India.

Batra et al. (2005) compared the efficacy of nets treated with Bifenthrin formulations (ME and SC) with Lambdacyhalothrin formulation (CS) against anopheline & culicine mosquitoes. Alten et al. (2003) evaluated the efficacy of Deltamethrin-impregnated bednets in malaria control and in reducing the biting nuisance caused by Anopheles sacharovi in endemic area of malaria in the surrounding rural settlements of Turkey. Hougard et al. (2003) carried out a comparative laboratory study under standardized conditions to test efficacy of Bifenthrin EW against susceptible and pyrethroid-resistant An. gambiae and Cx quinquefasciatus, in comparison with Alpha-cypermethrin SC, Cyfluthrin EW, Deltamethrin SC, Etofenprox EW, Lambda-cyhalothrin CS and Permethrin EC formulations. Lenhart et al. (2008) studied the effect of Insecticide-treated bed nets to control dengue vectors.
Washing and Persistency

Elissa and Curtis (1995) studied efficacy and persistence of various formulations of Deltamethrin and one of Permethrin, for treatment of mosquito nets. Pinder et al. (1999) studied the wash resistance of mosquito net treated with Permethrin 10% EC, 1 Deltamethrin % SC and Deltamethrin WT formulations in a village trial. Prasittisuk et al. (1995) compared the residual life of three dosages of Etofenprox EC (100, 200 and 400 mg/m²) on cotton and nylon mosquito nets by using standard bioassay with 3 minutes exposure. Elissa and Curtis (1995) studied efficacy and persistence of various formulations of Deltamethrin and one of Permethrin, for treatment of mosquito nets. Persistence of Permethrin in Olyset Nets was examined after seven months of practical use inside and outside houses (exposed to sunlight) in northern Vietnam (Itoh and Okuno, 1996). Hougard et al. (2000) studied wash resistance and dynamics of the insecticide in Olyset Nets under the laboratory conditions. Rajendra et al. (2005) evaluated the persistence and wash resistance of Deltamethrin tablet formulation (K O Tab) against An. culicifacies and Anopheles stephensi under field condition. Gimnig et al. (2005) studied the laboratory wash resistance of long-lasting insecticidal nets and compared the same with the conventionally treated mosquito nets. Graham (2005) compared the wash resistance of conventionally treated nets and wash resistant Perma nets against Anopheline and Culicine mosquitoes. Lindblade et al. (2005) studied the efficacy of LLIN, Olyset Net and wash resistance of LLIN and compared the same with nets that were treated with a combination of Permethrin and Cyclodextrin (a starch) to render the nets more wash-resistant and were compared to conventional nets treated with Deltamethrin (K-O Tab). Sharma et al. (2006) studied the wash resistance and bioefficacy of Olyset
Net against malaria vector and other non target species like bed bugs, cockroach, and housefly.

12. Blood feeding inhibition

Elissa and Curtis (1995) compared the blood feeding inhibition of various formulations of Deltamethrin and one of Permethrin, against An. gambiae females employing tunnel test. Ability of Ae.aegypti and An. maculatus to pass through treated netting to feed on the host was studied in laboratory by Vythilingam et al. (1996).

Hougaard et al. (2003) compared performance of pyrethroids in terms of mortality and blood feeding inhibition against both Anopheles and Culex. Corbel et al. (2004) studied the effect of Permethrin treatment under laboratory conditions using the tunnel test technique against susceptible, heterozygous and homozygous genotypes of Anopheles gambiae. Vatandoost et al. (2006) studied the efficacy of torn nets treated with Lambda cyhalothrin and Bifenthrin against the malaria vector, Anopheles stephensi by tunnel test method. Chouaibou et al. (2006) studied the blood feeding inhibition exhibited by Bifenthrin impregnated bed nets at different doses.

13. Irritability

Over a short period of time, pyrethroid chemicals have taken on greater importance in vector control worldwide, despite the increased reporting of resistance to a general class of compounds (Malcolm, 1988). The irritant effect of an insecticide is an important characteristic to be considered, as it modifies the tarsal time with the treated substrate (WHO, 2006). The irritant property of some insecticides can cause a proportion of insects to leave sprayed surfaces before a lethal dose; hence the repeated contact is required before mortality occurs.
The term repellency (more often excito-repellency) is sometimes related to this phenomenon. Repellency is the prevention of the insect from approaching the insecticide. This irritability would produce heightened activity in the landing mosquito and will only remain on the treated surface for a short period of time. The irritability responses of vectors were interpreted to have a negative impact on control efforts. Insecticide repellency could prevent vectors from entering human habitations treated with the insecticides. In the long run this is likely to cause reduction in endophilic mosquitoes and an increase in the pedophilic populations. Pyrethroids may repel insects due to air-borne repellency or contact, which raises the possibility of the behavioural response, an important attribute of pyrethroid use. Irritancy may lead to survival of a species in the treated houses, but the reason is often attributed to the reduced intrinsic toxicity of insecticide or occurrence of physiological resistance. The irritability to insecticides may reduce the effectiveness of residual applications of the insecticides. Careful monitoring of both physiological and behavioural responses to pyrethroids is essential in the evaluating the pyrethroids (Vatandoost, 2001).

Irritability occurs when insects actually make physical contact with chemical residues before eliciting a stimulus-mediated response, whereas repellency is defined as stimulus acting from a distinct distance, without actual physical contact to an insecticide-treated surface, deterring insects from entering treated areas or otherwise disrupting normal patterns of behaviour. Assessment of insecticide irritability and repellency in mosquitoes is generally affected by a variety of factors covering environmental, biological and actual test conditions, many of the same factors which presumably play under natural conditions. These include type and formulation of insecticide, mosquito species and origin (laboratory or wild-caught), nutritional and physiological conditions of mosquitoes at time of test, number of mosquitoes used in
the effect of crowding), time of test and ambient environmental factors such as temperature and illumination (Chareonviriyaphap et al., 1997). Several reports have shown irritability is depressed after taking a recent blood meal (Roberts et al., 1984). Blood-fed mosquitoes have shown less irritability than unfed females (Hecht et al., 1960; Qutubuddin, 1967) that may result in delayed avoidance responses.

The degree of irritability is known to vary depending on the type of insecticides used (Busvine, 1964). It is undeniable that irritancy and repellency produced in the presence of an insecticide can have a dramatic impact on the effectiveness of chemical control of mosquito vectors, thus profoundly impacting the local transmission of disease. The Irritability of Deltamethrin, Permethrin, Lambda-cyhalothrin and Cyfluthrin at the diagnostic dose were studied by Vatandoost (2001). Differences in irritancy, excito-repellency, and knockdown properties may have a significant impact on the overall efficacy of an insecticide (Roberts et al., 2000). In addition, this impact might differ, depending on how insecticides are used e.g., indoor residual spraying, treatment of mosquito nets, or space spraying (Hougardi et al., 2002).

Synthetic pyrethroids and DDT have two types of toxic effect on insects - an initial rapid knock down effect, followed by a lethal effect. DDT and pyrethroids also show an excito-repellent effect. This effect may be regarded as a disadvantage as it could drive away an insect before a lethal dose has been absorbed. However, there are beneficial features of this characteristic, because mosquitoes can be driven away from hosts to bite animals, or to rest in less favourable outdoor sites and they may be prevented from biting through a treated net or from exploring a treated net for long enough to find a hole in it (Hodjati et al., 2003). In some cases DDT or pyrethroid
Resistant genes cause reduced irritability. Selection for DDT resistance in the spotted
maggot, *Euxesta notata*, resulted in decreased irritability (Hooper *et al.*, 1965, a,
b); Brown (1964) reported that many DDT-resistant strains of mosquitoes showed less
irritability than susceptible strains. Hodjati and Curtis (1997, 1999a, b) reported the
same for Permethrin and pyrethroid resistant *An. stephensi*. Chandre *et al.* (2000)
reported that Permethrin was more irritant to the susceptible strain than to the resistant
mosquitoes carrying the *kdr* gene. Darriet *et al.* (1999) reported that pyrethroid
impregnated bednets in laboratory and experimental hut tests provided good levels of
protection against biting and high mortality of *kdr* resistant strains of *An. gambiae*.

Behavioral insecticide avoidance can be separated into two categories, namely
contact irritancy and non contact repellency, collectively termed “excito-repellency”
(Roberts *et al.*, 1997). There have been numerous problems encountered in accurately
and objectively accessing these two related, but distinct, forms of behavioral response.
Repellency was a far weaker response compared to contact irritancy
(Chareonviriyaphap *et al.*, 2004). Together with a general lack of understanding of
the importance of behavioral phenomena related to mosquito control, the primary
obstacle until recently was the lack of an appropriate test system and method of data
clear behavioral responses have been documented among numerous species of
anophelines and *Aedes aegypti* using the excitorepellency test chambers but no similar
investigation has been performed with *Cx. Quinquefasciatus*. Sathantriphop *et al.*
(2006) quantitatively assessed Irritancy and repellency responses of *Cx.
Quinquefasciatus* against NIH strain, Nonthaburi strain, Mae Sot strain using the
improved excito-repellency (ER) test system.
Chareonviriyaphap et al. (1997) using an experimental escape chamber system. Provided information on both contact irritability and non-contact repellency for behavioral response tests on Anopheles albimanus under laboratory and field conditions. Chareonviriyaphap et al. (2001) evaluated the behavioral responses of a laboratory colony and field populations of Anopheles minimus to DDT, Deltamethrin and Lambdacyhalothrin using the modified chamber. To overcome the technical problems, an improved version of the excito-repellency test chamber design was developed as described in this report by Chareonviriyaphap et al. (2002). Bangs et al. (2004) studied the behavioural responses of nine Aedes aegypti strains, six from recent field collections and three from the long-established laboratory colonies under laboratory-controlled conditions by using an excito-repellency test system. Guessan et al. (2001) studied the insecticidal repellency of Olyset Nets.

14. Resistance to pyrethroids

Strong insecticidal properties and low mammalian toxicity make pyrethroids the most suitable class of insecticides for bed net impregnation; however, development of pyrethroid resistance in malaria vectors may threaten the sustainability of insecticide-treated nets (ITNs).

The two major mechanisms for resistance include target-site mechanisms, which interfere with binding of the insecticide to its target, and metabolic mechanisms, which prevent the insecticide from reaching its site of action Manisha (2006). Resistance against pyrethroids, the preferred class of insecticides for ITN use, has been recorded from countries in Asia, Africa and South America. Resistance is expressed as reduced excito-repellency and mortality of mosquitoes exposed to insecticide-treated materials. Pyrethroid resistance in insects is complex and presents
at different levels. Because pyrethroids can cause three effects – mortality, repellency and exiting behaviour – resistance mechanisms can develop against each of these. The relationship between these effects is not clear and resistance may operate independently (Takken, 2002).

Resistance to the insecticides used for impregnation may be a limiting factor to impregnated nets in vector control. Pyrethroid resistance of the most important African malaria vector *Anopheles gambiae* s.s. is already widespread in several West African countries (Chandre *et al.*, 1999). A common resistance is caused by the *kdr* mutation that occasionally is found at very high frequency (>90%) (Diabaté *et al.*, 2003). The predominant *kdr* mechanism apparently does not prevent the efficacy of pyrethroid-treated bed nets (Darriet *et al.*, 2000).

In Africa, resistance was first reported in Ivory Coast (Elissa *et al.*, 1993) and soon found to be widespread in that country (Curtis *et al.*, 1998 and Guillet *et al.*, 2001). Apart from malaria vector *Anopheles gambiae*, such resistance has also been reported in other anophelines and in different geographical areas (*An. funestus* (Hargreaves *et al.*, 2000), *An. albimanus* (Malcolm, 1988), *An. stephensi* (Omer *et al.*, 1980). It is unlikely that the selection for pyrethroid resistance in *An. gambiae* has occurred because of ITN use, as these have not yet been used blanket wise over very large areas. However, where pyrethroids have been used on a large scale as indoor sprays, as in South Africa, this route may have been the path to selection for resistance. As only pyrethroids are used for bednet impregnation and resistance against other groups of insecticides in relation to ITN use does not apply (Zaim *et al.*, 2000).
In contrast with the kdr-based resistance in *A. gambiae* sensu stricto (s.s.), the pyrethroid resistance of *A. funestus* in South Africa is based on elevated levels of mixed function oxidases, and this species is still susceptible to DDT (Brooke *et al.*, 2001). It is not well understood why, after exposure to same-class insecticides, the reported resistance mechanisms are different. Future research may reveal the reasons for these observations (Takken, 2002).

In areas where resistance against pyrethroids has been reported, ITNs may remain effective in affording protection because the reduced excito-repellent effect causes prolonged contact with the insecticide and therefore mosquitoes are killed. However, in areas where the mortality of mosquitoes landing on the treated nets is still high, caused by longer exposure times (Chandre *et al.*, 2000), the overall density of the mosquitoes are reduced, causing fewer infectious bites. Therefore, a protective effect against malaria may still be present even when a high degree of pyrethroid resistance is present. Trials to assess the impact of resistance of *Anopheles gambiae* to Permethrin and Deltamethrin on the efficacy of insecticide-treated bednets were carried out by Darriet *et al.*, 1998.

Comparative studies of Chlorpyrifos-methyl (CM), an organophosphate with low mammalian toxicity, and Lambdacayhalothrin (L), a pyrethroid, were conducted in experimental huts in Côte d'Ivoire, West Africa. *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes from the area are resistant to pyrethroids and organophosphates (*kdr* and insensitive acetyl cholinesterase). Several treatments and application rates on intact or holed nets were evaluated, including single treatments, mixtures, and differential wall/ceiling treatments were evaluated against *Anopheles gambiae* and *Culex quinquefasciatus* (Asidi, 2005). Josiane *et al.* (2004) studied the

Chouaibou (2006) studied Bifenthrin treated nets against resistant *Anopheles gambiae* and *An. funestus*. The efficacy of a mixture of a repellent (N,N-diethyl toluamide [DEET]) and a non-pyrethroid insecticide (Porous) was investigated under laboratory conditions against both pyrethroid-susceptible and pyrethroid-resistant mosquitoes with the knockdown resistance (*kdr*) mutation (Pennetier *et al.*, 2005). Studies confirmed experimental hut observations that pyrethroid-treated mosquito nets Olyset Net could significantly reduce man/vector contact even against pyrethroid-resistant *An. gambiae* (WHO, 2001).