EFFECT OF IMPREGNATED NETS ON MORTALITY AND BEHAVIOUR OF MOSQUITOES

by

MD. ISMAIL HOSSAIN
B.Sc. (Hons), M.Sc., Dhaka University, Bangladesh.

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University of London.

Department of Entomology,
London School of Hygiene
and Tropical Medicine.

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ABSTRACT

The effectiveness of impregnated fabrics on mosquitoes has been studied in the laboratory with a view to improving the means for communities to protect themselves against vectors of malaria and other diseases.

Di-ethyl toluamide (deet) was found to be more effective against mosquitoes when impregnated cotton nets were placed at a distance from the bait rather than close to the bait. Deet at a dose of 6.25 ml/m^2 gave complete protection against An. gambiae for 2 weeks.

When various types of netting and sheeting were dipped in permethrin emulsion at normal temperature, the amount of insecticide absorbed was generally proportional to the weight of liquid taken up, i.e. there was no evidence of selective absorption. However, selective absorption of more permethrin than expected was observed when nets were impregnated at 97°C and acid pH. Diffusion of permethrin did not occur between pieces of netting and sheeting sewn together.

The LD_{50} of permethrin on cotton nets was found to be about three times greater than on nylon nets. Ae. aegypti was found to be more susceptible to permethrin than An. gambiae, which was more susceptible than C. quinquefasciatus.

PP321 (Icon) and cypermethrin were found to be the most effective of 9 pyrethroids tested. Hand washing with cowfat soap reduced the amount of all the pyrethroids remaining on the nets, ageing in tropical condition did not have such an effect. The effectiveness of permethrin remained constant for 30 weeks when impregnated into a thick cotton net and evaluated in a "tunnel" against An. gambiae.

No clear cut effect of temperature on the toxicity of permethrin against An. gambiae was detected within the range of 16 to 28°C.

Mosquitoes resistant to various insecticides (one of them to DDT) did not show cross-resistance to permethrin; only two strains showed some tolerance. A prolonged exposure of one of the tolerant strain to permethrin did not increase permethrin resistance level. When a part of the same strain was exposed to DDT, resistance developed quickly but with no cross-resistance to permethrin. The present WHO method for detecting resistance in adult mosquitoes is not satisfactory. Short exposure of mosquitoes to impregnated surfaces may be a solution.

Although for other groups of insecticides variation in time and dose have equivalent effects, for permethrin this was not found to be true, i.e. on halving the exposure time the LD_{50} was not doubled.

Mosquitoes frequently find their way into, or bite through, untreated bednets. When mosquitoes were released in a room and a human subject sat under a permethrin impregnated bednet with an arm pressing against the net, mosquitoes failed to bite through the net. All the mosquitoes trying to bite through or entering the net through holes cut in it were knocked down within 30 minutes of release and ultimately died.

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CHAPTER 1. INTRODUCTION

The factors responsible for the frequent failure of mosquito control by spraying residual insecticides have been identified by Curtis and Lines (1985) as (1) physiological resistance to the insecticides, (2) mosquito species or varieties which do not rest for long enough in houses to pick up a lethal dose, (3) refusal by house holders of the entry of spray teams and (4) the rising cost of transport for centrally organized spray teams. Thus interest has been developing for improving personal protection against mosquitoes, as well as other disease vectors and nuisance insects. Personal protection can be achieved either by using a chemical, e.g. a repellent, or by putting a physical barrier between the human and the mosquito, e.g. by the use of bednets.

Topical repellents that are used on skin feel oily and some have an unpleasant odour. Sometimes they may cause irritation to skin and eyes, or they may cause allergy. Repellent treated garments may, therefore, be chosen as an alternative. The problem with repellent impregnated garments is that they cannot be used all the time, especially while one is sleeping. Bednets are the best protective measure against mosquitoes while sleeping. Yet they have some shortcomings: – (1) when a bednet becomes badly torn it does not protect from mosquitoes (it is very difficult to keep a bednet completely free of holes for long), (2) sometimes bednets are not properly tucked in, thus mosquitoes can easily find their way into the nets, (3) often part of the body remains in contact with the net through which mosquitoes can bite easily, and (4) bednets prevent circulation of air which is uncomfortable in tropical countries. Thus the idea of impregnating bednets (including wide-mesh bednets) with repellents has developed.

Another alternative to keep a house mosquito free, so that people remain safe even before going to bed, is to use treated curtains on windows, eaves or any other place through which mosquitoes can enter the house.

The residual activity of volatile repellents, e.g. diethyl toluamide (deet), is not very long and they do not kill insects, so they may simply divert them from a protected person to an unprotected person. Thus use of

pyrethroids, such as permethrin, which are toxic to insects, for impregnating bednets appears more suitable.

The idea of pyrethroid impregnation of bednets seems a very attractive one but a number of practical questions still remain to be solved.

One important requirement was to find an easy but reliable method for impregnating bednets. Schreck and Self (1985a)recommended calculation of the amount of pyrethroid necessary to give a target dose and then to soak the net with that amount of pyrethroid. But people working in the field raised objections against this method as it was laborious and it was also difficult to soak the whole net properly (Loong et al. 1985; Snow et al. 1987).

Bednets can be made of various materials, such as nylon, cotton, polyester, etc. It is important to find out which of the materials is best as a medium for pyrethroid impregnation in terms of mosquito killing.

Owing to their potency for insect control, numerous pyrethroids have been marketed by different manufacturers. It is important to determine which are the most effective pyrethroids against mosquitoes. To be a good impregnating chemical on bednets, a pyrethroid should be persistent and able to withstand washing of the nets.

Resistance to insecticides is one of the major setbacks to mosquito control using residual insecticides. Sometimes mosquitoes resistant to one insecticide are also cross-resistant to other groups of insecticides. Thus it is important to determine the extent of cross-resistance to pyrethroids, e.g., permethrin, a widely used pyrethroid, of mosquitoes resistant to other insecticides. It is also important to determine the potentiality of mosquito populations to develop permethrin resistance as a result of selection by that insecticide.

The present WHO method for pyrethroid resistance detection in adult mosquitoes is not satisfactory. In the present system mosquitoes are exposed for one hour and the test kits are recommended to be kept in a horizontal position so that of mosquitoes are knocked down before the exposure time is over they do not fall off the impregnated paper. However, when lying on the paper they offer more surface area to the paper than when they stand on it. It seems desirable to develop a more satisfactory test method.

Although work has been done on the effect of temperature on the toxicity of pyrethroids against some agricultural pests, only one or two studies have been reported on this question in mosquitoes and more information is needed.

Most of the research on pyrethroid impregnated nets has been done in the field and very little work has been done in the laboratory to investigate in detail the behaviour of mosquitoes towards such nets. Thus a study was undertaken to investigate the behaviour of mosquitoes and the killing effect of permethrin impregnated nets in relation to several variables. In the course of this study mosquito behaviour due to the use of deet or permethrin on wide-mesh netting was also investigated.

CHAPTER 2. LITERATURE REVIEW

2.1. Vapour repellents:

Repellents can be defined as compounds that cause insects to make oriented movements away from the source (Dethier et al., 1960). Researchers involved in the development of repellent substances define a repellent in a different way - as chemicals which elicit a combination of behavioural responses whose net result is the prevention of biting by insects (Davis, 1985). Although insects have been specified here, repellents have been used successfully against other groups of invertebrates, such as, ticks and mites (Bertram et al., 1967; Gouck and Gilbert, 1955, 1960; Kochhar et al., 1974) and leeches (Kochhar et al., 1974; Kumar et al., 1984; Saxena and Khalsa, 1967).

Citronella oil is reported to has been the most widely used repellent from about 1901 until World War II and was the repellent against which new compounds were compared (Christophers, 1947). The other three repellents that were considered as standard at that time were dimethyl phthalate (DMP), Indalone and Rutgers 612 (2-ethyl hexanediol-1,3) (Dethier, 1956). During World War II and afterwards thousands of compounds have been screened for repellency in various laboratories throughout the world, especially in the United States. In 1954 a very promising repellent, N,N-diethyl-m-toluamide (deet), was synthesized (McCabe et al., 1954) and was evaluated in the field by Gilbert et al. (1955) against some mosquito species. From that time deet has been used as reference repellent for the evaluation of other newly synthesized repellents.

Since its synthesis deet has been used so extensively that Rutledge et al. (1978b) published a bibliography on this compound compiling 350 different reports published up to that time. Its popularity among people is indicated in a report of Buescher et al. (1983) that in the USA alone there were 250 different chemical formulations of deet marketed with concentrations of active ingredients ranging from two to 100 percent.

Deet has been evaluated by applying it to bare skin (Altman, 1969; Altman and Smith, 1955; Gilbert, 1957; Gilbert and Gouck, 1955; Gilbert et al., 1955, 1966, 1970; Kumar et al., 1984; Reifenrath and Akers, 1981; Schmidt, 1977;

Schmidt and Schmidt, 1969; Schreck and McGovern, 1985), by impregnating wide-mesh net jackets (Frommer et al., 1975; Gorham, 1974; Grothaus and Adams, 1972; Mulrennan et al., 1975; Schiefer et al., 1976; Schreck et al., 1979; Sholdt et al., 1975; Zaugg, 1978), by impregnating other garments, such as, military uniforms (Gilbert, 1957; Gouck and Gilbert, 1955, 1960; Saxena and Khalsa, 1967; Schreck et al., 1978b), by impregnating canvas tents in Kenya and spraying interiors of mud and grass house in Ethiopia to attempt to achieve dwelling space repellency (Sholdt et al., 1976, 1977). For skin application it is formulated as an aerosol (Blume et al., 1971; Dremova et al., 1969), as a stick, lotion or cream (Curtis and Maxwell, 1987, unpublished report) or in a soap formulation along with permethrin (Yap, 1986).

Deet has been reported to be effective both in the laboratory and in the field not only against mosquitoes but also sand flies, Lutzomyia longipalpis (Buescher et al., 1982), Lutzomyia spp. (Zaugg, 1978), Phlebotomus papatasi (Schmidt and Schmidt, 1969), blackflies, Simulium spp. (Frommer et al., 1975), S. damnosum (Schmidt, 1977), tsetse fly, Glossina morsitans (Schmidt, 1977, Sholdt et al., 1975), stable fly, Stomoxys calcitrans (Blume et al., 1971), biting midges, Culicoides furens, Cu. mississippiensis, Cu. hollensis and Cu. barbosai (Schreck et al., 1979a, 1979b), Culicoides spp. (Zaugg, 1978), ticks, Amblyoma americanum (Gouck and Gilbert, 1955, 1960), Riphicephalus sanguineus and Ornithodorus sayignyi (Kochhar et al., 1974), mites, Ornithonyssus bacoti (Bertram et al., 1967), the rat flea, Xenopsyla cheopis (Dremova, et al., 1969), water leech, Hirudo medicinalis (Kochhar et al., 1974) and the land leeches, Haemadispa zeylanica (Kumar et al., 1984) and Ha. sylvestris (Saxena and Khalsa, 1967).

Granett (1940) proposed the concept "time until the first bite", which he called the "repellent time", as measure of the effectiveness of a repellent. He proposed that a measured amount of a repellent should be evenly distributed on the fore arm or leg and one should then expose the limb to mosquitoes. One major defect of this method is that the result depends on whether there are a few highly tolerant mosquitoes in a population, and the "repellent time" obtained is not a true representation of the susceptibility of the whole population to that repellent. Also it confounds:- (a) effectiveness per unit concentration when freshly applied, (b) rate of loss from the skin. Thus a median effective dose (ED_{50}) based on number of bites or landings on a series of concentrations of repellents and an untreated control may be preferred as a system of evaluating the effectiveness of a repellent (Curtis et al., 1987).

Rutledge et al. (1978a) observed that the ED_{50} values varied by as much as 1.75 times among two strains of Ae. aegypti. In their experiment effectiveness of deet was measured by applying it to a membrane and allowing mosquitoes to feed on blood through the membrane. A 'choice' was offered between sections of membrane with various concentrations of deet (Rutledge et al., 1976). Other investigations also revealed that some species of mosquitoes are more susceptible to deet than other species. Rutledge et al. (1983) and Schreck (1985) showed that An. albimanus is not as susceptible to deet as Ae. aegypti. Zhogolev (1968) observed An. pulcherrimus to be less susceptible to deet than Ae. vexans and Ae. caspius caspius. Curtis et al. (1987) showed that An. stephensi is more susceptible to deet than An. gambiae, An. albimanus and An. pulcherrimus.

Several investigators attempted to explain the mode of action of repellents in mosquitoes. It was observed that when a female mosquito enters an airstream containing host-related stimuli she makes no apparent response but when she leaves the host airstrean she turns so as to re-enter it. Furthermore wind tunnels show that mosquitoes have olfactory senses by which they can differentiate between a repellent and host-related stimuli (Kellogg, 1970; Kellogg, et al., 1968; Rayner and Wright, 1966; Simpson and Wright, 1967). Wright (1975) stated that insect repellents function by blocking the pores on the antennal sensilla, thereby preventing the mosquitoes from detecting host-related signals. But Davis (1985) argued that if the repellents simply blocked the sensilla pores, neither the repellent nor the host odour would have been perceived by a mosquito and she would have flown through and out of the airstrean with no apparent response. Davis (1985) reviewed literature related to the responses of mosquitoes to host-stimuli and repellents and came to the conclusion that "no clear picture emerges about how female mosquitoes find a host and how insect repellents work".

2.2. Synthetic pyrethroids – history, residual spraying, etc.

Pyrethrum, pyrethrin and pyrethroid – are the three terms often encountered when one studies the history of pyrethroid insecticides. The term **pyrethrum** or "insect powder" refers to the dried and powdered flower heads of the plant Chrysanthemum cinerariaefolium. The active constituents of the pyrethrum extract are collectively referred to as **pyrethrins**. **Pyrethroids** are

the synthetic analogues of the pyrethrins.

Although the use of natural pyrethrum and pyrethrin have a long history, the first synthetic pyrethroids to show higher or equal killing power to pyrethrin, resmethrin and bioresmethrin, were reported only 20 years ago (Elliott et al., 1967). These new insecticides were unstable in air and sunlight, which is a characteristic of the natural pyrethrins. This is an advantage in situations where short persistence is essential, but strictly limits applications in many other situations. This problem was overcome by Elliott et al. (1973), who synthesised the first photostable pyrethroid, permethrin. The synthesis of permethrin was a breakthrough in the history of insect control. In addition to its photostable nature, permethrin is highly toxic to insects but it has a low mammalian toxicity (Kadota et al., 1976). Following the synthesis of permethrin large numbers of pyrethroids have been synthesized by scientific organisations or insecticide firms, some of which are much more toxic than permethrin, for example, cypermethrin and deltamethrin. Pyrethroids are the second most widely used class of insecticides with world wide sale of one billion dollars(Scott, 1987).

Permethrin and other pyrethroids have been evaluated as residual sprays against vectors including mosquitoes. Coosemans and Sales (1977) evaluated permethrin and deltamethrin against mosquitoes in the field in Burkina Faso. Permethrin was sprayed at a dose of 0.5 g/m^2 and deltamethrin at 0.025 g/m^2. Mortality among wild mosquitoes collected in experimental huts and verandah traps was very low despite the good mortality and residual effectiveness shown in bioassay cones attached to the walls. This inconsistency may be due to the fact that these two synthetic pyrethroids are highly irritant, causing the mosquitoes to leave the deposit before they had absorbed a lethal dose.

One hundred percent mortality of C. p. molestus was obtained in 8 hour bioassays repeated over at least six months on permethrin treated whitewashed walls at a dose of 0.1 g/m^2 in a trial in Czechoslovakia (Rettich, 1980a). But as we know that mosquitoes do not spend more than a few minutes on a pyrethroid treated surface, whether bioassays for such a long exposure time have any practical value is questionable. Permethrin at a dose of 0.125 g/m^2 on mud walls in Zimbabwe substantially reduced house resting densities of anopheline mosquitoes due, at least partly, to an irrito-repellent effect which caused the mosquitoes to leave treated surfaces (Taylor et al., 1981).

Permethrin was also proved to be very effective against the vectors of Chagas' disease, Triatoma infestans, when sprayed on mud walls and under the

roofs of houses in Brazil. Pinchin et al. (1981) freed houses of infestation for at least 520 days when he sprayed with permethrin at 2 g/m² and 300 days when sprayed at 1 g/m².

Permethrin was used as a residual lawn spray in the U.S.A. by Helson and Surgeoner (1983) for adult mosquito control. The residual effectiveness, as measured by bioassaying mosquitoes on grass cut from the lawn, of permethrin was much greater than that achieved with chlorpyriphos, carbaryl, methoxychlor, iodophenphos or malathion.

Permethrin, cypermethrin and deltamethrin were used successfully over two years against tsetse fly in Nigeria. Insecticides were applied to fly resting sites on vegetation using either pressurised knapsack sprayer or helicopters (Spielberger et al., 1979).

Permethrin and other pyrethroids have been evaluated successfully against mosquito larvae by many researchers both in the laboratory and in the field (Darwazeh et al., 1978; Mulla et al., 1975, 1978a, 1980, 1981, 1982; Rettich, 1980b; Rubio-Moran, et al., 1981; Thompson and Meisch, 1977; Varma and Rahman, 1984a, 1984b). Data on the effect of the pyrethroids on non-target invertebrates were inconclusive (Mulla et al., 1975, 1978a, 1980, 1982; Rettich, 1980b), but they were found to be safe against smaller fishes (Mulla et al., 1978b). In a recent study it was observed that organophosphate resistant Simulium damnosum s.l. larvae showed up to 11-fold increased susceptibility to permethrin as compared with organophosphate susceptible populations (Kurtak et al., 1987). Rettich (1980c) observed that when permethrin was sprayed at doses of 0.02 - 0.75 g/m² on dried-up breeding sites in forests in Czechoslovakia before the breeding season in autumn it prevented or substantially reduced the occurrence of Aedes cantans larvae the following spring.

Attempts have been made to determine the mode of action of pyrethroid insecticides on insects. Narahashi (1971) stated that hyper-excitation and tremors followed by paralysis of an insect are the results of pyrethroid intoxication which imply that pyrethroids act primarily on the neuromuscular system. Burt and Goodchild (1974) stated that all poisoning symptoms (knockdown and advanced intoxication) resulted from actions of pyrethroids on the central nervous system of insects. In contrast to this Clements and May (1977) argued that knockdown is so fast in pyrethroid intoxication that there is not time for penetration of a compound into the central nervous system, and it is due to the excessive sensory hyperactivity in the peripheral nervous

system. Narahashi (1982) concluded that all pyrethroids have a common mode of action, the sodium channel. Pyrethroids produce lesions in the motor nerve terminals of a variety of insect species (Salgado et al., 1983a, 1983b). Miller et al. (1983) reported the results for the examination of about two dozen species of Diptera, Lepidoptera and Coleoptera and in every case the nerve terminal sensitivity was lower in the pyrethroid resistant strains than in the susceptible strains. It it is now clear that pyrethroids act on the nervous system; but we are not yet at a position to draw a conclusion about the exact mode of action of this group of insecticides. Miller and Salgado (1985) reviewed in detail the work done so far on the mode of action of pyrethroids and came to the conclusion that "there is no universally held theory of pyrethroid mode of action."

2.3. Impregnation of bednets with chemicals:

Bednets whether intact or with small tears can protect people at least partly from mosquitoes (Port and Boreham, 1982). In Papua New Guinea significantly more Anopheles were collected from the walls of a room containing people without bednets than that with people protected by untreated bednets. The number of blood-fed culicines was significantly higher in a room without a bednet although the total number of culicines in the rooms was not significantly affected by the presence of a mosquito net (Charlwood, 1986).

In China a significant difference in malaria infection rate between populations using and not using bednets was reported (Lin, 1985). A retrospective study in The Gambia indicated a marked protective effect against morbidity from malaria due to the use of bednets in children (Bradley et al., 1986; Campbell et al., 1987). But another retrospective study by Snow (1987) in the same country revealed no significant effect on the splenomegaly of children who slept under bednets (34%) or not (38%). He argued that the positive effect of the use of bednets in the previous two surveys might be due to the fact that in those cases most of the children in the study area (79% and 67%) slept under bednets, thus diverting the infected mosquitoes to the minority of unprotected children. In his survey Snow (1987) observed that only 13% children slept under bednets. Thus it seems that the use of unimpregnated bednets may not have any effect on the overall malaria situation, but on the other hand it may increase the suffering of under-privileged people without

nets. In contrast to this a pyrethroid impregnated bednet, as will be seen later, offers partial protection to a nearby child who is not sleeping under the net due, it is supposed, both to the irrito-repellency and killing effect of pyrethroids (Lines et al., 1987).

In addition to protection from mosquitoes and malaria, people use bednets for privacy, protection from other smaller insects, dust, rats, etc. (MacCormack and Snow, 1986). Despite all these positive benefits the use of bednets has some problems, (i) the nets soon become torn, (ii) sometimes they are not properly tucked in, (iii) mosquitoes can bite through them if the body touches the net, (iv) they prevent air circulation and (v) if most of the people in a particular area use bednets, the few people without bednets may be more vulnerable to malaria than if there were no bednets. So the idea of impregnating bednets with insecticides developed.

Impregnation of bednets with DDT started during World War II. Harper et al. (1947) reported that bednets were impregnated with DDT in kerosene for the use of allied forces in the South Pacific against Anopheles farauti in 1942 - 45. Bioassays were done by releasing mosquitoes inside impregnated nets either for 10 minutes or for one hour and nearly 100% mortality was achieved. When mosquitoes were released into an impregnated bednet with a man inside, they tried to bite within the first few minutes but there were no bites from 5 minutes after the release. From a review it appears that the German army also used DDT impregnated bednets against sandflies at almost the same time (Nauck et al., 1948).

Impregnation of bednets with insecticides has re-started recently. Hervy and Sales (1980) reported the results of bioassays carried out with females of Ae. aegypti made to rest on permethrin and deltamethrin impregnated fabrics for one hour. Cotton netting was found to be better than synthetic netting when impregnated with deltamethrin, whereas both types of netting gave similar results when impregnated with permethrin. Impregnated sheeting was not as good as impregnated netting. This may be due to the fact that the number of fibres in a unit area is much more in sheeting than in netting, thus the amount of insecticide per unit length of fibre, where a resting mosquito must place its tarsae, is much less in sheeting than in netting.

The first of the recent field work on impregnated nets was carried out in China during 1976 - 77 (Zhao et al., 1984). The authors performed a simple experiment whereby they counted the number of mosquitoes found to rest on nets impregnated with 0.2 g/m^2 permethrin. This number was compared with the number

resting on control nets. The authors reported 95% reduction in the number of mosquitoes resting on bednets due to impregnation. This apparent reduction may be due to the fact that mosquitoes rest for a very short time, sometimes a couple of seconds, on permethrin treated nets, thus mosquitoes that landed on the treated nets might have been escaped the observer's notice.

The first of the recent extensive field work on impregnated nets was done by Darriet et al. (1984) in Burkina Faso. The authors observed that when cotton bednets were impregnated with permethrin at a dose of 0.08 g/m^2 the entry into experimental huts of An. gambiae and An. funestus was reduced by about 70% . Due to the irritating effect of permethrin 97% of the mosquitoes were caught in the exit traps of the huts containing impregnated nets, compared to only 25% in the untreated control. By comparison with control huts, impregnation of the mosquito nets reduced the engorgement rate (the ratio of the number of engorged females and the total number of females caught) by 20% for An. gambiae and 10% for An. funestus. The mosquitoes entering the huts with impregnated nets had an overall mortality of 17% . The residual activity, determined by bioassaying Ae. aegypti for one hour, observed under the conditions of normal use was at least 5 months.

At about the same time there was another field study run in Mali by Ranque et al. (1984a, 1984b) who impregnated semi-synthetic bednets with deltamethrin at a dose of 0.008 g/m^2. They came to the conclusion that: (1) the nets were well accepted by the people, (2) one impregnation of deltamethrin remained effective throughout the 6 months duration of the experiment, (3) mosquitoes almost completely disappeared from houses with impregnated nets (this observation may be explained by the irrito-repellency of deltamethrin), (4) a lower prevalence of malaria parasites was observed among people using impregnated nets than among people using untreated control nets, but the difference was not statistically significant. However, a significant difference was observed between the splenic index of children of 0 - 9 years of age in the control group (69.2%) and in the protected group (28.7%).

Field studies in Tanzania showed that permethrin treated bednets killed some mosquitoes and increased the tendency of survivors to exit during the night. In their studies Lines et al. (1985) observed that treated cotton nets did not perform so well as the treated nylon nets, as determined on the basis of catches of fed mosquitoes in experimental huts. An impregnated bednet in which holes had been cut, to simulate a torn net, reduced the number of

mosquitoes which fed and survived approximately as well as an intact net (Curtis and Lines, 1985; Lines et al., 1985, 1987). Treated curtains around the eaves of experimental huts did not perform so well as bednets, but caused considerable reductions in the number of mosquitoes which fed and survived. This may be due to the fact that the eave curtains acted more as resting place than as a barrier to entry of mosquitoes. There was no such effect when a narrow strip of treated netting was placed around the eaves of a dwelling house. This may be due to the fact that in a dwelling house there are more available resting places, other than the eave curtain, than in an experimental hut. Lines et al. (1987) observed that when one child slept under a treated net and another slept outside the net in the same hut, the number of bites on the latter child was less than if neither child had been under a net. The reason may be that when there is a treated net in the house mosquitoes are irritated and repelled as they may rest on the treated net before having a blood meal.

In Papua New Guinea Schreck and Self (1985b) obtained negligible mortality of mosquitoes by using permethrin impregnated bednets made from finely woven cloth with no openings for air circulation. Nylon nets, on the other hand, have a wider mesh and may be more attractive to mosquitoes trying to reach a human host inside, thus detectable vector mortality could probably be achieved by using a conventional bednet. Another reason for this observed low mortality may be that, as already stated, the dose per unit length of fibre must be much higher on netting than sheeting with same dose per square metre.

In another study in Papua New Guinea the incidence of Plasmodium falciparum was significantly reduced in 0 - 4 year olds in villages with impregnated nets compared to those with unimpregnated nets, leading to reduced prevalence of P. falciparum in this age group. No effect of permethrin impregnated nets on incidence or prevalence of P. falciparum in 5 - 9 year olds or on P. vivax in either age group were observed (Graves et al., 1988, in press). The reason for there being no significant reduction in Plasmodium infection among the senior group of children may be that they go to bed late, and may be infected before they go to bed. P. vivax is known to relapse and this was thought to be the reason for there being no significant difference in incidence and prevalence rate between control and treated villages for this parasite. It may be mentioned that the authors eliminated parasites from blood of all children in the village using chloroquine plus Fansidar but this would

not have eliminated the P. vivax from the liver.

There were also reports that head lice disappeared from many people using bednets and that annoyance from bed bugs decreased (Charlwood and Dagaro 1988, in press). Whole night human landing catches and a capture-recapture experiment in Papua New Guinea revealed that due to the introduction of permethrin impregnated bednets in a whole village the biting population of An. farauti decreased by about 30% and the oviposition cycle became irregular, although survival rates were not significantly affected (Charlwood and Graves 1987). The human blood index of the engorged females was found to be decreased significantly in the same experiment, due to introduction of impregnated nets.

In The Gambia when permethrin treated bednets were compared with placebo treated bednets it was observed that there was an almost complete absence of mosquitoes inside the treated nets (total 10) whereas a considerable number were found inside placebo treated net (total 265). Significantly fewer unfed female mosquitoes were found inside the rooms containing treated nets and there was a higher rate of exophily in rooms containing permethrin treated nets than in rooms containing placebo treated nets. However, the proportion fed and the mortality in the exit traps were not significantly affected by permethrin treatment. Bioassays showed that the toxicity varied between four different fabric types; hand washing severely reduced the toxicity and approximately halved the permethrin content (Snow et al., 1987a). In the same field trial it was also observed that children who slept under treated nets had significantly fewer episodes of clinical malaria than control children (Snow et al., 1987b). However, at the end of rains there was no significant difference in the prevalence of splenomegaly or parasitaemia or in the mean packed cell volume (PCV) between the groups. As an explanation of why the reduction in the incidence of clinical malaria had no effect in the reduction of splenomegaly it was suggested that there was difference of time spent by mosquitoes during probing in the two groups. Mosquitoes were thought to spend less time biting a child through a permethrin treated net than through a placebo treated net. Thus both groups were supposed to receive a similar number of infective bites and hence a similar amount of splenomegaly, but the children sleeping under placebo treated nets were thought to receive larger sporozoite inocula and thus were more prone to clinical malaria.

In China cotton nets were impregnated with deltamethrin at 0.025 g/m^2 and DDT at 2.0 g/m^2 to study their relative efficacy and persistence against mosquitoes (Li, 1986; Li et al., 1987). Bioassays for 30 minutes using An.

dirus resulted in more than 98% mortality 18 months after treatment on deltamethrin impregnated nets, whereas about 95% mortality was obtained on DDT impregnated nets only up to eight days after impregnation. The authors performed a field trial during the peak season of An. sinensis for three years. A bednet was hung around a cow in an experimental hut, the lower margin of the net either touched the floor (closed) or there was a 50 cm gap in between (semi-closed). About 95% mortality of An. sinensis, adding dead mosquitoes in the window trap to those on the floor, was obtained two years after impregnation in the "closed" net and 81% in the "semi-closed" net. In the case of the "closed" net there was much more mortality in the window trap than on the floor, whereas in the case of the "semi-closed" type the situation was reversed. This was apparently due to the fact that in the "semi-closed" net more mosquitoes got into the net, fed, rested on the treated net and ultimately died. It was also observed that impregnation of netting with deltamethrin and DDT did not affect the strength of the netting material. Xu et al. (1988, in press) observed 100% mortality 19 weeks after treatment when culicine and anopheline mosquitoes were exposed to 0.5 g/m^2 permethrin treated nets for one hour. The authors did not notice any difference in performance between cotton and nylon nets. Impregnation greatly reduced the number of mosquitoes in and on the nets.

Kurihara et al. (1986) carried out a field test in Japan in which 4x4 cm or 1x1 cm mesh nylon nets were treated with the pyrethroid, phenothrin, and placed around pigsties, so that the nets formed "walls" around pigs, but no roof. There was a large gap between the netting "walls" and the roof of the sty. Light traps inside the walls were used to collect mosquitoes. It was observed that the total number of mosquitoes caught when using a treated 1x1 cm mesh net was much less than half that with untreated nets, the number of mosquitoes collected with treated large mesh net was more than that with the untreated net. However, both of the treated nets reduced the proportion of surviving mosquitoes to a negligible level and they also greatly reduced the proportion of mosquitoes which fed. The high mortality among trapped mosquitoes may by due to either (i) the effect of pyrethroid that they might have picked up during passage through the net meshes, or (ii) the effect of pyrethroid vapour or dust after being trapped.

Hii et al. (1987) reported the results of a trial of permethrin impregnated bednets in 5 villages in Sabah, Malaysia. The nylon nets were impregnated at a dose of 0.062 g/m^2. At the time of distributing bednets,

there was mass drug administration with Fansidar plus primaquine to the human population to clear all parasitaemias due to P. falciparum. The parasite rate in children declined significantly in villages containing impregnated bednets. However, after about 2 months the parasite rate started to increase and in 4 - 6 months the rate in the villages having bednets approached the rate in the control village without impregnated nets. The reasons for this failure within 2 months of combined bednet use and mass drug administration were identified as (1) use of a low permethrin dose, (2) damage of bednets due to use, (3) the tendency of the people of either going to bed very late at night or not using the nets at all. It was observed that people did not like to use bednets as they stopped air circulation during the hot season.

Few studies have been reported on mosquito behaviour in relation to impregnated netting, but Kurihara (1984), Kurihara et al. (1985) and Kurihara and Umino (1987) conducted laboratory studies in Japan in which wide-mesh nylon nets were impregnated and interposed between two cages, one containing hungry mosquitoes and the other one a bait. It was observed that phenothrin had a deterrent effect preventing mosquitoes from getting through the nets. Fed mosquitoes were driven out of the bait cage by the irritant effect of the insecticide. When mosquitoes were introduced into bait cages they tended to move into the other cage without feeding. C. p. pallens was found to be less deterred and An. albopictus was less inhibited from feeding than An. stephensi.

Itoh et al. (1986) compared the impregnation of fenitrothion, d-phenothrin, fenvalerate, permethrin, cyphenothrin, prallethrin and fenpropathrin on to wide-mesh nylon netting. Cyphenothrin was found to be the most effective after 9 months of ageing. The authors used a 24 hour exposure of mosquitoes to treated nets which is unrealistic in the sense that mosquitoes spend only a couple of minutes on a treated surface in nature. When the mesh size of the netting was less than the width of a mosquito's wing span, mosquitoes were found to rest on the netting before they passed through, thus allowing them time to pick up a lethal dose of the insecticide.

Schreck and Self (1985a) recommended treatment of bednets by dipping them in the amount of emulsion that is needed just to wet the net without any running off. The concentration of the emulsion has to be adjusted so that the desired amount of pyrethroid is taken up per unit area of netting. But Loong et al. (1985) found this method of impregnation to be cumbersome, time consuming and it was difficult to completely soak the inner layers of a folded

net. So they adopted a method of making an emulsion which was twice the amount required to soak the net completely. The nets were then dipped into the emulsion and the excess emulsion was wrung out. Curtis (1987) suggested a similar method of impregnation. Snow et al. (1987) also commented that the method suggested by Schreck and Self (1985a) would not be suitable at the community level and an easier method should be adopted.

A few field studies on repellent impregnated widemesh bednets were carried out in the late 1960s and early 1970s. Deet, the most widely used repellent, was found to give protection against different species of mosquitoes when impregnated into cotton bednets (Gouck et al., 1967, 1971; Gouck and Moussa, 1969; Smith et al., 1970). Good results were also obtained in other experiments using either deet treated nets and/or nets treated with other repellents (Grothaus et al., 1972; McDonald and Grothaus, 1973; Grothaus et al., 1974).

2.4. Impregnation of fabrics other than bednets with pyrethroids:

Fabrics, including military uniform, netting jackets and window net-curtains, have been impregnated in the past few years with pyrethroids (in most cases permethrin) to evaluate their efficacy against various blood sucking arthropods including mosquitoes. Schreck et al. (1978a) impregnated pieces of either cotton or cotton-polyester military uniform with permethrin at a series of doses and studied the insecticidal effect and its durability. Twelve different species of insects were exposed for either 10 secs or 30 secs to the impregnated fabrics in WHO test kits, mortality was scored either after 15 minutes or after 1 hour. All but one species were killed at doses of 0.08 - 2.5 g/m^2. Only the Lone Star Tick, Amblyomma americanum, required 2 minutes exposure to obtain 100% mortality with a dose of 0.16 g/m^2. Clothes treated with similar doses were effective after one month exposure to outdoor weathering. Schreck et al. (1978b) observed that people who wore permethrin treated military uniforms and deet on their bare arms obtained 50% longer protection time than when they wore M-1960 (a repellent mixture) treated uniform and deet on their bare arms. There was no difference in the average mosquito landing rate before and after the test when people wore repellent treated uniforms or untreated uniforms, whereas the landing rate was reduced by 72% when the people wore permethrin treated uniforms. This reduction may be due to the combined toxic and irritant effect of the permethrin. Some of the

mosquitoes might have been killed and some others might have left the place due to the irritant effect of the permethrin.

The combined use of permethrin treated fabric and deet on exposed skin was found to give much better protection against Ae. taeniorhynchus than either of the protective methods alone. The mosquito landing rate was found to be reduced by more than 90% at the end of a 9 hour experiment due to the use of permethrin treated jackets (Schreck et al., 1984). Schreck et al. (1982b), however, did not achieve protection against phlebotomine sandflies using permethrin treated clothing. Sandfly behaviour and resistance to quick knockdown appeared to be responsible for this failure.

Permethrin treated military fatigue uniform was found to give very good protection against the Lone Star Tick, Amblyomma americanum, over a 3-month period, during which the uniform had accumulated wear of 100 hours (Schreck et al., 1980a). Permethrin was more effective than deet for this purpose. It was observed that when permethrin was sprayed on fabrics from a pressurized aerosol dispenser less permethrin was required for complete protection compared to other methods of impregnation. This is presumably due to the relatively slight penetration of the insecticide into the fabric when an aerosol is used and thus easy availability to ticks (Schreck et al., 1982b; Mount and Snody, 1983).

When a choice was given to ticks to crawl either on permethrin treated clothing or on untreated clothing, most of them were found to move towards untreated surfaces within 1 - 2 minutes. This initial repellency wore off within 4 - 15 minutes depending on the species of tick (Lane and Anderson, 1984). The observed number of Dermacentor occidentalis ticks collected from humans walking through infested grassland was 14% less on treated clothing than on untreated, but this difference was not statistically significant. The difference in the morbidity/mortality one day later of ticks removed from the treated and untreated overall (60% vs 3%) was highly significant (Lane and Anderson, 1984).

Neither permethrin treated jackets nor resmethrin treated jackets proved to be as good as deet treated jackets in reducing the number of attacking insects at the beginning of an experiment in a given place. However, after sitting for about 10 minutes the pyrethroid treated jackets were found to give good protection because a large proportion of the hungry population of insects had been killed by momentary contact with the impregnated jackets and the number of attacking insects in the surrounding area was reduced to a low level

(Schreck et al., 1977; Lindsay and McAndless, 1978).

Impregnated window curtains greatly reduced the mosquito entry into an experimental hut in Tanzania and also caused most of those that entered to exit after feeding (Lines et al., 1985). A combination of permethrin impregnated curtains made of cotton on doorways, windows and eaves greatly reduced the number of mosquitoes found resting indoors, for about a year at a dose of 1 g/m^2 in Burkina Faso (Majori et al., 1987). This may have been due to driving of the mosquitoes out of doors rather than reduced entry. Observations with a verandah trap showed that among those which did enter there was an increased exit rate and mortality rate. Whole-night man-biting catches both indoors and outdoors for 5 nights on three different occasions showed that permethrin impregnation of curtains greatly reduced indoor biting populations of mosquitoes, while the outdoor biting population remains unchanged. There was more than 98% reduction of indoor man-biting population of mosquitoes three months after installation of the curtains. After 11 months the reduction of mon-biting population was still 54.4%.

Bry et al. (1976) impregnated 100% woollen cloth with permethrin at a dose of 0.09% by weight of the fabric to test the performance of this insecticide for moth-proofing. The impregnation was carried out at a temperature of 100°C and pH 4.5 keeping the pieces of cloth in the hot bath for one hour. Permethrin treatment by the above method was found to protect wool satisfactorily against Black Carpet Beetle, Attagenus megatoma, and Webbing Clothes Moth, Tineola biselliella, when the treated cloth was subjected to 20 machine washings, 20 dry cleanings, abrasion or exposure to ultraviolet light. At the concentration used permethrin was not toxic to Black Carpet Beetle larvae, however, mortality among the Webbing Clothes Moth ranged from 86 to 100%. Chemical analysis showed that 30 to 37% of the initial dose of permethrin was present in the fabric after 20 machine washes and drycleanings respectively.

Duffield (1977) showed that dye-bath application of permethrin to wool and wool-nylon blends showed good fastness to light, drycleaning, pressing and high temperatures. Carter and Duffield (1977) also showed that when permethrin was applied with various dyestuffs, it had no significant effect on dye performance.

Bry et al. (1979) sprayed aqueous or oil formulations of permethrin on to 100% woollen clothes and evaluated them against three species of insects. Oil formulations at a dose of 0.08% and aqueous formulation at a dose of 0.01% by

weight of the cloth were found to be very effective against all 3 species of insects resulting in 100% mortality for 7 days exposure 6 months after impregnation. When the insects were exposed for 24 hours 100% of A. megatoma and T. biselliella were knocked down by both formulations but only 58% and 23% of the Furniture Carpet Beetle, Antrenus flavipes, were knocked down by oil and aqueous formulations respectively. No A. megatoma or A. flevipes were killed after 24 hours exposure but 50% and 73% of T. biselliella were killed by oil and aqueous formulations respectively.

2.5. Persistence of vapour repellents, permethrin and other pyrethroids on various materials:

Experiments have been done to evaluate the persistence of permethrin when applied to plywood, various types of walls, fabrics etc, under various conditions. Thompson and Meich (1978) concluded that the dose of permethrin which gave 90% mortality on plywood in the rice field mosquitoes, An. quadrimaculatus and Psorophora columbiae, was about 0.125 and 0.50 g/m^2 indoors and outdoors, respectively, after 11-weeks ageing in ambient conditions. In these experiments mosquitoes were exposed for one hour. Rettich (1983) found permethrin at a dose of 0.1 g/m^2 to be effective against C. p. molestus for more than 2 months on plywood, whitewashed or limewashed surfaces or ceramic tiles. The author's criterion of success was 100% knockdown within 90 minutes and he continuously exposed the mosquitoes, unless all of them were knocked down, to determine KT100. This method may give misleading results because after knockdown the insects are no longer in contact with the insecticide. Mosquitoes pick up a lethal dose of pyrethroid very quickly, although they may not be knocked down immediately. Therefore it sems desirable that mosquitoes should be exposed for a very short time and knock down may be scored one hour post-exposure.

The half life of permethrin,when applied on a sample mud block and kept at 25°C and 80% RH, was found to be 88 days, but at 20% RH the half life of the same material was found to be only 53 days,thus emphasizing that persistence of permethrin on mud is positively correlated with humidity (Barlow et al., 1977).

In Malaysia Loong et al. (1985) observed that permethrin impregnated cotton and nylon bednets remained effective for one year giving 100% mortality

when 50 An. maculatus were released for 30 minutes under a bednet which had been impregnated at a dose of 0.2 g/m². Similar result was obtained when cotton nets were impregnated with DDT at a dose of 2.0 g/m².The net was kept hung in an open corridor throughout the year. Bioassay test in a WHO test kit gave similar results after 10 minutes exposure. Even after one soapy wash after 42 weeks there were similar results.

Gas chromatographic analysis showed that 24 - 48% of the permethrin, which had been applied, remained on pieces of cotton-polyester military uniforms after impregnation with 1.25 g/m² and 10 - 30 days of wearing (Schreck et al., 1980b). The impregnated patches of cloth were worn, with the help of elastic tapes, on the lower leg below the knee under the trousers so that they remain in close contact with the skin. The patches were in contact with the body throughout the observation period. The unworn patches were stored at 22°C wrapped in aluminium foil. Bioassays with Ae. aegypti and An. quadrimaculatus showed that the knock down time of worn patches was approximately 5-fold longer than that for unworn patches after 30 days. Both chemical and biological analysis showed that the greatest loss of permethrin from the patches occurred within the first 10 days.

Polyester-cotton fabric, when impregnated at a dose of 3.0 g/m² permethrin, in boiling water at acid pH, withstood 5 machine washes with soap and cold water and caused 90% or more mortality after 30 secs exposure of Ae. aegypti. Nylon-cotton fabric when impregnated at a dose of 0.77 g/m² caused 90% or more mortality for the same exposure time of Ae. aegypti and An. quadrimaculatus even after 9 machine washes (Schreck et al., 1982a).

A permethrin treatment rate of 1.25 - 2.0 g/m² of military uniform was found to give 100% protection from attack of Amblyomma americanum through 132 hours of accumulated wearing and 3 machine washes (Schreck et al., 1982d). Laboratory bioassays with adult Ae. aegypti and An. quadrimaculatus showed a slight loss of activity in worn clothing and much reduced activity in washed clothing. The gas chromatographic analyses showed 5% loss of permethrin in clothing after 132 hours of wear and 49% loss after 4 washes. In a previous field study Schreck et al. (1980b) achieved 89% protection from Amblyomma americanum by treating military uniform at a dose of 1.25 g/m² for over 100 hours of accumulated wearing.

The persistence of the repellent effect of deet on skin varies with the concentration and amount used, the species of attacking insects and probably temperature, wind, etc. Altman (1969) observed that when one ml of deet was

applied at 75% concentration to the fore arms it protected the subjects for 2.15 hours, the maximum observation period, against An. albimanus. When pure deet was applied at 0.25 mg/cm^2 on arms and legs the protection times achieved were 6 hours against C. quinquefasciatus. 5.5 hours against Simulium himalayense abd 5.5 hours against Haemadispa zeylanica. The protection times at 0.5 mg/cm^2 were 7 hours against C. quinquefasciatus, 6.75 hours against S. himalayense and 7.25 hours against H. zeylanica (Kumar et al., 1984). The repellency time of pure deet at 0.4 mg/cm^2 on arms against An. freeborni were found to be in the range of less than 4 hours to 10 hours with different testing subjects (Reifenrath and Akers, 1981). More than 7 hours of protection were achieved against S. damnosum by applying one ml of 40% deet to the fore arms, whereas the same amount of 50% deet gave less than 2 hours protection against Glossina morsitans (Schmidt, 1977). Schmidt and Schmidt (1979) achieved 4.5 hours of protection against Phlebotomus papatasi by applying one ml of pure deet to the fore arms. In the laboratory one ml of deet was found to give 3.8 hours of protection when applied at 12.5% concentration and 7.7 hours of protection when applied at 25% concentration against Mansonia mosquitoes, whereas in the field 25% deet of the same amount gave only 4.3 - 4.8 hours of protection against a mixed population of mosquitoes, mostly Mansonia (Schreck and McGovern, 1985). The shorter protection in the field was apparently due to physical factors, such as, wind, temperature, etc.

Deet was found to give a protection for 21 days against Ae. taeniorhynchus when impregnated into a four-mesh-per-inch cotton nets at a dose of 0.5 g/g netting (Gouck et al., 1967, 1971). In another experiment deet treated net gave protection for 16 and 17 weeks against Ae. aegypti and C. quinquefasciatus respectively (Gouck and Moussa, 1969; Smith et al., 1970).

On an impregnated net jacket, deet was found to remain effective for 3 to 14 days (Gorham, 1974; Mulrennam et al., 1975; Sholdt et al., 1975).It was found to remain effective on widemesh cotton netting for 64 days when put around a CDC miniature light trap and evaluated against biting midges and sand flies (Zaugg, 1978). In field trials in Tanzania deet, when impregnated into window curtains of thick wide-mesh cotton netting at a dose of 18.5 ml/m^2, persisted only 2 to 3 days (Lines et al., 1985). However, in the laboratory when sleeves made out of the same material were impregnated with deet at 20 ml/m^2 repellency persisted 42 days, as determined by weekly landing counts for 30 secs, the nets being hung in the laboratory in between the periods of use (Curtis et al., 1987). This difference in action may be due to the draught

through the window in the field test which removed the deet vapour quickly.

Deet impregnated military uniforms were found to remain effective for 4 to 5 weeks against Lone Star Tick (Gouck and Gilbert, 1955) and for 5 to 19 days against land leeches (Saxena and Khalsa, 1967). When canvas tents were impregnated with deet at a dose of 54 g/m^2 it gave more than 99% protection even after 4 weeks of ageing in open air (Sholdt et al., 1977). Deet impregnated anklets gave about 84% protection against C. quinquefasciatus for 80 days after one impregnation, in a trial in which the anklets were brought out of sealed storage and tested for 2 hours nightly (Curtis et al., 1987).

2.6. Effect of temperature on efficacy of pyrethroids:

Most of the pyrethroids tested so far for their insecticidal effect in relation to temperature have shown a negative temperature coefficient, i.e., a lower kill at higher temperature. Some of them, however, have shown a positive temperature coefficient or a neutral coefficient (no effect of temperature). The same chemical may have a negative temperature coefficient against some insects and positive against other insects. Sometimes the effect of temperature was found to be dependent on the nature of the test. Schmidt and Robertson (1986) observed that when the Hornfly, Haematobia irritans, was exposed to permethrin treated cloth a positive temperature coefficient was observed between 21°C and 27°C but temperature had no significant effect between 27 and 32°C On the other hand when permethrin was applied topically it showed a negative temperature coefficient throughout the range of temperatures. As we know that all chemical reactions go faster at higher temperature, a negative temperature coefficient of an insecticide implies that degredation of the insecticide is more temperature sensitive than the insecticidal effect.

Permethrin was found to be 3.63 times more toxic (defined in terms of ratio of LD_{50} values) at 20°C than at 30°C against Ae. aegypti larvae (Cutkomp and Subramanyam 1986). Permethrin also showed a negative temperature coefficient against the Boll Weevil, Anthonomus grandis grandis (Sparks et al., 1983), Cabbage Looper, Trichoplusia ni, Fall Armyworm, Spodoptera frugipereda, Tobacco Budworm, Heliothis virescens (Harris et al., 1978; Sparks et al., 1982) and House Fly, Musca domestica (Scott and Georghiou, 1983).

Fenvalerate and deltamethrin exhibited either neutral or positive

temperature coefficients against Fall Armyworm, and the Tobacco Budworm but, on the other hand, these two chemicals showed a negative temperature coefficient against Cabbage Looper, (Sparks et al., 1982), Boll Weevil, (Sparks et al., 1983) and Tobacco Cutworm, S. litura (Hirano, 1979).

Burgess and Hinks (1986) observed that cypermethrin is more toxic as a contact spray to the adult Flea Beetle, Phyllotrela crucifera, at 32°C than at 21 or 10°C. The difference in its toxicity between the latter two temperatures was not significant. The resistant strain of Blattella germanica had a negative temperature coefficient towards cypermethrin, whereas the susceptible strain of the same species had a negative temperature coefficient for knockdown but a positive temperature coefficient for mortality (Scott, 1987). However, cypermethrin exhibits a negative temperature coefficient of toxicity against H. virescens (Sparks et al. 1982), Melanoplus sanguinipes (Ewen et al., 1984), S. littoralis (Riskallah, 1984), and M. domestica (Scott and Georghiou, 1983).

Subramanyam and Cutkomp (1987) observed that d-phenothrin had a positive temperature coefficient for the Confused Flour Beetle, Tribolium confusum, but a negative temperature coefficient for H. virescens (Sparks et al., 1983).

All other pyrethroids, tested so far, have shown a negative temperature coefficient (Yoke and Sudderuddin, 1975; Harris and Kinoshita, 1977; DeVries and Georghiou,1979; Cutkomp and Subramanyam,1986; Subramanyam and Cutkomp, 1987; Scott, 1987).

Apart from the work of Cutkomp and Subramanyam (1986) on Ae. aegypti larvae there is no published report on the effect of temperature on the toxicity of pyrethroids against mosquitoes. Almost all field tests of pyrethroid impregnated nets (and perhaps some laboratory tests) have taken no account of temperature but variation in this might explain discrepancies between different studies. Also bioassays are done by day but bednets are required to be effective at night when temperature may be 10°C less. In view of these facts it is important to carry out more extensive work on the effect of temperature on the toxicity of pyrethroids against different species of mosquitoes and also to keep records of temperatures of all pyrethroid experiments.

2.7. Time-dose-response relationship of insecticides to insects:

Mortality is generally equally a function of the time of exposure to a poison and the concentration in the environment. Thus, the dosage and exposure time for a given effect may be interchangeable, that is, c.t = k (Busvine, 1971). Busvine (1958) showed that LC_{50} x time = constant for dieldrin against Ae. aegypti. This simple relationship between concentration of insecticide and exposure time has later been demonstrated by other authors.

Garms and Rehm (1961) found that doubling the exposure time from one hour to two hours or from two hours to four hours had the effect of halving the LC_{50} value found for DDT against An. atroparvous.

Pennell et al. (1964) reported that in WHO bioassay tests the pick-up of dieldrin by a Culex quinquefasciatus strain homozygous for dieldrin resistance was a linear function of the concentration on the paper and also of the time of exposure. A similar observation was made by Romgsriyam and Busvine (1973) for C. quinquefasciatus against carbamate and organophosphate insecticides. They determined c.t value in two different ways:- from LT_{50} x concentration or from LC_{50} x time. The values estimated in these two different ways were not substantially different. A similar result was obtained by Hamon (1963) after exposing Ae. aegypti to dieldrin or malathion impregnated papers, by Ariaratnam and Brown (1969) for C. quinquefasciatus against DDT, Sales and Mouchet (1973) for C. quinquefasciatus and Ae. aegypti against carbamate and organophosphorus insecticides.

A slightly different observation was made by Wickham et al. (1974) regarding knockdown-time-dose relationship for different formulations of insecticides and different insects. With aerosol formulations against houseflies, although a linear regression was found regardless of the technique used, at a dose of a given formulation, when the maximum performance was approached during the time of observation, the knockdown curve became less steep and began to level out. By the term "maximum performance" the authors apparently meant the ability of the insecticide to knockdown most of the insects in the population, except a few very tolerant individuals. When the insects were exposed to a surface from which the insecticide was picked up continuously throughout the exposure period, a distinctly curvilinear regression line of increasing slope was obtained.

2.8. Pyrethroid cross-resistance studies and selection for permethrin resistance in mosquitoes:

So far 8 Anopheles and 2 culicine mosquito populations have been reported to be resistant to pyrethroids. These are An. albimanus (El Salvador), An. arabiensis (Sudan), An. culicifacies (India, Sri Lanka), An. pseudopunctipennis (Guatemala), An. sacharovi (Turkey) and An. stephensi (India), Ae. aegypti (Malaysia, Thailand, Guyana, USA), and C. quinquefasciatus (India, USA) (Brown, 1986). In all these cases pyrethroid resistance was found to be associated with DDT resistance. In the cases of Ae. aegypti, An. gambiae and C. quinquefasciatus it was shown genetically that these are cases of cross-resistance conferred by a single gene.

Ae. aegypti was the first mosquito species to have shown a detectable amount of pyrethroid resistance due to DDT resistance. Prasittisuk and Busvine (1977) observed that 7 out of 8 strains of Ae. aegypti studied having high levels of DDT resistance had low levels of cross-resistance to permethrin. One strain from East Coast Demerara, Guyana, having a DDT resistance level 73 times the susceptible strain had a permethrin cross-resistance of 30 times the susceptible strain, whereas another strain from El Salvador having a similar DDT resistance level had only slight permethrin resistance (2.3 times the susceptible strain).

A follow-up study by Chadwick et al. (1977) confirmed that Ae. aegypti from Bangkok and Jakarta with high levels of DDT resistance had cross-resistance to pyrethroids. In both the above situations it was observed that the DDT resistance in this species was partly due to dehydrochlorination and involvement of the microsomal oxidase system. It was suggested that an additional unknown mechanism associated with DDT resistance confers a low level cross-resistance to pyrethroids. Before the laboratory investigation of Chadwick et al. (1977) it was observed that, during field trials in Bangkok, bioresmethrin failed to kill Ae. aegypti although the performance of this insecticide against houseflies and other insects was normal. The laboratory studies confirmed that the failure of bioresmethrin in killing Ae. aegypti in the field was due to the cross-resistance of the mosquito to this insecticide.

Malcolm and Wood (1982a) selected a homogeneous permethrin resistant strain of Ae. aegypti, which was derived from the same strain as was studied

by Chadwick et al. (1977), by applying single family sib-selection. Their attempt to establish a similar strain by mass selection failed, probably due to the nature of the WHO test kits used, in which knockdown removes the insect from contact with the insecticide impregnated paper before the intended exposure time has been completed. Early knockdown of less resistant insects could result in their picking up an insufficient dose to cause mortality. They could recover during the holding period and contribute towards the next generation.

Chadwick et al. (1984) performed an experiment to study the cross-resistance between DDT and pyrethroids in Ae. aegypti. DDT selection over 14 generations raised the resistance to DDT so far that no accurate LC_{50} values could be determined. Selection with permethrin raised the tolerance to 7 -10 times the original. Permethrin selection of the mosquitoes raised resistance to other pyrethroids more than DDT selection, but also increased DDT resistance.

An. gambiae and An. quadrimaculatus having low level DDT resistance (about 2 times the susceptible strain) were found to possess some cross-resistance to permethrin (about 1.5 times the susceptible strain) (Prasittisuk and Busvine, 1977). When An. gambiae s.s. was selected in the laboratory for DDT resistance, the resistance was increased to 5 times the initial level, as determined on the basis of LT_{50} value and an appreciable cross-resistance to permethrin was produced (2 times the initial level) (Prasittisuk and Curtis, 1982). In the same study it was observed that when An. gambiae was selected for permethrin resistance, with an increase of the resistance to 5 times its initial level, there was no change of DDT resistance.

An. stephensi from Pakistan was selected by Omer et al. (1980) in California. This species was initially slightly resistant to DDT but susceptible to permethrin. Larval selection with DDT induced a cross-resistance to trans-permethrin (12-fold) and cis-permethrin (18-fold)(cis- and trans- are the two optical isomers of permethrin). After four generations a substrain of the DDT selection line was selected with trans-permethrin for two generations. This increased resistance to trans-permethrin and cis-permethrin 15-fold and 20-fold respectively relative to the initial level. An. albimanus resistant to organophosphates, carbamates and DDT were found to possess a low level of cross-resistance to some of the pyrethroids (Priester et al., 1981). In India An. culicifacies resistant to DDT, dieldrin and malathion was found to be susceptible to deltamethrin (Das et al., 1986).

C. quinquefasciatus, resistant to propoxur and DDT, was found to possess a low level of cross-tolerance to some of the 26 pyrethroids tested (Priester et al., 1981). When a high level of resistance to trans-permethrin of greater than 4000-fold was selected in larvae of C. quinquefasciatus they were found to have cross-resistance to cis-permethrin (1021-fold) and various other pyrethroids (Priester and Georghiou, 1978). It was suggested that non-metabolic mechanisms, such as reduced sensitivity of the target site, may be the primary source of resistance. C. quinquefasciatus selected with trans- and cis-permethrin were examined in the larval stage for cross-resistance to 30 pyrethroids, DDT, dieldrin, temephos, propoxur and two organotin compounds (Priester and Georghiou, 1980). The trans-permethrin-R strain and cis-permethrin-R strain were found to be cross-resistant to all pyrethroids tested as well as DDT. However, they were not significantly cross-resistant to dieldrin, temephos, propoxur or the two organotin compounds. Gaaboub and Abu-Hashish (1981) observed that DDT-resistant Culex pipiens in Egypt has cross-resistance to permethrin.

2.9. Genetics of pyrethroid resistance in mosquitoes:

It was suggested by Chadwick et al. (1984) that two major independent resistance mechanisms existed in the DDT selected strain of Ae. aegypti, a dehydrochlorinase affecting DDT alone, and an unknown mechanism, probably nerve insensitivity (kdr) affecting both DDT and pyrethroids.

The term kdr (knockdown resistance) was first introduced by Milani (1954) to refer to the type of DDT resistance gene in Musca domestica in the presence of which knockdown was delayed when the fly was exposed to DDT. He reported that the kdr gene is present on chromosome III in M. domestica. Later the same author described another gene kdr-0 in another DDT resistant strain of housefly (Milani, 1960). Farnham (1973) isolated another kdr gene from a strain of M. domestica selected for resistance with natural pyrethrins and named it kdr-NPR. This was also reported to be present on chromosome III, to delay the knockdown effect of pyrethrins, and to give resistance to all pyrethroids and also cross-resistance to DDT. Farnham (1977) studied the genetics of resistance to pyrethroids and found that all three kdr resistance factors are identical.

Genetic analysis of permethrin resistance in Ae. aegypti was carried out by Malcolm and Wood (1982b) and Malcolm (1983a, 1983b). It was observed that the major part of pyrethroid resistance in a strain of Ae. aegypti, BKPM3 - homogeneous for permethrin resistance, is controlled by a single gene. It was also observed that the factors responsible for DDT resistance in this strain occur on both chromosomes II and III. However, the major gene responsible for pyrethroid resistance was found to be present on chromosome III only and it was closely linked or allelic to the chromosome III DDT resistance factor.

In an attempt to select permethrin resistance from DDT-resistant An. stephensi a reduction was achieved in susceptibility to what the author described as larval knockdown (2 hours exposure) of 17-fold, but only 1.6-fold to kill (24 hours exposure) (Malcolm 1988, in press). Genetic analysis revealed that several interacting genetic factors were collectively responsible for the reduced larval susceptibility to knockdown. These were only maintained together by selection pressure and the effect was lost quickly in the absence of selection or with outcrossing. The 30- to 40-fold DDT resistance found in the parental strain was barely altered by permethrin selection, suggesting no relationship with the major source of DDT resistance.

Priester and Georghiou (1980) while studing the cross-resistance between DDT and pyrethroids observed limited synergism of pyrethroids and DDT which suggested that some non-metabolic mechanism, such as kdr, may be an important component of resistance to pyrethroids as well as to DDT in this mosquito. The mode of inheritance of permethrin resistance in C. quinquefasciatus was studied by Priester and Georghiou (1979). The authors calculated the degree of dominance (D) of resistance in the F_1 following Stone (1968)

$$D = \frac{2LC_{50}\ (RS) - LC_{50}\ (RR) - LC_{50}\ (SS)}{LC_{50}\ (RR) - LC_{50}\ (SS)}$$

The value thus derived indicates whether resistance is fully recessive (D = -1), co-dominant (D = 0), fully dominant (D = 1) or at some other point between the two extremes. Resistance was found to be co-dominant (D = -0.014 to 0.14) in the trans- selected strain and partially recessive (D = -0.15 to -0.35) in the cis- selected strains of this species. The dose-response line for the back cross progeny suggested that permethrin resistance in this species is of

polyfactorial origin. Further genetic analysis of permethrin resistance in the permethrin selected C. quinquefasciatus was done by Halliday and Georghiou (1985a, 1985b). kdr was found to be the predominant gene causing larval resistance to permethrin in a permethrin resistant strain of this species. These authors indicated that resistance to permethrin segregates monofactorially in the backcross to resistant parents, although there is evidence for additional factors influencing resistance. The kdr gene was found to be present on the 2nd chromosome of this species and at a distance of 35±4 map units from the marker gene, y, responsible for yellow larvae.

CHAPTER 3. MATERIALS AND GENERAL METHODS

3.1. Mosquito species and strains studied:

Fifteen strains of mosquitoes belonging to 5 species and 3 genera were used in these studies. The details of each of the strains are as follows:

Aedes aegypti (L.)

AE AE - A strain susceptible to insecticides, originated in West Africa in 1926 and has been maintained in the insectary of LSHTM since then without exposure to insecticides.

BKPM12 - Obtained from University of Manchester through Dr.R.J. Wood. This strain was mass-selected with permethrin by Malcolm (1981) from BKPM which was also mass-selected with permethrin from BKK, a strain established in Wellcome Research Laboratories, Berkhamsted, from eggs collected from Bangkok (Chadwick *et al.* 1977). BKPM12 was homogeneous for permethrin resistance at the time of selection.

Anopheles albimanus Wiedemann

PALB - A subculture of a colony from Liverpool School of Tropical Medicine. Originated from Panama. Maintained in LSHTM since 1968. This strain is susceptible to insecticides.

FEST - Originated at Fernando, El Salvador. Originally colonized in LSHTM in 1974, then lost, and re-obtained from J.A. Seawright, USDA, Gainsville, U.S.A. It is a strain with broad spectrum organophosphate and carbamate resistance. The broad spectrum resistance is due to decreased sensitivity of the insect's acetylcholinesterase (AChE) to inhibition by insecticides (Ayad and Georghiou 1975).

Anopheles gambiae s. s. Giles

KWA - Originated from Kwale, 35 Km north of Tanga, Tanzania, from pooled eggs. Colonized in LSHTM in 1975. This strain is susceptible to organophosphates, carbamates and DDT.

16cSS - Selected as a strain homozygous for collarless, a genetic marker, and susceptible to all insecticides. Derived from LAGOS strain in 1974, which was originated in Lagos, Nigeria and was colonized in LSHTM in 1951.

G3 - Collected in The Gambia in 1986 and colonized in LSHTM in the same year. Susceptible to all insecticides.

Z11 - A DDT susceptible strain derived by single family selection from ZANU, a strain which originated from Zanzibar, Tanzania, in 1982.

ZANDS - A DDT resistant strain selected from the same ZANU wild population as Z11 and maintained under selection pressure.

IANP20 - Selected for permethrin resistance over 20 generations (Prasittisuk, 1979), derived from IAN, a strain which originated from Iworo, Nigeria and was colonized in LSHTM in 1975. Carries genes for DDT and dieldrin resistance. During the present investigation permethrin resistance was not detected.

MU - Originated from pooled catches in Muheza, Tanzania, and colonized in LSHTM in 1980. Resistant to 4% dieldrin.

Anopheles stephensi Liston

STLASS - An insecticide susceptible strain from Lahore, Pakistan.

STMAL - Originated from Pakistan, colonized in 1974. Resistant to malathion and dieldrin. Malathion resistance is based on an increased metabolism of malathion to the monocarboxylic acids. Rowland (1985) selected the malathion resistant strain for dieldrin resistance and designated it originally as MALDIEL and later as STMAL.

Culex quinquefasciatus Say

CfCA - Originated in Colombo, Sri Lanka, and colonized in LSHTM in 1950s. Susceptible to insecticides (CfCA is the abbreviated form of Culex fatigans Colombo strain A).

DAR82 - Broad spectrum organophosphate and carbamate resistance due to the involvement of both high esterase and altered acetylcholinesterase resistance mechanisms. Originated from Dar es Salaam, Tanzania, and colonized in LSHTM in 1982.

3.2. Chemicals used:

DEET - N,N-diethyl-m-toluamide, Pfizer Chemical Co., U.K.

Permethrin - 3-phenoxybenzyl-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate. cis:trans isomer ratio 25:75; 10% and 20% emulsifiable concentrate; Wellcome Research Laboratories, U.K. Also, cis:trans isomer ratio 40:60; 25% emulsifiable concentrate; ICI Plant Protection Division, U.K.

Cypermethrin- α-cyano-3-phenoxybenzyl-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate. ICI Plant Protection Division, U.K.

Cyfluthrin - α-cyano-(4-fluro-3-phenoxyphenyl)-methyl-3-(2,2-dichloroethenyl) -2,2-dimethyl-cyclopropane carboxylate. Bayer, FRG.

Deltamethrin- α-cyano-3-phenoxybenzyl-3-(2,2-dibromovinyl)-2,2-dimethyl cyclopropane carboxylate. Roussel Uclaf, France.

Fenpropathrin - α-cyano-3-phenoxybenzyl-2,2,3,3-tetramethylcyclopropane carboxylate. Sumitomo Chemical Co., Japan.

Fenvalerate - α-cyano-3-phenoxybenzyl-2-(4-chlorophenyl)-3-methyl butyrate. Sumitomo Chemical Co., Japan.

d-phenothrin- 3-phenoxybenzyl-2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane carboxylate. Sumitomo Chemical Co., Japan.

Cyphenothrin- α-3-phenoxybenzyl-2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane carboxylate. Sumitomo Chemical Co., Japan.

PP321 - (Icon) α-cyano-3-phenoxybenzyl-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl cyclopropane carboxylate. ICI Plant Protection Division, U.K. Now designated lambda cyhalothrin.

4% DDT and 0.25% permethrin impregnated papers were obtained from WHO.

3.3. Bednets and other fabrics used:

Various types of synthetic and cotton netting and sheeting were used. The details of the fabrics used are as follows:

Widemesh cotton netting - Green coloured, made as military camouflage netting, from Fryma Fabrics, Nottingham, 8 mm mesh size, thickness of the threads 1.0 mm.

Cotton netting - One of a few types used to make mosquito-nets, 2 mm mesh size, the thickness of the threads is 0.25 mm.
(J.C.Small & Tidmas code no. 111).

Nylon netting - One of a few types used to make mosquito-nets, 1.5 mm mesh size, the threads were thinner than those of cotton netting. (J.C.Small & Tidmas code no. 202).

Polyester netting - Structurally similar to nylon netting but synthetised from polyester, 1.5 mm mesh size.
(J.C.Small & Tidmas code no. 2012).

Cotton sheeting - Finely woven sheeting, 30 threads per cm.

Nylon sheeting - Finely woven sheeting.

Polyester sheeting - Finely woven sheeting.

Poly-cotton sheeting - A combination of 40% polyester and 60% cotton, finely woven sheeting, 35 threads per cm, thicker than either cotton or polyester sheeting.

3.4. Rearing of mosquitoes:

Mosquitoes were reared and maintained in the insectaries of LSHTM. Most of the insectaries of the School are in the old vaults under Mallet street and Gower street, where Anopheles mosquitoes are kept. A smaller insectary is located on the roof of the School, where Culex and Aedes are reared. A brief account of the conditions of the insectaries and mosquito rearing methods is given here.

Environmental conditions:

Although temperature, relative humidity and daily light-dark regime (the three important physical parameters for mosquito studies) are supposed to be under accurate control in a good insectary, in fact this was not found to be always true during the present investigation. The ways in which these three

parameters deviated from ideal conditions were described in detail by Webb (1987). Similar problems were faced by me. However, I always tried to perform my experiments in as ideal conditions as possible. When very high temperatures occurred, I stopped my experiments for some time until the conditions again became normal.

The following ideal conditions were always aimed at in the insectaries: a temperature of 27±2°C, maintained using electric radiators and thermostats, a relative humidity of 70±5% maintained by steam supplied from a humidifier was controlled by a hygrometer. A 12-hour light-dark regime were maintained using a sharp automatic on/off switch (light on at 08:00 hours and off at 20:00 hours). Light was supplied by standard AC white fluorescent tubes.

Rearing methods:

Larvae were were reared in 25 cm diameter plastic bowls in tap water. The depth of the water was maintained at about 5 cm. A piece of mud and grass was used in each bowl containing Anopheles and Culex larvae. This was used as an inoculum of microorganisms, to provide trace minerals and to help buffer the pH of the water (Laurence 1964). The bowls were covered by a netting lid to avoid any accidental "contamination" of the strain or escape of any emerged adult.

Anopheles larvae were fed "Farex" baby food daily. For younger larvae very fine particles of Farex were provided and the older ones were given normal Farex powder. Culex larvae were fed guinea pig pellets and Aedes larvae were provided with dried liver powder. About 400 to 500 larvae were reared in each bowl. Rearing bowls were thoroughly cleaned with hot water without using any detergent and dried between successive uses.

Either the pupae were picked up from the rearing bowl with a pipette and transferred to adult cages or newly emerged adults were transferred with the help of a mouth aspirator or a battery operated aspirator. The adult cages were of two sizes, either 30x30x30 cm or 45x45x45 cm. Generally the larger cages were used to maitain stocks and the smaller ones to keep experimental mosquitoes. Each cage was made out of a steel frame and fine mesh mosquito nets having a sleeved opening at one side.

Adult mosquitoes were provided with a 10% glucose solution on a lint wick. The glucose solutions were changed weekly. In addition to glucose feeding the females in stock cages were also blood fed twice a week. Anopheles and Aedes were provided with anaesthetised guinea pigs and Culex with chicks. Guinea pigs were anaesthetised by injecting 0.35 to 0.8 ml of "Sagatal", depending on age and tolerance of the guinea pig, subcutaneously. The anaesthetised guinea pigs were laid on top of the cages for 15 to 30 minutes with their abdomen downward.

On the evening of the second day after blood feeding an oviposition bowl was put into each cage. These were enamel bowls, 10 cm in diameter, lined with a filter paper and partially filled with tap water. Egg bowls were collected two days later. In the cases of Anopheles and Culex they were covered with perspex slabs until the eggs hatched and the larvae were then transferred to the rearing bowls. In the case of Aedes the filter papers with the eggs were dried and then placed in the rearing bowl to hatch.

CHAPTER 4. RESULTS WITH DETAILED METHODS AND DISCUSSION

4.1. Repellency of deet against the CfCA and KWA strains:

4.1.1. Introduction:

Repellent impregnated fabrics can be broadly categorized into those which remain in close contact with the body, e.g. garments and bednets, and those remaining at a distance from the human body, e.g. window and eave curtains. It is important to see: (i) if one type is more effective than the other, which may depend partly on whether closeness of the host to the repellent has any influence, (ii) whether there is a species difference in response. A simple method was developed to investigate these questions under laboratory conditions.

4.1.2. Methods:

A "tunnel" was constructed out of six 30 cm cubic cages placed in a line (fig. 1). The cages were made out of wooden frames and fibre glass netting. The two end cages were open on one side only, whereas the other four cages were open on two opposite sides. A bait was placed at one end of the tunnel and mosquitoes were released at the other end. One strain of An. gambiae (KWA) and one strain of C. quinquefasciatus (CfCA) were used to study the relative responses of these two species of mosquitoes. An anaesthetised guinea pig was used as a bait for KWA and a chick in a small wire-cage (to restrict its movements) was used as a bait for CfCA. These different baits were used because An. gambiae prefers a mammal and C. quinquefasciatus prefers a bird for blood feeding.

A small hole was made in the netting on one side of each of the cages through which mosquitoes could be released into the cages and collected out of the cages using a mouth aspirator. The holes were closed with pieces of cotton wool during the experiments. There was provision to place a piece of netting between any two adjacent cages, so that the distance of the net from the bait could be adjusted as required. In the present experiment the net was placed either between the 2nd and 3rd cage or between the 5th and 6th cage, numbering

from the mosquito releasing cage to the cage containing the bait. So the distance of the net from the bait was either 105 cm or 15 cm, assuming that the bait was in the centre of the 6th cage. These positions were chosen to simulate the natural conditions of the above mentioned two categories of impregnated fabrics. A 30 cm square cardboard sheet having an 16 cm diameter circular hole was placed between the adjacent cages to make the joints mosquito-proof and also as a support to hold the netting in position. The nets were pinned to the pieces of cardboard.

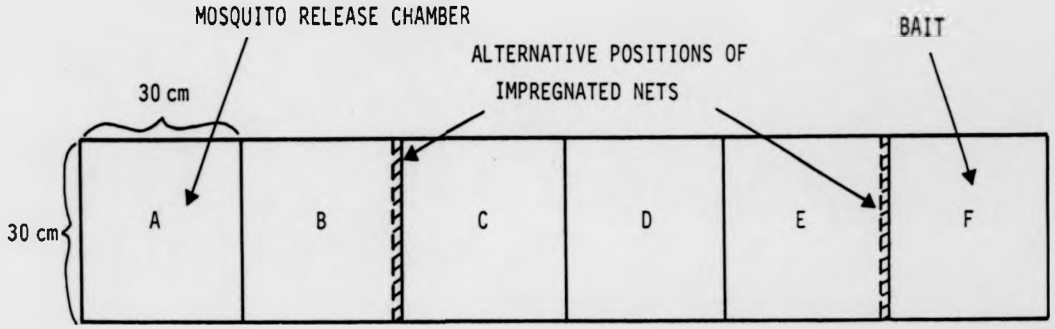
For experiments with CfCA, mosquitoes were exposed for about 17 hours, whereas KWA were exposed for about 6 hours. This difference in exposure time was for two reasons - (i) Culex mosquitoes do not respond to a host as quickly as Anopheles, so a longer time should be allocated to Culex than Anopheles in a feeding experiment, (ii) chicks were available for feeding overnight whereas guinea pigs were available only for about 7 hours during day time. For the experiments with KWA a 12-hour light-dark cycle was used (as with all other experiments), but in this case the lights were set to go off at 1030 hours and come on at 2230 hours, so that the experiments could conveniently be done in the scotophase. The mosquitoes used in these experiments were reared with the same light-dark cycle. Generally the first stage larvae were transferred to this lighting system. Experiments with CfCA were done with the usual light:dark system of the insectary, i.e. lights on at 0800 hours and off at 2000 hours.

Pieces of 20x20 cm wide-mesh cotton netting having 0.8 cm mesh-size were impregnated with a series of doses of deet. The doses used were 0.05, 0.15, 0.25, 0.50, 1.0 or 2.0 ml of deet per piece of netting. The required amount of deet was added to acetone to give a total volume of 12 ml, which was necessary to soak the netting properly. After soaking in a polythene bag, the netting pieces were dried in a fume cupboard for about 15 minutes. The nets were then sealed in polythene bags until used, except in the case of persistence studies, where the nets were hung in the laboratory. Each of the pieces of impregnated netting was generally used only once, but in the case of persistence tests the same net was used repeatedly.

About 25 three to five day old hungry female mosquitoes were released at a time. Results were scored by counting the mosquitoes fed or unfed, and having passed the curtain or not having passed it.

Figure 1

A "Tunnel" of cages designed to study mosqutio behaviour in relation to impregnated nets.



4.1.3. Results: Effect of distance of the repellent from the host and comparative susceptibility of CfCA and KWA to deet:

Responses of the CfCA strain of C. quinquefasciatus released in a "tunnel" containing a piece of deet-treated wide-mesh cotton netting are presented in table 1 and those of the KWA strain of An. gambiae in table 2. A maximum dose of 0.50 ml/400 cm^2 of netting (or 12.5 ml/m^2) was necessary to completely stop CfCA from getting through the nets. Complete protection was obtained against KWA using 0.25 ml/400 cm^2. In most cases a substantial number of mosquitoes did not pass through the untreated netting. Only when the net was at a distance of 15 cm from the bait did all the mosquitoes pass through the net but more than 15% of them did not take a blood meal. To determine the effect of the deet alone, results were corrected for untreated control nets and were plotted on a log-dose/probit repellency graph. The ED_{50} and ED_{90} values were determined using a computer program provided by Dr. C. Schofield. Regression lines were drawn joining ED_{50} and ED_{90} and are presented in figures 2 to 5. In the figures 95% confidence limits from the binomial distribution (Fisher and Yates, 1974) of repellency at each of the doses are shown. Figures 2 and 3 show that netting at a distance of 105 cm from the bait is significantly more effective than when it was at 15 cm from the bait. Deet is significantly more effective against KWA than against CfCA (Figures 4 and 5).

Table 1. Responses of the CfCA strain of C. quinquefasciatus when released into a "tunnel" containing a chick. A piece of deet-treated or untreated wide-mesh cotton netting was interposed between the chick and the mosquitoes.

Dose per 400 cm^2 netting	Distance of curtain from bait(cm)	% of mosquitoes passed curtain	% not passed curtain corrected for control*	% of fed mosquitoes	% unfed corrected for control*	Total number of mosquitoes
Control	15	100	-	84.8	-	105
"	105	77.8	-	57.6	-	99
0.05 ml	15	83.1	16.9	70.4	17.0	71
"	105	33.0	57.6	18.0	68.8	100
0.15 ml	15	60.5	39.5	38.7	54.4	119
"	105	7.1	90.9	4.1	92.9	98
0.25 ml	15	18.1	81.9	9.7	88.6	72
"	105	8.6	91.4	2.8	95.1	70
0.50 ml	15	0	100	0	100	73
"	105	0	100	0	100	70

$$*100 - \left[\frac{\%\ \text{success in treated}}{\%\ \text{success in control}} \times 100\right]$$

Table 2. Responses of the KWA strain of An. gambiae when released into a "tunnel" containing a guinea pig. A piece of deet-treated or untreated wide-mesh cotton netting was interposed between the guinea pig and the mosquitoes.

Dose per 400 cm^2 netting	Distance of curtain from bait (cm)	% of mosquitoes passed curtain	% not passed curtain corrected for control*	% of fed mosquitoes	% unfed corrected for control*	Total number of mosquitoes
Control	15	66.3	-	57.9	-	95
"	105	10.5	-	3.2	-	95
0.05 ml	15	24.3	63.3	15.7	72.9	70
"	105	-	-	-	-	-
0.15 ml	15	10.3	84.5	9.6	83.4	73
"	105	-	-	-	-	-
0.25 ml	15	0	100	0	100	75
"	105	-	-	-	-	-

$$* 100 - \left[\frac{\% \text{ success in treated}}{\% \text{ success in control}} \times 100\right]$$

- = not recorded

Figure 2

Comparative repellency (corrected for control) of deet impregnated wide-mesh cotton nets to the CfCA strain of C.quinquefasciatus placed at two different distances from the bait.

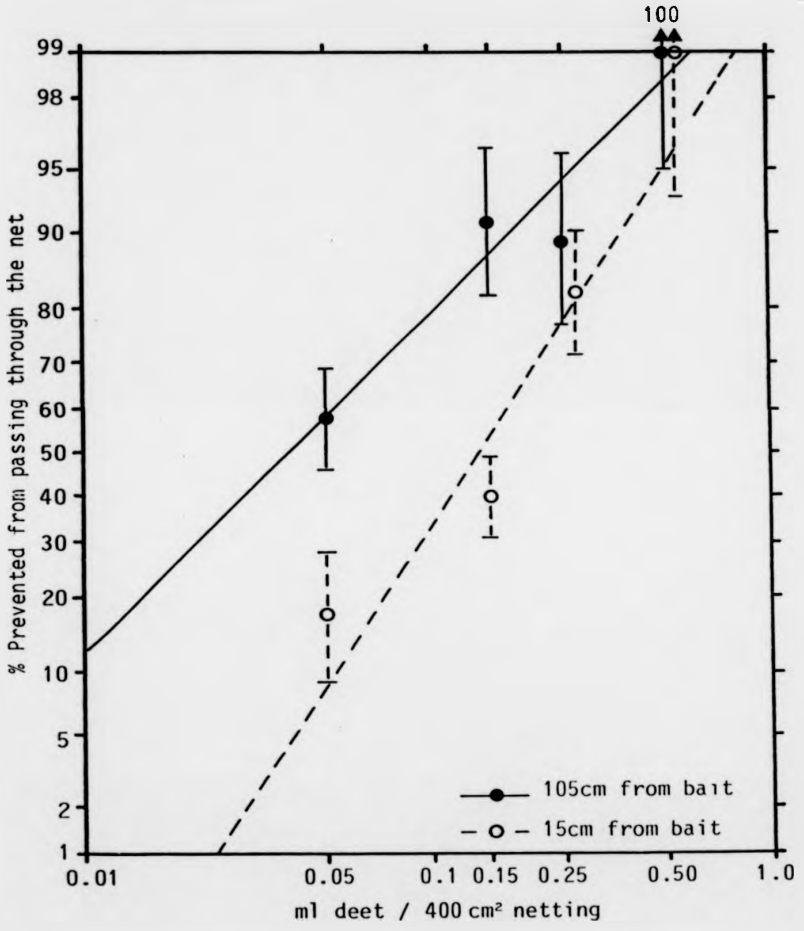


Figure 3

Percentage unfed C.quinquefasciatus(CfCA strain) due to the use of deet impregnated wide-mesh cotton nets at two different distances from the bait (results were corrected for control).

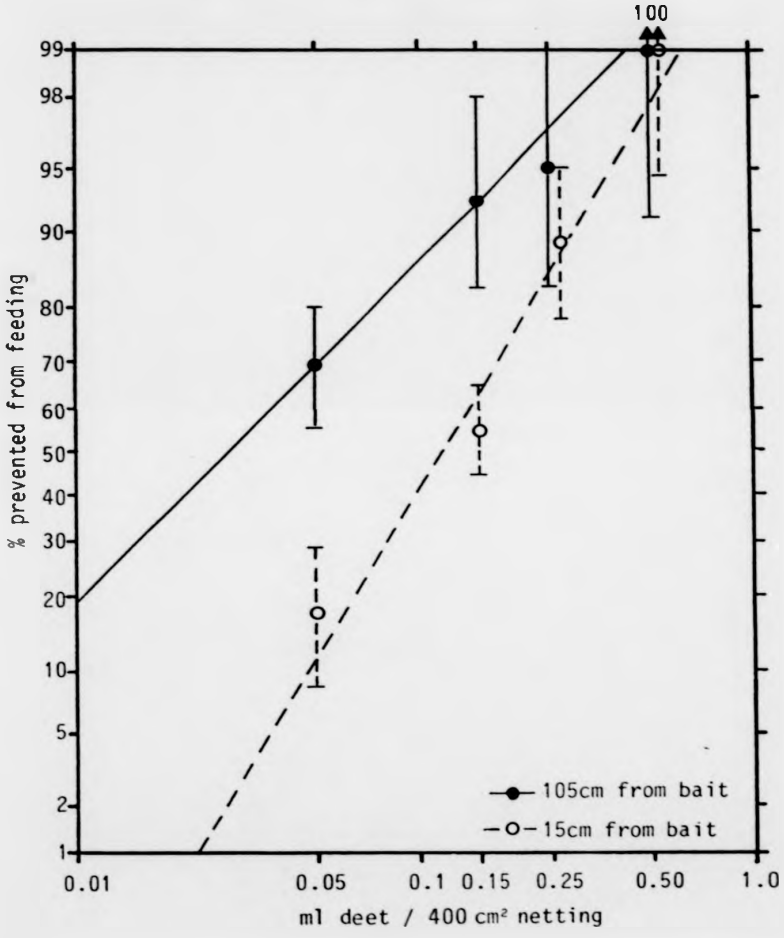


Figure 4

Comparison of responses of the CfCA and KWA strains to deet impregnated wide-mesh cotton nets (results were corrected for control).

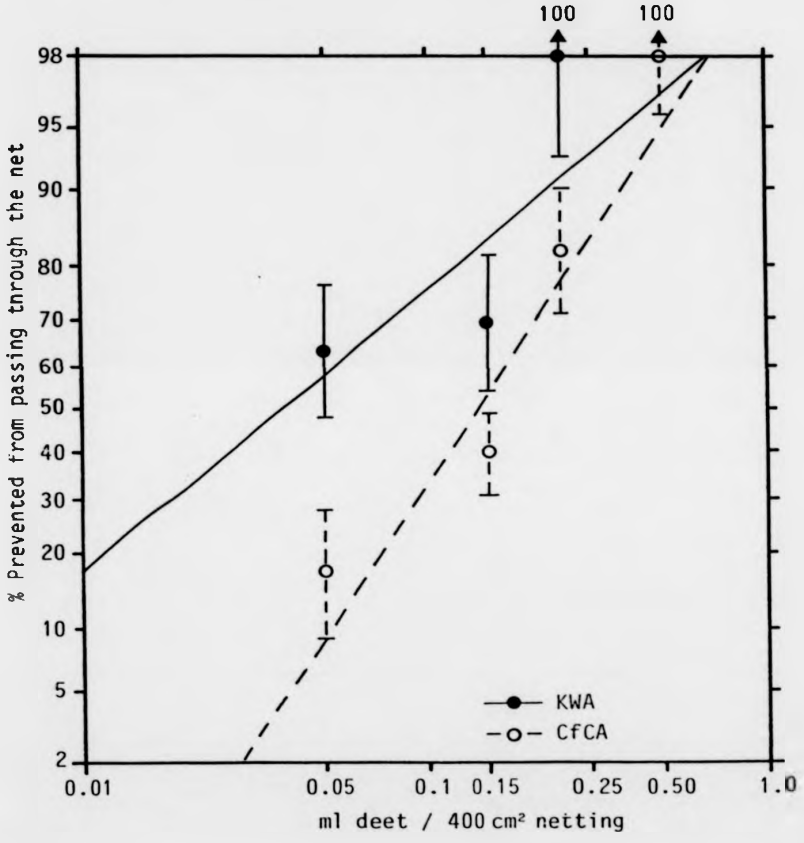
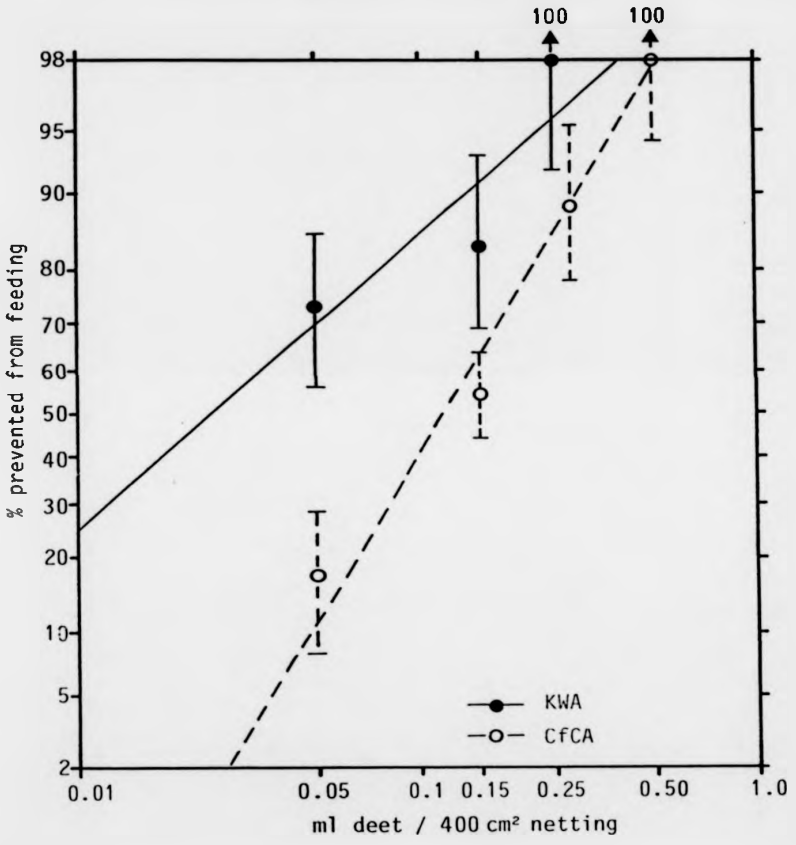


Figure 5

Comparison of blood feeding of the CfCA and KWA strains in the presence of deet impregnated wide-mesh cotton nets (results were corrected for control)



4.1.4. Discussion:

Previous investigations have shown that deet is effective against mosquitoes when impregnated into wide-mesh netting and used as jackets or bednets (Gorham, 1974; Gouck et al., 1967, 1971; Gouck and Moussa, 1969; Mulrenum et al., 1975; Sholdt et al. 1975; Smith et al. 1970) The present investigation indicates that deet impregnated netting is likely to be more effective at a given dosage when it is placed at some distance from the bait. It seems reasonable that when mosquitoes come closer to the bait they are more attracted by it and they try harder to cross any barrier. From the present findings it seems that, other things being equal, impregnation of deet would be more effective on window- and eave-curtains than on bednets.

In the present experimental set up it is difficult to compare the susceptibility of KWA and CfCA to deet, because the two strains were exposed for different exposure periods and different baits were used for reasons stated in the methods section. But the importance of these differences are minimized by using a control test in each case. The results were corrected for control before performing regression analysis. From the present data it is concluded that An. gambiae is significantly more susceptible to deet than C. quinquefasciatus. Table 2 also shows that when the netting was used in the farther position from the bait very few An. gambiae passed through the untreated netting. Therefore, no experiment using treated netting at that position was done with An. gambiae. Earlier experiments revealed that the effectiveness of deet depends upon the species of mosquito and different strains of the same species differ in their response to deet (Curtis et al. 1987, Rutledge et al. 1978, 1983, Schreck 1985 and Zhogolev 1968). Curtis et al. (1987) also found that deet impregnated anklets were better against C. quinquefasciatus than An. gambiae perhaps because Culex is more rigidly programmed to bite the ankles.

4.1.5. Results of persistence tests of deet on cotton netting:

The results, obtained by exposing KWA to pieces of deet impregnated wide-mesh cotton netting at various intervals after impregnation, are presented in table 3. It was observed that deet at 0.25 ml/400 cm^2 gives 100% protection for only 2 weeks. Complete protection was achieved up to 7 weeks using 1.0 ml deet/400 cm^2 of netting. When netting with the same dose was sealed in a polythene bag, it was 100% effective even one year after impregnation.

4.1.6. Discussion:

The effectiveness of deet impregnated netting is lost quickly if the netting is left exposed. A dose of 1.0 ml per 400 cm^2 netting, which was 4 times the ED_{100}, continued to give complete protection for 7 weeks. The impregnated netting was kept in a room with still air. It is probable that the loss of deet would be quicker if the netting was kept in natural air currents. Lines et al. (1985) observed that when similar netting was impregnated at a dose of 18.5 ml/m^2 and used as a window curtain in Tanzania, it failed to protect against mosquitoes and the smell of deet persisted for only 2 to 3 days. When the dose was doubled, netting gave good protection but the authors did not mention how long deet persisted. The dose 1 ml/400 cm^2 is between the two doses used by Lines et al. (1985).

The results on the netting that was sealed for one year show that if impregnated fabrics can be sealed between occasions when they are needed the persistence of deet can be increased. It was observed in Tanzania that deet impregnated anklets, made out of thick cotton netting, gave about 84% protection against wild C. quinquefasciatus for 80 days after one impregnation (Curtis et al., 1987). In their tests the anklets were brought out of sealed storage and tested for 2 hours nightly. However, it would not be feasible to seal window curtains up during the day time. Therefore, although deet would be more effective in window curtains than in bednets, it seems impractical to use deet in window curtains because of its short persistence.

Table 3. Persistence of deet on pieces of wide-mesh cotton netting as measured in a "tunnel" test.

Dose per 400 cm^2 netting (ml)	Time for which impregnated netting was aged	% of mosquitoes failed to pass curtain	% failed to pass corrected for control	% of unfed mosquitoes	Percentage unfed corrected for control	Total number of mosquitoes
Control	-	15.8	-	22.4	-	76
0.25	2 Weeks	100	100	100	100	54
0.25	4 "	60.9	53.5	67.4	58.0	46
0.50	3 "	100	100	100	100	46
0.50	7 "	23.3	8.9	13.3	-11.7	60
1.0	7 "	100	100	100	100	50
1.0	9 "	89.4	87.4	89.4	86.3	47
1.0	11 "	36.4	24.5	38.6	20.9	44
1.0*	1 Year	16.7	1.1	29.2	8.8	24
1.0**	1 "	100	100	100	100	25
2.0*	1 "	8.0	-9.3	4.0	-23.7	25

* netting used only once.

** netting preserved in polythene bag.

4.2. Impregnation of fabrics with permethrin:

4.2.1. Introduction:

An easy but reliable method for impregnating bednets with pyrethroids is desirable. With this intention in mind various pieces of netting and sheeting were impregnated with permethrin in the laboratory. It was thought possible that permethrin would be absorbed selectively by fabrics. If so, the remaining emulsion after an impregnation would contain a lower concentration than it had before impregnation. An experiment was done to evaluate this suggestion.

In the construction of bednets highly absorptive sheeting is often sewn to relatively non-absorptive netting. Therefore the questions arise - (i) whether permethrin can diffuse from one fabric type to another, if they are joined together, during the process of drying after impregnation, (ii) whether permethrin can move from one fabric type to another by a "creeping" process during ageing of impregnated fabrics joined together. An experiment was done to evaluate these two points.

4.2.2. Methods:

Various types of netting and sheeting, either single or sewn in different combinations, were impregnated in the laboratory. Doses, in terms of g/m^2, were calculated on the assumption that uptake is passively dependent on the amount of liquid taken up without any special affinity of the fabric for the permethrin. Chemical analysis of the impregnated materials were done by Wellcome Research Laboratories, Berkhamsted, U.K., to determine the actual amount of permethrin absorbed per square metre. For chemical analysis the pieces of fabric samples were extracted with acetone and the resulting solution was analysed by Gas Liquid Chromatography. The impregnation and calculation of the expected dose was done as follows.

A measured piece of fabric was weighed with an electronic balance, dipped into water, excess water was squeezed out and the piece was then weighed again. The weight of water absorbed was calculated by subtracting the first weight from the second. By repeating the process a few times an average weight was obtained, which was taken as the weight of water absorbed by that piece of fabric. It was assumed that the piece would absorb the same amount of

permethrin emulsion as water. Then the amount of permethrin E.C. required to give a certain dose was calculated by the formula:

$$\text{Permethrin E.C. required (ml)} = \frac{D \times A \times 100}{\text{E.C. \%}}, \text{ where:}$$

D = intended dose,

A = area of fabric.

The calculated amount of E.C. was then added to water to give an emulsion that would be required just to soak the fabric. As there are some problems in using exactly the amount of emulsion required to just soak a piece of fabric, the amount of emulsion prepared was always at least five times that required to soak one piece of fabric. Once the emulsion was prepared, the pieces of fabrics were dipped into the emulsion. Excess emulsion was squeezed out by pressing between the fingers. The fabric pieces were then dried overnight spreading them horizontally on a piece of polythene sheet.

To evaluate the suggestion that permethrin would be absorbed selectively by various fabric types, 7 different fabrics, both netting and sheeting, were impregnated by dipping them one after another into a permethrin emulsion. The same material was dipped at the beginning and also at the end of the series. The total amount of emulsion was about double the amount all the pieces of fabric could absorb. The amount of emulsion absorbed by each of the fabrics was measured by weighing the fabric before and after impregnation. Calculation of the expected permethrin content, on the assumption of non-selective absorption, was done on the basis of amount of emulsion absorbed by the fabric and was determined by the formula:-

$$\text{Expected dose (g/m}^2\text{)} = \frac{P \times w}{W \times F}, \text{ where:}$$

P = total permethrin active ingredient in the emulsion,

w = weight of emulsion absorbed by the fabric,

W = total weight of emulsion,

F = area of fabric.

Again, P was determined by the formula

$$P = \frac{\text{Amount of E.C. added X E.C. \%}}{100}$$

After drying, the impregnated fabrics were wrapped in aluminium foil and sent to Wellcome Research Laboratories for chemical analysis.

To evaluate the questions regarding the diffusion or "creeping" of permethrin between two fabric types the following experiment was done. Pieces of three different types of netting were sewn to pieces of four different types of sheeting in such a way that each of the netting types was paired with each of the sheeting types. Thus altogether there were 12 different combinations. The netting and sheeting were also impregnated unjoined to other material. All the separate and joined pieces were impregnated by dipping them, one after another, into an emulsion of known concentration. The same netting type was impregnated at the beginning and the end of the series. The permethrin content expected in each of the separate netting and sheeting pieces was calculated by applying the same formula as before. But the amount of permethrin expected to be absorbed by the components of a composite piece of netting and sheeting was determined by applying another formula:

$$\text{Permethrin expected to be absorbed by netting} = \frac{ns \times N}{N + S}, \text{ where:}$$

ns = permethrin absorbed by composite piece of netting and sheeting (obtained from weight of emulsion absorbed by composite piece and emulsion percentage)

N = permethrin absorbed by separate piece of netting,

S= permethrin absorbed by separate piece of sheeting,

$$\text{Permethrin expected to be absorbed by sheeting} = \frac{ns \times S}{N + S}$$

After drying each of the separate and composite pieces of fabric were cut into two sections. Immediately after drying one section was divided into its two components by unpicking the stitches joining them together and each was sent to Wellcome Research Laboratories immediately after drying for chemical analysis. The other section was hung in the laboratory for ageing. After 14 weeks that section was also unpicked and sent for chemical analysis.

4.2.3. Results of chemical analysis:

The expected permethrin content of each of the fabric types was calculated as described above and it was also measured by chemical analysis. The expected and observed doses in g/m^2 are shown in tables 4 and 5 for the fabrics impregnated on two different occasions. Calculation of the expected permethrin content, on the assumption of non-selective absorption, was done on the basis of the amount of emulsion absorbed. Table 4 shows that the permethrin content, observed by chemical analysis, of each of the fabric types was similar to that expected. Only in the case of cotton-polyester sheeting was the observed permethrin content much less than expected. The permethrin content of the nylon netting dipped at the end of the series (serial number 8) was almost identical to that dipped at the beginning of the series (serial number 1).

Table 5 shows that the permethrin contents of freshly impregnated fabrics are almost the same as of fabrics aged for 14 weeks after impregnation. The observed values of the permethrin contents of various fabrics are more or less similar to the expected values. The permethrin content of the nylon netting that was dipped at the end of the series (serial no. 20) was not less than that at the beginning of the series. Both the tables show that the observed permethrin content of each of the fabric types was approximately proportional to the weight of emulsion absorbed.

Table 4. Expected and observed permethrin content of fabrics impregnated by dipping a series of pieces in a permethrin emulsion (strength of emulsion was 0.417%)

Serial number of dipping	Fabric type	Weight of emulsion absorbed (g/m^2)	Permethrin content (g/m^2)	
			Expected	Observed
1	Nylon netting	45.0	0.188	0.269
2	Polyester netting	40.9	0.171	0.174
3	Cotton netting	124.4	0.520	0.568
4	Cotton sheeting	217.6	0.908	0.782
5	Cotton poplin sheeting	181.2	0.756	0.581
6	Polyester sheeting	126.5	0.528	0.690
7	Cotton-Polyester sheeting	243.2	1.015	0.446
8	Nylon netting	49.8	0.207	0.289

Table 5. Permethrin content of various types of separate and composite fabrics impregnated by dipping in a permethrin emulsion.

Serial number of dipping in the emulsion (1)	Fabric type (2)	Joined to: (3)	Permethrin content (g/m^2) Expected (4)	Observed: Freshly impregnated (5)	Observed: Aged for 14 weeks (6)
1	Nylon netting	-	0.162	0.389	-
8	"	Nylon sheeting	0.224	0.325	0.436
9	"	Cotton "	0.223	0.430	0.447
10	"	Polyester "	0.193	0.351	0.362
11	"	Cotton-Poly. "	0.192	0.250	0.198
20	"	-	0.219	0.511	-
2	Cotton netting	-	0.597	0.885	-
12	"	Nylon sheeting	0.712	0.529	0.490
13	"	Cotton "	0.733	0.531	0.607
14	"	Polyester "	0.671	0.700	0.558
15	"	Cotton-Poly. "	0.672	0.523	0.360
3	Polyester netting	-	0.239	0.472	-
16	"	Nylon sheeting	0.257	0.282	0.314
17	"	Cotton "	0.294	0.415	0.363
18	"	Polyester "	0.261	0.436	0.097
19	"	Cotton-Poly. "	0.269	0.060	0.059

(Contd. to next page)

Table 5 (Contd.)

(1)	(2)	(3)	(4)	(5)	(6)
4	Nylon sheeting	-	0.770	0.635	-
8	"	Nylon netting	1.605	0.438	0.422
12	"	Cotton "	0.918	0.691	0.511
16	"	Polyester "	0.826	0.373	0.313
5	Cotton sheeting	-	0.935	1.221	-
9	"	Nylon netting	1.291	0.918	0.624
13	"	Cotton "	1.147	0.797	0.551
17	"	Polyester "	1.148	0.833	0.714
6	Polyester sheeting	-	0.730	0.612	-
10	"	Nylon netting	0.871	0.458	0.311
14	"	Cotton "	0.868	0.484	0.384
18	"	Polyester "	0.796	0.843	0.413
7	Cotton-Polyester	-	1.143	1.632	-
11	sheeting	Nylon netting	1.357	2.027	0.967
15	"	Cotton "	1.286	1.371	0.904
19	"	Polyester "	1.283	1.091	1.285

4.2.4. Discussion:

It is easy to impregnate fabrics by dipping in an excess of emulsion and then shaking or squeezing off the liquid which has not been absorbed. Schreck and Self (1985b) suggested impregnation of bednets using a measured amount of emulsion equal to that required just to wet the net without any running off. The main objection to this system is that it is very difficult to soak the whole net thoroughly. Another problem in this system is that it is necessary to impregnate each net individually which is a very laborious job. Loong et al. (1985) working in Malaysia found similar problems and so impregnated their nets by dipping them in excess emulsion. Snow et al. (1987b) also expressed the opinion that the method suggested by Schreck and Self is not suitable at the community level.

It has been suggested that permethrin might be absorbed selectively by certain fabrics, but this proved not to be the case with the present method of impregnation using cold water. This is confirmed by the fact that there was no less permethrin than expected in the pieces of nylon netting that were dipped at the ends of the series (serial no. 8 in Table 4 and serial no. 20 in Table 5) and these amounts resembled those absorbed at the beginnings of the series. It may be mentioned that the amount of emulsion used to impregnate the fabrics was only about double that necessary to just wet all the fabrics. So, if there was any process of selective absorption, there would be considerably less permethrin in the nylon netting that was dipped at the end of the series than that dipped at the beginning. However, it will be seen in a later section (section 4.5) that if impregnation is done using hot emulsion at acid pH permethrin is absorbed selectively.

There was no evidence (Table 5) for diffusion of permethrin between pieces of fabrics during impregnation nor creeping during drying and ageing of fabrics. Thus in dipping composite bednets (bednets having a sheeting border) one can assume that each material will take up and hold permethrin proportionally to its absorption of liquid.

As the sheeting absorbs more emulsion (thus more chemical) than netting, to impregnate a composite bednet one should make up an emulsion that would give the target dose to the netting. A bednet impregnated in this way will contain more permethrin in the sheeting border, but this is probably desirable because it is that part of the bednet which is most vulnerable to losing the

impregnated chemical due to abrasion.

The differences between observed and expected values in one or two cases may be due to error either in chemical analysis or during weighing. Unfortunately it was not possible to arrange for repeat analyses to be done in these cases. In Table 4 cotton polyester sheeting showed a serious deviation from expectation but this was not repeated in Table 5. In Table 5 it was polyester netting joined to polyester sheeting and cotton-polyester sheeting which showed the largest deviation from expected.

4.3. Short exposure of mosquitoes to permethrin impregnated netting:

4.3.1. Introduction:

Three different strains of mosquitoes were exposed to various types of permethrin impregnated netting for various exposure times to determine: (i) the relationship of exposure time to knockdown and mortality of these strains of mosquitoes, (ii) the killing effect of permethrin on different types of netting and (iii) the susceptibility of different strains of mosquitoes to permethrin.

4.3.2. Materials and Methods:

Bioassays were performed using three to five day old adult mosquitoes of both sexes in WHO bioassay test kits. Mosquitoes were exposed to impregnated netting instead of the papers commonly used in the WHO test for insecticide resistance in adults. Mosquitoes were exposed to various types of netting, e.g., nylon, cotton and polyester netting (details of these materials are given in Chapter 3.3); in addition to these three netting types another synthetic netting was used in these tests. This netting was brought from The Gambia where it is in common use. It appeared neither like nylon nor polyester

that was being studied in this laboratory. Later this net was identified as polyester by ICI Fibres Division, Harrogate, U.K. This net has been designated as Gambian polyester in the description.

For the purpose of bioassays a method was developed following Schreck et al. (1978a). Netting pieces, 12 x 15 cm, were impregnated with permethrin at a series of doses ranging from 0.025 to 5.0 g/m^2. After drying, the pieces of netting were fixed to similar sized filter papers with Sellotape. Filter papers were used for two reasons - (i) to prevent permethrin from coming in contact with the plastic surface of the test kit, because plastic absorbs permethrin, (ii) to keep the netting in position. Care was taken that as little Sellotape as possible was on the exposure surface of the filter paper to minimize the non-insecticidal surface available to the mosquitoes.

As the exposure times were very short (mostly 30 seconds to 2 minutes), it was important to transfer all the mosquitoes very quickly from the holding tube to the exposure tube and back to the holding tube. Therefore only about 10 mosquitoes were exposed on each occasion. Knockdown was scored one hour post-exposure and mortality was scored 24 hours post-exposure. Mosquitoes that were lying on their back were counted as knocked down. A piece of cotton wool soaked with glucose solution was provided at the end of the holding tube on the nylon gauze during the 24 hour holding period.

Three strains of mosquitoes, namely, the KWA strain of An. gambiae, the CfCA strain of C. quinquefasciatus and the AE AE strain of Ae. aegypti, were exposed for 30 seconds and two minutes to permethrin impregnated nylon, cotton and polyester netting. In addition to 30 seconds and two minutes exposures, CfCA mosquitoes were also exposed for 15 seconds, one minute, four minutes and eight minutes.

4.3.3. Results: Time-dose-response relationship of permethrin to knockdown and mortality of mosquitoes:

For a series of doses and times of exposures the raw data including the number of mosquitoes tested, number knocked down, number dead and number alive are presented in appendices 1 to 35 and the computed KD_{50}, KD_{90}, LD_{50} and LD_{90} and heterogeneity chi square values about the regression line with degrees of freedom are presented in appendices 36 to 39. The KD_{50}, KD_{90}, LD_{50} and LD_{90} values were multiplied by the time of exposure to give a series of c.t values

(Busvine 1971). The c.t values for the KWA and the AE AE are presented in Table 6 and those for the CfCA are presented in Table 7. Table 6 shows that the c.t value for two minutes exposure is always higher than that for 30 seconds exposure. Table 7 shows in general an increase in c.t value with increasing exposure time; almost the only exceptions were between the longest times tested - 4 and 8 minutes.

4.3.4. Discussion:

In the present investigation it was observed that ED_{50} or ED_{90} x exposure time is not constant for permethrin: rather this value increases along with the increase of exposure time. This is contradictory to other authors' work with other groups of insecticides where dose and time are "interchangable," i.e. doubling the dose and simultaneously halving the time of exposure leads to the same insecticidal effect. For example, Busvine (1958) showed that LC_{50} x time = constant for dieldrin against Ae. aegypti. Rongsriyam and Busvine (1973) observed that the c.t. values of carbamate and organophosphate insecticides against C. quinquefasciatus are constant. Similar results were obtained by other authors using DDT, dieldrin, carbamates and organophosphates against Ae. aegypti and C. quinquefasciatus (Ariaratnam and Brown 1969, Hamon 1963, Sales and Mouchet 1973).

The results of the present investigation indicate that permethrin is relatively more effective at a shorter exposure time than at a longer exposure time. This presumably indicates that mosquitoes do not absorb permethrin at the same rate throughout the exposure period, rather the rate of absorption decreases as the exposure time increases, apparently because of the saturation of some part of the absorption mechanism.

Table 6. Time-dose-response relationship for mosquitoes of the KWA and AE AE strains when exposed to permethrin impregnated netting.

Mosquito strain	Exposure time	Netting type	KD_{50}xTime	KD_{90}xTime	LD_{50}xTime	LD_{90}xTime
KWA	30 secs	Nylon	0.236	0.573	0.241	0.626
"	2 mins	"	0.380	1.298	0.446	1.586
"	30 secs	Cotton	0.814	1.333	0.658	1.351
"	2 mins	"	1.658	3.638	1.402	3.868
"	30 secs	Polyester	0.283	0.611	0.343	0.902
"	2 mins	"	0.518	0.996	0.482	1.132
AE AE	30 secs	Nylon	0.033	0.291	0.088	1.009
"	2 mins	"	0.132	0.424	0.330	1.244
"	30 secs	Polyester	0.144	0.320	0.176	0.509
"	2 mins	"	0.246	0.606	0.302	0.940

Table 7. Time–dose–response relationship for C. quinquefasciatus (CfCA strain) when exposed to pieces of permethrin impregnated netting.

Exposure time	Netting type	KD_{50}xTime	KD_{90}xTime	LD_{50}xTime	LD_{90}xTime
15 secs	Nylon	0.131	0.541	0.137	0.599
30 secs	"	0.316	1.262	0.442	2.932
1 min	"	0.444	1.377	0.541	2.046
2 mins	"	0.594	2.168	0.822	4.008
4 mins	"	0.620	2.756	0.840	4.684
8 mins	"	0.616	2.488	0.952	5.400
15 secs	Cotton	0.583	1.426	0.831	2.330
30 secs	"	0.839	2.027	1.049	4.440
1 min	"	1.279	5.826	1.426	5.734
2 mins	"	1.752	5.760	2.350	13.266
4 mins	"	4.572	17.380	5.996	35.620
8 mins	"	5.720	25.928	4.888	23.440
15 secs	Polyester	0.374	1.907	0.419	3.202
30 secs	"	0.561	2.204	0.657	3.722
1 min	"	0.750	2.466	0.956	4.166
2 mins	"	0.942	3.326	1.336	6.594
4 mins	"	1.240	4.132	1.476	7.556
8 mins	"	1.528	5.416	1.960	8.248
15 secs	Gambian	0.421	1.194	0.420	1.499
30 secs	synthetic	0.737	2.616	0.684	3.169
1 min	"	0.891	3.144	1.019	5.547
2 mins	"	1.120	4.092	1.468	9.300
4 mins	"	2.120	7.732	3.044	18.904
8 mins	"	1.120	7.488	1.424	16.728

4.3.5. Results: Comparison of the killing effect of permethrin on different types of netting and different species of mosquitoes:

The LD_{50} values of permethrin for 4 different types of netting materials and 3 different strains of mosquitoes at two exposure times are presented in Tables 8 and 9. The tables are extracted from appendices 36 to 39. Table 8 shows that nylon is the most effective and cotton is the least effective as a medium for permethrin impregnation. Nylon is about 3 times more effective than cotton on the basis of LD_{50} values. The values for polyester netting and Gambian polyester netting are generally slightly higher than that of nylon.

Table 9 shows that with only one exception AE AE is the most susceptible to permethrin of the 3 strains studied. CfCA is the least susceptible.

Table 8. Comparative effectiveness of various netting types as media for permethrin impregnation.

Netting type	Mosquito strain	Exposure time	LD_{50} (g/m^2)	LD_{50} value relative to nylon
Nylon	KWA	30 secs	0.481	1
Cotton	KWA	30 "	1.315	2.7
Polyester	KWA	30 "	0.685	1.4
Nylon	KWA	2 mins	0.223	1
Cotton	KWA	2 "	0.701	3.1
Polyester	KWA	2 "	0.241	1.1
Nylon	AE AE	30 secs	0.176	1
Polyester	AE AE	30 "	0.352	2.0
Nylon	AE AE	2 mins	0.165	1
Cotton	AE AE	2 "	0.878	5.3
Polyester	AE AE	2 "	0.151	0.9
Nylon	CfCA	30 secs	0.884	1
Cotton	CfCA	30 "	2.098	2.4
Polyester	CfCA	30 "	1.314	1.5
Gambian polyester	CfCA	30 "	1.367	1.5
Nylon	CfCA	2 mins	0.411	1
Cotton	CfCA	2 "	1.175	2.9
Polyester	CfCA	2 "	0.668	1.6
Gambian polyester	CfCA	2 "	0.734	1.9

Table 9. Comparative susceptibility of three species of mosquitoes to permethrin.

Mosquito strain	Netting type	Exposure time	LD_{50} (g/m^2)	Ratios of LD_{50} values to KWA
KWA	Nylon	30 secs	0.481	1
AE AE	Nylon	30 "	0.176	0.4
CfCA	Nylon	30 "	0.884	1.8
KWA	Nylon	2 mins	0.223	1
AE AE	Nylon	2 "	0.165	0.7
CfCA	Nylon	2 "	0.411	1.8
KWA	Cotton	30 secs	1.315	1
CfCA	Cotton	30 secs	2.098	1.6
KWA	Cotton	2 mins	0.701	1
AE AE	Cotton	2 "	0.878	1.3
CfCA	Cotton	2 "	1.175	1.7
KWA	Polyester	30 secs	0.685	1
AE AE	Polyester	30 "	0.352	0.5
CfCA	Polyester	30 "	1.314	1.9
KWA	Polyester	2 mins	0.241	1
AE AE	Polyester	2 "	0.151	0.6
CfCA	Polyester	2 "	0.688	2.6

4.3.6. Discussion:

Bioassay results show that permethrin impregnated synthetic netting is better than similarly impregnated cotton netting against all the mosquito species tested at both exposure times. Lines et al. (1985) also observed that permethrin impregnated synthetic netting performed better than permethrin impregnated cotton netting against wild populations of malaria vectors in Tanzania. Hervy and Sales (1984), however, did not observe any difference in performance between permethrin impregnated synthetic and cotton netting. The authors also reported that deltamethrin impregnated cotton netting was better than deltamethrin impregnated synthetic netting when bioassayed with Ae. aegypti. However, the authors exposed mosquitoes for one hour which is less realistic than the short exposures used in the present work.

The better performance of synthetic netting over cotton netting may be due to their different texture. The threads of synthetic netting are much smoother than those of cotton (Fig. 6) and it seems likely that most of the permethrin remains on the outer surface of synthetic fibres and hence easily available to mosquitoes. It seems likely that some permethrin is 'lost' in the crevices on rough cotton fibres.

To study the comparative susceptibility of different species of mosquitoes 3 susceptible strains belonging to different genera were selected. It is generally accepted that C. quinquefasciatus is more tolerant to most insecticides than anopheline mosquitoes. For detection of resistance WHO recommended exposure of C. quinquefasciatus to 0.25% permethrin for 3 hours but anophelines for one hour to the same concentration (WHO 1986). There is no such recommendation of difference in discriminating dose or discriminating time between anopheline and Aedes mosquitoes. The difference in susceptibility to permethrin of An. gambiae and Ae. aegypti as revealed in the present investigation may be due to the fact that An. gambiae is more irritated than Ae. aegypti by permethrin. Thus a part of the short exposure period was spent flying within the tube by KWA, which was not true for AE AE. So it is possible that Ae. aegypti picked up more permethrin than An. gambiae.

Figure 6. Photographs of three different types of netting. The specimens were gold sputted coated before examining in the S.E.M. Magnification of prints x34.

Nylon

Cotton

Polyester

4.4. Persistence of permethrin on impregnated netting under various conditions as measured by bioassays:

4.4.1. Introduction:

Two experiments were performed to study the persistence of permethrin on nylon netting. The first experiment was done to see the effect of ageing and exposure of mosquitoes to impregnated netting. The second experiment was performed to study the effect of washing and/or drying in sunlight on the persistence of permethrin on nylon netting.

4.4.2. Methods:

For the first experiment eight sets of nylon netting were impregnated at a series of doses. Bioassays for 2 minutes were performed on these netting samples using the KWA strain of An. gambiae. Knockdown was scored one hour post-exposure and mortality 24 hour post-exposure as before. KD_{50} with 95% confidence limits, KD_{90}, LD_{50} with 95% confidence limits, LD_{90} and heterogeneity chi square values about the regression lines were determined using a computer program. One set of netting was used every week throughout the 12 week period, except weeks 8 and 9 due to non-availability of mosquitoes. Each of the other sets were used for one week only, e.g. set 2 in week 3, set 3 in week 4, and so on. Netting of set 2 to set 8 was hung in a warm room (25°C) until they were used, netting of set 1 was kept in similar conditions in between their repeated exposures.

For the second experiment four sets of netting were impregnated with permethrin at doses of 0.4 and 0.8 g/m^2. After impregnation one of these sets was dried outdoors in direct sunlight on a roof in London on a sunny day in the month of July at a temperature of about 27°C. The other three sets were dried indoors. After drying indoors two sets were washed by hand, using laundry soap (Puritan, Lever Brothers, U.K.). Netting pieces, 12 x 15 cm, were rubbed between the fingers for two minutes in soapy water and rinsed for one minute with cold tap water. One set was then dried outdoors in sunlight and the other set was dried indoors. Bioassays were done using the KWA strain. A similar process of washing and drying was performed using cow fat soap from The Gambia.

4.4.3. Results: Effect of ageing and exposure of mosquitoes:

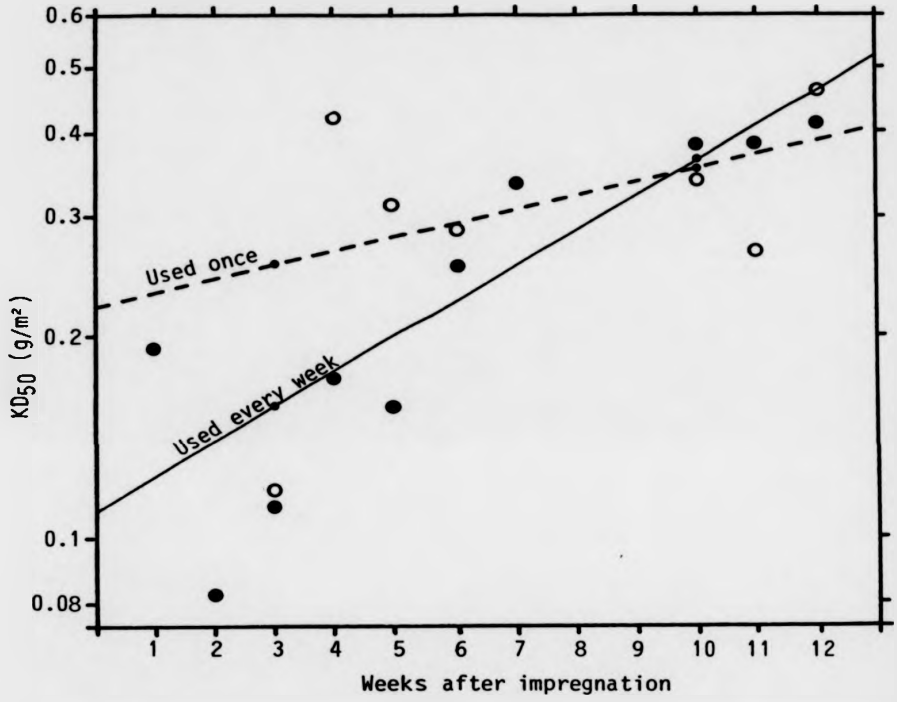
The KD_{50}, KD_{90}, LD_{50} and LD_{90} values from exposure of An. gambiae to both the sets of netting are marked on log-dose graph paper and are presented in Figures 7 to 10. The heterogeneity chi square values about the regression lines are significant in six cases out of the seven netting sets that were used only once. Thus confidence limits could not justifiably be attached to the estimates of ED_{50}. A type II regression was done of the estimated ED_{50} and ED_{90} values against number of weeks since impregnation. The percentage mortality with 95% confidence limits at each of the doses are plotted on log-dose/probit-mortality scales for weeks 3, 4, 5, 6, 10, 11 and 12 for both the sets of netting ; these are presented in Appendices 40 to 46.

4.4.4. Discussion:

The analyses of variance in Figures 9 and 10 show that there was no significant loss of insecticidal activity over 12 weeks of use, during which mosquitoes were exposed to one of the sets of netting two to three times each week for 10 weeks. The change of KD_{90} value with time was also found non-significant as was that of KD_{50} value for netting samples used once only. However, the KD_{50} and KD_{90} values for the netting samples used repeatedly did show a significant rise with time (Figs 7 and 8).

Figure 7

The KD_{50} values of permethrin impregnated nylon netting in relation to ageing and use for testing mosquitoes. The regressions were calculated and the lines are shown on the graph. Analyses of variance of the significance of the regressions are also shown.(● = netting used every week for testing, O = netting used once).



Netting used every week

	d.f	S.S	M.S	F
Regression	1	0.0115	0.0115	44.07**
Residual	8	0.0021	0.0002	
Total	9	0.0136		

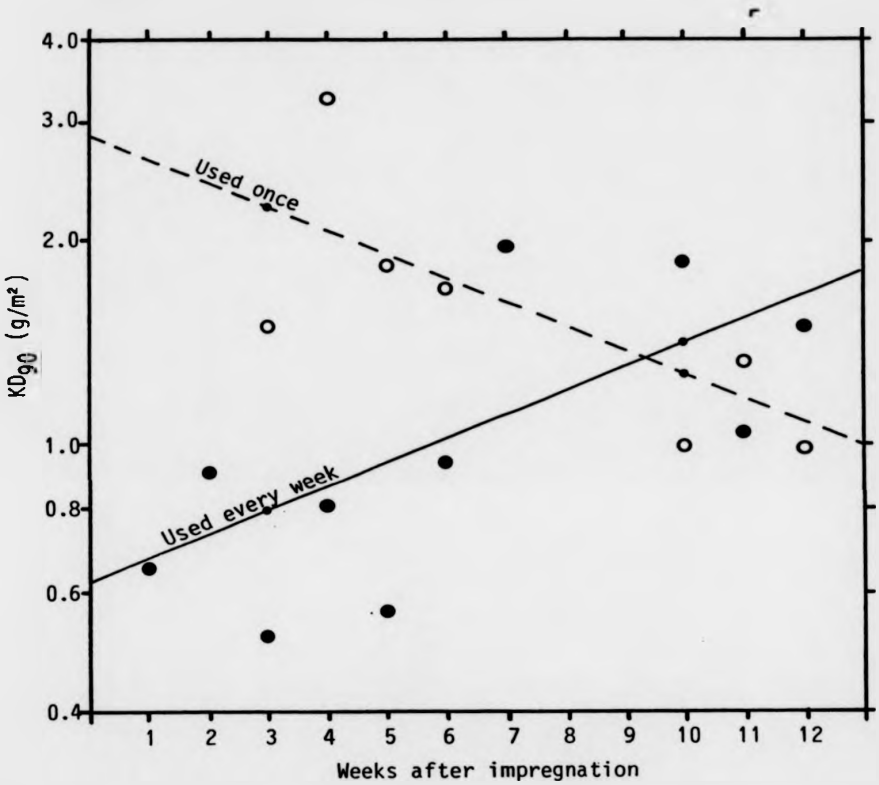
* significant

Netting used once

	d.f	S.S	M.S	F
Regression	1	0.0015	0.0015	1.32
Residual	5	0.0060	0.0012	
Total	6	0.0075		

Figure 8

KD_{90} values from the same tests as those referred to in Fig.7.



Netting used every week

	d.f	S.S	M.S	F
Regression	1	0.1048	0.1048	5.92*
Residual	8	0.1418	1.7728	
Total	9	0.2466		

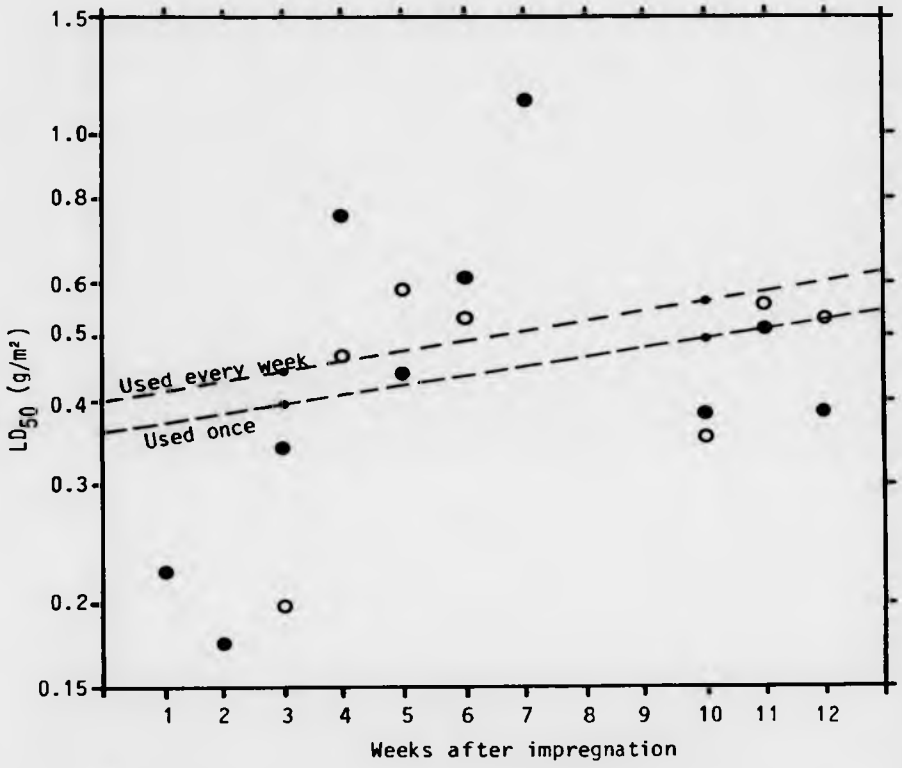
* significant

Netting used once

	d.f	S.S	M.s	F
Regression	1	0.1591	0.1591	3.84
Residual	5	0.2073	0.0414	
Total	6	0.3664		

Figure 9

LD_{50} values from the same tests as those referred to in Fig.7.



Netting used every week

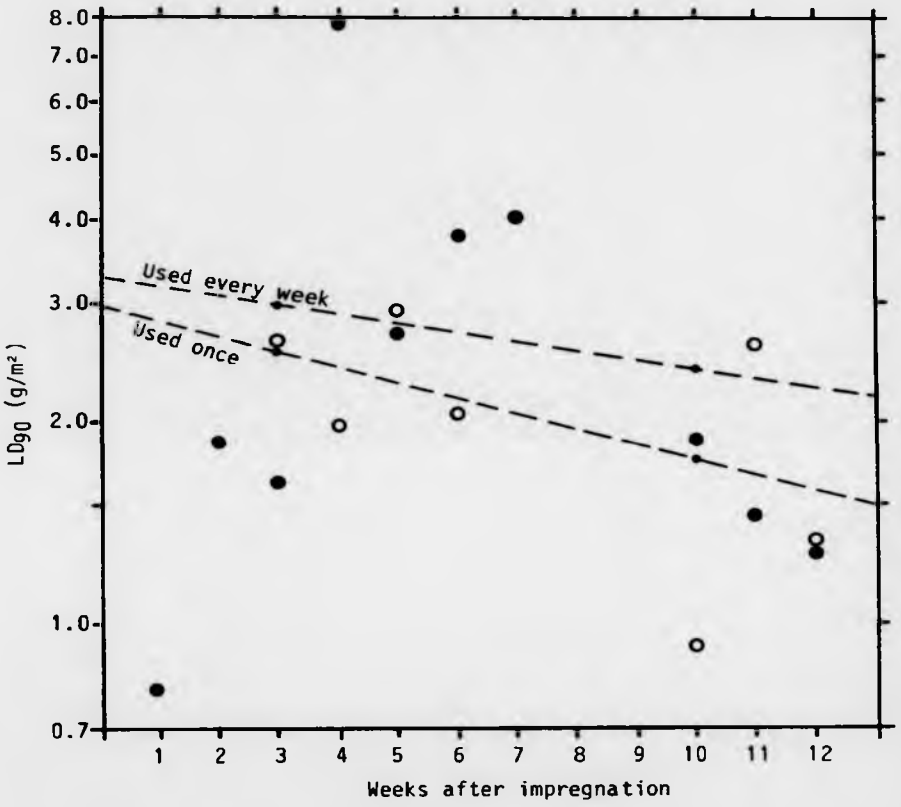
	d.f	S.S	M.S	F
Regression	1	0.0381	0.0381	0.46
Residual	8	0.6521	0.0815	
Total	9	0.6902		

Netting used once

	d.f	S.S	M.S	F
Regression	1	0.0095	0.0095	2.12
Residual	5	0.0224	0.0044	
Total	6	0.319		

Figure 10

LD_{90} values from the same tests as those referred to in Fig.7.



Netting used every week

	d.f	S.S	M.S	F
Regression	1	0.0958	0.0958	0.20
Residual	8	3.9187	0.4898	
Total	9	4.0145		

Netting used once

	d.f	S.S	M.S	F
Regression	1	0.0953	0.0953	2.12
Residual	5	0.2245	0.0449	
Total	6	0.3198		

4.4.5. Results: Effect of washing and drying:

Results of bioassays on impregnated netting that was unwashed or washed using soap bought in London are presented in Table 10 and Figure 11; that of another series of experiments with unwashed netting or netting washed with Gambian cow fat soap are presented in Table 11 and Figure 12. The 95% confidence limits about the mortality estimates are also shown in the figures. No significant effect of washing was observed when netting was washed using Puritan soap bought in London. However, there was a significant difference in mortality after a 2 minutes exposure to 0.4 g/m^2 between washed and unwashed netting when they had been washed using Gambian cow fat soap. With 30 secs exposure there was a significantly lower mortality with netting dried outside exposed to sunlight.

4.4.6. Discussion:

Sometimes it is necessary to wash bednets and in some communities, e.g. the Mandinka tribe in The Gambia, it is customary to do so about every two weeks (MacCormack and Snow 1985). Studies have been performed by various authors to evaluate the effect of washing on the persistence of permethrin on impregnated netting, the results are equivocal. Schreck and his colleagues found on different occasions that permethrin when impregnated into military uniforms withstood 3 to 4 machine washes with detergent and gave very good protection against mosquitoes and ticks after washing (Schreck et al. 1980a, 1982d). However, Snow et al. (1987a) observed in The Gambia that hand washing of permethrin impregnated bednets severely reduced the toxicity and approximately halved the permethrin content.

In view of the above findings it was necessary to find out whether the loss of permethrin was due to the effect of handwashing with soap, or the effect of drying in the sun (in The Gambia nets were dried in the sun), or a combined effect of both. Therefore, an experiment was carried out to investigate these questions. It was observed that washing and/or drying in the sun has no effect on the persistence of permethrin when the netting was washed using 'Puritan' soap bought in London. So, the question arose whether the loss

of permethrin, as observed by Snow et al. (1987a), was due to the use of the particular cow fat soap available in Gambian markets. Therefore, a second experiment was performed with such soap. As it is well known that permethrin is relatively photostable (Elliot et al. 1973) and as in the first experiment it was observed that there was no effect of sunlight on permethrin, unwashed netting dried after impregnation outdoors was not evaluated in the second experiment.

Very high mortality was obtained using 0.8 g/m^2 with two minutes exposure and no effect of washing and drying could be detected in this case. There was a significant difference in knockdown and mortality between washed and unwashed netting impregnated at 0.4 g/m^2 when the mosquitoes were exposed for two minutes. When mosquitoes were exposed for 30 seconds to either dose, it was observed that there was a significant difference in knockdown and mortality between netting dried indoors (both washed and unwashed) and netting washed and dried outdoors. It was observed that there was no significant difference between washed and unwashed netting, both dried indoors, when mosquitoes were exposed for 30 seconds though the observed mortality was less among mosquitoes exposed to washed netting than among those exposed to unwashed netting. Therefore, on the basis of above findings it may be concluded that there are indications that washing with Gambian cow fat soap and drying for 6 hours in sunlight reduces the effectiveness of impregnated netting.

In conclusion it can be said that this is a preliminary observation and more work should be done in this field using different varieties of soap and drying methods. If it is proved that some soap has a severe effect on the persistence of permethrin on netting, it would be necessary to analyse which particular constituent of a soap is responsible. Ultimately it may be necessary to recommend the use of laundry soap without that constituent. The apparent effect of 6 hours drying in sunlight suggests that one should minimize the time that impregnated nets are left out for drying especially in very strong tropical sunlight.

Table 10. Effect of washing with soap (Puritan brand) bought in London and drying indoors or outdoors on persistence of permethrin on pieces of nylon netting as measured by bioassays.

Dose (g/m^2)	Netting treatment*	Exposure time	Knockdown (%)	Mortality (%)	Total number of mosquitoes
0.4	Washed/Outdoors	30 secs	47.1	47.1	34
0.4	Unwashed/Outdoors	30 "	51.7	55.2	29
0.4	Washed/Indoors	30 "	44.1	52.9	34
0.4	Unwashed/Indoors	30 "	29.4	23.5	34
0.8	Washed/Outdoors	30 "	86.7	90.0	30
0.8	Unwashed/Outdoors	30 "	100	100	30
0.8	Washed/Indoors	30 "	100	100	32
0.8	Unwashed/Indoors	30 "	85.3	91.2	34
0.4	Washed/Outdoors	2 mins	91.3	100	23
0.4	Unwashed/Outdoors	2 "	94.7	100	19
0.4	Washed/Indoors	2 "	85.7	90.5	21
0.4	Unwashed/Indoors	2 "	80.0	85.0	20
0.8	Washed/Outdoors	2 "	100	100	22
0.8	Unwashed/Outdoors	2 "	100	100	25
0.8	Washed/Indoors	2 "	100	100	23
0.8	Unwashed/Indoors	2 "	100	100	24

*Outdoors = dried out of doors in the sun at about 27°C for 6 hours
Indoors = dried indoors

Figure 11

Mortality of the KWA strain of An.gambiae exposed to pieces of pemethrin impregnated nylon netting either unwashed or washed with soap (Puritan brand) bought in London. The netting pieces were dried either indoors or outdoors in the sunlight. Confidence limits were obtained from the binomial distribution.

Washed/unwashed	W	U	W	U	W	U	W	U	W	U	W	U	W	U	W	U
Drying	OUT	OUT	IN	IN	OUT	OUT	IN	IN	OUT	OUT	IN	IN	OUT	OUT	IN	IN
Dose(g/m²)	0.4	0.4	0.4	0.4	0.8	0.8	0.8	0.8	0.4	0.4	0.4	0.4	0.8	0.8	0.8	0.8
Exp. time(min)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0

W = washed, U = unwashed, OUT = outdoors, IN = indoors

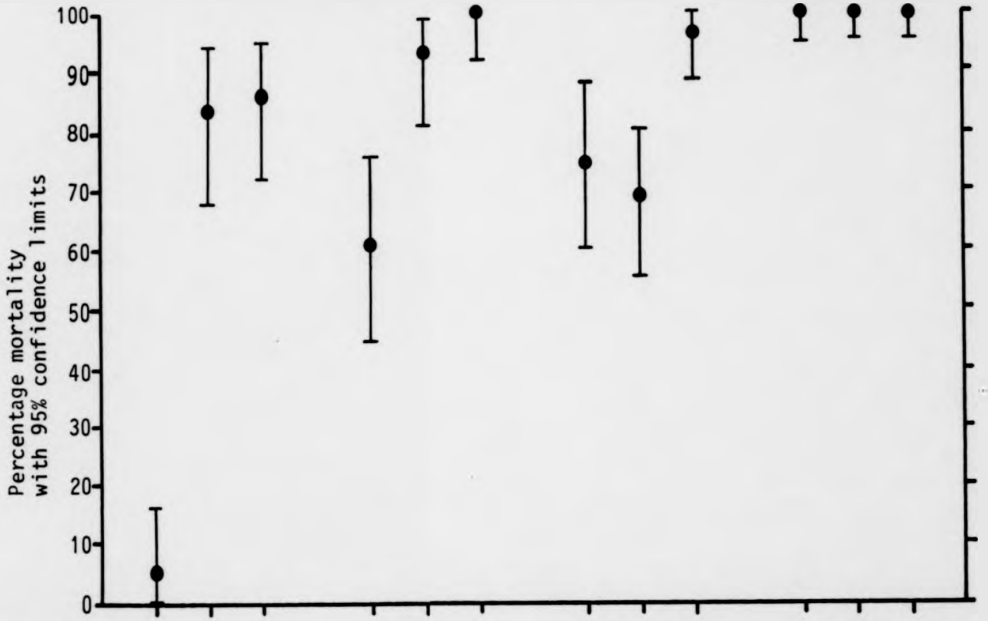
Table 11.Effect of washing with Gambian cow fat soap and drying indoors or outdoors on persistence of permethrin on pieces of nylon netting as measured by bioassays.

Dose (g/m^2)	Netting treatment*	Exposure time	Knockdown (%)	Mortality (%)	Total number of mosquitoes
0.4	Washed/Outdoors	30 secs	0	4.8	42
0.4	Washed/Indoors	30 "	59.5	83.8	37
0.4	Unwashed/Indoors	30 "	81.4	86.1	43
0.8	Washed/Outdoors	30 "	51.2	61.0	41
0.8	Washed/Indoors	30 "	86.7	93.3	45
0.8	Unwashed/Indoors	30 "	100	100	42
0.4	Washed/Outdoors	2 mins	71.4	76.2	42
0.4	Washed/Indoors	2 "	60.3	69.0	58
0.4	Unwashed/Indoors	2 "	100	96.7	61
0.8	Washed/Outdoors	2 "	94.6	100	69
0.8	Washed/Indoors	2 "	100	100	73
0.8	Unwashed/Indoors	2 "	100	100	70

*Outdoors = dried in the sun out of doors for 6 hours at about 27°C
Indoors = dried indoors

Figure 12

Mortality of the KWA strain of An.gambiae exposed to pieces of pemethrin impregnated nylon netting either unwashed or washed with gambian cow fat soap. The netting pieces were dried either indoors or outdoors in sunlight. Confidence limits were obtained from the binomial distribution.



Washed/unwashed	W	W	U	W	W	U	W	W	U	W	W	U
Drying	OUT	IN	IN	OUT	IN	IN	OUT	IN	IN	OUT	IN	IN
Dose(g/m²)	0.4	0.4	0.4	0.8	0.8	0.8	0.4	0.4	0.4	0.8	0.8	0.8
Exp.time(min)	0.5	0.5	0.5	0.5	0.5	0.5	2.0	2.0	2.0	2.0	2.0	2.0

W = washed, U = unwashed, OUT = outdoors, IN = indoors

4.5. Effectiveness of different pyrethroids:

4.5.1. Introduction:

Pieces of polyester netting were impregnated by Drs S. Lindsay and C.F. Curtis with nine different pyrethroids to study their comparative effectiveness against mosquitoes and persistence of these chemicals under the conditions of washing and ageing. The main motivation for this work was the report by Snow et al. (1987a) of a very serious effect of washing on the effectiveness of permethrin and the hope that another formulation of permethrin or another pyrethroid would avoid this problem.

4.5.2. Methods:

The chemicals and the target doses used were as follows:-

Cyfluthrin	0.1 g/m^2
Cyfluthrin	0.2 g/m^2
Cypermethrin	0.1 g/m^2
Cypermethrin	0.2 g/m^2
Cyphenothrin	0.1 g/m^2
Cyphenothrin	0.2 g/m^2
Deltamethrin	0.025 g/m^2
Deltamethrin	0.05 g/m^2
d-phenothrin	0.2 g/m^2
d-phenothrin	0.5 g/m^2
Fenpropathrin	0.1 g/m^2
Fenpropathrin	0.2 g/m^2
Fenvalerate	0.1 g/m^2
Fenvalerate	0.2 g/m^2

Permethrin (Wellcome, 20% E.C., cis:trans = 25:75)	0.2 g/m^2
Permethrin (Wellcome, 20% E.C., cis:trans = 25:75)	0.5 g/m^2
Permethrin (Wellcome) + Agral	0.2 g/m^2
Permethrin (Wellcome) + Agral	0.5 g/m^2
Permethrin (Wellcome, treated at 97°C and pH 3.4)	0.2 g/m^2
Permethrin (Wellcome, treated at 97°C and pH 3.4)	0.5 g/m^2
Permethrin (ICI, 25% E.C., cis:trans = 40:60)	0.2 g/m^2
Permethrin (ICI, 25% E.C., cis:trans = 40:60)	0.5 g/m^2
PP 321	0.025 g/m^2
PP 321	0.05 g/m^2

Each of the chemicals was used at two doses. The doses were chosen to be lower in the case of those compounds known to be more insecticidally active (Leahy, 1985), so that approximately equal mosquito kills were expected with each compound. Permethrin was used from two manufacturers and that from Wellcome was used in three different conditions.

All the insecticides were impregnated into polyester netting following the system described earlier, except the hot water treatment of permethrin in acid pH. This treatment was done following the system of Bry et al. (1976) for moth-proofing wool. A permethrin emulsion was prepared, the concentration of which was adjusted in such a way that it would give a dose of either 0.2 or 0.5 g/m^2 to impregnated netting if treated cold. Reagent grade glacial acetic acid was then added to the emulsion so that the pH was adjusted to 3.4 . This emulsion was then boiled and netting was impregnated as the emulsion began to cool. The temperature of the emulsion during impregnation was 97°C.

Two sets of netting were impregnated with each of the insecticides and each of the doses. After impregnation all the netting samples were hung in a hut at the Medical Research Council Farafenni field station in The Gambia. One of the sets of netting was washed by a Gambian housewife using local cow fat soap once every two weeks for six weeks (first wash - two days after treatment). One piece from each of the impregnated samples (both washed and unwashed) was cut at week 0, week 2 and week 6 and brought to London for bioassay. Bioassays were also done on unwashed netting three months after

impregnation. The G3 and AE AE strains were used for the bioassays. Chemical analysis was done by the ICI Plant Protection Division to determine the chemical content of the netting. Chemical analysis was done of all the samples aged for two weeks (both washed and unwashed) and most of the freshly impregnated samples (both washed and unwashed). Chemical analysis of fenvalerate impregnated netting, however, was not performed. The nets were impregnated with this chemical later than those with the other chemicals. So, it was not possible to arrange for chemical analysis of these nets.

4.5.3. Results:

4.5.3.1. Freshly impregnated netting:

The mortality of Ae. aegypti (AE AE) and An. gambiae (KWA) following 30 second and three minute exposures to freshly impregnated netting are shown in Figures 13 to 16. Results of chemical analysis of freshly impregnated netting and netting aged for two weeks, both washed and unwashed, are presented in Tables 12 and 13. The expected dose of each of the chemicals are also shown in the same tables.

Percentage knockdown and mortality of both the species at both the exposure times and both the doses are shown in appendices 47 and 48. The total number of mosquitoes at each of the exposure times and each of the doses are also shown.

Cypermethrin and PP321 were found to be the most effective of all the chemicals, resulting in almost 100% mortality to both the mosquito species at both the exposure times and both the doses. These two chemicals were followed by Wellcome permethrin when impregnated in hot water at acid pH. All other tests of permethrin impregnation gave more or less similar results. Cyfluthrin was also found to be very good, giving similar or better performance than permethrin. Fenpropathrin showed good performance against An. gambiae but was not so good against Ae. aegypti. d-phenothrin was found to be the worst of all the chemicals resulting in only negligible mortality in both the species of mosquitoes.

Figure 13

Mortality of the AE AE strain of Ae.aegypti exposed to the lower dose of each of the pyrethroids for 30 seconds and 3 minutes. Mosquitoes were exposed to freshly impregnated unwashed netting.

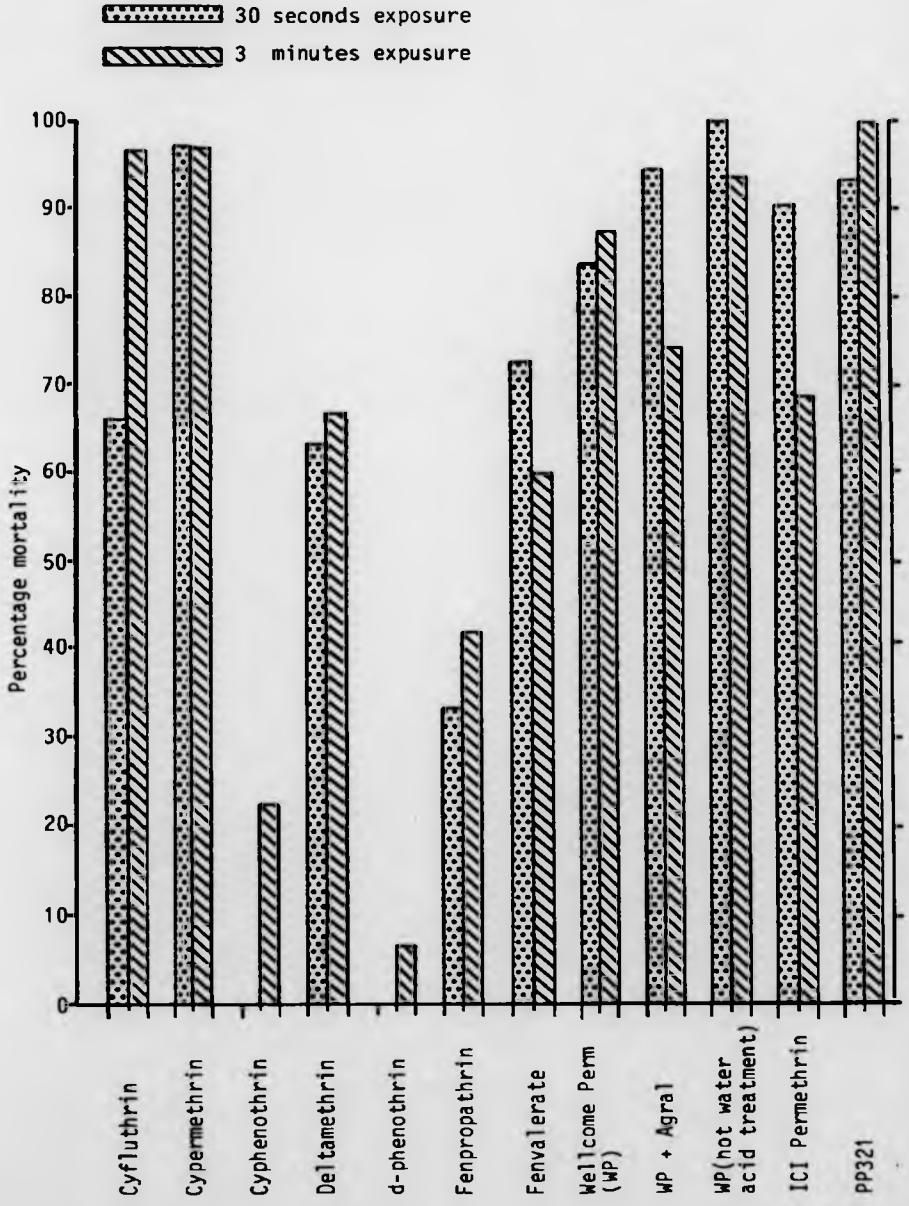


Figure 14

Mortality of the AE AE strain of Ae.aegypti exposed to the higher dose of each of the pyrethroids for 30 seconds and 3 minutes. Mosquitoes were exposed to freshly impregnated unwashed netting.

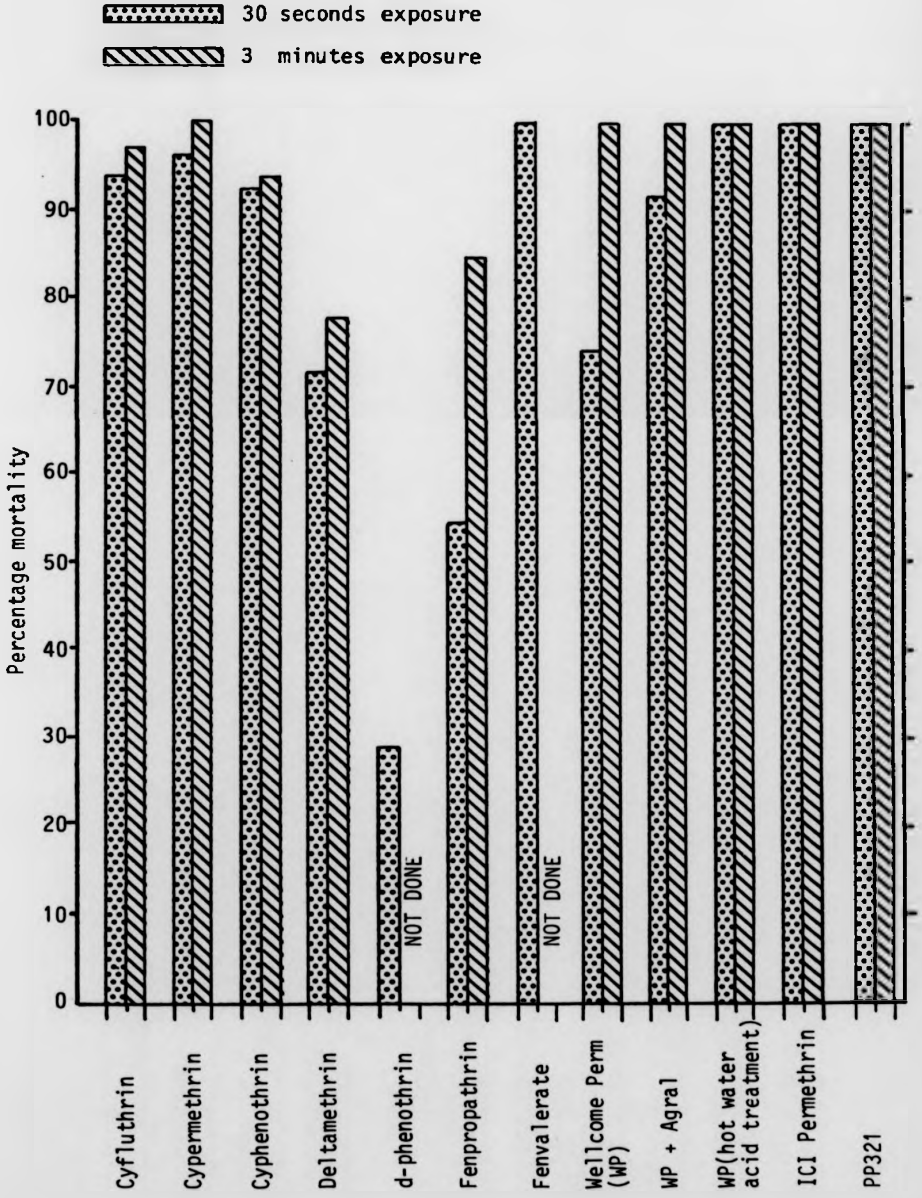


Figure 15

Mortality of the G3 strain of An.gambiae exposed to the lower dose of each of the pyrethroids for 30 seconds and 3 minutes. Mosquitoes were exposed to freshly impregnated unwashed netting.

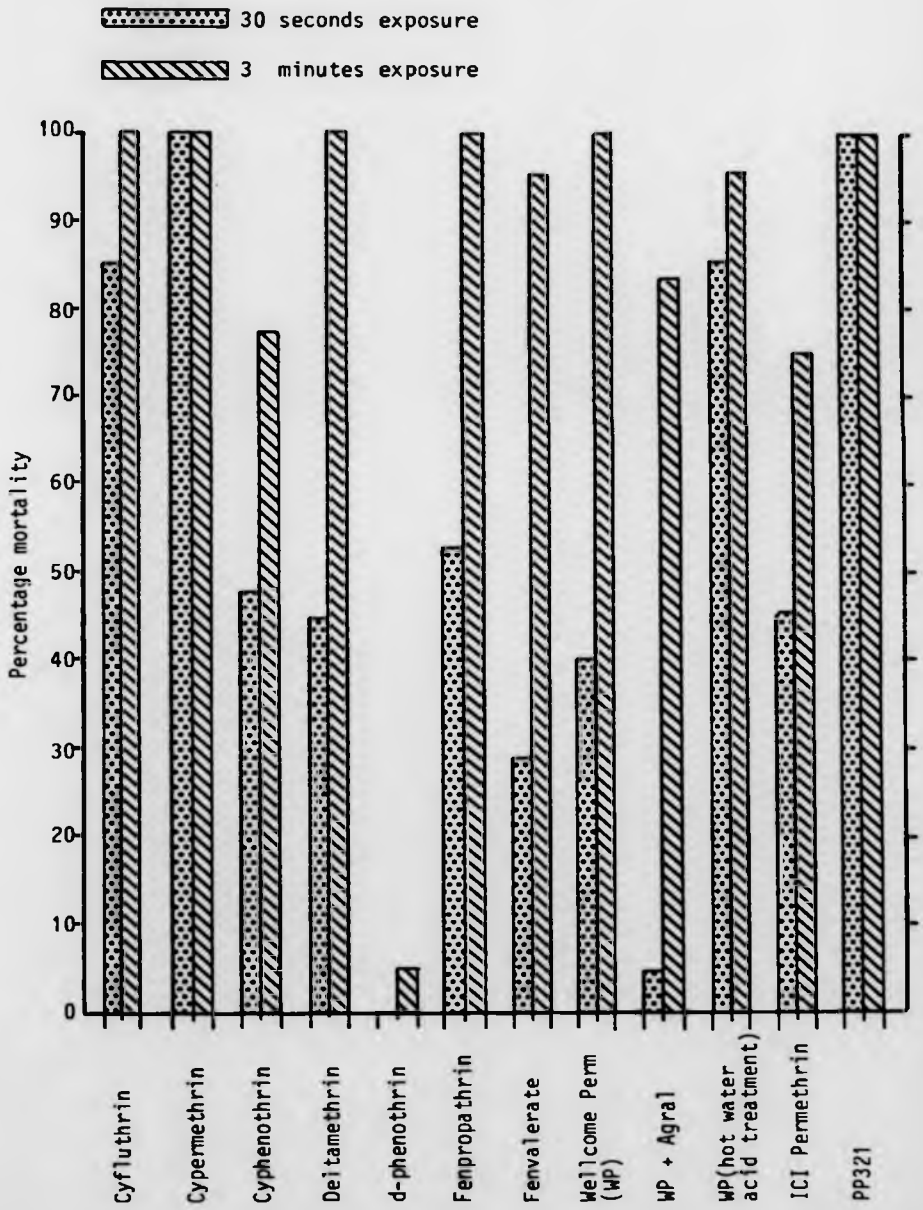


Figure 16

Mortality of the G3 strain of An.gambiae exposed to the higher dose of each of the pyrethroids for 30 seconds and 3 minutes. Mosquitoes were exposed to freshly impregnated unwashed netting.

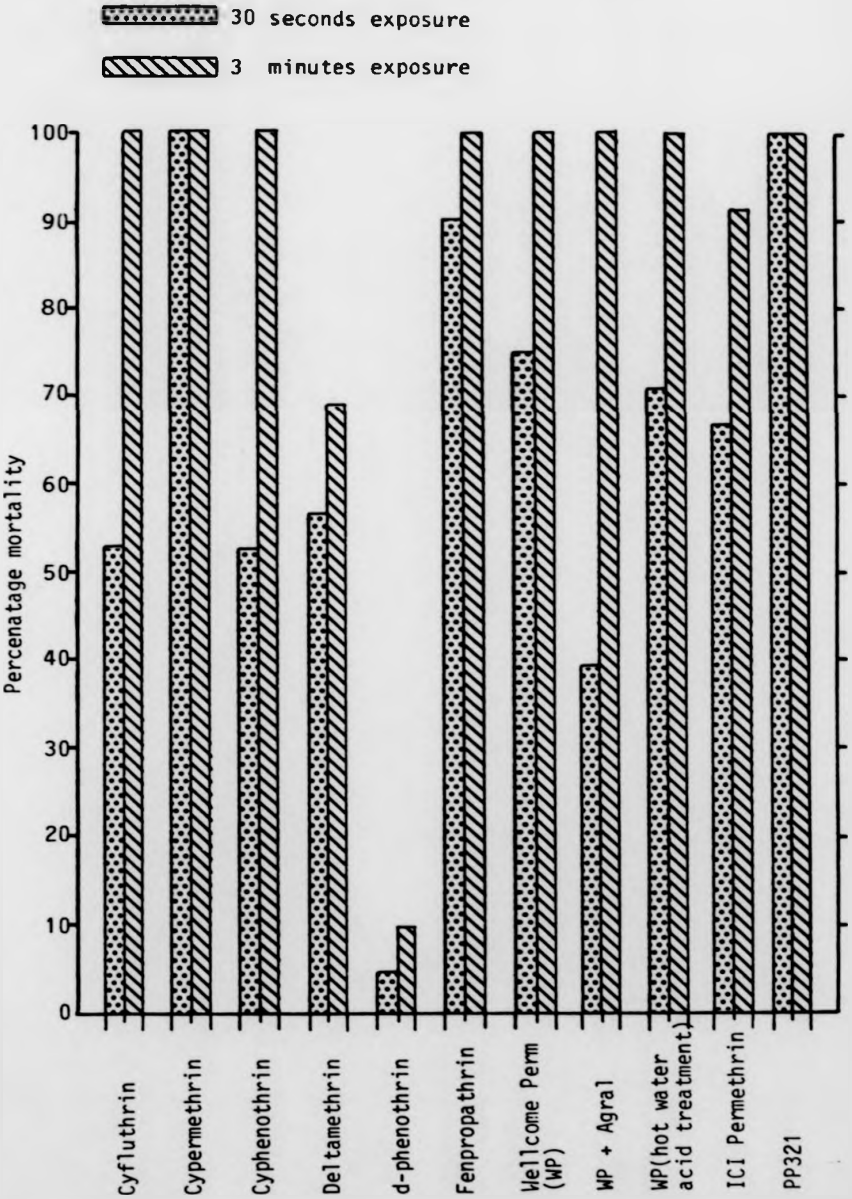


Table 12. Chemical content of netting impregnated with various pyrethroids (lower dose).

Insecticide	Washing information	Expected dose (g/m^2)	Observed dose (g/m^2) Week 0	Week 2
Cyfluthrin	Unwashed	0.1	0.11	0.11
"	Washed		0.03	0.02
Cypermethrin	Unwashed	0.1	0.09	0.08
"	Washed		0.03	0.02
Cyphenothrin	Unwashed	0.1	0.01	None
"	Washed		0.005	None
Deltamethrin	Unwashed	0.025	0.02	0.03
"	Washed		0.007	0.003
d-phenothrin	Unwashed	0.2	0.04	None
"	Washed		0.01	None
Fenpropathrin	Unwashed	0.1	0.1	0.09
"	Washed		0.02	0.05
Wellcome permethrin	Unwashed	0.2	-	0.15
"	Washed		-	0.08
Wellcome permethrin +Agral	Unwashed	0.2	-	0.15
	Washed		-	0.04
Wellcome permethrin (Hot water treatment at acid pH)	Unwashed	0.2	0.66	0.66
	Washed		0.39	0.4
ICI permethrin	Unwashed	0.2	0.19	0.19
"	Washed		0.09	0.07
PP321	Unwashed	0.025	0.022	0.029
"	Washed		0.012	0.01

Table 13. Chemical content of netting impregnated with various pyrethroids (higher dose).

Insecticide	Washing Information	Expected dose (g/m^2)	Observed dose (g/m^2) Week 0	Week 2
Cyfluthrin	Unwashed	0.2	-	0.19
"	Washed		-	0.02
Cypermethrin	Unwashed	0.2	-	0.15
"	Washed		-	0.05
Cyphenothrin	Unwashed	0.2	-	None
"	Washed		-	None
Deltamethrin	Unwashed	0.05	-	0.06
"	Washed		-	0.01
d-phenothrin	Unwashed	0.5	-	None
"	Washed		-	None
Fenpropathrin	Unwashed	0.2	-	0.16
"	Washed		-	0.02
Wellcome permethrin	Unwashed	0.5	-	0.47
"	Washed		-	0.15
Wellcome permethrin + Agral	Unwashed	0.5	-	0.38
	Washed		-	0.14
Wellcome permethrin (Hot water treatment at acid pH)	Unwashed	0.5	-	1.1
	Washed		-	0.69
ICI permethrin	Unwashed	0.5	-	0.27
"	Washed		-	0.17
PP321	Unwashed	0.05	0.074	0.076
"	Washed		0.041	0.041

4.5.3.2. Effect of washing and ageing on persistence of different pyrethroids:

The mortality of Ae. aegypti (AE AE) and An. gambiae (G3) exposed to netting impregnated with various pyrethroids and aged for various periods of time, either washed or unwashed, are shown as histograms. Figure 17 shows the mortality of AE AE exposed for 30 seconds to netting freshly impregnated with the lower dose of each pyrethroid and aged for two weeks, six weeks and three months. Figure 18 shows the mortality of G3 exposed for three minutes to the lower dose of each of the pyrethroids when the netting was aged for similar periods of time.

Percentage mortality of AE AE and G3 for both the exposure times and both the doses exposed to impregnated netting aged for various periods of time are presented in Appendices 49 to 52.

The mortality of AE AE and G3 exposed to impregnated netting washed at week 0, week 2 and week 6 are shown as histograms and are presented in Figures 19 to 22 and Appendices 53 to 56. Mortalities on unwashed freshly impregnated netting are also presented in the same histograms so that the effect of washing (and also ageing) of the impregnated netting on mortality of mosquitoes can be compared directly. Percentage knockdown and mortality of both the species of mosquitoes for both the exposure times and both the doses are presented in Appendices 57 to 60.

The mortality of AE AE and G3 exposed for three minutes to netting impregnated at the lower of the doses and aged for two weeks (both washed and unwashed) are presented in Table 14 along with expected and observed doses. Table 15 shows the mortality of AE AE and G3 exposed for 30 seconds to two week old (both washed and unwashed) netting impregnated at the higher dose of each of the chemicals.

Chemical analysis shows that there was no loss of most of the pyrethroids due to ageing of the impregnated netting for two weeks (Tables 12 and 13). However, cyphenothrin and d-phenothrin had undergone serious degradation and were completely absent from netting at week 2. With a few exceptions bioassay results also showed that pyrethroids on impregnated netting were fully effective for three months (Figures 17 and 18). However, ICI permethrin and Wellcome permethrin plus agral were much less effective at month 3 than at

week 0. There was no mortality when mosquitoes were exposed to cyphenothrin and d-phenothrin after week 0.

Washing had a severe effect on all the pyrethroids. Tables 12 and 13 show that there was a five to 10 times reduction in cyfluthrin, deltamethrin and fenpropathrin as a result of two washes. There was a three to four times reduction of cypermethrin due to two washes. Permethrin and PP321 were reduced to half to one third by two washes.

Bioassay results also show a reduction in mortality due to washing of netting (Figures 19 to 22). Four washes of the netting caused a large reduction in almost all cases. Reduction in mortality was not so marked, except in one or two cases, after two washes. However, fenpropathrin was ineffective after two washes resulting in almost no mortality.

Figure 17

Mortality of the AE AE strain of Ae.aegypti exposed to the lower dose of each of the pyrethroids for 30 seconds. Mosquitoes were exposed to unwashed netting that was aged for various periods of time.

Figure 18

Mortality of the G3 strain of An.gambiae exposed
for 3 minutes. Mosquitoes were exposed to unwashe

to the lower dose of each of the pyrethroids
d netting that was aged for various periods of time.

Figure 19
Mortality of the AE AE strain of Ae.aegypti exposed to the lower
Mosquitoes were exposed to impregnated netting that was washed a
impregnated unwashed netting are also shown.

dose of each of the pyrethroids for 30 seconds.
t various intervals of time. Results of freshly

Figure 20
Mortality of the AE AE strain of Ae.aegypti to the higher
Mosquitoes were exposed to impregnated netting that was w
of freshly impregnated unwashed netting are also shown.

dose of each of the of the pyrethroids for 3 minutes.
ashed at various intervals of time. Results

Figure 21
Mortality of the G3 strain of An.gambiae exposed to the
Mosquitoes were exposed to netting that was washed at va
impregnated unwashed netting are also shown.

lower dose of each of the pyrethroids for 3 minutes.
rious intervals of time. Results of freshly

Figure 22
Mortality of the G3 strain of An.gambiae exposed to the higher dose of each of the pyrethroids for 30 seconds. Mosquitoes were exposed to netting that was washed at various intervals of time. Results of freashly impregnated unwashed netting are also shown.

Table 14. Mortality of the AE AE and G3 strains exposed for three minutes to pyrethroid impregnated netting which had been aged for two weeks and had undergone two hand washes with soap and cold water. (Expected and observed pyrethroid content of netting are also shown).

Insecticide	Washed/ Unwashed	Expected dose(g/m^2)	Observed dose(g/m^2)	Percentage mortality AE AE	G3
Control	Unwashed	-	-	6.7	0
"	Washed	-	-	0	5.0
Cyfluthrin	Unwashed	0.10	0.11	100	100
"	Washed		0.02	89.2	90.5
Cypermethrin	Unwashed	0.10	0.08	100	100
"	Washed		0.02	94.4	100
Cyphenothrin	Unwashed	0.10	None	0	0
"	Washed		None	0	0
Deltamethrin	Unwashed	0.025	0.030	89.6	95.8
"	Washed		0.003	33.3	66.7
d-phenothrin	Unwashed	0.20	None	0	0
"	Washed		None	0	0
Fenpropathrin	Unwashed	0.10	0.09	0	0
"	Washed		0.05	0	0
Wellcome permethrin	Unwashed	0.20	0.15	100	65.0
"	Washed		0.08	69.7	4.6
Wellcome + Agral	Unwashed	0.20	0.15	87.0	52.2
"	Washed		0.04	48.5	4.4
Wellcome (hot water treatment at acid pH)	Unwashed	0.20	0.66	100	96.0
	Washed		0.40	100	100
ICI permethrin	Unwashed	0.20	0.19	100	100
"	Washed		0.07	68.8	31.8
PP321	Unwashed	0.025	0.029	100	100
"	Washed		0.012	18.8	100

Table 15. Mortality of the AE AE and G3 strains exposed for 30 seconds to pyrethroid impregnated netting which had been aged for two weeks and twice washed by hand with soap and cold water. (Expected and observed pyrethroid content of netting are also shown).

Insecticide	Washed/ Unwashed	Expected dose(g/m^2)	Observed dose(g/m^2)	Percentage mortality AE AE	G3
Control	Unwashed	-	-	0	0
"	Washed	-	-	0	0
Cyfluthrin	Unwashed	0.20	0.19	100	81.0
"	Washed		0.02	61.3	4.4
Cypermethrin	Unwashed	0.20	0.15	100	100
"	Washed		0.05	90.9	95.5
Cyphenothrin	Unwashed	0.20	None	0	0
"	Washed		None	0	0
Deltamethrin	Unwashed	0.05	0.06	71.9	37.5
"	Washed		0.01	14.7	8.0
d-phenothrin	Unwashed	0.50	None	0	0
"	Washed		None	0	0
Fenpropathrin	Unwashed	0.20	0.16	33.3	95.7
"	Washed		0.02	4.8	0
Wellcome permethrin	Unwashed	0.50	0.47	97.1	13.0
"	Washed		0.15	84.2	4.4
Wellcome permethrin	Unwashed	0.50	0.38	93.3	30.0
+ Agral	Washed		0.14	70.6	0
Wellcome permethrin	Unwashe	0.50	1.10	100	76.2
(hot water treatment	Washed		0.69	93.9	44.0
at acid pH)					
ICI permethrin	Unwashed	0.50	0.27	100	56.5
"	Washed		0.17	100	22.7
PP321	Unwashed	0.05	0.08	100	100
"	Washed		0.04	55.6	83.3

4.5.4. Discussion:

As all the chemicals were not used at the same dose, it is not possible to compare the toxicity of all these pyrethroids directly. But while describing the methods it was mentioned that the doses were chosen according to the "toxicity rating" of each of the chemicals and it was expected that all the chemicals should be more or less equally toxic at the doses chosen.

In the present investigation it has been seen that cypermethrin and PP321 are the most toxic chemicals. PP321 was used at doses which were one fourth the doses of cypermethrin, so it can be said that PP321 is the most toxic of all the chemicals used against Ae. aegypti and An. gambiae. Deltamethrin was used at the same dose as PP321, but it was not found to be so effective as PP321. Cyfluthrin was used at the same dose as cypermethrin and was found to be very effective against AE AE and G3. Fenpropathrin was not found to be very effective against these two species of mosquitoes.

d-phenothrin gave very poor results. Chemical analysis showed that most of the active ingredient was broken down and only a fraction of the expected amount of the chemical was present on the netting. The active ingredient of cyphenothrin was also broken down and only one tenth of the targeted dose was present on netting during chemical analysis. Bioassay results were not so bad. This may be due to the fact that most of the cyphenothrin active ingredients were broken down between the time of the bioassay and chemical analysis. The reasons for degradation of these two chemicals are not known.

Wellcome permethrin when impregnated at acid pH in hot water was found to be much more insecticidal than both Wellcome and ICI permethrin when impregnated in cold water at neutral pH. Chemical analysis showed that when netting was impregnated in hot water much more permethrin was picked up from the solution and the netting contained two to three times the expected dose of permethrin. Thus there is no reason to suppose that the treatment system increases the insecticidal effectiveness of a given quantity of permethrin. One major problem of impregnating nets in this system would be that in large scale treatment the nets impregnated at the beginning of a batch dipped in the same emulsion would pick up more permethrin, resulting in the emulsion becoming less and less concentrated. Thus for the nets that would be impregnated towards the end of the series there would be very little permethrin left to pick up. Wellcome permethrin and ICI permethrin were found

to give more or less similar results. Addition of agral to permethrin did not increase the effectiveness of permethrin.

Pyrethroids were not found to be very much affected by ageing of impregnated netting. Bioassays showed that only one or two pyrethroids partly lost their performance due to ageing for three months. Chemical analysis was not done on three month old netting, so it is not possible to say exactly what proportion of the chemicals are lost over that time. However, chemical analysis of two week old netting showed no loss of chemicals except in the cases of cyphenothrin and d-phenothrin.

Itoh et al. (1986) found cyphenothrin to be the most persistent of all the six pyrethroids tested over a nine month period. However, the authors used Culex pipiens pallens, a different species of mosquito, and 24 hour bioassays. In the present investigation, the active ingredients of both cyphenothrin and d-phenothrin were broken down quickly. It was not possible to find out the reason for this degradation.

Chemical analysis was done on all the samples aged for two weeks and washed twice. Discussion of the chemical content on the netting will be based mainly on netting two weeks after impregnation. Cyfluthrin, deltamethrin and fenpropathrin were the worst affected by washing. Bioassay results showed that fenpropathrin became almost ineffective after the second wash. Cyfluthrin and deltamethrin, however, were still effective against mosquitoes, although their toxicity was reduced. As in unwashed netting, cyphenothrin and d-phenothrin were completely absent from netting washed twice and only a fraction of the initial content of these compounds was present on netting washed only once.

Although the chemical content of cypermethrin was reduced by three to four times due to two washes, its toxicity to mosquitoes was not so much reduced. This chemical was found to cause high mortality even after four washes. So this chemical can be considered as one of the promising pyrethroids for impregnating bednets, subject to satisfactory reports on human toxicity risks.

Both Wellcome and ICI permethrin were found to lose about 50% of their chemical content due to two washes (averaging the lower and higher doses). Permethrin plus agral was found to lose more (68%) and permethrin when impregnated in hot acid conditions lost less (38%) than the normal ambient temperature impregnation. Snow et al. (1987a) also observed that handwashing of permethrin impregnated bednets of four different types almost halved the permethrin content. In a different part of the present investigation it was

observed that washing carried out in London of permethrin impregnated nylon nets with Gambian cowfat soap gave a much less clear cut reduction in the effectiveness of the impregnated nets (Chapter 4.4, Table 11) and when similar nets were washed with soap bought in London there was no evidence of reduction in effectiveness in killing of mosquitoes (Table 10). It may be mentioned that the pieces of netting, being discussed in this chapter, were also washed using Gambian cow fat soap. It seems probable that washing done by a Gambian housewife was more vigorous than that done by the author.

Schreck et al. (1982d) observed that military uniform treated with permethrin at 1.25 to 2.0 g/m^2 lost about 49% of the chemical content after four machine washes. However, the uniform gave 100% protection against Lone Star Tick, Amblyomma americanum. The reason may be that the dose was so high that even after about 50% reduction there was enough chemical to completely prevent ticks from feeding. For the same reason, in the present investigation it was observed that permethrin when impregnated in hot water at acid pH was very effective even after washing.

Although two hand washes almost halved the chemical content of PP321 impregnated netting, it was still very effective against both AE AE and G3. From the present investigation this chemical seems to be the best of all the pyrethroids used. Further investigation of this chemical using other species of mosquitoes and more realistic testing methods should be encouraged.

4.6. Cross-resistance tests of various strains of mosquitoes:

4.6.1. Introduction:

Before introducing pyrethroid impregnated bednets in the field, the susceptibility status of the vector populations to the pyrethroid concerned ought to be determined. Sometimes, mosquitoes resistant to one insecticide are also cross-resistant to other insecticides. Various strains of mosquitoes colonized at LSHTM resistant to various insecticides were bioassayed on permethrin impregnated nylon netting to determine whether they are cross-resistant to permethrin. Strains of the same species known to be susceptible to insecticides were used as controls.

4.6.2. Methods:

The cross-resistance test was performed in two steps. At the initial step four resistant strains belonging to four species of mosquitoes were exposed to permethrin. The susceptible strains of the same species were used as controls. One strain of An. gambiae with dieldrin resistance was found to be significantly more tolerant to permethrin than the susceptible control. In the second step seven different strains of mosquitoes (three strains resistant to various insecticides and four susceptible strains) all belonging to An. gambiae, were bioassayed on permethrin impregnated netting. The netting samples were impregnated at a series of doses. The bioassays on each strain were replicated three times. Mosquitoes were exposed for two minutes and knockdown was scored one hour post-exposure and mortality was scored 24 hours post-exposure. The results were plotted on log-dose/probit-mortality paper. LD_{50} with 95% confidence limits and LD_{90} were calculated using the same computer program as before.

4.6.3. Results:

The results of the first cross-resistance test are presented in table 16. It was observed that there was no difference in the permethrin resistance level between a strain of An. albimanus resistant to organophosphates, carbamates and to dieldrin (FEST) and a strain susceptible to these insecticides (PALB). Furthermore, permethrin susceptibility was similar in organophosphate resistant and susceptible strains of An. stephensi (STMAL and ST LASS).

There was a slight difference in the level of permethrin resistance between organophosphate resistant and susceptible strains of C. quinquefasciatus (DAR 82 and CfCA); the difference was not marked in the KD_{50} value but it was more than three fold with respect to the LD_{50} value.

A pronounced difference in the level of permethrin resistance was observed between a susceptible strain of An. gambiae (KWA) and a dieldrin resistant strain (MU).

It was therefore decided to carry out a more careful investigation of this pair of strains and also other strains of An. gambiae. The results of these further cross-resistance tests are presented in table 17. On the basis of the KD_{50} values it was observed that there was no difference in the level of susceptibility to knockdown by permethrin between KWA, 16cSS, G3 and Z11, the four strains of An. gambiae known to be susceptible to all other insecticides.

MU was found to have a significantly higher level of resistance to knockdown than these four strains. ZANDS was slightly more tolerant than the susceptible strains but more susceptible than the MU strain. IAN P20, which had previously been selected for permethrin resistance by Prasittisuk and Curtis (1982), was found now to be more susceptible to permethrin than all the other strains.

The LD_{50} values indicate significantly higher permethrin tolerance in Z11, ZANDS and MU compared with all other strains.

Table 16. Results of the exposure of various strains of mosquitoes, resistant to various insecticides, to pieces of permethrin impregnated nylon netting. Susceptible strains of the same species were used as controls. Mosquitoes were exposed for 15 seconds.

Mosquito species	Strain	Known susceptibility status	KD_{50}(95% C.L.) (g/m^2)	χ^2(d.f.) about regression line	LD_{50}(95% C.L.) (g/m^2)	χ^2(d.f.) about regression line
An. albimanus	PALB	Susceptible	0.20 (?)[a]	*16.33 (6)	0.89 (?)[a]	***31.73 (6)
"	FEST	OP & Carb.-R	0.21 (0.17 - 0.27)	5.64 (6)	0.76 (0.59 - 1.04)	5.45 (6)
An. gambiae	KWA	Susceptible	0.73 (0.55 - 1.01)	5.63 (6)	0.44 (0.29 - 0.70)	9.77 (6)
"	MU	DLN & Partially DDT-R	2.66 (1.78 - 5.44)	2.50 (6)	12.55 (2.86-1967.53)	6.12 (6)
An. stephensi	STLASS	Susceptible	0.12 (?)[a]	*13.31 (6)	0.02 (?)[a]	**20.51 (6)
"	STMAL	MAL & DLN-R	0.12 (0.09 - 0.16)	8.51 (6)	0.04 (0.01 - 0.06)	12.47 (6)
C. quinque-fasciatus	CfCA	Susceptible	1.05 (0.88 - 1.24)	0.19 (6)	0.27 (?)[a]	***45.92 (6)
	DAR 82	OP &Carb.-R	1.45 (?)[a]	*20.99 (6)	0.92 (0.60 - 1.66)	9.27 (6)

*** P < 0.001

** P < 0.01

* P < 0.5

[a] Where there was a significant heterogeneity χ^2 about the regression line, 95% confidence limits could not be attached to ED_{50} estimates.

Table 17. Results of the exposure of various strains of An. gambiae for 2 minutes to permethrin impregnated nylon netting samples.

Strain	Known susceptibility ststus	KD_{50} (95% C.L.) (g/m^2)	χ^2 (d.f.) about regression line	LD_{50} (95% C.L.) (g/m^2)	χ^2 (d.f.) about regression line
KWA	Susceptible	0.190 (0.157 - 0.230)	3.3 (5)	0.223 (0.184 - 0.270)	4.1 (6)
16cSS	"	0.188 (?)[a]	*11.7 (4)	0.168 (?)[a]	*13.9 (5)
G3	"	0.125 (?)[a]	**13.3 (4)	0.291 (?)[a]	***25.3 (5)
Z11	"	0.187 (0.159 - 0.220)	6.8 (5)	0.680 (0.551 - 0.846)	6.2 (5)
ZANDS	DDT-R	0.239 (?)[a]	**16.9 (6)	0.888 (?)[a]	**23.6 (8)
IAN P20	Per.-R	0.095 (0.072 - 0.122)	3.4 (6)	0.213 (0.158 - 0.284)	11.4 (6)
MU	DLN & partially DDT-R	0.315 (0.261 - 0.379)	4.1 (7)	0.816 (?)[a]	***45.0 (7)

*** P < 0.001
** P < 0.01
* P < 0.05

[a] Where there was a significant heterogeneity χ^2 about the regression line, 95% confidence limits could not be attached to the ED_{50} estimates.

4.6.4. Discussion:

Studies by other authors indicate that mosquitoes resistant to DDT may show cross-resistance to permethrin. Generally mosquitoes resistant to other groups of insecticides are not cross-resistant to permethrin . Priester and Georghiou (1980) observed that C. quinquefasciatus selected for permethrin resistance was not cross-resistant to dieldrin, temephos, propoxur or organotin compounds. Thus, it was not unexpected that FEST and STMAL were not cross-resistant to permethrin.

It was surprising that organophosphate resistant DAR 82 and dieldrin resistant MU showed some degree of tolerance to permethrin. DAR 82 is known to have about a 5 to 6 times larval resistance to permethrin relative to CfCA (Hemingway, personal communication). The permethrin tolerance of MU found in the first test (Table 16) was confirmed in the screening of seven An. gambiae strains (Table 17). IAN P20 selected for permethrin resistance 5 years ago has lost all trace of resistance.

The strongly DDT resistant ZANDS is no more tolerant to permethrin than is the MU strain. Furthermore, ZANDS is no more tolerant than is the DDT susceptible Z11 strain, which like ZANDS, is derived from the ZANU strain collected in Zanzibar in 1982. It is concluded that the DDT resistance of the ZANDS strain is not due to the kdr resistance mechanism, which is well known to cause cross-resistance to permethrin. The larval DDT resistance mechanism of this strain was found to be mainly due to enhanced glutathione S-transferase (Hemingway et al. 1985).

4.7. Selection of MU stock for DDT and permethrin resistance:

4.7.1. Introduction:

As shown in the previous chapter the MU stock of An. gambiae was found to possess a significant level of tolerance to permethrin. This strain was known to be resistant to dieldrin (which is unlikely to cause cross-resistance to pyrethroids) and there was no record of DDT resistance. When this strain was exposed to 4% DDT papers for one hour, about 25% survived. Therefore, it was thought that permethrin tolerance of this species may be due to a low level of a kdr type of resistance to DDT. A study was, therefore, undertaken to select a population from the MU stock for DDT resistance and another population for permethrin resistance. It was intended to carry out cross-resistance tests between the two selected strains.

4.7.2. Methods:

Selection for DDT resistance was done by exposing three to five day old mosquitoes of both sexes to 4% DDT papers in WHO test kits. Mosquitoes were sexed in the pupal stage or within the day of emergence, thus, only virgin females were used for the tests. The parent population was exposed for one hour, but as the resistance increased in the following generations the exposure times were also increased reaching 10 hours towards the end of the selection process. Mosquitoes of four generations (P, F_1, F_6 and F_9) were exposed to DDT at a range of exposure times to estimate the LT_{50} values so that the level of resistance could be compared. LT_{50} values were determined using the same computer program which was also used for analysis of dose/mortality data.

Adult mosquitoes of both sexes were exposed to 0.8 g/m^2 permethrin impregnated nylon netting in WHO test kits for permethrin selection. Only virgin females were used as before. Initially the mosquitoes were exposed for one minute. The exposure time was increased to four minutes towards the end of the selection process. The same pieces of netting were used throughout .

4.7.3. Results:

The results of the exposure of the MU strain of An. gambiae to DDT are presented in Table 18. The raw data from which the LT_{50} values were calculated are presented in appendices 61 to 64. Table 18 shows that when the population of the MU strain was put under DDT selection presssure, the level of resistance increased until at the F_9 the LT_{50} value was more than 25 times that of the parent population. After F_9 there was no increase in the level of resistance, as indicated by the survival rate at 10 hours although the population was under strong selection pressure.

Results of the exposure of MU to 0.8 g/m^2 permethrin impregnated netting are presented in table 19. The raw data from which the LD_{50} values were calculated are presented in Appendices 65 and 66. There was no increase in permethrin resistance over 9 generations of selection, as was indicated by the LD_{50} values of the parent population and that of the F_9 generation. Table 20 shows that the permethrin resistance level of MU, selected for DDT resistance over 11 generations, had not increased.

4.7.4. Discussion:

A rapid increase in the level of DDT resistance indicates that genes for DDT resistance were present in the parent MU population. The static level of resistance after F_9 suggests that the population had attained homozygosity for a resistance gene. The selected DDT resistant strain did not show any cross-resistance to permethrin (Table 20) unlike the results of Prasittisuk and Curtis (1982) on IAN. Therefore, it seems that the DDT resistance of this population is not due to the presence of a kdr gene, but some other mechanisms are involved.

The attempt to select a permethrin resistant strain was not successful. When F_9 was exposed to a series of doses of permethrin, it was observed that the KD_{50} and LD_{50} were not significantly different from the parent population (Table 20) indicating no increase in the level of resistance. The apparent increase during selection might be due to the fact that the same piece of netting was being used for more than one year and it now seems likely that the insecticide was wearing off.

Table 18. Results of the exposure of the MU strain to 4% DDT impregnated papers in an attempt to select a population for DDT resistance.

Mosquito generation	Exposure time (hours)	Number of mosquitoes			Percentage mortality	LT_{50} (hours)
		Alive	Dead	Total		
Parent	1	348	1119	1503	74.45	0.43
F_1	1	487	374	861	43.44	0.98
F_2	2	302	1398	1700	82.24	-
F_3	2	296	467	763	61.21	-
F_4	3	160	284	444	63.96	-
F_5	4	417	633	1050	60.29	-
F_6	7	416	1127	1543	73.04	2.3
F_7	7	246	849	1095	77.53	-
F_8	6	371	183	554	33.03	-
F_9	10	702	1092	1794	60.87	10.8
F_{10}	10	540	926	1466	63.17	-
F_{11}	10	189	419	608	68.91	-

- = not recorded

Table 19. Results of the exposure of the MU strain to pieces of nylon netting impregnated with permethrin at a dose of 0.8 g/m².

Mosquito generation	Exposure time (minute)	Number of mosquitoes			Percentage mortality	LD_{50} (g/m²)
		Alive	Dead	Total		
Parent	1	305	504	809	62.3	0.816
F_1	2	302	525	827	63.48	-
F_2	2	174	527	701	75.18	-
F_3	1	65	66	131	50.38	-
F_4	1	235	126	361	34.9	-
F_5	2	227	135	362	37.29	-
F_6	4	258	517	775	66.71	-
F_7	4	155	216	371	58.22	-
F_8	4	257	495	752	65.82	-
F_9	4	631	474	1105	42.9	0.715

- = not recorded

Table 20. Exposure of three strains of An. gambiae for 2 minutes to permethrin impregnated nylon netting samples at a series of doses.

Strain	Known susceptibility status	KD_{50} (95% C.L.) (g/m^2)	χ^2 (d.f.) about regression line	LD_{50} (95% C.L.) (g/m^2)	χ^2 (d.f.) about regression line
MU	DLN & partially DDT-R	0.315 (0.261 - 0.379)	4.1 (7)	0.816 (?)[a]	***45.0 (7)
MU/DDT F_{11}	DDT-R	0.412 (?)[a]	***32.1 (3)	0.565 (?)[a]	***17.5 (3)
MU/PER F_9	partially DDT-R	0.420 (0.350 - 0.502)	7.4 (5)	0.714 (0.598 - 0.850)	5.9 (4)

*** P < 0.001

[a] Where there was a significant heterogeneity χ^2 about the regression line, 95% confidence limits could not be attached to the ED_{50} estimates.

4.8. Methodology for resistance detection – paper or netting:

4.8.1. Introduction:

It is more reasonable to expose mosquitoes to a material which wild mosquitoes would encounter (e.g. impregnated netting) than to a material only loosely simulating a real one (e.g. impregnated papers simulating a sprayed wall surface). But WHO has long recommended exposure of mosquitoes to impregnated papers for resistance detection in adults. It was therefore decided to evaluate whether impregnated netting would be more suitable than papers for detecting resistance in the adult mosquitoes.

WHO recommends exposure of Anopheles mosquitoes to 0.25% permethrin impregnated papers for one hour for resistance detection. But it was observed that mosquitoes were knocked down long before the completion of the exposure period. Thus mosquitoes did not remain in contact with the insecticide throughout the exposure period. Therefore, WHO (1986) recommended putting the test-kits in a horizontal position during the exposure period. It was decided to investigate whether test-kits in the horizontal or vertical position are more suitable for resistance detection.

4.8.2. Methods:

Various strains of mosquitoes were exposed to 0.25% permethrin impregnated papers or to permethrin impregnated nylon netting at a series of doses in order to develop a suitable method that can be used to detect permethrin resistance in mosquitoes easily and reliably in the field. The papers were supplied by WHO and impregnation of netting was done in the laboratory.

Mosquitoes were exposed to impregnated papers in batches of about 25 for one hour. The test kits were kept either in a horizontal position (WHO, 1986) or in a vertical position. Knockdown and mortality was scored immediately after the exposure period and after 24 hours respectively. The mosquitoes that were knocked down were separated from those not knocked down to see if there was any recovery among those knocked down.

To compare the effectiveness of the two test-kit positions significance tests were performed on dead and alive mosquitoes. Chi square tests were done in two of the four cases. In two other cases Fisher's exact test of significance was performed, because the data were not suitable for a chi square test.

About 10 mosquitoes were exposed at a time to permethrin impregnated netting for two minutes. Knockdown was scored one hour post-exposure and mortality 24 hour post-exposure. LD_{50} with 95% confidence limits and LD_{90} were calculated by using a computer program as before.

4.8.3. Results:

The results of the exposure of different strains of mosquitoes to 0.25% permethrin papers are presented in Table 21. The LD_{50} values of the same strains of mosquitoes exposed to permethrin impregnated nylon netting at a range of doses are presented in the previous chapter (Tables 17 and 20). Table 21 shows that one hundred percent mortality was not achieved even in the susceptible strain. Less survival was observed when the tubes were kept horizontal, but the difference was statistically significant in one of the four cases only. In one case, with the vertical position of the test-kit, 3 out of 11 surviving mosquitoes were knocked down during the one hour exposure period but recovered later.

Table 21. Exposure of various strains of mosquitoes to 0.25% permethrin impregnated papers.

Mosquito strain	Test kit position	Number of mosquitoes				
		Knockdown	Deaths(%)	Alive	Total	P value*
KWA	Vertical	308	325 (99.7)	1	326	
KWA	Horizontal	164	174 (99.4)	1	175	0.22
						(Fisher's exact test)
MU	Vertical	296	335 (96.8)	11**	346	
MU	Horizontal	124	151 (99.3)	1	152	0.08
						(Fisher's exact test)
MU/DDT-F_{11}	Vertical	-	83 (92.2)	7	90	
MU/DDT-F_{11}	Horizontal	-	143 (97.3)	4	147	>0.05
						(χ^2 test)
MU/PER-F_9	Vertical	-	175 (82.2)	38	213	
MU/PER-F_9	Horizontal	-	256 (93.8)	17	273	<0.01
						(χ^2 test)

* Test of heterogeneity between two test kit positions.

** Three of the 11 surviving mosquitoes were knocked down during the one hour exposure period but recovered later.

4.8.4. Discussion:

When KWA, a susceptible strain of An. gambiae, was exposed to 0.25% permethrin impregnated papers, more than 99% mortality was achieved. Therefore, according to the definition of WHO (1986) this strain is susceptible to permethrin. Mortality among MU/DDT-F_{11} and MU/DDT-F_9 varied between 82 and 98% and thus according to WHO these strains should be suspected of having resistance and verification is required.

Among MU/PER-F_9 mortality was significantly higher using the horizontal position of the test-kit than the vertical position. In all other cases although there was a higher mortality among mosquitoes in the horizontal test-kit than in the vertical test-kit, the difference was not statistically significant. The WHO recommendation for pyrethroid resistance detection is to put the test-kits in a horizontal position (WHO 1986) during the exposure period. However, it is questionable whether this is a complete solution to the problem of knockdown during the exposure period. In this system mosquitoes when knocked down, sometimes long before the completion of the exposure period, lie on their backs. Thus they offer a larger surface for absorption of insecticide than in an insect standing on a deposit. In the present investigation 3 out of 604 mosquitoes recorded to be knocked down recovered later, when the test-kit was in a vertical position.

Among the mosquitoes of the MU strain there was more than 99% mortality when the test-kit was in a horizontal position, suggesting the strain to be susceptible to this insecticide. However, when the test-kit was in a vertical position the mortality was 96.8% indicating that the strain should be suspected of being resistant. Results of the exposure of this strain to impregnated netting also suggest that this strain has a significantly higher level of tolerance to permethrin than KWA (Table 17).

No discriminating dose of impregnated netting has been proposed yet for resistance detection. In the present investigation it was observed that a dose of 2.5 g/m^2 on permethrin netting killed all the susceptible An. gambiae exposed for two minutes. However, the same dose also killed all the individuals of the permethrin tolerant strain (MU) of this species with the same exposure time. No strongly permethrin resistant An. gambiae was available. Thus it was not possible to investigate whether strongly resistant individuals survive this dose. In the absence of a single discriminating dose

one may compare mortality by using a range of doses but this requires many mosquitoes which are not always available in the field.

On the basis of the above discussion, it may be suggested that permethrin impregnated papers and the test-kits in their vertical position is the best method for detecting permethrin resistance in adult mosquitoes. The problem of knockdown during the exposure period could be solved by choosing the exposure period in such a way that no mosquito is knocked down within the period of exposure. Hemingway (1980a, b) suggested removal of mosquitoes that are knocked down within the first 20 minutes of exposure during a selection process, for which it is necessary to modify the present WHO test-kit. It is hoped that if the exposure period is chosen according to the above suggestion, there will be no need of this extra complication.

4.9. Effect of temperature on knockdown and mortality of mosquitoes:

4.9.1. Introduction:

People use bednets at night when environmental temperature is lower than in the day. Bioassays, to see the effectiveness of a chemical on bednets against mosquitoes, are generally done by day. It was there felt important to determine whether variation of temperature by a few degrees has any effect on the toxicity of pyrethroids against mosquitoes. An experiment was undertaken to evaluate this idea in the laboratory using permethrin impregnated nylon nets.

4.9.2. Methods:

Bioassays using KWA, a susceptible strain of An. gambiae, were done on a series of pieces of permethrin impregnated nylon netting at 16°C, 22°C and 28°C to study the effect of temperature on knockdown and mortality of this species of mosquito. As before knockdown and mortality were scored one-hour and 24-hour post-exposure respectively. Exposures at each of the temperatures

were replicated 6 to 9 times. KD_{50}, KD_{90}, LD_{50}, LD_{90} and heterogeneity chi square values were calculated using a computer program as before.

4.9.3. Results and Discussion:

Percentage mortality at each of the doses and the regression lines are plotted on log-dose/probit-mortality paper in figures 23 and 24 for knockdown and mortality respectively. The KD_{50}, KD_{90} and chi square values are presented in table 22 and the LD_{50}, LD_{90} and chi square values in table 23. The raw data from which the above values were computed are presented in appendices 67 to 69.

Considering KD_{50} values it seems that there is a negative temperature coefficient between 16°C and 22°C but there is no effect of temperature between 22°C and 28°C. When LD_{50} values are considered the results become more complicated: higher mortality was observed at 16 and 28°C than at 22°C. When knockdown and mortality at individual doses are considered, it was found that in most cases there was no significant difference in knockdown or mortality between the three temperatures. It is, therefore, concluded that temperatures in the range 16 - 28°C have no strong effect on mortality of An. gambiae due to permethrin. In the lower part of this range there appears to be a negative temperature coefficient with respect to knockdown at one hour. This presumably indicates that the process of permethrin metabolism and de-activation proceeds more slowly at low temperatures.

Figure 23

Effect of temperature on knockdown of the KWA strain of A.gambiae as determined by bioassaying on pieces of permethrin impregnated nylon netting.

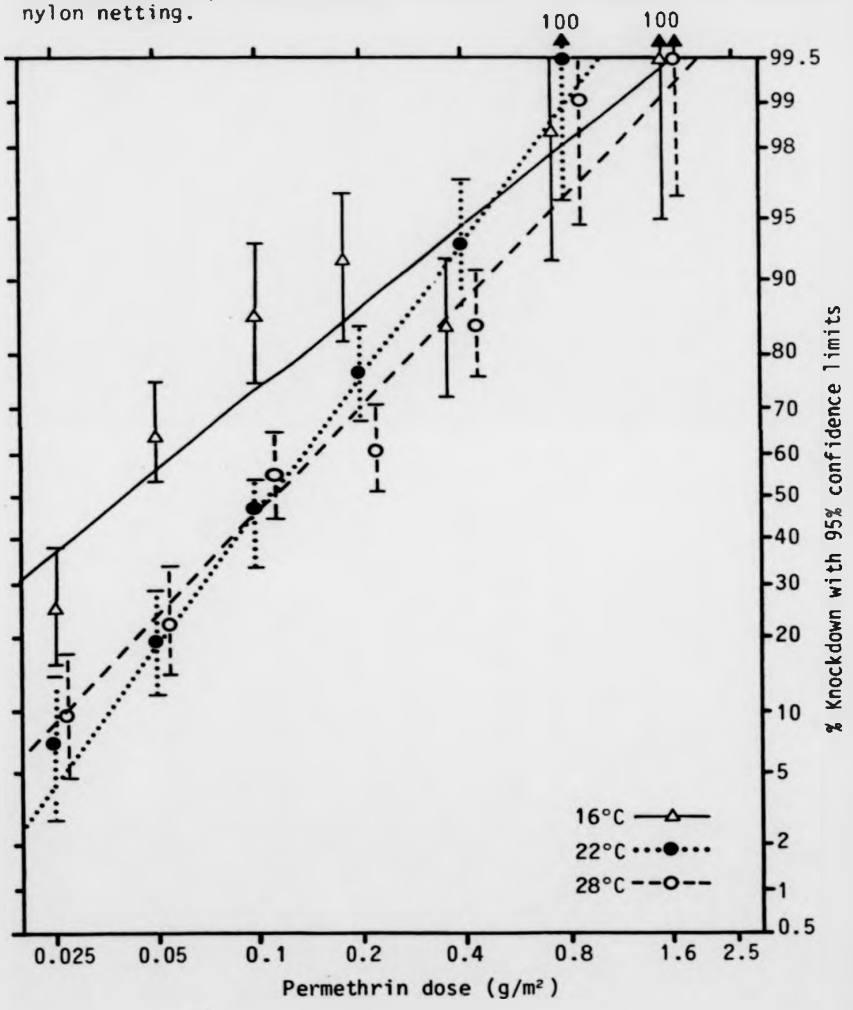


Figure 24

Effect of temperature on the mortality of the KWA strain of An.gambiae as determined by bioassaying on pieces of permethrin impregnated nylon netting.

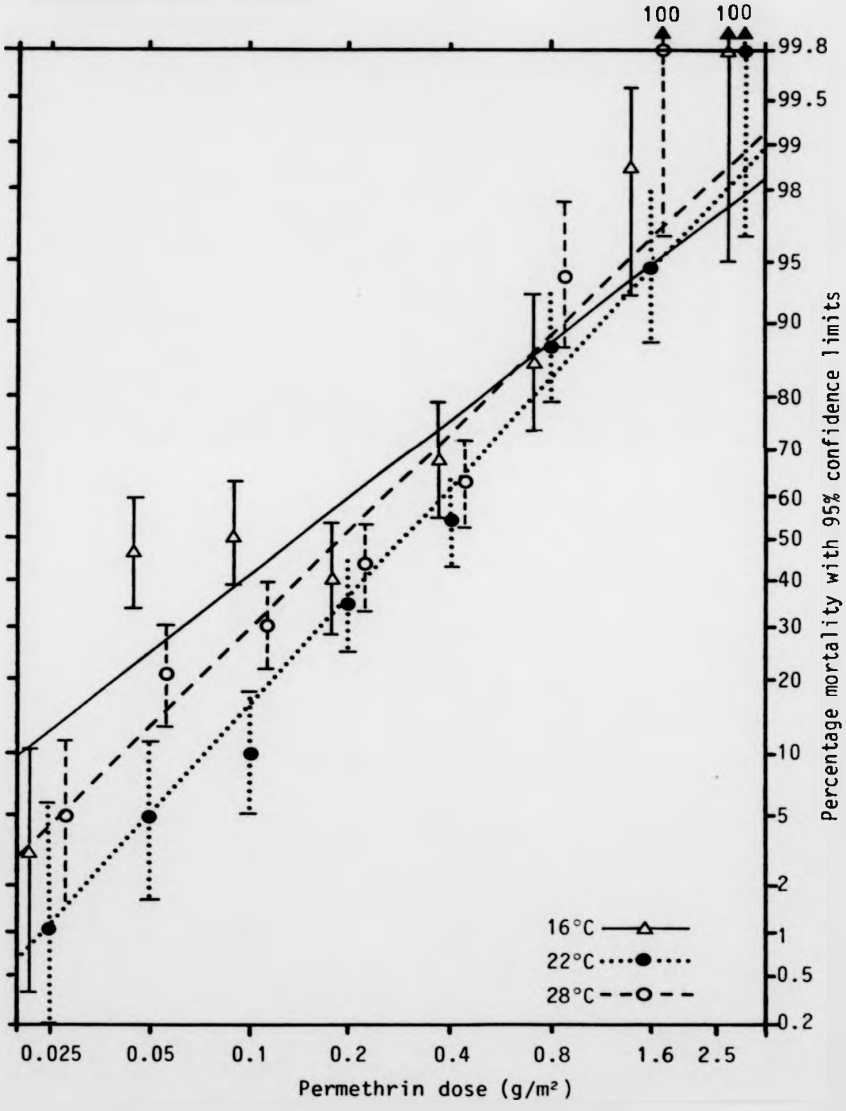


Table 22. Effect of temperature on knockdown of the KWA strain of An. gambiae exposed to pieces of permethrin impregnated nylon netting.

Temperature	KD_{50} (g/m²)	KD_{90} (g/m²)	Heterogeneity χ^2 (d.f.) about regression
16°C	0.04	0.25	22.4*** (5)
22°C	0.11	0.33	3.7 (4)
28°C	0.11	0.46	12.0* (5)

*** P< 0.001
* P< 0.05

Table 23. Effect of temperature on mortality of the KWA strain of An. gambiae exposed to pieces of permethrin impregnated nylon netting.

Temperature	LD_{50} (g/m²)	LD_{90} (g/m²)	Heterogeneity χ^2 (d.f.) about regression
16°C	0.14	0.96	41.7*** (6)
22°C	0.29	1.13	19.8** (6)
28°C	0.19	0.88	23.8*** (5)

*** P< 0.001
** P< 0.01
* P< 0.05

4.10. Behavioural and killing effects of permethrin as measured in "tunnel" tests:

4.10.1. Introduction:

An experiment was designed to simulate the natural conditions of permethrin impregnated wide-mesh bednets and their influence on the behaviour and mortality of mosquitoes. Pieces of wide-mesh cotton and nylon netting were impregnated with permethrin and evaluated in a "tunnel" of cages using a guinea pig as bait. Persistence of permethrin on wide-mesh cotton nets was also studied in the same experiment.

4.10.2. Methods:

Wide-mesh cotton netting used in these tests was the same as was used in the tests with deet (Chapter 4.1) and was impregnated with 0.2 or 0.5 g/m^2 permethrin. Three different types of nylon netting were used:- 0.4 and 0.6 cm mesh-size netting was impregnated at 0.2 g/m^2 and 1.8 cm mesh-size netting was impregnated at 0.5 g/m^2.

The tests were essentially the same as were done with deet. Here the impregnated netting was used between cages 5 and 6 only, i.e. the netting was at a distance of 15 cm from the bait (Fig. 1). The KWA strain of An. gambiae was used for these tests. At the end of the exposure period the fed and unfed mosquitoes were separately counted. Both fed and unfed mosquitoes were kept in paper cups to score mortality after 24 hours. Cotton soaked with glucose solution was given as a source of food during the holding period.

Tests of the above type were also conducted to study the persistence of permethrin on impregnated cotton netting. The netting was impregnated at 0.2 and 0.5 g/m^2 and placed at a distance of 15 cm from the bait. The tests were done every two to three weeks over a period of 30 weeks. Each test was replicated twice. Pieces of impregnated netting were hung in a hot (27°C) and humid (70% RH) room between the tests.

4.10.3. Results: Effect of mesh-size on protection from mosquitoes:

Results of the responses of An. gambiae released in a "tunnel" containing permethrin impregnated nylon netting of various mesh sizes are presented in table 24 and the distribution of mosquitoes among different cages of the "tunnel" are presented in appendix 70. The relative proportions of fed and unfed, and dead and alive, mosquitoes on either side of the netting are shown in Figure 25. Impregnated nylon netting of 0.4 and 0.6 cm mesh sizes were very effective against this species of mosquito. Netting of 1.8 cm mesh size was only slightly better than control, although a higher dose was used than with the 0.4 and 0.6 cm mesh. When corrected for control there was more reduction in feeding in the case of 0.6 cm mesh netting than 0.4 cm mesh netting. There was more mortality with 0.6 cm mesh nets than with 0.4 cm mesh nets. When 1.8 cm mesh netting was used, most of the mosquitoes left cage F after feeding and most of them were caught in cage A (Appendix 70).

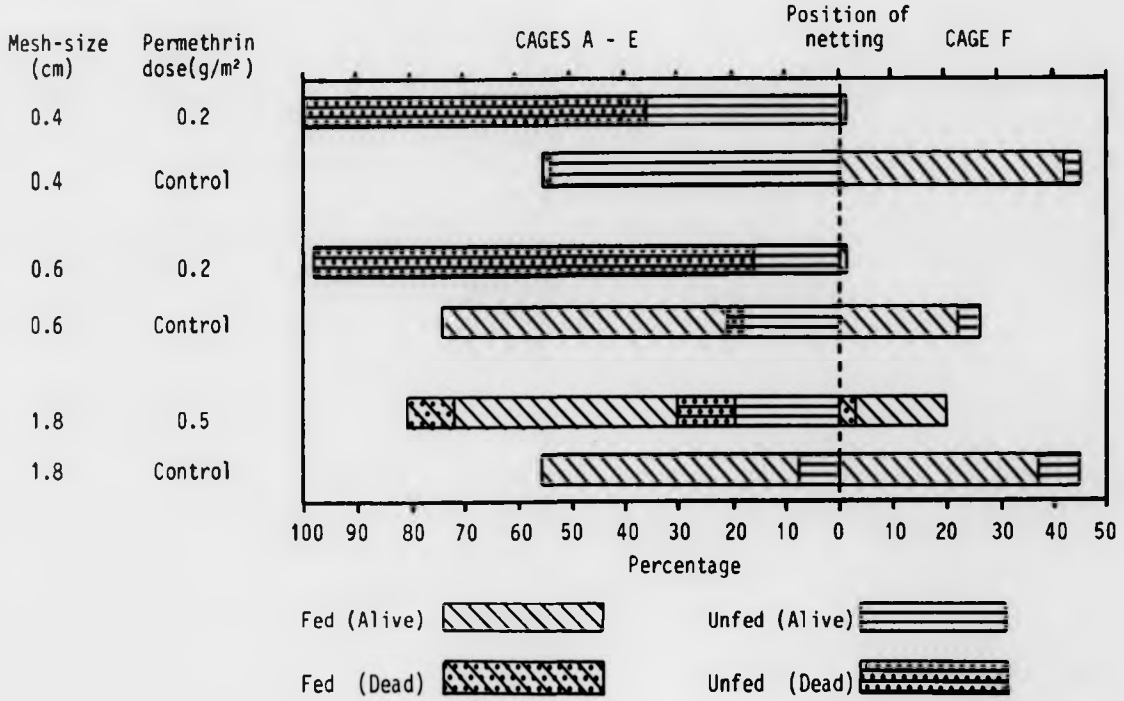
Table 24. Responses of the KWA strain of *An. gambiae* released in a "tunnel" of cages containing a guinea pig. Between the animal and the release point were various types of wide-mesh nylon netting impregnated with permethrin.

Mesh size and dose	Mosquitoes caught in cage F (%)	% excluded from cage F corrected for control*	Fed (%)	% reduction in feeding corrected for control*	Survival (%)	Mortality corrected for control*	Total no.
0.4 cm 0.2 g/m²	1.4	96.8	1.4	96.7	36.6	63.4	71
Control	44.4	-	43.1	-	100	-	72
0.6 cm 0.2 g/m²	1.3	95.0	1.3	98.3	17.1	82.4	76
Control	26.0	-	75.3	-	97.3	-	73
1.8 cm 0.5 g/m²	19.4	56.3	70.1	17.7	77.6	22.4	67
Control	44.4	-	85.2	-	100	-	27

$$* \; 100 - \left[\frac{\text{\% success in treated}}{\text{\% success in control}} \times 100 \right]$$

Figure 25

Distribution of fed and unfed An.gambiae (KWA strain) in the "tunnel" containing a guineapig in cage F and various types of wide - mesh nylon netting impregnated with permethrin placed in between cages E and F.



4.10.4. Discussion:

The very good protection afforded by the use of 0.4 and 0.6 cm mesh nets supports the findings of Itoh et al. (1986). Those authors found that when the mesh size of the netting was less than the width of a mosquito's wing span, mosquitoes rested on the netting before they passed through. Conversely 1.8 cm mesh impregnated netting failed to protect from mosquito bites. Thus it is possible that mosquitoes in the present investigation rested on impregnated netting of 0.4 and 0.6 cm mesh netting and either picked up a lethal dose of permethrin or they were irritated and failed to feed on the guinea pig.

Kurihara et al. (1986) obtained good protection using 1.0 cm mesh nets, but failed to get any protection, in terms of mosquito entry, by using 4.0 cm mesh nets. But with both nets a high mortality was achieved. In their experiment they obtained a very high mortality in the control experiment (65 to 82 %), so mortality with treated nets must have been due to a combination of natural hazards and insecticidal hazards. Thus it appears that to achieve a good protection against mosquitoes one should use an impregnated net whose mesh size is less than the length of mosquito's wing span.

Much more reduction in feeding due to impregnation was obtained using 0.6 cm mesh netting than using 0.4 cm mesh netting. This may be due to the fact that with 0.4 cm mesh netting there was already a great reduction in feeding with control unimpregnated nets. With 1.8 cm mesh netting most of the mosquitoes, both fed and unfed, were found in cage A which suggests an irrito-repellent action of permethrin. Kurihara et al. (1985) and Kurihara and Umino (1987) also observed that due to the irritant effect of phenothrin fed mosquitoes were driven out of the bait cages.

4.10.5. Results: Persistence of permethrin on wide-mesh cotton netting:

Results of a "tunnel" test to evaluate the persistence of permethrin on cotton netting are presented in tables 25 and 26. The distribution of mosquitoes over the "tunnel" is presented in appendices 71 and 72. When corrected for control, 80 - 100 % mosquitoes were excluded from cage F due to the impregnation of the netting. There was also more than 80 % reduction in feeding. In average about one quarter of the mosquitoes released were killed due to the toxic effect of permethrin. The effectiveness of the nets was almost the same throughout 30 weeks of observation. There was no marked difference in effectiveness between doses of 0.2 and 0.5 g/m^2. There was much higher mortality among unfed mosquitoes than fed mosquitoes. The largest number of mosquitoes were found in cage A when impregnated netting were used, whereas the largest number of mosquitoes were caught in cage F in experiments with most of the untreated control netting.

Table 25. Responses of the KWA strain of An. gambiae released in a "tunnel" of cages containing a guinea pig. Pieces of 0.2 g/m² permethrin impregnated 0.8 cm mesh cotton netting were interposed between the animal and the mosquito release point. The netting was retested at intervals to determine the persistence of the permethrin.

Weeks after impregnation	Mosquitoes found in cage F (%)	% excluded from cage F corrected for control*	Fed (% of total)	% reduction in feeding corrected for control*	Survival (%)	Mortality corrected for control*	Total no.
Control	84.6	-	84.6	-	100	-	52
0	12.8	84.9	12.8	84.9	51.3	48.7	39
3	12.8	84.9	12.8	84.9	85.1	14.9	47
6	9.5	88.8	2.4	97.2	81.0	19.0	42
10	0	100	0	100	88.2	11.8	51
14	6.3	92.6	4.2	95.4	64.6	35.4	48
17	17.8	79.0	13.3	84.3	73.3	26.7	45
20	4.3	94.9	2.1	97.5	78.7	21.3	47
26	11.8	86.1	3.9	95.4	74.5	25.5	51
30	13.7	83.8	9.8	88.4	68.6	31.4	51

$$* \; 100 - \left[\frac{\% \text{ success in treated}}{\% \text{ success in control}} \times 100 \right]$$

Table 26. Responses of the KWA strain of An. gambiae released in a "tunnel" of cages containing a guinea pig. Pieces of 0.5 g/m² permethrin impregnated 0.8 cm mesh cotton netting were interposed between the animal and the mosquito mosquito release point. The tests were repeated at intervals to determine the persistence of the permethrin.

Weeks after impregnation	Mosquitoes found in cage F (%)	% excluded from cage F corrected for control*	Fed (% of total)	% reduction in feeding corrected for control*	Survival (%)	Mortality corrected for control*	Total no.
Control	84.6	-	84.6	-	100	-	52
0	12.5	85.2	5.0	94.1	67.5	32.5	40
3	11.4	86.5	11.4	86.5	86.4	13.6	44
5	23.3	72.5	16.3	80.7	72.1	27.9	43
11	15.2	82.0	4.3	94.9	71.7	28.3	46
14	6.3	92.6	2.3	97.3	64.6	35.4	44
17	6.4	92.4	4.3	94.9	72.3	27.7	45
20	4.0	95.3	0	100	60.0	40.0	50
27	2.1	97.5	0	100	77.1	22.9	48
30	2.0	97.6	2.0	82.6	63.3	34.7	49

$$* \; 100 - \left[\frac{\%\text{ success in treated}}{\%\text{ success in control}} \times 100 \right]$$

4.10.6. Discussion:

Compared to the control very few mosquitoes were present in cage F when an impregnated wide-mesh cotton net was used. This may be either because they were prevented from passing through the net, or because some of the mosquitoes after entering cage F later returned to cage E due to the irritant effect of permethrin. Whatever may be the reason, the overall feeding rate was drastically reduced due to the use of impregnated netting.

Apparently there was no reduction in the effectiveness of the netting throughout a 30 week observation period. As it was not possible to continue the observation, it is not possible to say how long permethrin persists on this netting. The long persistence of permethrin may be due to the fact that the netting was hung in a still air and was not washed during the observation period.

At present most researchers in this field either use 0.2 or 0.5 g/m^2 of permethrin to impregnate bednets. But from the present investigation it is clear that there is no marked difference in effectiveness between these two doses. Lines et al. (1987) observed, in the field, that even if the dose was increased from 0.2 to 1.0 g/m^2, there was no significant difference in the effect of the permethrin. Thus it appears that an impregnation dose of 0.2 g/m^2 is enough to give protection against mosquitoes.

4.11. Tests of feeding inhibition due to impregnation:

4.11.1. Introduction:

It is important to determine whether impregnation of nets can prevent mosquitoes from feeding through them and also, if they try to feed, whether they survive, fed or unfed. To investigate this question experiments were performed allowing mosquitoes to feed on a human arm or mice through pieces of netting impregnated with permethrin at a series of doses.

4.11.2. Methods:

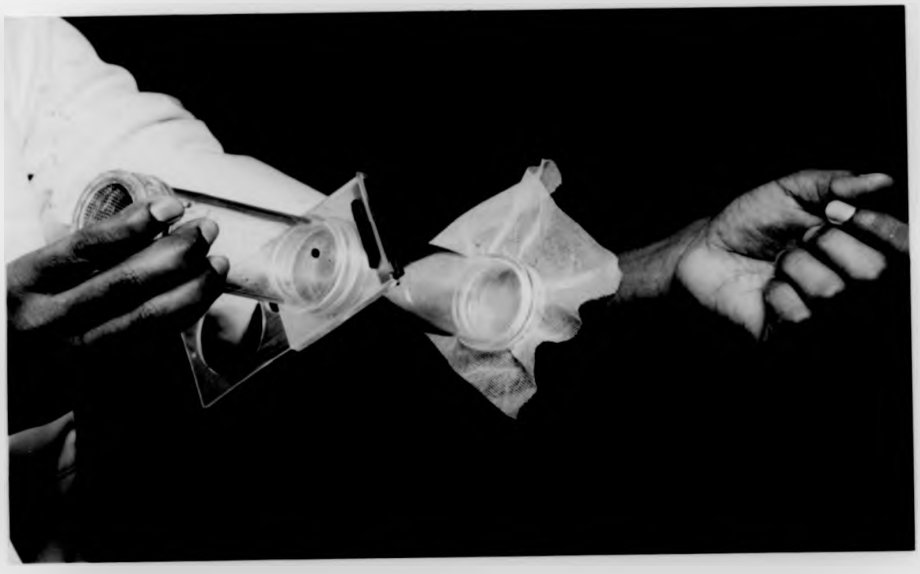
This experiment was performed in two ways - (i) inhibition of feeding by mosquitoes when they were given the opportunity to feed on a human arm, (ii) inhibition of feeding when they were given the opportunity to feed on mice.

(i) Feeding test on a human arm:- A WHO bioassay test kit with some modification was used for this purpose. The nylon gauze at the end of exposure tube was removed. Either cotton or nylon netting was impregnated with permethrin at a series of doses. After drying, impregnated netting was fastened to the end of the exposure tube. Five hungry female mosquitoes were put into the holding tube with an aspirator and the sliding door was closed. The holding tube was then connected with the exposure tube. The end of the exposure tube with impregnated netting was pressed against a human arm (Fig. 26). The sliding door was opened to allow mosquitoes to approach the arm. An. gambiae (KWA) and Ae. aegypti (AE AE) were used in this experiment. The mosquitoes were allowed the opportunity to feed for 5 minutes after which they were quickly transferred to the holding tube. The mosquitoes were then transferred to plastic cups having netting lids. Cotton wool soaked with 10% glucose solution was provided on the netting lids. Mortality was scored after 24 hours. In the case of AE AE knockdown was also scored one hour post-exposure. A control test using untreated net was run with each test. The test was replicated three times for most of the doses.

ORIGINAL

IN COLOUR

Figure 26. The WHO test-kit with a piece of netting at one end. The test-kit with the netting was pressed against an arm to see if mosquitoes can bite through permethrin impregnated netting.



(ii) Feeding test on mice:- This test was performed following Carter (1985). The test apparatus consists of plastic cups and a plywood platform. A set of plastic cups were used for holding mosquitoes and another set for exposing them to mice. A 2.5 cm hole was cut in the bottom of each cup which was covered by a piece of filter paper in the holding cup. The holding cups were covered with pieces of untreated netting and the exposure cups were covered with pieces of netting impregnated with permethrin at a series of doses. About 25 hungry female AE AE were introduced into each of the holding cups in the upright position. Each of the holding cups was then put on top of inverted exposure cups. A plywood platform was made having 10 holes 7 cm in diameter (Fig. 27). The height of the platform was such that the two plastic cups, one on top of another, just touched the platform.

Mice anaesthetised with Sagatal were placed, ventral side down, on the holes of the platform. The pairs of plastic cups were inverted. The filter papers at the bottom of holding cups were thus removed which allowed the mosquitoes to fly into the exposure cups. The cups were then placed under the holes of the platform, so that the impregnated nets were in contact with the mice (Fig. 28). Mosquitoes were allowed to feed on mice through impregnated netting for 30 minutes after which they were removed and counted for knockdown. Mosquitoes were then transferred to another set of plastic cups provided with cotton wool soaked with glucose solution. Mortality was counted 24 hour post-exposure. A control experiment with untreated net was run at the same time.

Figure 27. A plywood platform to study the effect of impregnated netting on feeding of mosquitoes.

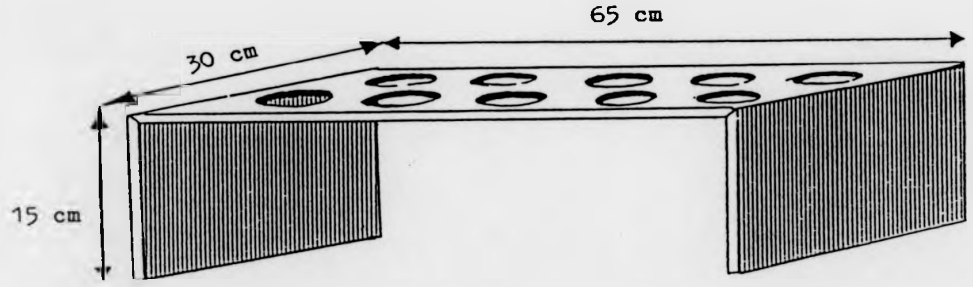
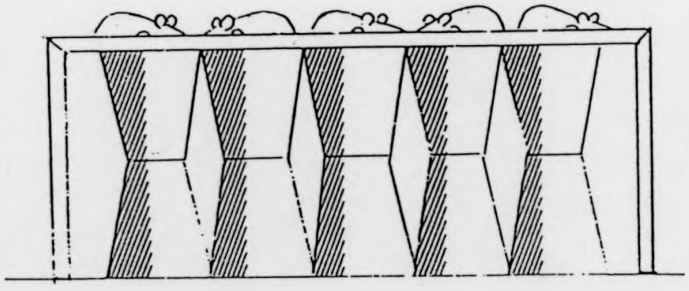


Figure 28. Anaesthetised mice upon the holes of the platform. Pairs of paper cups containing mosquitoes and covered with pieces of permethrin impregnated netting are seen under the mice.



4.11.3. Results:

Results of the test of feeding inhibition on a human arm are presented in Tables 27 and 28 and Figure 28. Results of the test on mice are presented in Table 29. A dose of 2.5 g/m^2 was required to prevent An. gambiae completely from feeding through nylon netting on a human arm. More than 45% of An. gambiae succeeded in feeding through cotton netting impregnated at the same dose. The survival rate was high among fed and unfed An. gambiae with cotton netting. There was a high survival rate among fed An. gambiae with both nylon and cotton netting. More than 50% of Ae. aegypti fed through 2.5 g/m^2 permethrin impregnated nylon netting, but none of the fed mosquito survived at this dose. In general there was more mortality among fed Ae. aegypti than fed An. gambiae. Table 29 shows that a dose of 0.05 g/m^2 was enough to prevent Ae. aegypti completely from feeding through nylon netting on mice. There was high mortality among those mosquitoes which were exposed to mice.

Table 27. Blood feeding of the KWA strain of An. gambiae through permethrin impregnated nylon and cotton netting pressed against the arm of a human subject for 5 minutes.

Netting type	Permethrin dose (g/m^2)	Percentage fed	% Mortality		% fed and survived	Total number of mosquitoes
			Unfed	Fed		
Nylon	Control	80.0	0	0	80.0	15
Cotton	Control	100	0	0	100	15
Nylon	0.025	80.0	0	6.7	73.3	15
Cotton	0.025	80.0	0	0	80.0	15
Nylon	0.4	73.3	13.3	13.3	60.0	15
Cotton	0.4	66.7	0	0	66.7	15
Nylon	0.8	60.0	20.0	20.0	40.0	5
Cotton	0.8	-	-	-	-	-
Nylon	1.6	20.0	80.0	0	20.0	5
Cotton	1.6	-	-	-	-	-
Nylon	2.5	0	93.3	0	0	15
Cotton	2.5	46.7	6.7	6.7	40.0	15
Nylon	5.0	-	-	-	-	-
Cotton	5.0	0	73.3	0	0	15

- = not recorded

Table 28. Blood feeding of the AE AE strain of Ae. aegypti through permethrin impregnated nylon netting pressed against the arm of a human subject for 5 minutes.

Permethrin dose (g/m^2)	Percentage fed	Knockdown in 1 h (%)	% Mortality		% fed and survived	Total number of mosquitoes
			Unfed	Fed		
Control	93.3	0	0	0	93.3	15
0.025	80.0	0	20.0	0	80.0	5
0.05	100	20.0	0	0	100	5
0.1	80.0	80.0	20.0	20.0	60.0	5
0.2	93.3	46.7	6.7	26.6	66.7	15
0.4	66.7	93.3	13.3	33.3	33.3	15
0.8	60.0	80.0	33.3	33.3	26.7	15
1.6	60.0	93.3	40.0	46.7	13.3	15
2.5	53.3	93.3	40.0	53.3	0	15

Figure 29
Percentage fed, not knocked down and survived when the AE AE strain of Ae.aegypti was allowed to feed through pieces of permethrin impregnated nylon netting on an arm.

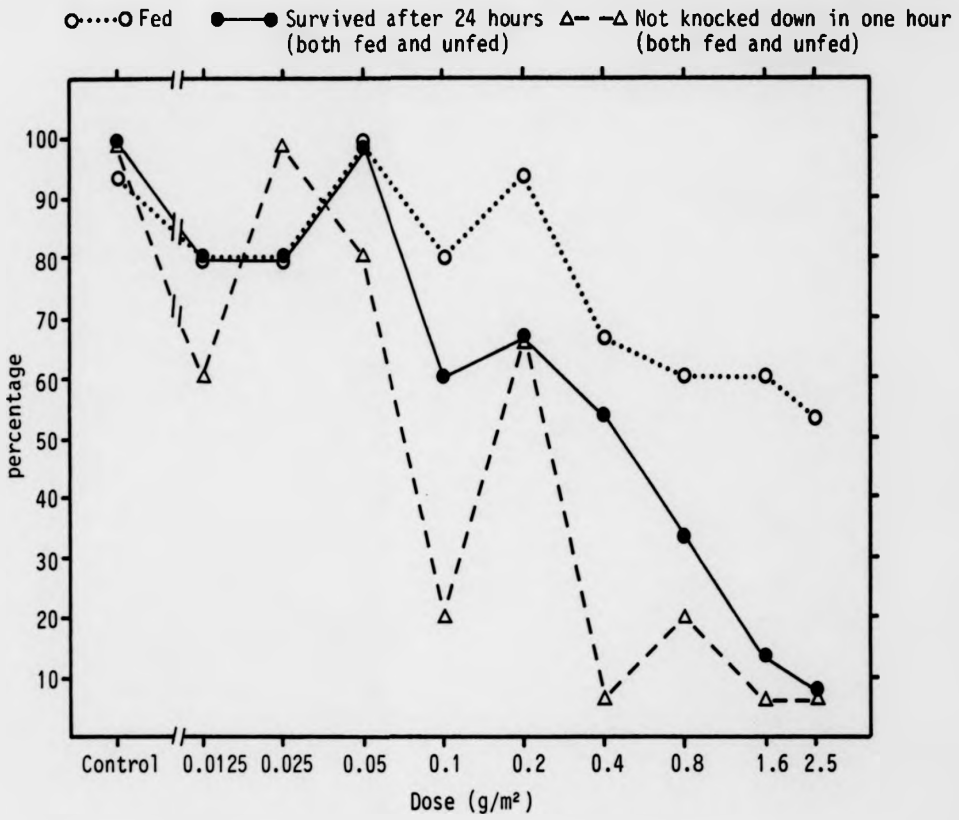


Table 29. Blood feeding of the AE AE strain of Ae. aegypti on anaesthetised mice through pieces of permethrin impregnated nylon netting. Exposure time was 30 minutes.

Permethrin dose (g/m^2)	Percentage fed	Knockdown in 30 mins (%)	% Mortality		% fed and survived	Total number of mosquitoes
			Unfed	Fed		
Control	54.5	0	20.2	3.1	51.4	37
0.025	22.2	31.1	44.5	2.2	20.0	45
0.05	0	92.0	100	0	0	25
0.1	0	96.0	96.0	0	0	25
0.2	0	85.7	90.5	0	0	21
0.4	0	97.7	100	0	0	44
0.8	0	100	100	0	0	39

4.11.4. Discussion:

When mosquitoes were exposed in a test kit pressed against an arm, a very high dose was required to prevent mosquitoes from feeding through netting. It would probably not be practicable to use such a high dose for impregnating bednets.

According to a report of Wellcome Research Laboratories, a dose of 0.2 g/m^2 was enough to prevent 85% of Ae. aegypti from feeding on mice and 90% of the mosquitoes were knocked down within 30 minutes of exposure (Carter 1983). In the present experiment a dose of 0.05 g/m^2 gave 100% protection to mice against Ae. aegypti. But more than 50% of these mosquitoes succeeded in feeding on an arm through netting impregnated at a dose which was fifty times higher (2.5 g/m^2). This paradox requires explanation. It may be mentioned that mosquitoes were exposed for 30 minutes to mice and only for 5 minutes to the arm. One reason for the difference in effectiveness of the impregnated netting may be that as the body of mice is covered with thick fur, it takes some time for the mosquitoes to find a suitable place to probe through. But within this short contact with the impregnated netting they pick up a dose which is enough to change their physiology and they fail to take any blood. From this experiment it appears that in studying chemicals intended for the protection of humans it is important to use a human subject, wherever possible, for this kind of test; use of an animal model may give a misleading result.

Most of the An. gambiae survived after feeding through an impregnated net. There was more mortality among unfed than among fed individuals. The reason may be that the insecticides are diluted when there is a large amount of blood in the abdomen. The practical significance of this finding is that the mosquitoes that succeed in taking a blood meal from a malaria or dengue patient through an impregnated bednet would still be capable of transmitting the disease. Comparatively fewer Ae. aegypti survived after taking a blood meal. This may be due to the feeding behaviour of this mosquito. While feeding through an impregnated net, not only the tarsae but also the abdomen came in contact with the netting, thus exposing more surface to insecticide. In most cases An. gambiae was found to touch the arm (and also the impregnated netting) with 2 or 4 of its legs keeping the other legs on the wall of the test kit.

The reason that Ae. aegypti succeeded in feeding at a higher dose may be due to the sluggish nature of this mosquito, i.e. Ae. aegypti seems to be less readily irritated than An. gambiae. In the comparison of the susceptibility of these two species in bioassays, it was shown in section 4.4 that Ae. aegypti was more susceptible than An. gambiae and the explanation may again be a lower tendency to be irritated in Ae. aegypti.

4.12. Effect of impregnated bednets on mosquito behaviour:

4.12.1. Introduction:

One of the intentions in impregnating bednets with chemicals is to reduce the mosquito population by killing approaching mosquitoes. Although a considerable amount of field work has been done, no laboratory work simulating natural conditions have yet been reported on the killing effect of a bednet on a known number of hungry mosquitoes. It is important to know how long mosquitoes spend on a bednet. Only after knowing this "resting" time of mosquito on a bednet can we try to determine a dose that is necessary to kill mosquitoes for that particular exposure period. No work had been done previously on this question. Therefore, an experiment was undertaken using bednets of various mesh-sizes and with or without large holes cut in them.

4.12.2. Methods:

Three types of bednets, namely, 1.5 mm mesh nylon bednets (normal mosquito nets), 0.4 cm mesh nylon nets and 0.8 cm mesh thick cotton nets, were impregnated with 0.2 g/m^2 permethrin. The bednets had no sheet border, instead they had folded cotton tapes along the border and along the joins. The size of the nets was 1 x 1 x 1 metre each. To test a net it was hung in a mosquito proof room. Care was taken to keep the room clean so that all the mosquitoes could be collected after the exposure period was over. Either two or four holes were cut in normal mesh-size nylon nets to simulate torn nets in the field. The sizes of the holes were either 5 x 10 cm or 10 x 20 cm.

In each test about 10 hungry female An. gambiae (KWA strain) were released in the room and the experimenter sat under the bednet either touching the nets with one of his arms or not. The experimenter directly observed the behaviour of mosquitoes for 30 minutes. The mosquitoes could feed either through the net, when the experimenter's arm was pressed against it, or after the mosquitoes had entered the net either through the holes which had been cut or through wide-meshes. After the exposure period was over the mosquitoes were recaptured using a mouth aspirator. The mosquitoes were scored as fed or unfed, knocked down or not knocked down. They were kept in paper cups with a piece of cotton wool soaked with glucose solution for 24 hours after which they were counted as dead or alive.

To study the effect of permethrin vapour on the mortality of mosquitoes the same experimental set up as above was used. About 25 KWA mosquitoes in a 30 x 30 x 30 cm cage were put under an impregnated bednet on the floor. Thus the mosquitoes were at a distance of about 50 cm from the impregnated netting. Normal mesh-size or 0.4 cm mesh-size nylon nets were used for this purpose. The wide-mesh nylon net was used within two weeks of impregnation but the other type was used between 75 and 85 days after impregnation. Glucose was provided inside the cage in an initial test but it was found in preliminary tests that many mosquitoes became stuck in the lint wick and died apparently due to the dry condition of the room. Therefore, in the actual experiments cotton wool soaked with glucose solution was put on the top of the cage. Mortality was counted at the end of fifteen hour exposure period. A control test was run in each case.

4.12.3. Results:

Tables 30, 31 and 32 show that the average time spent by mosquitoes on treated nets was much less than the average time spent by mosquitoes on control nets. On a treated net the longest time spent by a mosquito was three minutes, whereas, on a control net a maximum of 21 minutes was recorded as time spent resting and searching for a suitable place of entry. Table 30 shows that, compared with control, very few mosquitoes entered the net unless the holes were very large (a total of 800 sq cm in a net of surface area 5 sq m).

Among the mosquitoes which entered nets many of them failed to take a blood meal, the failure rate was more in treated then in untreated controls. This rate of failure was 100% when the mesh-size was 0.4 cm. Table 31 shows that no mosquito fed through permethrin impregnated nets on an arm pressed against the net, while in the control about 65% mosquitoes fed through the net. In all but one case there was 100% mortality among mosquitoes released into a room containing an impregnated net. However, there was only about 89% mortality when an impregnated thick cotton net was used.

Table 33 shows that when mosquitoes in a cage were put under an impregnated wide-mesh nylon net there was no significant mortality. There was, however, a significantly higher mortality among mosquitoes kept under an impregnated fine mesh nylon net.

Table 30. Behaviour of the KWA strain of An. gambiae released in a room containing nylon bednets (1.5 mm mesh) either untreated or treated with 0.2 g/m² permethrin and with a man sitting under the net but not touching it.

Description of nets	Geometric mean time spent by a mosquito on the net (range)	Number of mosquitoes which entered the net (%)	Number of mosquitoes which entered and fed (%)	Knockdown in 30 minutes	Deaths in 24 hours	Total number of mosquitoes
Control net with 2 holes (5 x 10 cm)	2 mins 11 secs (10 s - 21 m)	12 (40.0)	11 (36.7)	0	0	30
Impregnated net with 2 holes (5 x 10 cm)	13 secs (5 s - 1.5 m)	2 (7.1)	2 (7.1)	28	28	28
Control net with 4 holes (5 x 10 cm)	43 secs (5 s - 6.5 m)	24 (72.7)	23 (69.7)	0	0	33
Impregnated net with 4 holes (5 x 10 cm)	18 secs (5 s - 3 m)	2 (6.9)	1 (3.4)	29	29	29
Control net with 4 holes (10 x 20 cm)	2 mins (1 - 5 m)	12 (100)	9 (75.0)	0	0	12
Impregnated net with 4 holes (10 x 20 cm)	18 secs (5 s - 2 m)	16 (44.4)	9 (25.0)	35	36	36

Table 31. Behaviour of the KWA strain of An. gambiae released in a room containing a nylon bednet either untreated or treated with 0.2 g/m² permethrin. A man was sitting under the net and pressing one of his arms against the net.

Description of net	Geometric mean time spent by mosquitoes on net (range)	Number of mosquitoes which entered net (%)	Number of mosquitoes which entered and fed (%)	Number of mosquitoes which fed through net (%)	Knockdown in 30 mins	Deaths in 24 hrs	Total number of mosquitoes
Control net with 2 holes (5x10 cm)	2 mins 14 secs (30 secs - 10 mins)	6 (19.4)	6 (19.4)	20 (64.5)	0	0	31
Impregnated net with 2 holes (5x10 cm)	40 secs (5 secs - 2 mins)	5 (18.5)	1 (3.7)	0	27	27	27

Table 32. Behaviour of the KWA strain of An. gambiae released into a room containing a thick cotton net with 0.8 cm mesh or a nylon net with 0.4 cm mesh. The nets were either untreated or treated with 0.2 g/m² permethrin and a man sat under the net.

Description of nets	Geometric mean time spent by mosquitoes on net (range)	Number of mosquitoes entered net (%)	Number of mosquitoes entered and fed (%)	Knockdown in 30 minutes	Deaths in 24 hours	Total number of mosquitoes
Control thick cotton net	-	28 (66.6)	21 (50.0)	0	1	42
Impregnated thick cotton net	-	8 (17.8)	3 (6.7)	40	40	45
Control nylon net	44 secs (10 s - 8 m)	38 (55.9)	35 (51.5)	0	0	68
Impregnated nylon net	15 secs (5 s - 2.5 m)	16 (33.3)	0	48	48	48

- = not recorded

Table 33. Vapour effect of permethrin when impregnated into nylon nets and tested against the KWA strain of An. gambiae.

Type of net	Days after impregnation	Number of mosquitoes			Percentage mortality
		Dead	Alive	Total	
Control net	-	3	49	52	5.7
Impregnated net:					
(a) normal mesh (1.5 mm)	75 - 85	15	59	74	20.3
(b) wide-mesh (4 mm)	1 - 15	5	96	101	5.0

4.12.4. Discussion:

An experimental set up like the one used here has the advantages that we can calculate the mortality, knockdown, feeding rate, etc. but still there are some drawbacks. In the present set up there is no way for the mosquitoes to completely escape, so the same mosquito may repeatedly try to enter the bednet or feed through the net, and it thus may pick up a lethal dose and ultimately die. In the field some of the mosquitoes may actually die but some others may be irritated and repelled after first contact with the bed net. For the same reason it is quite possible that the same mosquito has been counted more than once as an individual spending time on a net, thus the actual time spent by some individuals may have been more than what was counted. With all these limitations if we look at the results certain conclusions are clear.

The geometric mean time spent by mosquitoes on impregnated nets was about 15 secs. Only when an arm was pressed against an impregnated net did the mosquitoes spend an average of 40 secs. This is due to the fact that the mosquitoes in this case tried to probe through the nets, thus spending more time on the nets. There was much variation in the time spent by mosquitoes on control nets. But in each of the cases mosquitoes spent much less time on impregnated nets than on control nets. The mosquitoes spent up to 21 minutes on a control net, while the maximum time spent by a mosquito on treated net was only 3 minutes. This must be due to the irritating effect of permethrin.

Only about 7% of mosquitoes entered the impregnated nets when there were two or four 5 x 10 cm holes on the nets. In the control nets 40% of mosquitoes entered the net when there were two holes and about 73% mosquitoes entered when there were four holes. When the holes were quadrupled in area and there were 4 holes in a net, the number of mosquitoes entering was reduced due to impregnation but still 45% of mosquitoes entered the nets, which cannot be taken as a good achievement for practical purposes. Port and Boreham (1982) showed that bednets with small tears still can afford some protection. Lines et al. (1985) showed that unimpregnated bednets with eight 10x20 cm holes provided no protection, whereas similar nets impregnated with 0.2 g/m^2 permethrin provided some protection.

When an arm was pressed against a net, very few mosquitoes entered the

net, both in the control and treated nets presumably due to the easy availability of a blood source even when the mosquitoes were outside the net. Comparatively fewer mosquitoes entered an impregnated 0.8 cm mesh thick cotton net than a 0.4 cm mesh nylon net. But this difference was not statistically significant ($\chi_1^2 = 3.47$, $P > 0.05$).

None of about 33% mosquitoes that entered permethrin impregnated 0.4 cm mesh nylon net were fed. This may be due to the fact that mosquitoes spent some time on the net before entering, thus picking up a dose of permethrin. Itoh et al. (1986) observed that when the mesh-size of the nets is less than the length of the mosquito's wing-span, the mosquitoes have to land on the net before passing through. It was observed that mosquitoes tried different meshes before finding a suitable mesh to pass through. Sometimes the mosquitoes pushed up to its thorax into a mesh and then withdrew itself. Perhaps this is due to the irritating effect of permethrin. When finally they succeeded in passing through the nets, they appeared to be uncoordinated. Some of the mosquitoes after entering flew out of the nets without feeding.

Although feeding was not completely inhibited in cases other than 0.4 cm mesh-size nylon nets, there was a reduction in feeding among mosquitoes that entered impregnated nets compared with those that entered the control net. The difference existed in the case of wide-mesh cotton nets, nets with 4 small holes and nets with two small holes when an arm was pressed against them. Only two mosquitoes entered the net with two small holes when no part of the body touched the net, and both of them fed.

One interesting finding of this experiment is that when an arm was pressed against an impregnated net none of the mosquitoes fed through the net, although the net was impregnated at a dose of only 0.2 g/m^2. In a previously described experiment (Table 27) it was observed that An. gambiae succeeded in feeding even if the dose was 1.6 g/m^2 applied to nets on the ends of testing tubes in which the mosquitoes were contained. This difference may be due to the fact that with free flying mosquitoes and a bednet the mosquitoes required some time to find a suitable place to probe through, but within this time they picked up a dose of permethrin, became uncoordinated and were therefore unable to feed. From this finding it can be concluded that impregnation of bednets can prevent mosquitoes from feeding through them on the limb of a sleeper which accidentally touches the net.

Although the mortality among mosquitoes put under an impregnated fine-mesh nylon net was not as high as was obtained by Ree (1986), the difference from control was statistically significant ($\chi_1^2 = 5.1$, $P < 0.05$). Ree obtained more than 90% mortality using nylon bednets treated with the same dose of permethrin as in the present experiment (i.e., 0.2 g/m^2). However, he exposed mosquitoes within 4 - 6 days of the treatment of the net. In the present investigation the nets were used between 75 and 85 days after impregnation, which may be a reason for the low mortality. But there was no effect of vapour in killing of mosquitoes when they were kept under the 0.4 cm mesh nylon net, although this net was freshly impregnated (used within 15 days of impregnation). It may be mentioned that 100% feeding inhibition was obtained using the same net. Recently J.E. Miller (personal communication) obtained 84% mortality keeping mosquitoes at a distance of one cm from 0.4 g/m^2 permethrin impregnated cotton netting for 24 hours.

CHAPTER 5. GENERAL DISCUSSION AND RELEVANCE OF THE WORK TO VECTOR CONTROL

Use of pyrethroid impregnated bednets and repellent impregnated net curtains is a fairly new idea in the field of vector control. While trying to implement this idea in the field, researchers have had to consider several problems:

1. What is the easiest and most reliable method of impregnation of bednets with pyrethroids?

2. How long does a pyrethroid, e.g. permethrin, persist on a net under various adverse conditions, e.g. washing, drying in the sun, exposure of the net to the tarsi of many mosquitoes, etc.?

3. Among various types of bednets (e.g. cotton, nylon, polyester) which one is the best as a medium for pyrethroid impregnation and gives the best protection against mosquitoes?

4. Is there a difference in response of various species of mosquitoes towards a pyrethroid, e.g. permethrin?

5. Of the various pyrethroids available on the market which ones are the most effective against mosquitoes?

6. Which pyrethroid best withstands washing and ageing in tropical conditions?

7. How long do mosquitoes spend on a bednet? Is this time enough for the mosquitoes to pick up a lethal dose of pyrethroid from the bednet?

8. What is the relationship between the exposure time and dose of a pyrethroid and the insecticidal effect?

9. Can a pyrethroid impregnated wide-mesh bednet protect a sleeper from mosquito bites? What should be the maximum mesh-size of a bednet if it is to be effective against attacking mosquitoes?

10. What is the prospect of using wide-mesh net curtains impreganted with a volatile repellent, e.g. deet, against mosquitoes?

11. Can a torn but pyrethroid impregnated bednet protect a sleeper from mosquito bites? Can the mosquitoes bite through impregnated nets when some part of the body touches the net?

12. Is the present WHO method for detection of pyrethroid resistance in adult mosquitoes reliable? If not, what are the other possibilities?

13. What is the extent of pyrethroid cross-resistance among the populations of mosquitoes resistant to other insecticides?

14. If there is a gene for pyrethroid tolerance in any particular target mosquito population, what is the potentiality of the population to develop resistance to pyrethroids due to the use of impregnated nets?

15. Is there any effect of temperature on pyrethroid action against mosquitoes?

These were the questions studied in the present project and the following main conclusions were reached:-

Impregnation of fabrics by dipping them into an excess volume of emulsion of permethrin is an easier method to carry out then the W.H.O. recommended method of measuring out the volume required for each net (Schreck and Self, 1985a). Chemical analysis showed that the permethrin content of impregnated fabrics was similar to that expected on the assumption of passive absorption of only the permethrin in the weight of liquid taken up, without any removal of permethrin from the liquid not taken up. But when the nets were impregnated by dipping them into permethrin emulsion near its boiling point and at acid pH the nets picked up about double the amount of permethrin contained in the volume of liquid absorbed. For impregnating several nets from the same emulsion this method would not be suitable because nets dipped at the beginning of the batch would pick up more permethrin, resulting in the emulsion becoming less and less concentrated. Thus very little permethrin would be left for the nets impregnated towards the end of the series.

Permethrin was found to persist well on impregnated nets if they were not washed. Permethrin impregnated wide-mesh cotton nets offered very good protection against mosquitoes for at least 30 weeks, when evaluated in a "tunnel" of cages. Bioassays were done on pieces of permethrin impregnated nylon netting over a period of 12 weeks. There was no significant reduction of effectiveness even when the same pieces of netting were used for repeated exposure to mosquitoes and they were kept in a hot humid room between their repeated exposures. There were, however, indications of reduction of effectiveness when impregnated nylon nets were washed in the laboratory using Gambian cow fat soap and dried in the sun. This effect was not seen when the nets were washed using soap bought in London. If further investigation proves that certain soap has a severe effect on persistence of permethrin, then it would be important to try to persuade people to avoid such soap when washing nets. Hand washing by a Gambian housewife, with cow fat soap, of nets impregnated with 9 different pyrethroids reduced the chemical content of all

the pyrethroids to various degrees. The toxicity of the nets against mosquitoes was also reduced.

The LD_{50} of permethrin on cotton nets was found to be about three times greater than on nylon. Lines et al. (1987) also achieved better protection against wild mosquitoes in Tanzania by impregnating nylon nets with permethrin than similarly impregnating cotton nets. However, Hervey and Sales (1980) did not see any difference in performance between permethrin impregnated nylon and cotton netting when bioassayed for one hour using Ae. aegypti. In some countries cotton nets are cheaper than nylon nets because the latter requires scarce foreign exchange. It was not possible to calculate whether the price of the extra permethrin required for cotton nets would compensate for the difference of the price of cotton and nylon nets. We also do not know whether permethrin persists longer on cotton or on nylon nets. Only after taking into account all these factors can we decide whether it is better to use cotton or nylon bednets in any particular country.

Ae. aegypti was found to be the most susceptible to permethrin of the three mosquito species studied. C. quinquefasciatus was the most tolerant and An. gambiae was intermediate between them. Ae. aegypti is mainly a day biting mosquito and there is not much prospect of using impregnated bednets against this species. C. quinquefasciatus is mainly an urban mosquito and there is yet no published report of using impregnated bednets in an urban environment. If anybody in future plans to introduce pyrethroid impregnated bednets in an area predominently infested with C. quinquefasciatus, it may be necessary to use a higher dose than that is being used against Anopheles mosquitoes.

PP321 (Icon) and cypermethrin were found to be the most effective of 9 pyrethroids tested. These two chemicals were very toxic against mosquitoes even after the impregnated nets were washed with soap and aged in a tropical country. There is as yet no report of using these two pyrethroids in the field for mosquito control. It is highly desirable that field tests of these insecticides on bednets are carried out.

WHO recommends exposure of Anopheles mosquitoes for one hour and C. quinquefasciatus for 3 hours to 0.25% permethrin impregnated papers for detection of resistance of adult mosquitoes (WHO, 1986). It is also recommended to keep the test kits in a horizontal position during exposure period, so that mosquitoes knocked down before the end of the exposure period still remain in contact with the insecticide. But a problem with this system is that mosquitoes, when they are knocked down, lie on their back on

impregnated papers, thus offering more surface to insecticide than when they stand on their tarsi. In the present investigation a strain of An. gambiae (MU) would be classified as susceptible to permethrin using data from the WHO method, but data with WHO papers and the test kit in the vertical position or with impregnated netting showed that this strain was more tolerant than the susceptible strain of the same species. However with netting there was no single dose which reliably discriminated the tolerant and the susceptible strain. Thus it is suggested that exposure of mosquitoes to permethrin impregnated papers in the vertical position of the test-kits is the best available method of resistance detection provided the exposure time is so adjusted that mosquitoes are not knocked down within the exposure period.

Out of 5 strains belonging to 4 different species and resistant to various insecticides, two strains showed some tolerance to permethrin. One of the other three strains was highly resistant to DDT but it was as susceptible to permethrin as a DDT susceptible strain of the same species. One of the permethrin tolerant strains (which was known to be resistant to dieldrin) was put under permethrin selection pressure for 9 generations. There was no increase in the permethrin resistance level, indicating the absence of a gene for strong permethrin resistance from this population. When a part of the population was put under DDT selection pressure the level of DDT resistance increased rapidly and within 9 generations the DDT resistance level of this strain became very high (LT_{50} value more than 25 times than that of parent population). However, there was no cross-resistance to permethrin.

It has been reported by other authors (Malcolm and Wood, 1982b; Chadwick et al.,1984) that among DDT resistant mosquitoes there may be at least two mechanisms involved:- (i) dehydrochlorination which does not cause cross-resistance to permethrin, and (ii) central nervous system insensitivity due to the presence of the kdr gene which is responsible for cross-resistance to permethrin; this mechanism was not detected in any of the strains during the present investigation.

Permethrin impregnated wide-mesh nylon and cotton bednets (0.4 and 0.8 cm mesh sizes respectively) performed very well against hungry An. gambiae. However, nylon nets having 1.6 cm mesh size did not protect from mosquito bites. Itoh et al. (1986) observed that when the mesh-size of a net is smaller than a mosquito's wing span it lands on the net before passing through the mesh. It was observed in the present investigation that although mosquitoes entered 0.4 cm mesh bednets which were impregnated with 0.2 g/m^2 permethrin,

none of them fed. This was apparently due to the fact that mosquitoes before entering nets rested on them and thus picked up a dose of permethrin which resulted in feeding inhibition and ultimately death. The effectiveness of impregnated wide-mesh nets is a very important finding for tropical countries where one of the reasons for people not being willing to use bednets is that they prevent air circulation (Hii et al., 1987).

Deet impregnated wide-mesh cotton net curtains performed better when they were placed at a distance from the bait. So, it seems that deet impregnated window and eave curtains might be more effective than deet impregnated bednets. But deet does not persist long on fabrics when left exposed to the atmosphere. Lines et al. (1985) observed in the field in Tanzania that the smell of deet on impregnated eave-curtains persists only 2 to 3 days after which the curtains fail to repel mosquitoes. Thus it would probably not be acceptable in practice to use deet or other vapour repellents for impregnation of either bednets or window- or eave-curtains.

Permethrin impregnated nets with holes cut in them gave very good protection against mosquitoes. Thus permethrin impregnation of bednets can increase the effective life of a bednet, which would amply compensate for the cost of permethrin. Mosquitoes cannot bite through impregnated nets even if part of the body remains in contact with the impregnated net. In developing countries, such as Bangladesh, people often sleep in groups under the same bednet. Thus almost always some parts of the body of the persons who sleep on the outside remain in contact with the net, and are thus bitten by mosquitoes. Permethrin impregnation of bednets can solve this problem.

No effect of temperature within the range of 16°C and 28°C on the toxicity of permethrin against An. gambiae was detected during the present investigation. Cutkomp and Subramanyam (1986) observed permethrin to be 3.63 times more toxic at 20°C than at 30°C against Ae. aegypti larvae. There is no published report on the effect of temperature on the toxicity of pyrethroids against adult mosquitoes.

The exposure time of mosquitoes to permethrin and the dose of permethrin are not interchangable, e.g. doubling the dose and simultaneously halving the exposure time increases the insecticidal effect. Thus permethrin is comparatively more effective at shorter exposure times than at longer exposure times. This contrasts with other groups of insecticides where in some cases doubling the dose and simultaneously halving the exposure time produces an unchanged insecticidal effect.

This rapid uptake of permethrin is fortunate because mosquitoes spend very little time (less than a minute on any one occasion) on an impregnated net, but this is enough for them to pick up a lethal dose. Almost all the mosquitoes released in a room containing a permethrin impregnated bednet died. In Papua New Guinea whole night human biting catches and a capture-recapture experiment revealed that due to the introduction of permethrin impregnated bednets in a whole village the biting population of An. farauti decreased by about 30% (Charlwood and Graves, 1987). Li et al. (in press) also reported a decreased vector density (as measured by outdoor biting catches on human baits) by about 50% compared with the pre-treatment sample in studies using deltamethrin treated bednets in China. As this project was highly successful in the initial stage with 3,500 people, it has been extended to include about 40,000 people and other very large scale operations are reported from China. The entomological data indicate that widespread impregnation of bednets can be expected to protect not only sleepers inside the nets but also the whole community by reducing the number and lifespan of mosquitoes.

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Appendix 1. Results of the exposure of the AE AE strain of Ae. aegypti to pieces of permethrin impregnated nylon netting for 30 secs at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	32
0.025	11 (33.3)	6 (18.2)	33
0.05	15 (44.1)	12 (35.3)	34
0.1	21 (60.0)	14 (40.0)	35
0.2	22 (66.7)	14 (42.4)	33
0.4	28 (80.0)	14 (40.0)	35
0.8	30 (96.8)	27 (87.1)	31
1.6	34 (100)	34 (100)	34
2.5	35 (100)	35 (100)	35

Appendix 2. Results of the exposure of the AE AE strain of *Ae. aegypti* to pieces of permethrin impregnated nylon netting for 2 minutes at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	35
0.025	3 (8.6)	0	35
0.05	15 (37.5)	7 (17.5)	40
0.1	35 (83.3)	19 (45.2)	42
0.2	34 (82.9)	15 (36.6)	41
0.4	42 (95.5)	35 (79.6)	44
0.8	36 (100)	35 (97.2)	36
1.6	35 (100)	35 (100)	35
2.5	35 (100)	35 (100)	35

Appendix 3. Results of the exposure of the AE AE strain of *Ae. aegypti* to pieces of permethrin impregnated cotton netting for 2 minutes at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	35
0.025	1 (3.1)	0	32
0.05	6 (15.5)	2 (5.3)	38
0.1	10 (27.8)	4 (11.1)	36
0.2	13 (35.1)	5 (13.5)	37
0.4	15 (40.5)	7 (18.9)	37
0.8	24 (64.9)	15 (40.5)	37
1.6	26 (66.7)	17 (43.6)	39
2.5	36 (94.7)	35 (92.1)	38
3.2	41 (100)	41 (100)	41

Appendix 4. Results of the exposure of the AE AE strain of Ae. aegypti to pieces of permethrin impregnated polyester netting for 30 secs at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	41
0.025	0	0	49
0.05	0	0	44
0.1	3 (6.1)	1 (2.0)	49
0.2	11 (22.4)	13 (26.5)	49
0.4	38 (77.6)	33 (67.3)	49
0.8	46 (90.0)	43 (86.0)	50
1.6	47 (100)	42 (89.4)	47
2.5	48 (100)	48 (100)	48

Appendix 5. Results of the exposure of the AE AE strain of Ae. aegypti to pieces of permethrin impregnated polyester netting for 2 minutes at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	44
0.025	3 (6.1)	3 (6.1)	49
0.05	4 (7.4)	3 (5.6)	54
0.1	12 (26.1)	14 (30.4)	46
0.2	37 (78.7)	30 (63.8)	47
0.4	48 (98.0)	42 (85.7)	49
0.8	43 (100)	42 (97.7)	43
1.6	54 (100)	54 (100)	54

Appendix 6. Results of the exposure of the KWA strain of *A. gambiae* to pieces of permethrin impregnated nylon netting for 30 secs at a range of doses.

Permethrin dose (g/m²)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	42
0.025	0	0	45
0.05	1 (2.6)	1 (2.6)	38
0.1	0	0	40
0.2	6 (13.6)	6 (13.6)	44
0.4	9 (23.7)	11 (28.9)	38
0.8	33 (80.5)	32 (78.0)	41
1.6	46 (100)	45 (95.7)	46
2.5	36 (100)	36 (100)	36

Appendix 7. Results of the exposure of the KWA strain of *A. gambiae* to pieces of permethrin impregnated nylon netting for 2 minutes at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	42
0.025	2 (4.9)	1 (2.4)	41
0.05	3 (6.3)	3 (6.3)	48
0.1	9 (22.5)	10 (25.0)	40
0.2	22 (52.4)	17 (40.5)	42
0.4	31 (75.6)	28 (68.3)	41
0.8	34 (94.5)	32 (88.9)	36
1.6	40 (100)	40 (100)	40
2.5	41 (100)	41 (100)	41

Appendix 8. Results of the exposure of the KWA strain of *A. gambiae* to pieces of permethrin impregnated cotton netting for 30 secs at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	36
0.025	0	0	36
0.05	0	0	38
0.1	0	0	36
0.2	0	0	38
0.4	0	2 (5.9)	34
0.8	3 (7.9)	6 (15.8)	38
1.6	13 (34.2)	20 (52.6)	38
2.5	31 (88.6)	31 (88.3)	35
3.2	37 (100)	37 (100)	37

Appendix 9. Results of the exposure of the KWA strain of *A. gambiae* to pieces of permethrin impregnated cotton netting for 2 minutees at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	35
0.025	0	0	30
0.05	0	0	38
0.1	0	0	37
0.2	0	2 (5.1)	39
0.4	7 (18.4)	13 (34.2)	38
0.8	14 (40.0)	16 (45.7)	35
1.6	32 (82.1)	31 (79.5)	39
2.5	40 (100)	40 (100)	40

Appendix 10. Results of the exposure of the KWA strain of *A. gambiae* to pieces of permethrin impregnated polyester netting for 30 secs at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	36
0.025	0	0	36
0.05	0	0	38
0.1	0	0	37
0.2	1 (2.8)	0	36
0.4	14 (38.9)	14 (38.9)	36
0.8	23 (59.0)	20 (51.3)	39
1.6	36 (100)	29 (80.6)	36
2.5	35 (100)	35 (100)	35

Appendix 11. Results of the exposure of the KWA strain of *A. gambiae* to pieces of permethrin impregnated polyester netting for 2 minutes at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	38
0.025	0	0	40
0.05	0	1 (3.0)	33
0.1	1 (2.8)	3 (8.3)	36
0.2	12 (34.3)	12 (34.3)	35
0.4	32 (76.2)	33 (78.6)	42
0.8	37 (100)	36 (97.3)	37
1.6	34 (100)	34 (100)	34

Appendix 12. Results of the exposure of the CfCA strain of *C. quinquefasciatus* to pieces of permethrin impregnated nylon netting for 15 secs at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	33
0.025	0	0	33
0.05	0	0	33
0.1	4 (12.9)	3 (9.7)	31
0.2	4 (14.3)	6 (21.4)	28
0.4	7 (25.9)	7 (25.9)	27
0.8	26 (83.9)	24 (77.4)	31
1.6	23 (85.9)	23 (85.9)	27
2.5	28 (93.3)	27 (90.0)	30
3.2	28 (93.3)	27 (90.0)	30

Appendix 13. Results of the exposure of the CfCA strain of C. quinquefasciatus to pieces of permethrin impregnated nylon netting for 30 secs at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	31
0.025	0	0	33
0.05	0	1 (3.2)	31
0.1	1 (3.9)	2 (7.7)	26
0.2	5 (18.5)	4 (14.8)	27
0.4	9 (33.3)	7 (25.9)	27
0.8	19 (61.3)	17 (54.8)	31
1.6	24 (72.7)	21 (63.6)	33
2.5	27 (93.1)	23 (79.3)	29
3.2	18 (94.7)	14 (73.7)	19

Appendix 14. Results of the exposure of the CfCA strain of C. quinquefasciatus to pieces of permethrin impregnated nylon netting for one minute at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	31
0.025	0	0	30
0.05	0	0	32
0.1	2 (6.5)	3 (9.7)	31
0.2	5 (16.1)	4 (12.9)	31
0.4	13 (46.4)	12 (42.9)	28
0.8	22 (78.6)	17 (60.7)	28
1.6	26 (86.7)	23 (76.7)	30
2.5	32 (100)	32 (100)	32

Appendix 15. Results of the exposure of the CfCA strain of *C. quinquefasciatus* to pieces of permethrin impregnated nylon netting for 2 minutes at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	30
0.025	1 (3.1)	1 (3.1)	32
0.05	2 (6.1)	1 (3.0)	33
0.1	6 (19.4)	4 (12.9)	31
0.2	7 (23.3)	8 (26.7)	30
0.4	11 (37.9)	13 (44.8)	29
0.8	28 (96.6)	22 (75.9)	29
1.6	32 (97.0)	27 (81.8)	33
2.5	33 (100)	31 (93.4)	33
3.2	13 (100)	13 (100)	13

Appendis 16. Results of the exposure of the CfCA strain of C. quinquefasciatus to pieces of permethrin impregnated nylon netting for 4 minutes at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	30
0.025	3 (9.4)	4 (12.5)	32
0.05	6 (18.2)	5 (15.2)	33
0.1	10 (32.3)	8 (25.8)	31
0.2	18 (51.4)	14 (40.0)	35
0.4	24 (75.0)	18 (56.3)	32
0.8	30 (100)	27 (90.0)	30
1.6	33 (100)	33 (100)	33
2.5	27 (100)	27 (100)	27

Appendix 17. Results of the exposure of the CfCA strain of *C. quinquefasciatus* to pieces of permethrin impregnated nylon netting for 8 minutes at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%	Total no. of mosquitoes
Control	0	0	31
0.025	6 (20.0)	5 (16.7)	30
0.05	10 (32.3)	8 (25.8)	31
0.1	18 (56.3)	14 (43.8)	32
0.2	24 (75.0)	19 (59.4)	32
0.4	28 (96.6)	21 (72.4)	29
0.8	35 (100)	35 (100)	35
1.6	38 (100)	38 (100)	38
2.5	33 (100)	33 (100)	33

Appendix 18. Results of the exposure of the CfCA strain of *C. quinquefasciatus* to pieces of permethrin impregnated cotton netting for 15 secs at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	30
0.025	0	0	33
0.05	0	0	33
0.1	0	0	30
0.2	0	0	31
0.4	1 (3.2)	1 (3.2)	31
0.8	2 (6.5)	1 (3.2)	31
1.6	7 (21.9)	4 (12.5)	32
2.5	15 (41.7)	9 (25.0)	36
3.2	29 (80.6)	21 (58.3)	36
5.0	29 (87.9)	24 (72.7)	33

Appendix 19. Results of the exposure of the CfCA strain of C. quinquefasciatus to pieces of permethrin impregnated cotton netting for 30 secs at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	29
0.025	0	0	31
0.05	0	0	31
0.1	0	0	30
0.2	0	1 (3.3)	30
0.4	3 (10.0)	2 (6.7)	30
0.8	8 (25.0)	9 (28.1)	32
1.6	8 (26.7)	7 (23.3)	30
2.5	19 (59.4)	15 (46.9)	32
3.2	29 (85.3)	25 (73.5)	34
5.0	29 (96.7)	25 (83.3)	30

Appendix 20. Results of the exposure of the CfCA strain of *C. quinquefasciatus* to pieces of permethrin impregnated cotton netting for one minute at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	30
0.025	0	0	21
0.05	0	0	23
0.1	0	0	21
0.2	3 (9.4)	2 (6.3)	32
0.4	6 (18.2)	5 (15.2)	33
0.8	10 (34.5)	9 (31.0)	29
1.6	12 (40.0)	10 (33.3)	30
2.5	20 (62.5)	20 (62.5)	32
3.2	29 (90.6)	29 (90.6)	32
5.0	10 (100)	10 (100)	10

Appendix 21. Results of the exposure of the CfCA strain of C. quinquefasciatus to pieces of permethrin impregnated cotton netting for 2 minutes at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	30
0.025	0	0	30
0.05	0	0	30
0.1	1 (3.3)	3 (10.0)	30
0.2	3 (10.3)	3 (10.3)	29
0.4	6 (19.4)	6 (19.4)	31
0.8	10 (31.3)	9 (28.1)	32
1.6	18 (62.1)	15 (51.7)	29
2.5	27 (96.4)	19 (67.9)	28
3.2	32 (97.0)	28 (84.9)	33
5.0	11 (100)	11 (100)	11

Appendix 22. Results of the exposure of the CfCA strain of *C. quinquefasciatus* to pieces of permethrin impregnated cotton netting for 4 minutes at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	30
0.025	0	0	30
0.05	0	0	34
0.1	1 (3.0)	2 (6.1)	33
0.2	4 (12.9)	4 (12.9)	31
0.4	5 (15.6)	6 (18.8)	32
0.8	8 (25.8)	6 (19.4)	31
1.6	7 (21.2)	11 (33.3)	33
2.5	33 (94.3)	21 (60.0)	35
3.2	34 (97.1)	30 (85.7)	35
5.0	11 (100)	11 (100)	11

Appendix 23. Results of the exposure of the CfCA strain of C. quinquefasciatus to pieces of permethrin impregnated cotton netting for 8 minutes at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	30
0.025	0	0	29
0.05	0	1 (3.3)	30
0.1	4 (13.3)	4 (13.3)	30
0.2	5 (16.7)	5 (16.7)	30
0.4	8 (25.8)	9 (29.0)	31
0.8	13 (43.3)	17 (56.7)	30
1.6	18 (60.0)	19 (63.3)	30
2.5	30 (93.8)	30 (93.8)	32
3.2	29 (100)	29 (100)	29

Appendix 24. Results of the exposure of the CfCA strain of *C. quinquefasciatus* to pieces of permethrin impregnated polyester netting for 15 secs at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	30
0.025	0	1 (3.0)	33
0.05	0	1 (3.5)	29
0.1	2 (7.4)	2 (7.4)	27
0.2	2 (6.1)	2 (6.1)	33
0.4	4 (13.8)	2 (6.9)	29
0.8	5 (15.2)	6 (18.2)	33
1.6	18 (56.3)	17 (53.1)	32
2.5	12 (52.2)	13 (56.5)	23
3.2	26 (89.7)	24 (82.8)	29

Appendix 25. Results of the exposure of the CfCA strain of C. quinquefasciatus to pieces of permethrin impregnated polyester netting for 30 secs at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	20
0.025	0	0	30
0.05	0	1 (3.2)	31
0.1	1 (3.5)	1 (3.5)	29
0.2	2 (6.7)	2 (6.7)	30
0.4	4 (13.8)	3 (10.3)	29
0.8	8 (28.6)	11 (39.3)	28
1.6	19 (63.3)	18 (60.0)	30
2.5	23 (82.1)	16 (57.1)	28
3.2	22 (84.6)	22 (84.6)	26

Appendix 26. Results of the exposure of the CfCA strain of *C. quinquefasciatus* to pieces of permethrin impregnated polyester netting for one minute at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	29
0.025	0	0	29
0.05	0	1 (3.1)	32
0.1	1 (3.3)	1 (3.3)	30
0.2	3 (10.3)	3 (10.3)	29
0.4	6 (20.0)	4 (13.3)	30
0.8	15 (50.0)	12 (40.0)	30
1.6	23 (76.7)	17 (65.7)	30
2.5	21 (87.5)	20 (83.3)	24
3.2	30 (100)	29 (96.7)	30

Appendix 27. Results of the exposure of the CfCA strain of C. quinquefasciatus to pieces of permethrin impregnated polyester netting for 2 minutes at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	30
0.025	1 (3.1)	1 (3.1)	32
0.05	1 (3.0)	1 (3.0)	33
0.1	3 (8.8)	3 (8.8)	34
0.2	5 (13.9)	5 (13.9)	36
0.4	9 (25.0)	7 (19.4)	36
0.8	22 (68.8)	18 (56.3)	32
1.6	32 (94.1)	25 (73.5)	34
2.5	29 (100)	27 (93.1)	29
3.2	30 (100)	28 (93.3)	30

Appendix 28. Results of the exposure of the CfCA strain of C. quinquefasciatus to pieces of permethrin impregnated polyester netting for 4 minutes at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	30
0.025	0	2 (6.9)	29
0.05	2 (5.9)	2 (5.9)	34
0.1	5 (16.7)	4 (13.3)	30
0.2	7 (21.9)	7 (21.9)	32
0.4	14 (48.3)	13 (44.8)	29
0.8	31 (91.2)	28 (82.4)	34
1.6	31 (100)	27 (87.1)	31
2.5	27 (96.4)	25 (89.2)	28
3.2	31 (100)	31 (100)	31

Appendix 29. Results of the exposure of the CfCA strain of C. quinquefasciatus to pieces of permethrin impregnated polyester netting for 8 minutes at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	30
0.025	1 (3.3)	2 (6.7)	30
0.05	3 (10.0)	3 (10.0)	30
0.1	7 (24.1)	5 (17.2)	29
0.2	16 (50.0)	11 (34.4)	32
0.4	21 (72.4)	18 (62.1)	29
0.8	30 (93.8)	27 (84.4)	32
1.6	28 (100)	28 (100)	28
2.5	25 (100)	25 (100)	25

Appendix 30. Results of the exposure of the CfCA strain of C. quinquefasciatus to pieces of permethrin impregnated Gambian polyester netting for 15 secs at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	30
0.025	0	0	31
0.05	0	0	32
0.1	0	0	32
0.2	0	1 (3.3)	30
0.4	1 (2.9)	2 (5.7)	35
0.8	8 (23.5)	8 (23.5)	34
1.6	16 (45.7)	17 (48.6)	35
2.5	18 (60.0)	17 (56.7)	30
3.2	27 (84.4)	26 (81.3)	32

Appendix 31. Results of the exposure of the CfCA strain of *C. quinquefasciatus* to pieces of permethrin impregnated Gambian polyester netting for 30 secs at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	32
0.025	0	0	33
0.05	0	0	32
0.1	0	1 (2.9)	34
0.2	0	2 (6.7)	30
0.4	4 (13.3)	5 (16.7)	30
0.8	8 (25.8)	8 (25.8)	31
1.6	16 (57.1)	14 (50.0)	28
2.5	19 (63.3)	19 (63.3)	30
3.2	26 (81.3)	28 (87.5)	32

Appendix 32. Results of the exposure of the CfCA strain of C. quinquefasciatus to pieces of permethrin impregnated Gambian polyester netting for one minute at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	30
0.025	0	0	30
0.05	0	1 (3.2)	31
0.1	1 (3.4)	2 (6.9)	29
0.2	3 (9.7)	4 (12.9)	31
0.4	6 (18.8)	5 (15.6)	32
0.8	11 (35.5)	12 (38.7)	31
1.6	23 (69.7)	18 (54.6)	33
2.5	23 (82.1)	20 (71.4)	28
3.2	30 (100)	29 (96.7)	30

Appendix 33. Results of the exposure of the CfCA strain of C. quinquefasciatus to pieces of permethrin impregnated Gambian polyester netting for 2 minutes at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	30
0.025	0	0	20
0.05	1 (3.6)	3 (10.7)	28
0.1	3 (9.7)	4 (12.9)	31
0.2	4 (13.3)	4 (13.3)	30
0.4	8 (25.0)	7 (21.9)	32
0.8	17 (57.1)	12 (37.5)	32
1.6	27 (90.0)	23 (76.7)	30
2.5	32 (100)	27 (84.4)	32
3.2	20 (100)	19 (95.0)	20

Appendix 34. Results of the exposure of the CfCA strain of C. quinquefasciatus to pieces of permethrin impregnated Gambian polyester netting for 4 minutes at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	29
0.025	0	1 (3.1)	32
0.05	2 (6.3)	1 (3.1)	32
0.1	2 (7.1)	3 (10.7)	28
0.2	6 (17.1)	6 (17.1)	35
0.4	6 (16.7)	7 (19.4)	36
0.8	22 (66.7)	18 (54.6)	33
1.6	28 (90.3)	19 (61.3)	31
2.5	32 (100)	27 (84.4)	32
3.2	21 (100)	20 (95.3)	21

Appendix 35. Results of the exposure of the CfCA strain of C. quinquefasciatus to pieces of permethrin impregnated Gambian polyester netting for 8 minutes at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	29
0.025	4 (13.8)	5 (17.2)	29
0.05	9 (27.3)	11 (33.3)	33
0.1	17 (50.0)	15 (44.2)	34
0.2	16 (51.6)	13 (41.9)	31
0.4	20 (60.6)	16 (48.5)	33
0.8	25 (86.2)	21 (72.4)	29
1.6	31 (100)	29 (93.6)	31
2.5	32 (100)	32 (100)	32
3.2	22 (100)	22 (100)	22

Appendix 36. The KD_{50} and KD_{90} values of the KWA and AE AE strains exposed to permethrin impregnated netting.

Mosquito strain	Exposure time	Netting type	KD_{50} g/m² (95% C.L.)	KD_{90} g/m²	χ^2(d.f.)
KWA	30 secs	Nylon	0.471[a] (?)	1.145	14.65* (5)
"	2 mins	"	0.190 (0.157 - 0.230)	0.649	3.32 (5)
"	30 secs	Cotton	1.627[a] (?)	2.665	8.02* (3)
"	2 mins	"	0.829 (0.707 - 0.966)	1.819	5.61 (3)
"	30 secs	Polyester	0.565[a] (?)	1.222	8.12* (3)
"	2 mins	"	0.259 (0.223 - 0.299)	0.498	1.66 (3)
AE AE	30 secs	Nylon	0.066 (0.043 - 0.091)	0.581	5.10 (5)
"	2 mins	"	0.066 (0.053 - 0.080)	0.212	8.60 (4)
"	30 secs	Polyester	0.187 (0.250 - 0.331)	0.640	3.46 (6)
"	2 mins	"	0.123[a] (?)	0.303	10.32* (4)

* $P < 0.05$

[a] Confidence limits can not be attached when there was a significant heterogeneity χ^2 about the regression line.

Appendix 37. The LD_{50} and LD_{90} values of the KWA and AE AE strains exposed to permethrin impregnated netting.

Mosquito strain	Exposure time	Netting type	LD_{50} g/m² (95% C.L.)	LD_{90} g/m²	χ^2 (d.f.)
KWA	30 secs	Nylon	0.481 (0.407 - 0.566)	1.251	7.75 (6)
"	2 mins	"	0.223 (0.184 - 0.270)	0.793	4.12 (6)
"	30 secs	Cotton	1.315 (1.136 - 1.504)	2.702	8.71 (4)
"	2 mins	"	0.701 (0.586 - 0.837)	1.934	9.08 (4)
"	30 secs	Polyester	0.685[a] (?)	1.803	9.01* (3)
"	2 mins	"	0.241 (0.203 - 0.284)	0.566	1.66 (5)
AE AE	30 secs	Nylon	0.176[a] (?)	2.018	24.07***(5)
"	2 mins	"	0.165[a] (?)	0.622	13.45* (5)
"	30 secs	Polyester	0.352 (0.300 - 0.413)	1.018	10.71 (5)
"	2 mins	"	0.151 (0.128 - 0.179)	0.470	4.86 (5)

*** $P < 0.001$

* $P < 0.05$

[a] Confidence limits can not be attached when there was a significant heterogeneity χ^2 about the regression line.

Appendix 38. The KD_{50} and KD_{90} values of the CfCA strain of *C. quinquefasciatus* exposed to pieces of permethrin impregnated netting at a series of doses.

Exposure time	Netting type	KD_{50} g/m² (95% C.L.)	KD_{90} g/m²	χ^2 (d.f.)
15 secs	Nylon	0.525 (0.415 - 0.660)	2.162	10.80 (7)
30 secs	"	0.631 (0.497 - 0.796)	2.523	2.06 (6)
1 min	"	0.444 (0.358 - 0.549)	1.377	3.50 (5)
2 mins	"	0.297 (?)	1.084	17.47* (7)
4 mins	"	0.155 (0.121 - 0.199)	0.689	6.84 (4)
8 mins	"	0.077 (0.059 - 0.089)	0.311	3.07 (4)
15 secs	Cotton	2.331 (1.999 - 2.717)	5.702	8.10 (5)
30 secs	"	1.677 (?)	4.054	12.00* (5)
1 min	"	1.279 (1.027 - 1.657)	5.826	11.84 (6)
2 mins	"	0.876 (0.710 - 1.070)	2.880	12.17 (7)
4 mins	"	1.143 (?)	4.345	45.32***(7)
8 mins	"	0.705 (?)	3.241	16.89** (6)
15 secs	Polyester	1.497 (?)	7.628	14.65* (6)
30 secs	"	1.126 (0.895 - 1.433)	4.408	2.55 (97
1 min	"	0.750 (0.605 - 0.924)	2.466	5.23 (6)
2 mins	"	0.471 (?)	1.663	16.76* (7)
4 mins	"	0.310 (0.250 - 0.382)	1.033	10.33 (7)
8 mins	"	0.191 (0.152 - 0.239)	0.677	1.98 (7)
15 secs	Gambian synthetic	1.682 (1.404 - 2.043)	4.775	2.50 (4)
30 secs	"	1.473 (1.186 - 1.856)	5.232	1.52 (4)
1 min	"	0.891 (0.721 - 1.100)	3.144	9.02 (6)
2 mins	"	0.560 (0.448 - 0.701)	2.046	11.88 (6)
4 mins	"	0.530 (?)	1.933	17.55* (6)
8 mins	"	0.140 (0.103 - 0.184	0.936	11.71 (7)

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

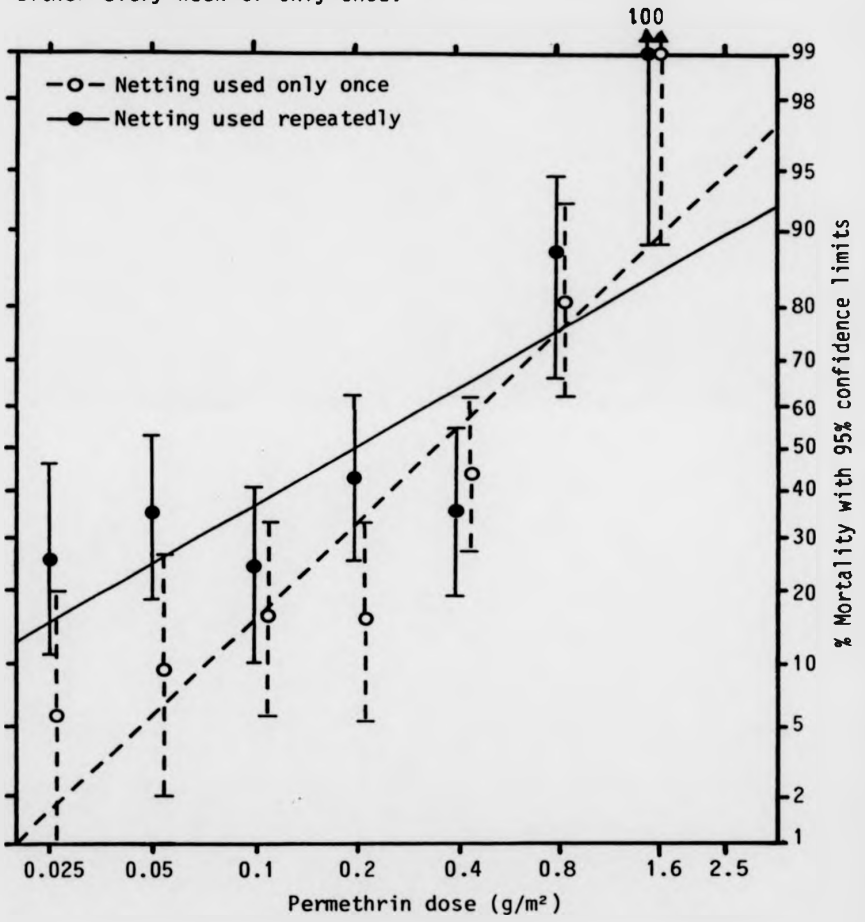
Appendix 39. The LD_{50} and LD_{90} values of permethrin impregnated netting as determined by exposing the CfCA strain of *C. quinquefasciatus* at a series of doses.

Exposure time	Netting type	LD_{50} g/m² (95% C.L.)	LD_{90} g/m²	χ^2 (d.f.)
15 secs	Nylon	0.549 (0.432 - 0.649)	2.396	6.55 (7)
30 secs	"	0.884 (0.666 - 1.207)	5.864	1.78 (7)
1 min	"	0.541 (0.429 - 0.685)	2.046	7.98 (5)
2 mins	"	0.411 (0.320 - 0.526)	2.004	3.46 (7)
4 mins	"	0.210 (0.161 - 0.274)	1.171	10.78 (5)
8 mins	"	0.119 (0.089 - 0.158)	0.675	8.12 (4)
15 secs	Cotton	3.323 (2.798 - 4.162)	9.320	6.88 (5)
30 secs	"	2.098 (1.687 - 2.673)	8.880	8.84 (6)
1 min	"	1.426 (?)	5.734	13.57* (6)
2 mins	"	1.175 (0.910 - 1.546)	6.633	9.72 (7)
4 mins	"	1.499 (?)	8.905	19.06** (7)
8 mins	"	0.611 (0.479 - 0.778)	2.930	12.83 (7)
15 secs	Polyester	1.677 (?)	12.807	15.06* (7)
30 secs	"	1.314 (1.002 - 1.803)	7.443	6.55 (7)
1 min	"	0.956 (0.755 - 1.224)	4.116	9.85 (7)
2 mins	"	0.668 (0.529 - 0.847)	3.297	9.02 (7)
4 mins	"	0.369 (0.287 - 0.471)	1.889	10.07 (7)
8 mins	"	0.254 (0.193 -0.311)	1.031	7.64 (7)
15 secs	Gambian polyester	1.679 (1.366 - 2.126)	5.994	2.52 (5)
30 secs	"	1.367 (1.074 - 1.792)	6.337	4.66 (6)
1 min	"	1.019 (0.792 - 1.339)	5.547	11.28 (7)
2 mins	"	0.734 (0.561 - 0.978)	4.650	12.69 (7)
4 mins	"	0.761 (0.587 - 1.007)	4.726	8.74 (7)
8 mins	"	0.178 (?)	2.091	14.89* (6)

** $P < 0.01$, * $P < 0.05$

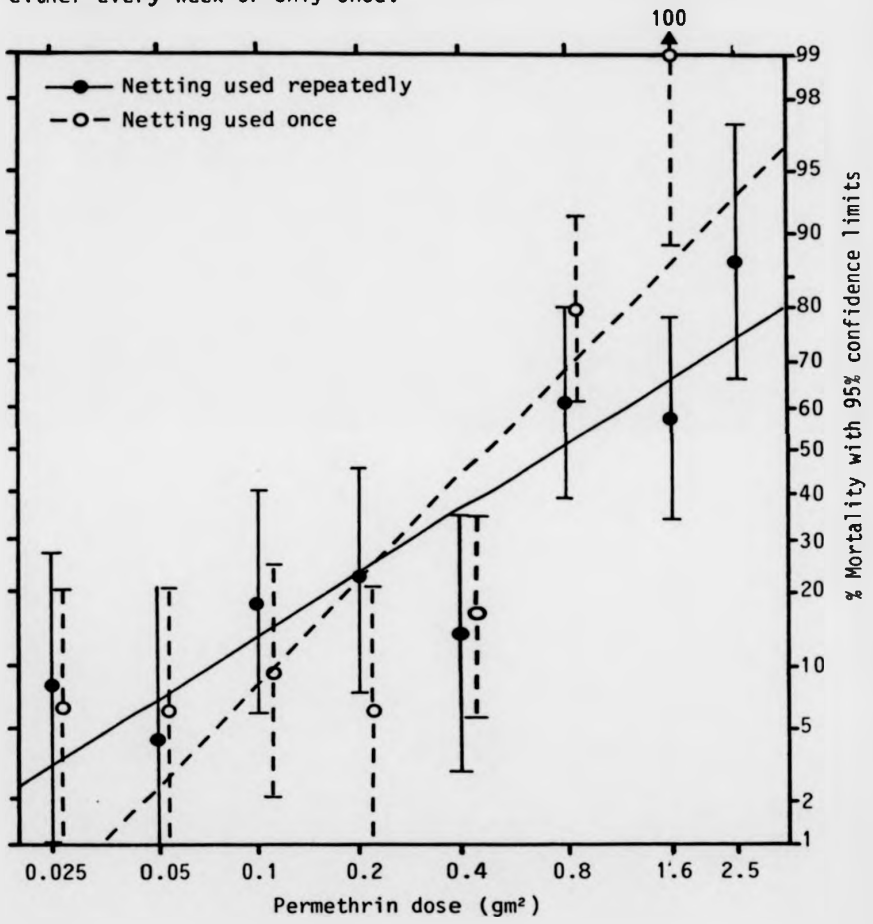
Appendix 40

Mortality of the KWA strain of An.gambiae exposed to pieces of permethrin impregnated nylon netting aged for 3 weeks and used either every week or only once.



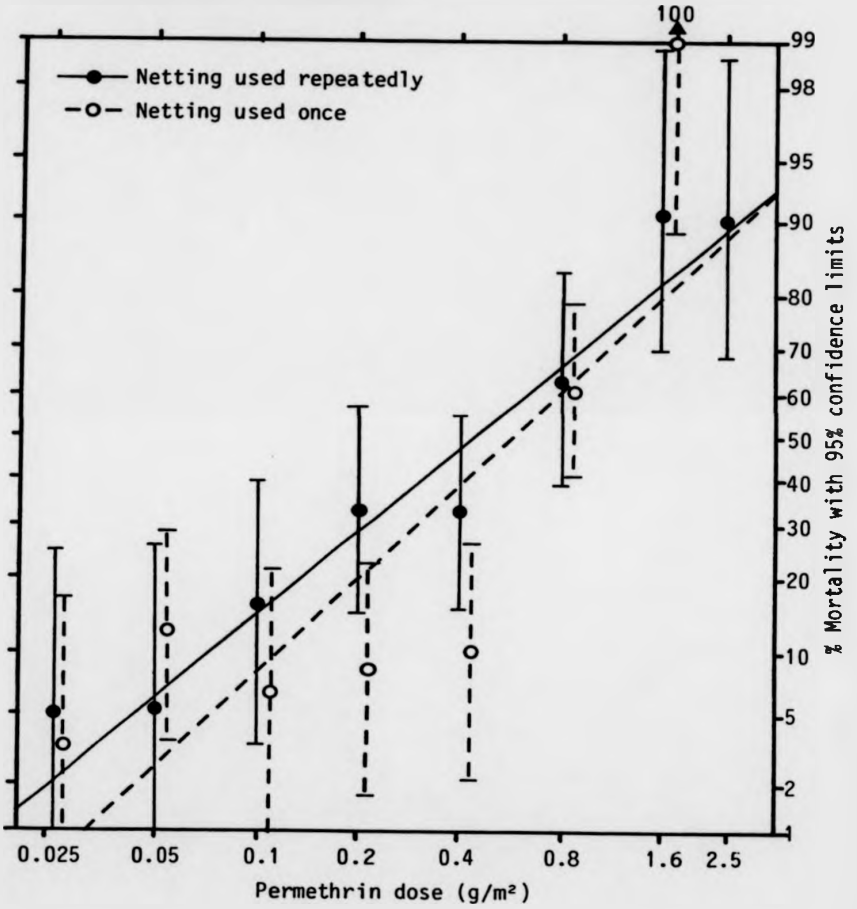
Appendix 41

Mortality of the KWA strain of An.gambiae exposed to pieces of permethrin impregnated nylon netting aged for 4 weeks and used either every week or only once.



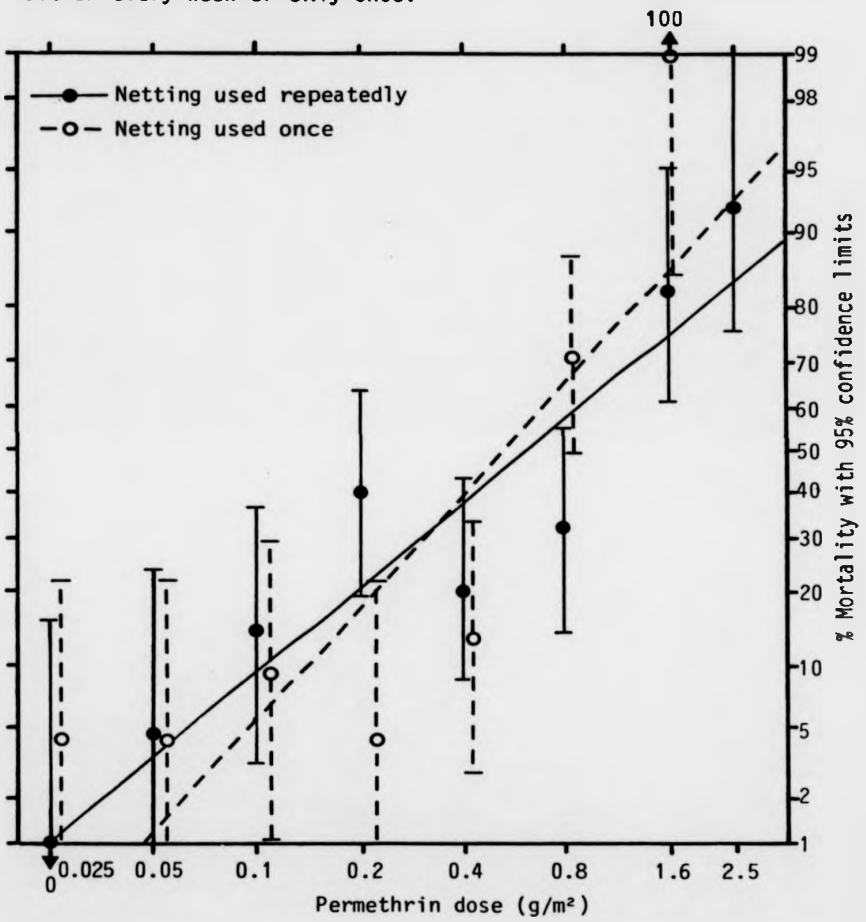
Appendix 42

Mortality of the KWA strain of An.gambiae exposed to pieces of permethrin impregnated nylon netting aged for 5 weeks and used either every week or only once.



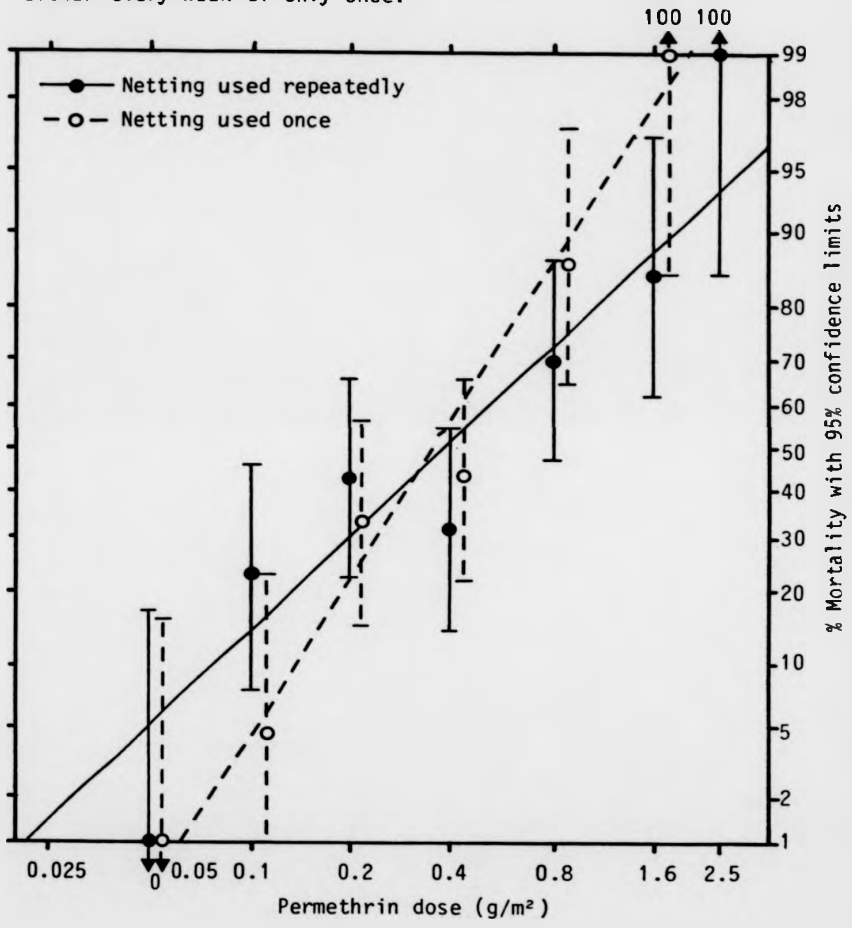
Appendix 43

Mortality of the KWA strain of An.gambiae exposed to pieces of permethrin impregnated nylon netting aged for 6 weeks and used either every week or only once.



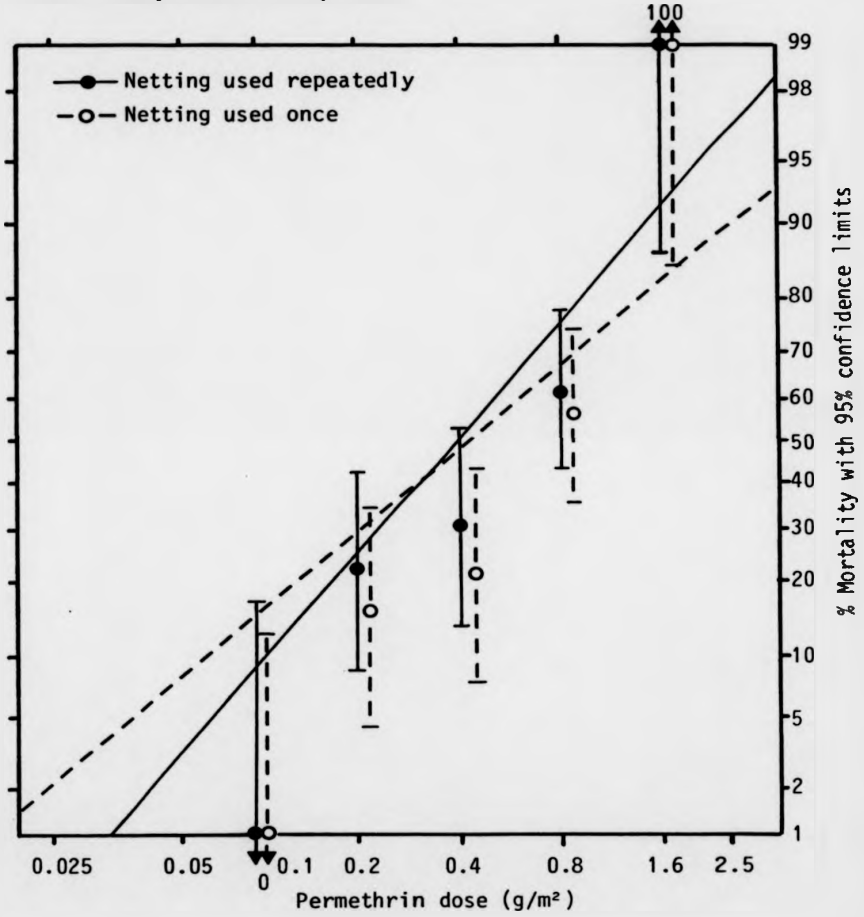
Appendix 44

Mortality of the KWA strain of An.gambiae exposed to pieces of permethrin impregnated nylon netting aged for 10 weeks and used either every week or only once.



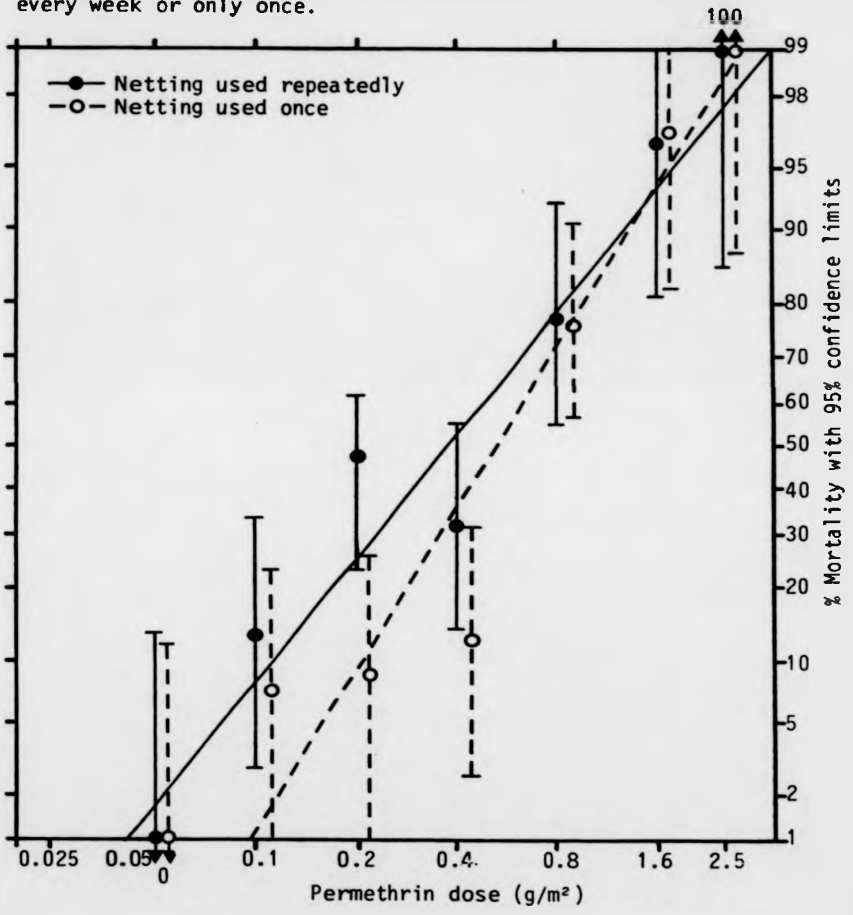
Appendix 45

Mortality of the KWA strain of An.gambiae exposed to pieces of permethrin impregnated nylon netting aged for 11 weeks and used either every week or only once.



Appendix 46

Mortality of the KWA strain of An.gambiae exposed to pieces of permethrin impregnated nylon netting aged for 12 weeks and used every week or only once.



Appendix 47. Exposure of the AE AE strain of Ae. aegypti to polyester netting impregnated with various pyrethroids.

Insecticide	Dose (g/m^2)	30 seconds exposure %KD	%Mortality	Total	3 minutes exposure %KD	%Mortality	Total
Control	–	0	6.1	33	0	0	26
Cyfluthrin	0.1	60.5	65.8	38	100	96.3	27
"	0.2	100	93.8	32	97.0	97.0	33
Cypermethrin	0.1	100	97.0	33	100	96.6	29
"	0.2	96.0	96.0	25	100	100	29
Cyphenothrin	0.1	0	0	11	33.3	22.2	27
"	0.2	92.3	92.3	13	100	93.3	30
Deltamethrin	0.025	42.1	63.2	38	88.9	66.7	27
"	0.05	67.9	71.4	28	85.2	77.8	27
d-phenothrin	0.2	0	0	11	9.7	6.5	31
"	0.5	14.3	28.6	14	0	0	26
Fenpropathrin	0.1	81.8	33.3	33	48.4	41.9	31
"	0.2	95.8	54.2	24	100	84.6	26
Fenvalerate	0.1	78.8	72.7	33	100	60.0	35
"	0.2	100	100	17	–	–	–
Wellcome permethrin	0.2	97.1	82.9	35	100	87.1	31
"	0.5	100	73.9	23	100	100	31
Wellcome permethrin	0.2	100	94.4	36	90.3	74.2	31
+ Agral	0.5	91.7	91.7	24	100	100	28
Wellcome permethrin	0.2	100	100	32	100	93.8	32
(hot water treatment at acid pH)	0.5	100	100	23	100	100	32
ICI permethrin	0.2	100	90.3	31	96.9	68.8	32
"	0.5	100	100	22	100	100	27
PP321	0.025	100	93.2	44	100	100	30
"	0.05	100	100	42	100	100	29

Appendix 48. Exposure of the G3 strain of An. gambiae to polyester netting impregnated with various pyrethroids.

Insecticide	Dose (g/m²)	30 seconds exposure %KD	%Mortality	Total	3 minutes exposure %KD	%Mortality	Total
Control	-	0	0	22	0	0	19
Cyfluthrin	0.1	100	85.0	21	100	100	22
"	0.2	57.1	52.4	21	100	100	26
Cypermethrin	0.1	100	100	22	100	100	23
"	0.2	100	100	23	100	100	25
Cyphenothrin	0.1	57.1	47.6	21	72.7	77.3	22
"	0.2	85.7	52.4	21	100	100	20
Deltamethrin	0.025	33.3	44.4	18	100	100	22
"	0.05	78.3	56.5	23	93.1	69.0	29
d-phenothrin	0.2	0	0	20	0	5.0	20
"	0.5	0	0	22	4.8	9.5	21
Fenpropathrin	0.1	100	52.4	21	100	100	20
"	0.2	100	90.0	20	100	100	21
Fenvalerate	0.1	52.4	28.6	21	100	95.0	20
"	0.2	-	-	-	-	-	-
Wellcome permethrin	0.2	15.0	40.0	20	100	100	26
"	0.5	84.4	75.0	32	100	100	22
Wellcome permethrin	0.2	22.7	4.6	22	88.9	83.3	18
+ Agral	0.5	43.5	39.1	23	100	100	25
Wellcome permethrin	0.2	81.0	85.7	21	100	95.7	23
(hot water treatment	0.5	91.7	70.8	24	100	100	24
at acid pH)							
ICI permethrin	0.2	54.6	45.5	22	95.0	75.0	20
"	0.5	75.0	66.7	24	100	91.3	23
PP321	0.025	100	100	21	100	100	25
"	0.05	100	100	21	100	100	24

Appendix 49. Effect of ageing on the persistence of various pyrethroids on impregnated polyester netting as determined by bioassaying the AE AE strain of Ae. aegypti for 30 secs.

Insecticide	Dose (g/m^2)	Week 0		Week 2		Week 6		Month 3	
		M	T	M	T	M	T	M	T
Control	-	6.1	33	0	34	0	32	0	32
Cyfluthrin	0.1	65.8	38	94.3	35	93.4	33	100	36
"	0.2	93.8	32	100	35	100	36	100	41
Cypermethrin	0.1	97.0	33	100	36	80.0	35	100	38
"	0.2	96.0	25	100	33	90.6	32	100	30
Cyphenothrin	0.1	0	11	-	-	-	-	-	-
"	0.2	92.3	13	0	21	-	-	-	-
Deltamethrin	0.025	63.2	38	86.1	36	72.7	33	84.6	39
"	0.05	71.4	28	71.9	32	48.6	35	67.4	43
d-phenothrin	0.2	0	11	-	-	-	-	-	-
"	0.5	28.6	14	0	21	-	-	-	-
Fenpropathrin	0.1	33.3	33	-	-	19.4	31	16.7	36
"	0.2	54.2	24	33.3	24	85.7	35	37.8	37
Fenvalerate	0.1	72.7	33	63.6	22	81.0	21	-	-
"	0.2	100	17	100	21	100	22	-	-
Wellcome	0.2	82.9	35	93.6	31	82.1	39	97.4	39
permethrin	0.5	73.9	23	97.1	35	93.8	32	90.3	31
Wellcome perm	0.2	94.4	36	97.0	33	62.9	35	62.1	29
+ Agral	0.5	91.7	24	93.3	30	82.1	39	100	34
Wellcome perm	0.2	100	32	100	36	94.7	38	100	37
(HAT)	0.5	100	23	100	32	100	35	100	38
ICI permethrin	0.2	90.3	31	100	34	47.4	38	45.7	35
"	0.5	100	22	100	33	86.5	37	76.3	38
PP321	0.025	93.2	44	97.6	42	-	-	-	-
"	0.05	100	42	100	44	-	-	-	-

M = % Mortality, T = Total number of mosquitoes.

HWT = Hot water treatment at acid pH.

Appendix 50. Effect of ageing on the persistence of various pyrethroids on impregnated polyester netting as determined by bioassaying the AE AE strain of Ae. aegypti for 3 minutes.

Insecticide	Dose (g/m^2)	Week 0		Week 2		Week 6		Month 3	
		M	T	M	T	M	T	M	T
Control	-	0	26	6.7	30	0	21	0	29
Cyfluthrin	0.1	96.3	27	100	36	93.9	33	100	36
"	0.2	97.0	33	93.0	43	100	37	100	36
Cypermethrin	0.1	96.6	29	100	32	79.3	29	100	35
"	0.2	100	29	98.0	49	56.0	25	100	33
Cyphenothrin	0.1	22.2	27	-	-	-	-	-	-
"	0.2	93.3	30	-	-	-	-	-	-
Deltamethrin	0.025	66.7	27	90.3	31	30.8	26	97.4	39
"	0.05	77.8	27	70.4	54	33.3	24	94.4	36
d-phenothrin	0.2	6.5	31	-	-	-	-	-	-
"	0.5	0	26	-	-	-	-	-	-
Fenpropathrin	0.1	41.9	31	-	-	17.2	29	60.0	35
"	0.2	84.6	26	41.9	43	23.3	30	60.6	33
Fenvalerate	0.1	60.0	35	81.3	32	73.1	26	-	-
"	0.2	-	-	96.4	28	93.0	27	-	-
Wellcome	0.2	87.1	31	100	34	79.9	26	100	34
permethrin	0.5	100	31	94.4	36	96.4	28	96.9	32
Wellcome perm	0.2	74.2	31	87.9	33	30.4	27	94.6	27
+ Agral	0.5	100	28	89.2	37	80.8	26	100	35
Wellcome perm	0.2	93.8	32	100	33	92.6	27	100	31
(HAT)	0.5	100	32	100	60	100	26	100	38
ICI permethrin	0.2	68.8	32	100	35	58.3	24	97.1	35
"	0.5	100	27	98.0	49	95.8	24	100	36
PP321	0.025	100	30	100	30	100	30	-	-
"	0.05	100	29	100	28	100	28	-	-

M = % Mortality, T = Total number of mosquitoes.

HWT = Hot water treatment at acid pH.

Appendix 51. Effect of ageing on the persistence of various pyrethroids on impregnated polyester netting as determined by bioassaying the G3 strain of An. gambiae for 30 secs.

Insecticide	Dose (g/m^2)	Week 0		Week 2		Week 6		Month 3	
		M	T	M	T	M	T	M	T
Control	-	0	22	0	23	0	21	0	20
Cyfluthrin	0.1	85.0	20	95.8	24	86.4	22	100	23
"	0.2	100	25	81.0	21	100	23	95.0	20
Cypermethrin	0.1	100	22	100	24	95.7	23	100	23
"	0.2	100	23	100	21	100	25	100	20
Cyphenothrin	0.1	47.6	21	-	-	0	22	-	-
"	0.2	52.4	21	-	-	0	23	-	-
Deltamethrin	0.025	44.4	18	58.3	24	60.0	25	57.1	21
"	0.05	56.5	23	37.5	24	27.3	22	55.0	20
d-phenothrin	0.2	0	20	-	-	0	22	-	-
"	0.5	0	22	-	-	0	22	-	-
Fenpropathrin	0.1	52.4	21	-	-	50.0	22	58.3	24
"	0.2	90.0	20	95.7	23	95.7	23	100	20
Fenvalerate	0.1	28.6	21	84.0	25	81.0	21	-	-
"	0.2	73.9	23	100	25	96.0	25	-	-
Wellcome	0.2	40.0	20	40.0	25	28.6	21	43.5	23
permethrin	0.5	75.0	32	13.0	23	54.2	24	45.0	20
Wellcome perm	0.2	4.6	22	4.8	21	26.1	23	9.5	21
+ Agral	0.5	39.1	23	30.0	20	79.2	24	54.6	22
Wellcome perm	0.2	85.7	21	81.8	22	82.6	23	36.4	22
(HAT)	0.5	70.8	24	76.2	21	90.9	22	83.3	24
ICI permethrin	0.2	45.5	22	55.0	20	16.7	24	4.8	21
"	0.5	66.7	24	56.5	23	76.0	25	4.2	24
PP321	0.025	100	21	100	21	-	-	-	-
"	0.05	100	21	100	22	-	-	-	-

M = % Mortality, T = Total number of mosquitoes.

HWT = Hot water treatment at acid pH.

Appendix 52. Effect of ageing on the persistence of various pyrethroids on impregnated polyester netting as determined by bioassaying the G3 strain of An. gambiae for 3 mins.

Insecticide	Dose (g/m^2)	Week 0		Week 2		Week 6		Month 3	
		M	T	M	T	M	T	M	T
Control	-	0	19	0	21	0	20	0	22
Cyfluthrin	0.1	100	19	100	24	100	21	100	22
"	0.2	100	26	100	22	100	21	100	24
Cypermethrin	0.1	100	23	100	21	100	21	100	26
"	0.2	100	25	100	20	100	21	100	23
Cyphenothrin	0.1	77.3	22	-	-	0	20	-	-
"	0.2	100	20	0	14	0	20	-	-
Deltamethrin	0.025	100	22	95.8	24	100	20	76.2	21
"	0.05	69.0	29	100	22	100	21	90.5	21
d-phenothrin	0.2	5.0	20	-	-	0	21	-	-
"	0.5	9.5	21	0	11	0	20	-	-
Fenpropathrin	0.1	100	20	-	-	100	21	88.9	18
"	0.2	100	21	100	21	100	21	92.0	25
Fenvalerate	0.1	95.0	20	100	25	92.3	26	-	-
"	0.2	100	25	100	24	100	22	-	-
Wellcome	0.2	100	26	65.0	20	100	22	88.5	26
permethrin	0.5	100	22	100	22	100	21	85.0	20
Wellcome perm	0.2	83.3	18	52.2	23	100	21	46.2	26
+ Agral	0.5	100	25	100	22	100	21	90.9	22
Wellcome perm	0.2	95.7	23	96.0	25	100	22	77.3	22
(HAT)	0.5	100	24	100	23	100	22	95.2	21
ICI permethrin	0.2	75.0	20	100	22	100	22	13.6	22
"	0.5	91.3	23	100	24	100	21	44.0	25
PP321	0.025	100	25	100	23	-	-	-	-
"	0.05	100	24	100	21	-	-	-	-

M = % Mortality, T = Total number of mosquitoes.

HWT = Hot water treatment at acid pH.

Appendix 53

Mortality of the AE AE strain of Ae.aegypti exposed to the lo
Mosquitoes were exposed to netting that was washed at various
unwashed netting are also shown.

wer dose of each of the pyrethroids for 3 minutes.
intervals of time. Results of freshly impregnated

Appendix 54

Mortality of the AE AE strain of Ae.aegypti exposed to the higher dose of each of the pyrethroids for 30 seconds. Mosquitoes were exposed to netting that was washed at various intervals of time. Results of the freshly impregnated unwashed netting are also shown.

Appendix 55

Mortality of the G3 strain of An.gambiae exposed to the lower dose of each of the pyrethroids for 30 seconds. Mosquitoes were exposed to netting that was washed at various intervals of time. Results of freshly impregnated unwashed netting are also shown.

Appendix 56

Mortality of the G3 strain of An.gambiae exposed to the higher dose of each of the pyrethroids for 3 minutes. Mosquitoes were exposed to netting that was washed at various intervals of time. Results of freshly impregnated unwashed netting are also shown.

Appendix 57. Effect of the combined action of washing and ageing of pyrethroid impregnated netting as determined by bioassaying the AE AE strain of Ae. aegypti for 30 secs.

Insecticide	Dose (g/m^2)	Week 0 (1st wash) %KD	Mortality	Total	Week 2 (2nd wash) %KD	Mortality	Total	Week 6 (4th wash) %KD	Mortality	Total
Control	-	0	0	33	0	0	35	0	0	21
Cyfluthrin	0.1	94.4	80.6	36	75.0	80.6	36	14.3	9.5	21
"	0.2	93.6	64.5	31	80.7	61.3	31	23.8	23.8	21
Cypermethrin	0.1	91.2	79.4	34	97.0	84.9	33	18.2	18.2	22
"	0.2	85.7	76.2	21	93.4	90.9	33	61.5	61.5	26
Cyphenothrin	0.1	0	0	11	-	-	-	-	-	-
"	0.2	62.5	62.5	16	0	0	23	-	-	-
Deltamethrin	0.025	34.4	40.6	32	11.4	34.3	35	13.6	9.1	22
"	0.05	39.1	56.5	23	5.9	14.7	34	0	0	24
d-phenothrin	0.2	0	0	10	-	-	-	-	-	-
"	0.5	10.0	10.0	10	0	0	23	-	-	-
Fenpropa-	0.1	0	0	32	-	-	-	0	0	22
pathrin	0.2	3.6	3.6	28	14.3	4.8	21	0	0	22
Fenvalerate	0.1	44.4	36.1	36	23.8	19.1	21	10.0	5.0	20
"	0.2	-	-	-	85.0	75.0	20	15.0	10.0	20
Wellcome	0.2	51.4	34.3	35	58.1	67.7	31	9.5	4.8	21
permethrin	0.5	78.3	60.9	23	100	84.2	38	18.2	13.6	22
Wellcome	0.2	56.3	43.8	32	25.0	41.7	36	0	4.8	21
+ Agral	0.5	82.6	73.9	23	82.4	70.6	34	9.5	4.8	21
Wellcome	0.2	100	100	30	96.8	96.8	31	69.6	60.9	23
(HWT)	0.5	95.8	95.8	24	90.9	93.9	33	77.3	68.2	22
ICI perme-	0.2	94.1	82.4	34	69.0	75.9	29	0	0	22
thrin	0.5	91.3	87.0	23	100	100	33	0	0	24
PP321	0.025	90.9	47.7	44	45.7	22.9	35	89.7	72.4	29
"	0.05	91.3	87.0	46	83.3	55.6	36	93.8	56.3	32

Wellcome = Wellcome permethrin, HWT = hot water treatment at acid pH

- = not bioassayed

Appendix 58. Effect of the combined action of washing and ageing of pyrethroid impregnated netting as determined by bioassaying the AE AE strain of Ae. aegypti for 3 minutes.

Insecticide	Dose (g/m²)	Week 0 (1st wash) %KD	Mortality	Total	Week 2 (2nd wash) %KD	Mortality	Total	Week 6 (4th wash) %KD	Mortality	Total
Control	-	0	0	33	0	0	35	0	0	21
Cyfluthrin	0.1	100	93.1	29	100	89.2	37	95.8	66.7	24
"	0.2	97.1	94.3	35	89.6	56.3	48	96.2	73.1	26
Cypermethrin	0.1	100	90.9	33	97.2	94.4	36	95.7	73.9	23
"	0.2	92.9	82.1	28	97.7	88.4	43	100	100	25
Cyphenothrin	0.1	26.9	7.7	26	-	-	-	-	-	-
"	0.2	80.0	23.3	30	-	-	-	-	-	-
Deltamethrin	0.025	74.1	63.0	27	27.3	33.3	33	83.3	75.0	24
"	0.05	89.3	71.4	28	89.1	62.2	37	61.9	57.1	21
d-phenothrin	0.2	0	0	28	-	-	-	-	-	-
"	0.5	0	0	30	-	-	-	-	-	-
Fenpropa-	0.1	37.0	33.3	27	-	-	-	0	0	25
pathrin	0.2	3.7	14.8	27	8.8	0	34	0	0	25
Fenvalerate	0.1	82.9	45.7	35	29.6	14.8	27	37.5	20.8	24
"	0.2	-	-	-	57.7	33.3	30	50.0	46.2	26
Wellcome	0.2	96.4	75.0	28	98.0	69.7	33	91.3	73.9	27
permethrin	0.5	100	92.0	25	94.6	81.1	37	100	95.8	24
Wellcome	0.2	58.6	51.7	29	87.9	48.5	33	0	0	27
+ Agral	0.5	100	96.6	29	88.1	78.2	42	95.7	91.3	23
Wellcome	0.2	100	89.3	28	100	100	34	100	96.2	26
(HWT)	0.5	96.7	93.3	30	100	97.1	35	100	100	27
ICI perme-	0.2	78.8	54.6	33	93.8	68.8	32	0	0	27
thrin	0.5	100	100	31	100	80.6	36	31.8	18.2	22
PP321	0.025	96.8	48.4	31	59.4	18.8	32	74.1	55.6	27
"	0.05	100	93.9	33	100	80.6	36	96.0	88.0	25

Wellcome = Wellcome permethrin, HWT = hot water treatment at acid pH

- = not bioassayed

Appendix 59. Effect of the combined action of washing and ageing of pyrethroid impregnated nets as determined by bioassaying the G3 strain of *An. gambiae* for 30 secs.

Insecticide	Dose (g/m²)	Week 0 (1st wash) %KD	Mortality	Total	Week 2 (2nd wash) %KD	Mortality	Total	Week 6 (4th wash) %KD	Mortality	Total
Control	-	0	0	33	0	0	35	0	0	21
Cyfluthrin	0.1	81.0	85.7	21	36.8	5.3	19	28.0	36.0	25
"	0.2	77.3	36.4	22	43.5	4.5	23	41.6	45.8	24
Cypermethrin	0.1	86.4	90.9	22	100	77.8	27	90.9	86.4	22
"	0.2	87.5	91.7	24	100	95.5	22	91.7	79.2	24
Cyphenothrin	0.1	15.0	10.0	20	-	-	-	-	-	-
"	0.2	58.3	20.8	24	-	-	-	-	-	-
Deltamethrin	0.025	52.9	52.9	17	28.6	23.8	21	79.3	75.9	29
"	0.05	44.0	32.0	25	24.0	8.0	25	73.1	76.0	25
d-phenothrin	0.2	0	0	21	-	-	-	-	-	-
"	0.5	0	0	22	-	-	-	-	-	-
Fenpro-	0.1	5.0	0	20	-	-	-	0	0	24
pathrin	0.2	19.1	0	21	20.8	0	24	0	9.5	21
Fenvalerate	0.1	31.8	13.6	22	15.0	15.0	20	14.3	9.5	21
"	0.2	-	-	-	33.3	23.8	21	13.6	9.1	22
Wellcome	0.2	0	0	22	0	0	24	18.1	27.3	22
permethrin	0.5	18.5	11.1	27	21.7	4.4	23	27.3	31.8	22
Wellcome	0.2	10.0	10.0	20	0	0	24	0	0	20
+ Agral	0.5	5.3	0	19	21.1	0	19	34.8	34.8	23
Wellcome	0.2	35.0	45.0	20	100	80.0	25	68.2	59.1	22
(HWT)	0.5	76.9	65.4	26	88.0	44.0	25	75.0	75.0	20
ICI perme-	0.2	0	8.3	24	0	5.0	20	0	0	24
thrin	0.5	23.8	19.1	21	45.5	22.7	22	8.3	8.3	24
PP321	0.025	100	87.5	24	84.0	76.0	25	78.3	30.4	23
"	0.05	100	100	23	100	83.3	24	100	65.0	20

Wellcome = Wellcome permethrin, HWT = hot water treatment at acid pH

- = Not done

Appendix 60. Effect of the combined action of washing and ageing of pyrethroid impregnated netting as determined by bioassaying the G3 strain of An. gambiae for 3 minutes.

Insecticide	Dose (g/m^2)	Week 0 (1st wash) %KD	Mortality	Total	Week 2 (2nd wash) %KD	Mortality	Total	Week 6 (4th wash) %KD	Mortality	Total
Control	-	0	0	33	0	0	35	0	0	21
Cyfluthrin	0.1	100	100	24	95.2	90.5	21	66.7	33.3	24
"	0.2	100	100	23	100	87.0	23	75.4	26.9	26
Cypermethrin	0.1	100	100	18	100	100	22	100	100	22
"	0.2	100	100	24	100	100	22	100	100	24
Cyphenothrin	0.1	85.0	70.0	20	-	-	-	-	-	-
"	0.2	100	100	20	0	0	12	-	-	-
Deltamethrin	0.025	95.8	91.7	24	76.2	66.7	21	100	95.5	22
"	0.05	95.0	70.0	20	94.7	78.9	19	92.3	96.2	26
d-phenothrin	0.2	0	5.0	20	-	-	-	-	-	-
"	0.5	0	10.0	20	0	0	12	-	-	-
Fenpropa-	0.1	100	68.2	22	-	-	-	0	0	23
pathrin	0.2	100	87.5	24	100	82.6	23	0	4.4	23
Fenvalerate	0.1	91.7	66.7	24	79.9	52.2	23	47.8	26.1	23
"	0.2	-	-	-	91.7	62.5	24	69.6	43.5	23
Wellcome	0.2	66.7	55.6	27	9.1	4.6	22	43.5	30.4	23
permethrin	0.5	92.0	88.0	25	90.9	68.2	22	76.0	64.0	25
Wellcome	0.2	52.4	47.6	21	4.4	4.4	23	0	0	24
+ Agral	0.5	43.5	30.4	23	76.0	48.0	25	77.3	72.7	22
Wellcome	0.2	100	95.0	20	100	100	24	100	100	23
(HWT)	0.5	100	100	21	100	100	22	100	100	25
ICI perme-	0.2	75.0	70.0	20	86.4	31.8	22	4.0	0	25
thrin	0.5	100	100	23	95.7	87.0	23	10.0	10.0	20
PP321	0.025	100	100	22	100	100	21	100	100	24
"	0.05	100	100	21	100	100	22	100	100	24

Wellcome = Wellcome permethrin, HWT = hot water treatment at acid pH

- = not bioassayed

Appendix 61. Exposure of the MU strain of *An. gambiae* to 4% DDT impregnated papers at a range of exposure times.

Exposure time (hours)	Number dead	Percentage mortality	Number alive	Total no. of mosquitoes
0.25	38	23.9	121	159
0.50	90	54.9	74	164
0.75	170	77.3	50	220
1	195	85.9	32	227
1.25	78	96.3	3	81
1.50	186	84.5	34	220
2	200	97.1	6	206
2.50	83	96.5	3	86
3	28	93.3	2	30
3.50	33	100	0	33

Appendix 62. Exposure of the MU/DDT F_1 strain of An. gambiae to 4% DDT impregnated papers at a range of exposure times.

Exposure time (hours)	Number dead	Percentage mortality	Number alive	Total no. of mosquitoes
0.5	12	36.4	21	33
1	11	42.3	15	26
1.5	16	61.5	10	26
2	19	79.2	9	28
2.5	16	66.7	8	24
3	43	86.0	7	50
3.5	42	89.4	5	47
4	25	100	0	25

Appendix 63. Exposure of the MU/DDT F_6 strain of An. gambiae to 4% DDT impregnated papers at a range of exposure times.

Exposure time (hours)	Number dead	Percentage mortality	Number alive	Total no. of mosquitoes
1	22	41.5	31	53
2	22	43.1	29	51
3	27	44.3	34	61
4	33	58.9	23	56
5	67	72.0	26	93
6	108	68.4	50	158
7	73	70.2	31	104
8	47	81.0	11	58
9	48	81.4	11	59

Appendix 64. Exposure of the MU/DDT F_9 strain of An. gambiae to 4% DDT impregnated papers at a range of exposure times.

Exposure time (hours)	Number dead	Percentage mortality	Number alive	Total no. of mosquitoes
6	10	20.0	40	50
7	15	28.3	38	53
8	11	25.0	33	44
9	13	37.1	22	35
10	12	54.5	10	22

Appendix 65. Exposure of the MU strain of An. gambiae to pieces of permethrin impregnated nylon netting for 2 minutes at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	31
0.025	0	1 (3.1)	32
0.05	0	2 (6.7)	30
0.1	1 (3.1)	2 (6.3)	32
0.2	11 (36.7)	2 (6.7)	30
0.4	16 (53.3)	2 (6.7)	30
0.8	29 (90.6)	9 (28.1)	32
1.6	36 (100)	24 (66.7)	36
2.5	30 (100)	30 (100)	30

Appendix 66. Exposure of the MU/PER F_9 strain of *An. gambiae* to pieces of permethrin impregnated nylon netting for 2 minutes at a range of doses.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	0	0	33
0.025	0	0	35
0.05	0	0	37
0.1	2 (5.7)	0	35
0.2	4 (11.7)	3 (8.6)	35
0.4	11 (36.7)	4 (13.3)	30
0.8	33 (91.7)	18 (50.0)	36
1.6	36 (97.3)	35 (94.6)	37
2.5	35 (97.2)	34 (94.4)	36

Appendix 67. Results of the exposure of the KWA strain of An. gambiae to pieces of permethrin impregnated nylon netting at a range of doses at 16°C.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	2 (3.1)	1 (1.5)	65
0.025	17 (25.8)	2 (3.0)	66
0.05	40 (64.5)	29 (46.0)	63
0.1	55 (85.9)	32 (50.0)	64
0.2	57 (91.9)	25 (40.3)	62
0.4	52 (83.9)	42 (67.7)	62
0.8	65 (98.5)	56 (84.9)	66
1.6	71 (100)	70 (98.6)	71
2.5	72 (100)	72 (100)	72

Appendix 68. Results of the exposure of the KWA strain of An. gambiae to pieces of permethrin impregnated nylon netting at a range of doses at 22°C.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	1 (1.0)	0	99
0.025	7 (7.1)	1 (1.0)	98
0.05	20 (19.6)	5 (4.9)	102
0.1	44 (43.6)	10 (9.9)	101
0.2	76 (76.8)	34 (33.3)	99
0.4	94 (93.1)	54 (53.5)	101
0.8	104 (100)	91 (87.5)	104
1.6	112 (100)	106 (94.6)	112
2.5	103 (100)	103 (100)	103

Appendix 69. Results of the exposure of the KWA strain of An. gambiae to pieces of permethrin impregnated nylon netting at a range of doses at 28°C.

Permethrin dose (g/m^2)	No. knocked down in 1 hour (%)	Number dead in 24 hours (%)	Total no. of mosquitoes
Control	1 (1.0)	0	100
0.025	10 (9.8)	5 (4.9)	102
0.05	22 (22.4)	20 (20.4)	98
0.1	55 (54.5)	30 (29.7)	101
0.2	60 (61.2)	42 (42.9)	98
0.4	85 (84.2)	63 (62.4)	101
0.8	98 (98.9)	93 (93.9)	99
1.6	100 (100)	100 (100)	100

Appendix 70. Distribution of the KWA strain of An. gambiae in the "tunnel" containing a guinea pig separated from the mosquito release point by different types of wide-mesh nylon netting impregnated with permethrin. The pieces of netting were positioned between cages E and F.

Mesh size and dose	Distribution of mosquitoes among different cages						% fed and survived	Total number of mosquitoes
	A	B	C	D	E	F		
0.4 cm 0.2 g/m²	39 UF	9 UF	10 UF	6 UF	6 UF	1 F	1.4	71
	(25 AUF, 45 DUF)					(1 AF)		
Control	22 UF	5 UF	4 UF	2 UF	5 UF	2 UF 30 F	43.1	72
	(39 AUF, 1 AF)					(2 AUF, 30 AF)		
0.6 cm 0.2 g/m²	35 UF	16 UF	6 UF	5 UF	13 UF	1 F	1.3	76
	(12 AUF, 63 DUF)					(1 AF)		
Control	11 UF 29 F	2 UF	1 UF 4 F	1 UF 5 F	1 F	3 UF 16 F	75.3	73
	(13 AUF, 2 DUF, 39 AF)					(3 AUF, 16 AF)		
1.8 cm 0.5 g/m²	15 UF 18 F	2 UF 2 F	1 UF 2 F	1 UF 7 F	1 UF 5 F	13 F	58.2	67
	(13 AUF, 6 DUF, 28 AF, 6 DUF)					(11 AF, 2 DF)		
Control	2 UF 2 F	2 F	0	2 F	7 F	2 UF 10 F	85.2	27
	(2 UF, 13 AF)					(2 AUF, 10 AF)		

UF = unfed. F = fed.
A = alive. D = dead.

Appendix 71. Distribution of the KWA strain of An. gambiae in the "tunnel" containing a guinea pig separated from the mosquito release point by 0.2 g/m² permethrin impregnated netting. The pieces of netting were positioned between cages E and F. The tests were repeated at intervals to determine the persistence of the permethrin.

Weeks after impregnation	Distribution of mosquitoes among different cages						Total number of mosquitoes
	A	B	C	D	E	F	
Control	3 UF	1 UF	1 UF	0	3 UF	44 F	52
0	15 UF	2 UF	5 UF	7 UF	5 UF	5 F	39
3	24 UF 1 F	2 UF	4 UF	2 UF	8 UF	5 F 1 UF	47
6	18 UF	7 UF	4 UF	4 UF	5 UF	1 F 3 UF	42
10	36 UF	8 UF	4 UF	3 UF	0	0	51
14	23 UF	11 UF	1 UF	3 UF	7 UF	2 F 1 UF	48
17	21 UF 1 F	2 UF	4 UF	2 UF	7 UF	6 F 2 UF	45
20	25 UF	4 UF	8 UF	5 UF	3 UF	1 F 1 UF	47
26	26 UF	7 UF	5 UF	2 UF	5 UF	2 F 4 UF	23
30	20 UF	11 UF	3 UF	5 UF	5 UF	5 F 2 UF	51

Overall mortality: fed = 3.4 (% of fed), unfed = 21.2 (% of unfed)

Overall fed and survived = 7.1 % (of total mosquitoes)

Appendix 72. Distribution of the KWA strain An. gambiae in the "tunnel" containing a guinea pig separated from the mosquito release point by 0.5 g/m² permethrin impregnated netting. The pieces of netting were positioned between cages E and F. The tests were repeated at intervals to determine the persistence of the permethrin.

Weeks after impregnation	Distribution of mosquitoes among different cages						Total number of mosquitoes
	A	B	C	D	E	F	
Control	3 UF	1 UF	1 UF	0	3 UF	44 F	52
0	14 UF	6 UF	6 UF	6 UF	3 UF	2 F 3 UF	40
3	14 UF	4 UF	1 UF	6 UF	14 UF	5 F	44
5	19 UF	6 UF	3 UF	0	5 UF	7 F 3 UF	43
11	28 UF	3 UF	1 UF	3 UF	4 UF	2 F 5 UF	46
14	21 UF	5 UF	4 UF	4 UF	7 UF	1 F 2 UF	44
17	23 UF	6 UF	4 UF	2 UF	7 UF	2 F 1 UF	45
20	28 UF	5 UF	8 UF	2 UF	6 UF	2 UF	50
27	32 UF	8 UF	3 UF	1 UF	3 UF	1 UF	48
30	33 UF	8 UF	1 UF	1 UF	5 UF	1 F	49

Overall mortality: fed = 5.0 (% of fed), unfed = 29.6 (% of unfed)

Overall fed and survived = 4.6 % (of total mosquitoes)

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