

Do insecticide-treated bednets have an effect on malaria vectors?

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Summary

The use of insecticide-treated bednets (ITNs) has been widely adopted as an important method for malaria control. Few data exist on effects of ITNs on mosquito biology and ecology, other than the development of insecticide resistance against the insecticides used. There is no hard evidence that the insecticide resistance recorded is the result of insecticidal use on bednets or from agricultural use. Resistance against pyrethroids, the preferred class of insecticides for ITN use, has been recorded from countries in Asia, Africa and South America. Resistance is expressed as reduced excito-repellency and mortality of mosquitoes exposed to insecticide-treated materials. In the absence of resistance, however, most studies on ITN effects report a reduced survival of adult mosquitoes as well as mass killing. Other effects are highly variable, and shifts in time of biting, feeding site and blood hosts have occasionally been reported, but not in proportion to the scale of ITN use. In general, a reduced sporozoite rate is recorded in ITN programmes. Because many of the anticipated behavioural effects caused by insecticidal use will be avoided by the use of untreated nets, studies on the efficacy of untreated nets are required. Examples are presented in which untreated nets provided a reasonable degree of protection against malaria.

keywords *Anopheles*, mosquito, insecticide impregnated bednet, malaria control, behaviour, resistance, survival, excito-repellency

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Introduction

Malaria is one of the most serious vector-borne diseases, affecting millions of people mainly in the tropics. In spite of major efforts undertaken for its control, through drug treatment and vector control, an increase in malaria incidence has occurred in the last 30 years, primarily caused by socio-economic underdevelopment and drug and insecticide resistance (White *et al.* 1999; Phillips 2001). In order to overcome the pending crisis in lack of adequate intervention methods, bednets treated with insecticides were re-introduced in the latter part of the 1980s. Bednets were to protect the user(s) against the bites of malaria-infectious mosquitoes, and hence contribute to a reduction of transmission risk. It was reported that untreated bednets did not provide adequate protection, presumably because the mosquitoes could bite the occupants through the netting, or nets would often be torn because of excessive use, thus giving mosquitoes easy access to a blood host (Burkot *et al.* 1990). Treatment of nets with a small

deposit of a long-lasting insecticide could overcome these problems, because mosquitoes landing on the net would be killed before having taken a blood meal, or they would be repelled by the insecticide, as is the case with synthetic pyrethroids. It was soon observed that the use of insecticide-treated bednets (henceforth termed ITNs) provided adequate protection against malaria infections, particularly in children (Lengeler & Snow 1996). The World Health Organization has adopted the use of ITNs as one of the main strategies for malaria control in their Roll Back Malaria programme (RBM 1999). At present ITNs are being applied in many malaria-endemic regions worldwide, and their use has replaced the use of indoor house spraying with insecticides in many countries.

The World Health Assembly advocated the large-scale use of insecticides for malaria control in 1955, and programmes were carried out to spray as many houses as possible with a residual deposit of insecticide [mostly organochlorine compounds such as dichlorodiphenyl-trichloroethane (DDT) and dieldrin]. Soon after it was

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discovered that malaria mosquitoes developed resistance against the insecticides directed against them, and before long insecticidal spraying was abandoned in many countries (Najera 1989; Georgiou 1990). The mechanisms of insecticide resistance are manifold: mostly these involve changes in the enzyme metabolism or neurotransmission of the insects (Soderlund & Bloomquist 1990; Hemingway & Ranson 2000), but behavioural changes of mosquitoes have also been reported (Grjebine 1956; Boreham & Garrett-Jones 1973). These included changes in biting behaviour, resting behaviour and avoidance of insecticide-treated rooms.

Indoor spraying with organochlorines has caused behavioural changes in several malaria vector species (Knols & Takken 1998), mostly due to the selective pressure placed on the target vector population. The effects of these changes can be manifold. For instance endophilic mosquitoes can be replaced by exophilic species which share larval habitats. A mass killing effect may create ecological niches for hitherto less relevant anophelines that subsequently may act as malaria vectors. In the extreme, one species may be replaced by another one, leading to even less malaria if the replacement species is not a vector. This paper reviews the status of research on the effects of ITNs on mosquitoes, with special reference to insecticide resistance and behavioural effects.

Bednets and insecticides for malaria control

Bednets have been used traditionally to protect people from the nuisance caused by nocturnally biting insects (Lindsay & Gibson 1988). In Gambia, West Africa, such nets are considered part of the cultural tradition, although it is not clear how long ago and for what reasons the people in this country started using nets (S.W. Lindsay, personal communication). After the introduction of synthetic insecticides during World War II, it was soon realized that bednets impregnated with insecticides might provide better protection against mosquitoes, and hence malaria infections, than untreated nets. The American navy experimented with the use of ITNs, mostly with the insecticide DDT (Harper *et al.* 1947). The protective effects of these experiments have not been clearly reported, but presumably as a result of the global adoption of indoor spraying with synthetic insecticides for malaria control in 1955, the potential effects of ITNs were ignored. The widespread reported development of insecticide resistance as well as the concern over the environmental effects of these substances placed the continuous use of insecticides, even for public health purposes, in a poor position. The development of synthetic pyrethroids, in the 1970s, as a new class of highly potent insecticides with a relatively low

toxicity for vertebrates and significantly fewer environmental effects compared with other classes of insecticides, caused renewed interest in the combined use of insecticides and bednets for malaria control. Darriet *et al.* (1984) reported the successful use of pyrethroid-treated mosquito nets for malaria control and Curtis and Lines (1985) compared the efficacy of different insecticides available for this specific purpose. These early reports created a rapid interest in this potential method for malaria control, and soon numerous trials were undertaken to test the efficacy of ITNs for malaria control (Rozendaal 1989). It was found that ITNs caused a significant reduction in malaria-attributable morbidity and mortality, especially in young children (Graves *et al.* 1987; Alonso *et al.* 1991; Lengeler & Snow 1996). However, there were few or no effects on malaria prevalence, suggesting that ITN users continued to receive infectious bites at times when they were outside the nets. The evidence of ITN use as a successful disease control method was so great that WHO adopted this method as one of the cornerstones for its Roll Back Malaria programme (RBM 1999; Carter *et al.* 2000). The insecticides used for this purpose belong to the class of synthetic pyrethroids and include permethrin, deltamethrin, lambdacyhalothrin and cypermethrin (Table 1). They share the property of a relatively long residual activity when kept out of daylight but break down rapidly under influence of UV-radiation. Their mammalian toxicity is low (see Table 1) but their effect on arthropods, including crustaceans, is generally serious (Takken *et al.* 1978). Because of their high toxicity for mosquitoes, coupled with a long-lasting residual activity on textiles, they are considered safe for use on mosquito nets (Curtis 1990; Naumann 1990; Fenn 1992; Smolen *et al.* 1999; Zaim *et al.* 2000; Barlow *et al.* 2001). In malaria endemic countries the use of ITNs is being promoted as an effective method for reducing malaria transmission risk. Although frequently debated, the question whether the grave errors

Table 1 Dose rates and oral LD₅₀ values of insecticides currently in use for ITN impregnation

Insecticide	Dosage (mg a.i./m ²)	Acute oral LD ₅₀ in rats (mg/kg body weight)
Alpha-cypermethrin	20–40	79
Bifenthrin	50	55
Cyfluthrin	50	250
Deltamethrin	15–25	135
Etofenprox	200	> 10 000
Lambdacyhalothrin	10–20	56
Permethrin	500	500

Source: Zaim *et al.* (2000), Guillet *et al.* (2001) and Chavasse and Yap (1997).

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in judgement made in 1955 concerning the development of insecticide resistance could have been avoided, is rarely being considered in the promotion of ITNs for malaria control (Curtis 1981; Curtis *et al.* 1993; Hemingway & Ranson 2000). Yet, insecticide resistance against pyrethroids has been reported in laboratory and field studies (Chandre *et al.* 1998; Guillet *et al.* 2001) and may threaten the basis for the presently adopted malaria control strategy.

Development of pyrethroid resistance and continuing efficacy of ITNs for malaria control

Pyrethroid resistance in insects is complex and presents at different levels. Because pyrethroids can cause three effects – mortality, repellency and exiting behaviour – resistance mechanisms can develop against each of these. The relationship between these effects is not clear and resistance may operate independently. The selection for pyrethroid resistance in anophelines was reported soon after the widespread use of these chemicals, mainly in agriculture (Georghiou 1990). In Africa, resistance was first reported in Ivory Coast (Elissa *et al.* 1993) and soon found to be widespread in that country (Curtis *et al.* 1998; Chandre *et al.* 1999a; Guillet *et al.* 2001). This concerns in particular the malaria vector *Anopheles gambiae*, but such resistance has also been reported in other anophelines and in different geographical areas [*A. funestus* (Hargreaves *et al.* 2000), *A. albimanus* (Malcolm 1988), *A. aquasalis* (Saume 1996), *A. stephensi* (Omer *et al.* 1980) and *A. sacharovi* (Kasap *et al.* 2000)]. Although there is no hard evidence how the pyrethroid resistance has occurred, there is a growing understanding that it may have resulted from agrochemical use of pyrethroids, in particular in connection with small-scale irrigation practices (Mouchet 1988; Chandre *et al.* 1999b). It is unlikely that the selection for pyrethroid resistance in *A. gambiae* has occurred because of ITN use, as these have not yet been used blanketwise over very large areas. However, where

pyrethroids have been used on a large scale as indoor sprays, as in South Africa, this route may have been the path to selection for resistance. Thus far only pyrethroids are used for bednet impregnation (Zaim *et al.* 2000) and resistance against other groups of insecticides in relation to ITN use does not apply. Cross-resistance of pyrethroids with organochlorines has been documented (Omer *et al.* 1980; Mallet 1989; Brogdon *et al.* 1999; Chandre *et al.* 1999b) and in some cases this may have accelerated the occurrence of pyrethroid resistance in mosquitoes. The resistance reported from West Africa is based on a knockdown (*kdr*) mechanism caused by mutation of the voltage-gated sodium channel gene (Martinez-Torres *et al.* 1998) and characterized by a reduced knockdown effect following exposure to the insecticide. Recently, a *kdr* resistance gene has also been identified from East Africa (Ranson *et al.* 2000) following reports on reduced susceptibility to pyrethroids by Vulule *et al.* (1994). It appears that the distribution of *kdr* resistance in East Africa is still sporadic. In contrast with the *kdr*-based resistance in *A. gambiae sensu stricto* (*s.s.*), the pyrethroid resistance of *A. funestus* in South Africa is based on elevated levels of mixed function oxidases, and this species is still susceptible to DDT (Brooke *et al.* 2001). It is not well understood why, after exposure to same-class insecticides, the reported resistance mechanisms are different. Future research may reveal the reasons for these observations.

In areas where resistance against pyrethroids has been reported, ITNs may remain effective in affording protection because the reduced excito-repellent effect causes prolonged contact with the insecticide (Chandre *et al.* 2000) and therefore mosquitoes are still killed. However, the effect on malaria morbidity and mortality following long-term use of ITNs in resistant areas is not yet known. Doannio *et al.* (1999) report from the Ivory Coast that the proportion of mosquitoes entering a room and taking a blood meal was not significantly different after ITNs were introduced compared with before net use (Table 2). The

	Mean biting rate (b/m/n)	Mean parous rate	Mean sporozoite rate	Mean entomological inoculation rate (inf. b/m/n)
Pre-intervention	77.4	40.6	0.99	0.7
Post-intervention (months)				
6	80.2	32	1.80	0.83
12	67.8	20.1	0.65	0.66
24	102.6	26.2	1.15	0.74

Table 2 Effects of ITN introduction in a village in the Ivory Coast

Source: Doannio *et al.* (1999).

One village, population size: $n = 867$; 352 bednets (net type *Olyset*), insecticide = permethrin; data for *Anopheles gambiae sensu lato* only.

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mean age of the mosquitoes, however, was reduced after installation of the ITNs, demonstrating an effect on survival. Unfed mosquitoes leaving the room by excito-repellent action of the pyrethroids, may cause high biting intensities at times when people are not in bed. In such cases the transmission rate of malaria parasites may not be much different compared with villages without nets. In Doannio's study the sporozoite rate and entomological inoculation rate (EIR) were not different before and after ITN introduction. However, in areas where the mortality of mosquitoes landing on the treated nets is still high, caused by longer exposure times (Chandre *et al.* 2000), the overall density of the mosquitoes may be reduced causing fewer infectious bites. Therefore, a protective effect against malaria may still be present even when a high degree of pyrethroid resistance is present.

Insecticide-induced changes in mosquito behaviour and survival

Insecticide resistance may render the pyrethroids ineffective for malaria control, but this is not necessarily the case with ITNs. On treated nets the pyrethroids work in three ways: first, they act as killing agent when the insect makes contact with the insecticide by landing on the net; secondly, pyrethroids have an irritating (excito-repellent) effect and the insect rests only briefly on the treated fabric and thirdly, the formulation in which the pyrethroid is presented contains volatiles that cause deterrence, leading to fewer mosquitoes entering a room where an ITN is present (Lindsay *et al.* 1991; Chandre *et al.* 2000). In Kenya *A. gambiae s.s.* and *A. arabiensis* did not enter houses in lower numbers in ITN-provided bedrooms compared with houses with untreated nets (Mathenge *et al.* 2001), but the proportion of unfed and exiting mosquitoes was significantly greater in treated houses than untreated ones. Thus, ITN users are protected because fewer mosquitoes land on the net and many will leave the house.

Other side-effects, however, are plausible. These may include changes in biting behaviour expressed by outdoor biting and/or time of biting. Mosquitoes may also express a change in host preference because favoured hosts can no longer be reached (they are under the ITN). Has evidence for such changes in mosquito behaviour been reported? An overview of studies that have investigated potential changes is presented in Table 3. All studies report a reduction in indoor biting in rooms where ITNs have been installed. It has to be stressed that the vectors covered by these studies concern endophilic mosquitoes, several of which prefer to bite humans. Therefore, in areas without vector control, such mosquitoes are mostly collected in bedrooms. The

excito-repellent effect of pyrethroids causes the mosquitoes to leave rooms for the outdoors, hence the observed reduction in indoor biting. Nevertheless many mosquitoes managed to make contact with the ITN, because several studies report mass killing as expressed by a significant reduction in mosquito densities in the treated area (e.g. Cuzin-Ouattara *et al.* 1999; Maxwell *et al.* 1999). It is unlikely that the mass killing would have resulted from reduced access to blood hosts. In spite of a strong anthropophilic tendency, *A. gambiae s.s.* can readily switch to other hosts should humans not be available (Diatta *et al.* 1998) and other human-biting species are similarly inclined to feed on other hosts. When a mass killing effect was reported, this was accompanied by a reduced survival, as expected. However, because survival rates in mosquitoes are calculated from the average population age structure, those individuals that survived the effects of ITN exposure may be the select group that contributes to the next generation. In this way any resistance gene will be rapidly spread through the remaining mosquito population. Some studies report a reduction in sporozoite rate, presumably as a result of reduced survival. However, other studies did not see an effect on the sporozoite rate. In these cases the mosquitoes may not have entered the ITN homes and fed elsewhere, also because no effect on survival was noted (Table 3). Most studies report some highly significant reductions in the EIR. This is to be expected when fewer mosquitoes bite man, and is of course the principal goal of ITN use.

Some effects of ITNs on the time of biting and the host choice of mosquitoes have been reported. In studies in Papua New Guinea and in Kenya a shift to outdoor biting was observed (Table 3). This was possibly also the case in a study in Tanzania (Magesa *et al.* 1991). In the Papua New Guinea and Tanzania studies shifts in host feeding and time of biting were also observed. Other hosts included pigs, dogs (Papua New Guinea) and cattle (Tanzania). Biting occurred earlier in the evening, presumably because the mosquito hosts had not yet gone to bed and were easily accessible. In the Kenya study (Mbogo *et al.* 1996) mosquitoes did not switch hosts, but they began biting earlier in the evening. The latter is the only study that reports reduced blood feeding. Because no effects on survival before and after ITN introduction were noted, we must assume that the mosquitoes must have fed elsewhere. It thus appears that the overall effects of both immediate and long-term use of ITNs on mosquitoes are variable. In many cases a reduced survival was observed as well as reduced sporozoite rates. With two exceptions, no shift to outdoor biting or non-human hosts has been recorded. In three studies mosquitoes started biting earlier in the evening. Because only few studies examined a comprehensive

W. Takken **Effects on ITNs on malaria vectors****Table 3** Review of the effect of ITNs on mosquitoes and malaria transmission potential

Country	Insecticide	Reduced indoor biting	Mass killing	Reduced survival	Shift to outdoor biting	Shift in host feeding	Shift in time of biting	Reduced % blood fed	Reduced sporoz. rate	Reduced EIR	Reference
Papua New Guinea	permethrin	+	no	no	yes	yes	yes			yes	Charlwood & Graves 1987
China	deltamethrin	+	yes	??							Zuzi <i>et al.</i> 1989
Ivory Coast	deltamethrin	+	yes	yes				63-73%		82-91%	Carnevale <i>et al.</i> 1988
Kenya	permethrin	+							n/a		Sexton <i>et al.</i> 1990
Tanzania	permethrin, lambda-dacyhalothrin	+	yes	yes	some*	no	yes*	yes		93.4-100%	Magesa <i>et al.</i> 1991
The Gambia	permethrin	+	no	no	yes	no		no		unclear	Lindsay <i>et al.</i> 1993
D.R. Congo (Zaire)	deltamethrin	+	yes	yes				no		98%	Karch <i>et al.</i> 1993
Solomon islands	permethrin	+	yes	yes				yes		43%**	Hii <i>et al.</i> 1995
China	alphacypermethrin	+	yes								Luo Dapeng <i>et al.</i> 1996
Kenya	permethrin	+	no	no	yes***	no	yes***	yes		8.30%	Mbogo <i>et al.</i> 1996
Ghana	permethrin	+	no							yes	Binka <i>et al.</i> 1996
Mexico	lambda-dacyhalothrin	+		yes							Arredondo-Jimenez <i>et al.</i> 1997
The Gambia	permethrin	+	no	no		no				yes	Quinones <i>et al.</i> 1998
Senegal	permethrin	+						76%		88%	Faye <i>et al.</i> 1998
India	lambda-dacyhalothrin	+	yes	yes	yes (cattle)	no				yes	Sampath <i>et al.</i> 1998
Burkina Faso	permethrin	+	yes							>95%	Cuzin-Ouattara <i>et al.</i> 1999
Ivory Coast	permethrin	+	no	yes				no		no	Doannio <i>et al.</i> 1999
Tanzania	alphacypermethrin, lambda-dacyhalothrin	+	yes	yes			yes	yes		>99%	Maxwell <i>et al.</i> 1999
Burkina Faso	permethrin	+	yes	no	??	??		yes		yes	Ilboudo-Sanogo <i>et al.</i> 2001
Kenya	permethrin	+	no				no****				Mathenge <i>et al.</i> 2001

* in one of two villages sign. shift to outdoor biting; in the other village sign. shift to earlier biting.

** based on calculations of expected infective life (EIL) values.

*** 30.3% caught biting outdoors, compared to 23.2% in non-intervention area; 12% of bites occurred before 22.00 hours in houses with ITN, compared to 7% in control houses.

**** differences were noted in biting times between *A. gambiae s.l.* and *A. funestus*.

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package of behavioural aspects related to ITN use, there is an urgent need to conduct such studies more often and in greater detail, in order to avoid long-term behavioural changes as have occurred following indoor spraying with organochlorines (Boreham & Garrett-Jones 1973; Knols & Takken 1998).

Protection afforded by untreated nets

An attractive alternative option of using bednets for malaria protection is the use of untreated nets, as was practiced for many centuries (Lindsay & Gibson 1988). Because the efficacy of insecticide impregnated nets was so much greater than of untreated ones (Snow *et al.* 1988), this option is rarely being considered. Its advantages are threefold – no negative effects of insecticide use, the avoidance of toxic chemicals at household level and financial savings (Guyatt & Snow 2002).

The behaviour of mosquitoes around untreated nets has shown that considerable protection is afforded provided the nets are tucked in, maintained in good condition and sufficiently large so that the sleepers do not make contact with the net (Lindsay *et al.* 1989). Where untreated nets have been used in village trials, they resulted in a reduction of malaria morbidity compared with areas without nets, but the protection was significantly lower compared with treated nets (Magesa *et al.* 1991; Maxwell *et al.* 1999). Yet, other studies report effects that are sufficiently encouraging to revisit this issue (Genton *et al.* 1994; Clarke *et al.* 2001; Hii *et al.* 2001; Guyatt & Snow 2002). In practice, most ITN users do not re-impregnate their nets unless a strongly coordinated action is put into place at the primary health care level (Clarke *et al.* 2001; Schellenberg *et al.* 2001). Therefore, the efficacy of untreated nets should be reconsidered not only from the aspect of malaria control but also from the point of sustainability. Untreated nets are clearly going to be of much longer use and easier and cheaper in use than treated ones. It is possible that behavioural changes in mosquitoes against the nets may develop, but these are unlikely to affect the protection against mosquito bites, unless a large shift in time of biting would occur. Such effects have hitherto not been clearly observed with large-scale use of ITNs and may therefore not occur with untreated nets. If children can sleep under a large enough net, the most vulnerable age groups for malaria disease would at least be well protected from mosquito bites.

The future of bednet use

The data in this review demonstrate an overwhelming success of the acceptance of ITNs for malaria prevention

and control. However, it is argued that the development of pyrethroid resistance, often linked to cross-resistance with organochlorine compounds, may put the concept of continuous ITN use in serious jeopardy. No consistent and large-scale effects on mosquito behaviour that would render the ITNs useless for malaria control have been observed. Thus far in this respect, ITNs may operate differently on mosquito populations than residual insecticides applied on walls and ceilings. The latter method caused rapid physiological and behavioural resistance in mosquitoes (Mallet 1989). In some cases these effects were still evident one decade after the last application of the insecticides (Boreham & Garrett-Jones 1973), demonstrating the extreme selection these mosquito populations underwent while being exposed to the insecticides. However, under the ITN strategy, mosquitoes that are repelled by pyrethroids may still be successful in finding a blood host and survive long enough to contribute to the gene pool of the population. Selection for resistance would thus be slow, provided the insecticides are used for public health measures only. Unfortunately this is often not the case, and farmers may have easy access to pyrethroids for the control of livestock and agricultural pests. The ideal strategy would be the exclusive reservation of pyrethroids for public health, but this is clearly not possible. Therefore, the use of untreated nets is being proposed as a reasonable alternative to ITNs for malaria control. In particular as many ITN users fail to re-impregnate their nets, untreated nets may be a better and more sustainable option in combination with early diagnosis and treatment. This strategy would have the advantage that one need not worry about re-impregnation, the safe storage of new stocks or the disposal of unused stocks of insecticides. In addition, the insects are being presented with a preventive strategy, which allows them to select for non-human hosts, which may be an unexpected bonus.

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