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Combined pyrethroid and carbamate 'two-in-one' treated mosquito nets: field efficacy against pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus*

P.^{Pierre} GUILLET*, R. N'GUESSAN, F.^{Midiatic} DARRIET,
M.^{Moumouni} TRAORE-LAMIZANA, F.^{Fabrice} CHANDRE and P.^{Pierre} CARNEVALE
Institut Pierre Richet, Bouaké, Côte d'Ivoire, and *WHO/CDS/CPE/PVC, Geneva, Switzerland

Abstract. A new approach is proposed in the treatment of mosquito nets, using a 'two-in-one' combination of pyrethroid and non-pyrethroid insecticides applied to different parts of bednets. The objectives are mainly to overcome certain limitations of pyrethroid-impregnated bednets currently recommended for malaria control purposes. Apart from developing alternatives to pyrethroid dependency, we sought to counteract pyrethroid irritant effects on mosquitoes (excito-repellency) and resistance to pyrethroids. The idea takes advantage of the presumed host-seeking behaviour of mosquitoes confronted by a net draped over a bed, whereby the mosquito may explore the net from the top downwards. Thus, nets could be more effective if treated on the upper part with residual non-irritant insecticide (carbamate or organophosphate) and with a pyrethroid on the lower part. Sequential exposure to different insecticides with distinct modes of action is equivalent to the use of a mixture as a potential method of managing insecticide resistance. We also intended to improve the control of nuisance mosquitoes, especially *Culex quinquefasciatus* Say (Diptera: Culicidae) that often survive pyrethroids, in order to encourage public compliance with use of insecticide-treated nets (ITNs).

Polyester bednets were pretreated with residual pyrethroid (bifenthrin 50 mg/m² or deltamethrin 25 mg/m²) on the lower half and with carbamate (carbosulfan 300 mg/m²) on the upper half to minimize contact with net users. Unreplicated examples of these 'two-in-one' treated nets were field-tested against wild mosquitoes, in comparison with an untreated net and bednets treated with each insecticide alone, including PermaNet™ wash-resistant formulation of deltamethrin 50 mg/m². Overnight tests involved volunteers sleeping under the experimental bednets in verandah-trap huts at Yaokofikro, near Bouaké in Côte d'Ivoire, where the main malaria vector *Anopheles gambiae* Giles, as well as *Culex quinquefasciatus* Say, are highly resistant to pyrethroids. Efficacy of these ITNs was assessed in the huts by four entomological criteria: deterrence and induced exophily (effects on hut entry and exit), blood-feeding and mortality rates (immediate and delayed).

Overall, the best impact was achieved by the bednet treated with carbo-sulfan alone, followed by 'two-in-one' treatments with carbo-sulfan plus pyrethroid. Blood-feeding rates were 13% *An. gambiae* and 17% *Cx. quinquefasciatus* in huts with untreated nets, but only 3% with carbo-sulfan ITNs, 7–11% with combined

Correspondence: Dr Pierre Guillet, WHO/CDS/CPE/PVC, Parasitic Diseases & Vector Control, Communicable Diseases Control Prevention & Eradication, World Health Organization, 20 Avenue Appia, CH-1211 Geneva 27, Switzerland. E-mail: guilletp@who.int

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ITN treatment, 6–8% *An. gambiae* and 12–14% *Cx. quinquefasciatus* with pyrethroid alone. Mosquitoes that entered the huts were killed sooner by nets with combined treatment than by pyrethroid alone. Mortality-rates in response to ITNs with carbosulfan (alone or combined with pyrethroid) were significantly greater for *Cx. quinquefasciatus*, but not for *An. gambiae*, compared to ITNs with only pyrethroid. About 20% of sleepers reported potential side-effects (headache and/or sneezing) from use of ITN treated with carbosulfan alone. Further development of this new 'two-in-one' ITN concept requires a range of investigations (choice of effective products, cost-benefit analysis, safety, etc.) leading to factory production of wash-resistant insecticidal nets treated with complementary insecticides.

Key words. *Anopheles gambiae*, *Culex quinquefasciatus*, *Mansonia*, bednets, carbamate, carbosulfan, combined treatment, insecticide resistance, insecticide-treated nets (ITNs), malaria vector, mixtures, mosquito nets, nuisance mosquitoes, pyrethroid, resistance management, vector control, verandah-trap, Côte d'Ivoire.

Introduction

Large-scale implementation of insecticide-treated mosquito nets (ITNs) is strongly advocated for malaria control, especially in sub-Saharan Africa, but faces various problems and limitations (Lines, 1996). Availability and cost of nets and insecticides are the most critical constraints. Operational difficulties include requirements for regular re-treatment of ITNs to maintain their efficacy against *Anopheles* malaria vectors and the need for better impact on pest mosquitoes such as *Culex* and *Mansonia* (N'Guessan et al., 2001). Experience shows that most users do not impregnate their nets correctly with pyrethroid insecticide when necessary and, after ITN projects progress from novelty to routine, the majority of nets found in place have not been treated or re-treated satisfactorily (Armstrong et al., 1999; Kachur, et al., 1999). Nuisance caused by *Cx. quinquefasciatus* is the main motivation for people to use ITNs in tropical towns and villages. This pest mosquito has become resistant to insecticides in many parts of the world, including Africa (Chandre et al., 1997), while remaining sensitive to the excito-repellent effects of pyrethroids (Chandre et al., 1998). Hence, ITNs have limited impact on *Cx. quinquefasciatus* populations, so people are not motivated to use or re-treat their nets.

Some manufacturers have developed more durably insecticidal treated bednets, such as the 'Olyset Net[®]' with permethrin incorporated in polyethylene fibre (N'Guessan et al., 2001) and the 'PermaNet[™]' of polyester with wash-proof deltamethrin treatment (WHO, 2000). Such 'long-lasting insecticidal wash-resistant mosquito nets' should be biologically active throughout the average life-expectancy of the net, perhaps 3–5 years of nightly use in typical village conditions. In delivering such ready-to-use products, the objective has been to adapt ITNs to the behaviour of users (occasional washing and no need for re-treatment), rather than trying to induce behavioural changes among users. Examples of durably insecticidal mosquito nets are currently under evaluation for malaria vector control by the WHO Pesticide Evaluation Scheme (WHO, 2000).

As pyrethroids are the only class of insecticide currently used for ITNs (Lengeler et al., 1996; Zaim et al., 2000), the occurrence of pyrethroid-resistant *Anopheles* could jeopardize the effectiveness of ITNs against malaria transmission. Pyrethroid resistance has developed in some populations of major malaria vectors, including *An. gambiae* s.s. in tropical Africa (Elissa et al., 1993; Chandre et al., 1999a, b; Ranson et al., 2000), *An. funestus* in southern Africa (Hargreaves et al., 2000), *An. albimanus* in Central America (Malcolm, 1988), *An. stephensi* in the Middle East (Omer et al., 1980) and *An. sacharovi* in Turkey (Kasap et al., 2000). Very high levels of resistance to pyrethroids in *An. gambiae* are associated with *kdr* mutations that confer so-called 'knockdown resistance' (Martinez-Torres et al., 1998; Fanello et al., 1999; Ranson et al., 2000), causing cross-resistance among pyrethroids (Chandre et al., 1999c), thus raising the need to consider alternative classes of insecticides for ITNs.

Likewise for mosquito nuisance control with ITNs the problem of pyrethroid resistance in *Cx. quinquefasciatus* needs circumvention, if this pest is not curtailed by other means (e.g. sanitation). The main alternative insecticides available for treatment of ITNs are carbamates and organophosphates (OPs), which would be generally active against pyrethroid-resistant and susceptible populations of both *Anopheles* and *Culex* (Curtis et al., 1998). When evaluated on ITNs against wild mosquitoes in experimental huts, OPs and carbamates were found to be non-excitorepellent and very active in killing *Anopheles*, *Culex* and *Mansonia* (Miller et al., 1991; Fanello et al., 1999; Kolaczinski et al., 2000). Because carbamates and OPs are potent inhibitors of cholinesterases, however, the toxicological risk implications of their use on ITNs should be considered in relation to human safety (WHO, 1986a, b).

In view of their non-excitorepellency to adult mosquitoes, OPs and carbamates used for large-scale ITN programmes might have more impact than pyrethroids on mosquito populations, by 'mass killing effect' reducing adult mosquito densities, survival rates and hence vectorial capacity (Curtis & Mnzava, 2000). Conversely, the use of these non-irritant insecticides is less likely to deter mosquitoes from blood-

feeding through a bednet, and so confers less immediate personal protection (Miller *et al.*, 1991; Kolaczinski *et al.*, 2000). We need to know much more about mosquito reactions to ITNs treated with various insecticides (Fanello *et al.*, 1999; Chandre *et al.*, 2000; Quiñones *et al.*, 2000) and their differential impact on malaria transmission.

In order to benefit from the relative advantages of pyrethroid and non-pyrethroid insecticides, mixtures of both could theoretically be used for treatment of ITNs. This would have cost and safety implications discussed below. We envisage a new approach based on the observed behaviour of mosquitoes flying around a bednet and occasionally settling on it. From sleepers under the bednet, heat and carbon dioxide emanate and move upwards thermally within the net, which acts like a chimney. Consequently, foraging female mosquitoes tend to begin exploring nets on the upper part, proceeding downwards, looking for any bloodmeal opportunity. Therefore, we designed and produced experimental bednets treated with pyrethroid on the lower part and with non-pyrethroid insecticide (OP or carbamate) on the upper part, intending that mosquitoes will contact both types of insecticide sequentially. This two-in-one treatment equates to the use of a mixture, in space and time, involving the mosquito contacting two (or more) different insecticides in rapid succession. Each insecticide is used at a concentration sufficient to (1) kill mosquitoes, including *Culex* and pyrethroid resistant anophelines (due to non-pyrethroid insecticide applied on the upper part of the net), and (2) prevent blood-feeding and rapidly neutralize mosquitoes (due to pyrethroid applied on the lower part of the net). With regard to safety, this treatment design limits the likelihood of human exposure to the more hazardous OP or carbamate insecticide.

Materials and methods

Bednets of knitted multifilament polyester: 100 denier, single size, 11.6 m² area (SiamDutch Mosquito Netting Co., Bangkok, Thailand) were of the type previously tested in our verandah-trap huts at Yaokofikro, near Bouaké, central Côte d'Ivoire (Fanello *et al.*, 1999; Kolaczinski *et al.*, 2000; Darriet *et al.*, 1999, 2000). Five treated nets were compared with an untreated control net, in six huts, following procedures described by N'Guessan *et al.* (2001). Nets were allocated to huts at random and used consistently in the same hut (to avoid cross-contamination between huts) by a sleeper every night from early June to late August 2000. To offset any personal bias (due to differential sleeping habits or relative attractiveness to mosquitoes), sleepers changed between huts sequentially for successive nights. Wild mosquitoes entered each hut nocturnally via the eaves and could exit into the verandah trap. Mosquito collections were made from the six huts on six consecutive nights per week, for 7 weeks, starting 2 weeks after introduction of the nets. Following each overnight test (20.00–05.00 hours), all female mosquitoes were collected from each hut plus verandah-trap at 08.00 hours and scored as alive or dead, and with regard to abdominal condition (unfed or blood-

fed). Surviving mosquitoes were kept in netted plastic cups (provided with 10% honey food) and delayed mortality was assessed after 24 h in the laboratory. By comparison with the numbers and conditions of mosquitoes collected from the hut with the untreated net (control), the effects of each ITN on mosquitoes were expressed in terms of: mortality rate (immediate and delayed); feeding rate: proportion of blood-fed mosquitoes (alive or dead); deterrence: relative number of mosquitoes entering hut with ITN; excito-repellency (exophily induced by insecticide): relative proportion of mosquitoes found in the veranda trap.

Insecticides tested on ITNs were: a carbamate, carbosulfan 2.5% suspension concentrate (25 SC) produced by FMC (Princeton, NJ, U.S.A.); two pyrethroids: bifenthrin 8% suspension concentrate (80 SC) produced by FMC; and deltamethrin in two presentations: 1% SC formulation, produced by Aventis (Frankfurt, Germany), and the wash-resistant PermaNet™ (Vestergaard Frandsen, Kolding, Denmark; www.permanet.dk) pretreated with deltamethrin (supplied by Phytagri, Geneva).

Carbosulfan was selected as the experimental non-pyrethroid for its efficacy against pyrethroid-resistant *An. gambiae* and *Cx. quinquefasciatus* (Darriet, 1998; Fanello *et al.*, 1999; Kolaczinski *et al.*, 2000). Bifenthrin was selected as the candidate pyrethroid as it is more potent than other pyrethroids for killing adult mosquitoes, including *Culex* (Guillet, unpublished). Deltamethrin SC was used as the standard pyrethroid (WHO, 1999a, b; Zaim *et al.*, 2000). In comparison, PermaNet™ with special deltamethrin formulation long-lasting insecticidal treatment was field-tested for the first time.

Nets with the following six treatments (unreplicated) were compared in the verandah-trap huts:

- bifenthrin 50 mg/m² alone;
- carbosulfan 300 mg/m² alone;
- bifenthrin 50 mg/m² plus carbosulfan 300 mg/m²;
- deltamethrin 50 mg/m² alone (PermaNet™);
- deltamethrin 25 mg/m² plus carbosulfan 300 mg/m²;
- untreated control.

For mono-treatment, each net was soaked in the required amount of insecticide diluted in water, then hung to dry. The procedure for treatment of nets with two insecticides involved dipping the upper and lower halves in separate baths of each insecticide, then drying that part. While each half of the net was dipped, the dry other half was held in a plastic bag – isolated by a tight knot. Target treatment concentrations on each part of the net were confirmed by chemical assays. People involved in this trial were recruited with informed consent and any perceived human side-effects of insecticides were surveyed by interview.

Mosquitoes monitored were the pest species *Cx. quinquefasciatus* and the malaria vector *An. gambiae* Savanna form identified by PCR (Scott *et al.*, 1993) and RFLP (Favia *et al.*, 1997), both highly resistant to pyrethroids due to *kdr* mutation (Martinez-Torres *et al.*, 1998, 1999) and resistant to carbamates due to acetylcholinesterase insensitivity (Chandre *et al.*, 1998; N'Guessan *et al.* in prep.). Abbott's correction was applied to data on mortality-rates (Swaroop, 1966).

Results

Mosquitoes collected from 252 hut-nights of sampling (7 weeks \times 6 days \times 6 huts) comprised 481 (17.3%) *An. gambiae*, 1287 (46.3%) *Cx. quinquefasciatus* and 1010 (36.4%) *Mansonioides* spp. (*Mansonia africana* and *Mn. uniformis*).

Against pyrethroid-resistant *An. gambiae* that entered the huts, nets treated with only deltamethrin (PermaNet™) or bifenthrin 50 mg a.i./m² gave high mortality-rates (81–86%), but had no significant impact on blood-feeding rates (Table 1). The net treated with only carbosulfan caused 90% mortality and significantly reduced blood-feeding success by 76% ($\chi^2=4.28$; $P=0.038$). Nets treated with the combination of carbosulfan and pyrethroids killed 87–92% but had no significant impact on blood-feeding. Nets treated with carbosulfan (alone or combined with pyrethroid) killed more *An. gambiae* faster (immediate 100% of the overall mortality 90–92%) than nets treated with pyrethroid alone (overall mortality 81–86% after 24 h). However, the overall mortality rates of 81–92% *An. gambiae* were significantly similar in huts with each of the five ITNs, compared to <10% with the untreated control net (Table 1). None of these ITNs induced significant deterrence or exophily (excito-repellency) of *An. gambiae*.

Against *Cx. quinquefasciatus*, the bifenthrin net caused significantly higher mortality than the deltamethrin-treated PermaNet™: 53% vs. 31% (χ^2 8.5, $P<0.01$), but neither reduced the blood-feeding rates (Table 2). The net treated with carbosulfan reduced blood-feeding by 80% (χ^2 18.85, $P<0.001$) and killed 98.5% without delay. Pyrethroid combinations with carbosulfan gave significantly higher mortality-rates than pyrethroids alone (80% vs. 31% for deltamethrin and 84% vs. 53% for bifenthrin). Rates of blood-feeding were also significantly less with 'two-in-one' combi-

nation ITNs compared to pyrethroid alone, especially for bifenthrin (7.2% vs. 14.3%: $\chi^2=15.9$, $P<0.001$). Only the PermaNet™ exerted significant deterrence against *Cx. quinquefasciatus*. Significantly increased exophily of *Cx. quinquefasciatus* resulted from ITNs with either pyrethroid alone, but not the other treatments (Table 2).

During the 252 man-nights of this study, sleepers experienced symptoms of headache (five cases) and sneezing (three bouts), overall frequency of potential side-effects ~20%, all reported by volunteers who slept under nets treated with carbosulfan alone. No such side-effects were perceived by people who treated or handled the nets.

Discussion

Differential efficacy of ITNs evaluated

The great majority of pyrethroid-resistant *An. gambiae* (81–92%) and *Cx. quinquefasciatus* (53–99%) died within 24 h of entering huts where each type of ITN was tested, with the exception of PermaNet™, which killed only 31% of *Cx. quinquefasciatus*. Mosquito mortality rates were higher and faster, especially of *Cx. quinquefasciatus*, in response to ITNs with carbosulfan treatment (alone or in combination with pyrethroid), than for nets treated with pyrethroid alone. With untreated nets >90% mosquitoes survived the test period. The 'two-in-one' treated nets consistently outperformed other treatments, except carbosulfan alone, for their impact on mosquito survival (Table 3). This bodes well for their potential use in resistance management.

With regard to other effects of ITNs, none of these treatments had as much deterrent effect on mosquito endophily as usually occurs with permethrin (Darriet *et al.*, 1984; Lines *et al.*, 1987; Lindsay *et al.*, 1991; Curtis *et al.*, 1996; N'Guessan

Table 1. *Anopheles gambiae* females (with proportions blood-fed and dead) found in verandah-trap huts after overnight use of a mosquito net (totals from 36 hut-nights during 7 weeks).

Mosquitoes	Bednet treatment					
	Untreated	Bifenthrin 50 mg/m ²	PermaNet™ (deltamethrin. 50 mg/m ²)	Carbosulfan 300 mg/m ²	Carbosulfan (300 mg) + deltamethrin (25 mg/m ²)	Carbosulfan (300 mg) + bifenthrin (50 mg/m ²)
Total	84 a,b	89 a	69 a,b	62 b	70 a,b	107 a
Deterrence	0 a	(-6%) a	18% a,b	32% b	17% a,b	(-27%) a
Exophily†	31 (37%) a	42 (47%) a	20 (29%)	16 (26%)	28 (40%)	39 (36%)
Blood-fed rate	11 (13.1%) a	7 (7.9%) a,b	4 (5.8%) a,b	2 (3.2%) b	8 (11.4%) a,b	7 (6.5%) a,b
Reduction	0 a	40% a,b	56% a,b	76% b	13% a,b	50% a,b
Mortality: immediate	7	73	57	56	62	99
Overall (rate*)	8 (9.5%) a	74 (81.4%) b	60 (85.5%) b	56 (89.5%) b	62 (87.4%) b	99 (91.7%) b

a,b In each row, values followed by different letters are significantly different, whereas those followed by the same letters are not significantly different.

†Verandah-trap collection

*Corrected according to Abbott's formula

Table 2. *Culex quinquefasciatus* females (with proportions blood-fed and dead) found in verandah-trap huts after overnight use of a mosquito net (totals from 36 hut-nights during 7 weeks).

Mosquitoes	Bednet treatment					
	Untreated	Bifenthrin 50 mg/m ²	PermaNet™ (deltamethrin. 50 mg/m ²)	Carbosulfan 300 mg/m ²	Carbosulfan (300 mg) + deltamethrin (25 mg)	Carbosulfan (300 mg) + bifenthrin (50 mg)
Total	236 a	209 a,b	193 b	211 a,b	202 a,b	236 a
Deterrency	0 a	11.4% a,b	18.2% b	10.6% a,b	14.4% a,b	0 a
Exophily†	81 (34%) a	102 (49%) b	93 (48%) b	65 (31%) a	78 (39%) a,b	85 (36%) a
Blood-fed rate	39 (16.5%) a	30 (14.3%) a	24 (12.4%) a	7 (3.3%) b	22 (10.9%) a	17 (7.2%) b
Reduction	0 a	13% a	25% a	80% b	34% a	56% b
Mortality: immediate	13	107	66	208	156	200
Overall (rate*)	19 (8%) a	118 (53%) b	70 (31%) c	208 (99%) d	164 (80%) e	203 (85%) d,e

a,b,c In each row, values followed by different letters are significantly different, whereas those followed by the same letters are not significantly different from each other.

†Verandah-trap collection

*Corrected according to Abbott's formula (Swaroop, 1966)

Table 3. Summary comparison of ITNs evaluated against wild pyrethroid-resistant *Anopheles gambiae* (An) and *Culex quinquefasciatus* (Cx) in verandah-trap huts at Yaokofikro: assessed by six criteria from Tables 1 and 2. Efficacy ranked 1–6 for each factor investigated, where 1 is best and 6 represents least effectiveness.

Net treatment	Deterrency		Exophily		Anti-feeding		Mortality				Rank mean value	Overall rank
							Immediate		Overall			
	An	Cx	An	Cx	An	Cx	An	Cx	An	Cx		
Carbosulfan	1	4	6	6	1	1	2	1	2	1	2.5	1
Carbosulfan + deltamethrin	3	2	2	3	5	3	3	3	3	3	3.0	2=
Carbosulfan + bifenthrin	6	5=	4	4	3	2	1	2	1	2	3.0	2=
Permanet™	2	1	5	2	2	4	4	5	4	5	3.4	4
Bifenthrin	5	3	1	1	4	5	5	4	5	4	3.7	5
Untreated	4	5=	3	5	6	6	6	6	6	6	5.3	6

= indicates two treatments of equivalent rank value in the same column.

et al., 2001). Exophily attributed to excito-repellency was greatest with the bifenthrin ITN, while the deltamethrin PermaNet™ was associated with significantly increased exophily of *Cx. quinquefasciatus* but not *An. gambiae*. Pyrethroid-treated nets generally had no significant impact on mosquito blood-feeding rates (except bifenthrin in combination with carbosulfan against *Cx. quinquefasciatus*). Nets treated with carbosulfan (alone or in combination with pyrethroid) reduced the blood-feeding success rates of both mosquito species (significantly in most cases) without affecting their hut entry or exit rates. In view of the various entomological advantages demonstrated by carbosulfan 300 mg/m² ITNs, it is disappointing that this carbamate when used alone was associated with side-effects (symptoms of

headache and/or sneezing) perceived by 20% of users. However, such effects were not experienced during a previous study in the same huts under the same test conditions (Kolaczinski *et al.*, 2000).

The 'two-in-one' concept for insecticide-treated nets (ITNs)

By combining pyrethroid insecticide, applied to the lower part of a bednet, together with non-pyrethroid insecticide on the upper part, it was possible to gain from the different powers of both insecticides against mosquitoes the protective (excitorepellent) effect provided by pyrethroids and the better killing by a carbamate (carbosulfan), even against highly

resistant mosquitoes: *An. gambiae* savanna populations with 96% knock-down resistance (Chandre *et al.*, 1999a, b, 2000) and multiresistant *Cx. quinquefasciatus* (Chandre *et al.*, 1997, 1998). 'Two-in-one' ITNs therefore have several potential advantages over nets treated with pyrethroid alone. Impact of the non-excitorepellent insecticide (OP or carbamate) on pest mosquitoes, such as *Mansonia* spp. in swampy areas and *Cx. quinquefasciatus* in urban situations, would greatly improve the popularity of ITNs and should be beneficial in reducing the transmission of filariasis as well as malaria. The greater killing effects of carbamate (Table 3) and OP insecticides (Curtis *et al.*, 1998; Kolaczinski *et al.*, 2000) should enhance the 'mass effect' of ITNs (Lengeler *et al.*, 1996; Howard *et al.*, 2000) on malaria vector populations, whether susceptible or resistant to pyrethroids. Most importantly, 'two-in-one' treated mosquito nets, combining different classes of insecticide, can be regarded as a potential tool for the implementation of resistance management.

The use of non-pyrethroid insecticides for bednet treatments

Pyrethroid-impregnated bednets provide an irritant barrier against susceptible mosquitoes, due to excito-repellency and knock-down effects of pyrethroids (Miller & Gibson, 1994). When treated with non-pyrethroids, such as OPs or carbamates, ITNs lack these immediately protective properties – although mosquitoes may die following exposure to them. Consequently, mosquitoes can readily bite through netting treated with non-irritant insecticides, and may enter such nets if they are holed or not tucked in during use, so infective vectors might transmit malaria before being killed (Kolaczinski *et al.*, 2000). In this case, personal protection will be no more than with untreated nets (Lindsay *et al.*, 1989). Even so, non-pyrethroid ITNs could effectively reduce malaria transmission through impact on vectorial capacity (survival rates and density), as traditionally achieved by house-spraying with residual insecticides (Curtis & Mnzava, 2000). This would require high rates of coverage as well as proper use of ITNs and timely re-treatment arrangements, difficult to facilitate currently in many malarious countries – particularly in Africa. To overcome these techno-logistical problems, and to improve compliance with use of ITNs having wash-resistant insecticidal efficacy, our ready-for-use 'two-in-one' treated bednets are designed to provide both personal protection and mass impact on populations of pest mosquitoes as well as malaria vectors.

Pyrethroid resistance and ITNs

In situations where *kdr* (knockdown resistance) frequencies are >90% in *An. gambiae*, ITNs with some pyrethroids (permethrin, deltamethrin, lambda-cyhalothrin) can still reduce malaria vectorial capacity in our experimental huts (Darriet *et al.*, 1999, 2000; N'Guessan *et al.*, 2001) and malaria transmission in village-scale trials (Henry *et al.*, 1999; Dossou Yovo *et al.*, 2000). The powerful *kdr* mechanism, due to point

mutation of a nerve receptor, paradoxically allows mosquitoes to be more exposed by tarsal contact with a lethal dose of pyrethroid (Chandre *et al.*, 2000). Even so, Kolaczinski *et al.* (2000) found that ITNs with some pyrethroids (etofenprox, alpha-cypermethrin) have reduced efficacy against *kdr* in *An. gambiae* in our experimental huts. In the present study, nets treated with only bifenthrin or deltamethrin (PermaNetTM) had good impact on *kdr* pyrethroid-resistant *An. gambiae* (giving >80% mortality) and these materials would be suitable for 'two-in-one' treated net production.

Enzyme-based pyrethroid resistance mechanisms in *Anopheles*, such as elevated oxidase or esterase (Vulule *et al.*, 1999), may be more of an obstacle to malaria vector control with ITNs. Examples with *An. albimanus* in Mexico (Penilla *et al.*, 1998) and *An. funestus* in South Africa (Hargreaves *et al.*, 2000) highlight the need for resistance management involving multiple insecticide classes. With current dependency on pyrethroids for ITNs (Lengeler *et al.*, 1996; Zaim *et al.*, 2000) and the increasing scale of bednet coverage, the selection pressures for pyrethroid resistance are bound to rise in most malarious countries, requiring alternative products and strategies to be invoked for malaria vector control (Curtis *et al.*, 1998).

Supposing that pyrethroids can be replaced entirely by other insecticide classes (only carbamates and OPs are available currently) for the treatment of bednets, insecticide resistance will remain of major concern and resistance management schemes would still have to be implemented. Various theoretical models have been proposed for the prevention and management of insecticide resistance (Hoy, 1998) without much real progress against this problem. The use of mixtures of insecticides acting on different targets, when feasible, is one potential strategy, as used with combination therapy for the management of drug resistance (e.g. White & Olliaro, 1996). The systematic use of organophosphate and pyrethroid insecticide mixtures against cotton pests in West and Central Africa has prevented or delayed for >20 years the development of pyrethroid resistance in the cotton bollworm, *Helicoverpa armigera* (Martin *et al.*, 2000). Treatment of ITNs with mixtures of insecticides, each at the necessary concentration, is probably impractical, costly, potentially unstable and might raise concerns for human safety in use. With 'two-in-one' treated nets, however, mosquitoes are likely to be exposed to two different insecticides – as they would be using a mixture.

Feasibility and safety concerns with 'two-in-one' ITNs

Pyrethroids on ITNs are regarded as relatively safe for human use, involving minimal exposure (WHO, 1999b; Zaim *et al.*, 2000). Considering the use of non-pyrethroid insecticides, however, human safety issues should be reviewed very carefully (WHO, 1986a, b). In putting forward this evidence for the utility of 'two-in-one' nets treated with carbamate plus pyrethroid, it is not intended to encourage the handling and storage of carbamates or OPs by householders; in any case, it would be unrealistic to expect villagers to treat their bednets with different insecticides on upper and lower parts.

Experience shows that, using only pyrethroids, large-scale ITN projects are hard to launch and difficult to sustain (Lines, 1996; Kachur *et al.*, 1999).

For reasons of safety and targeting, the routine application of acetylcholinesterase-inhibitor insecticides (OPs and carbamates) on ITNs is envisaged only if concentrated formulations are restricted for handling by operators who have been adequately trained and equipped, including provision of protective clothing, suitable containers for insecticides, containment facilities for working with them and ready access to professional therapy and antidotes in case of emergencies. This implies that 'two-in-one' treated nets are manufactured under suitably controlled safe factory conditions before their supply to the community and domestic installation. To preclude any need for re-treatment, 'two-in-one' nets would have to be treated primarily with a wash-resistant long-lasting insecticidal formulation (e.g. PermaNetTM) or incorporation (e.g. Olyset Net[®]) for application of the combination treatment involving two types of insecticide. This process should take care to limit human exposure to hazardous insecticides directly or indirectly. Using mechanical procedures (e.g. robots) in a controlled factory environment, netting could be pre-impregnated, cut and sewn to mass-produce the required 'two-in-one' ITNs. Automated production would be safer, quicker, more economical and better standardized than the customary sweatshop net-making procedures.

Acknowledgements

We thank the Companies Aventis, FMC, SiamDutch and Vestergaard for donating their products used in this study. Expert advice on mosquito behaviour was offered by Dr Bart Knols of ICIPE, Kenya. We are grateful to Dr Morteza Zaim, WHOPEs Manager, for his critical review of the manuscript.

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Accepted 17 January 2001



Volume 15 • Number 1 • March 2001

ISSN 0269-283X

Medical and Veterinary Entomology

Editors: G. B. White (Medical) and R. Wall (Veterinary)



Published for the Royal Entomological Society

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