

# Experimental hut comparisons of nets treated with carbamate or pyrethroid insecticides, washed or unwashed, against pyrethroid-resistant mosquitoes

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**Abstract.** The efficacy against mosquitoes (Diptera: Culicidae) of a bednet treated with carbamate insecticide [carbosulfan capsule suspension (CS) 200 mg/m<sup>2</sup>] was compared with four types of pyrethroid-treated nets in veranda-trap huts at Yaokoffikro near Bouaké, Côte d'Ivoire, where the malaria vector *Anopheles gambiae* Giles carries the *kdr* gene (conferring pyrethroid resistance) at high frequency and *Culex quinquefasciatus* Say is also pyrethroid resistant. Pyrethroids compared were lambdacyhalothrin CS 18 mg/m<sup>2</sup>, alphacypermethrin water dispersible granules (WG) 20 mg/m<sup>2</sup>, deltamethrin 50 mg/m<sup>2</sup> (Permanet™) and permethrin emulsifiable concentrate (EC) 500 mg/m<sup>2</sup>. Insecticidal power and personal protection from mosquito bites were assessed before and after the nets were used for 8 months and hand washed five times in cold soapy water. Before washing, all treatments except permethrin significantly reduced blood-feeding and all had significant insecticidal activity against *An. gambiae*. The carbosulfan net gave significantly higher killing of *An. gambiae* than all pyrethroid treatments except the Permanet. Against *Culex* spp., carbosulfan was more insecticidal and gave a significantly better protective effect than any of the pyrethroid treatments. After washing, treated nets retained various degrees of efficacy against both mosquito genera – but least for the carbosulfan net. Washed nets with three types of pyrethroid treatment (alphacypermethrin, lambdacyhalothrin, permethrin) gave significantly higher mortality rates of *Culex* than in huts with the same pyrethroid-treated nets before washing. After five washes, the Permanet™, which is sold as a long-lasting insecticidal product, performed no better than the other nets in our experimental conditions.

**Key words.** *Anopheles gambiae*, *Culex quinquefasciatus*, alphacypermethrin, bednets, carbosulfan, deltamethrin, insecticide-treated nets, *kdr*, knock-down resistance, lambdacyhalothrin, Permanet™, permethrin, pyrethroid resistance, Bouaké, Côte d'Ivoire.

## Introduction

In parts of tropical Africa, malaria vector anopheline mosquitoes have evolved high frequencies of genes causing resistance to pyrethroid insecticides, which are used for net treatment and house-spraying (Elissa *et al.*, 1993;

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WHO, 1995, 1998; Chandre *et al.*, 1999a; Vulule *et al.*, 1999; Hargreaves *et al.*, 2000). It seems important therefore to investigate alternative insecticides with different modes of action that could be effective on nets and/or for indoor residual spraying (Curtis *et al.*, 1998).

Miller *et al.* (1991) tested nylon bednets treated with the organophosphate pirimiphos-methyl, compared with pyrethroid-treated bednets, in experimental huts in The Gambia. Pirimiphos-methyl performed better than pyrethroids in killing *Anopheles gambiae* but was ineffective in preventing blood-feeding, presumably because of its lack of irritancy. Kolaczinski & Curtis (2001) evaluated in the laboratory and cited earlier work of Williams and Curtis (1996, unpublished observations) on the efficacy of fipronil, a phenyl-pyrazole insecticide, on netting against resistant and susceptible *Anopheles*. Fipronil was very slow to kill mosquitoes and the final mortalities achieved were no better than pyrethroids, even at a very high dose (500 mg/m<sup>2</sup>). In Tanzania, bendiocarb was used on eaves curtains and showed better performance than pyrethroids in killing *Culex* mosquitoes, but the manufacturer considered this carbamate too hazardous for use on bednets (Curtis *et al.*, 1996) and poor results were obtained in Sri Lanka by Weerasooriya *et al.* (1996), who tested curtains treated with bendiocarb or pyrethroids against *Culex quinquefasciatus*, the vector of Bancroftian filariasis. Among other organophosphates tested on nets, chlorpyrifos-methyl 100 mg/m<sup>2</sup> was effective against pyrethroid-resistant anophelines and culicines in veranda-trap huts at Bouaké, Côte d'Ivoire (N'Guessan *et al.*, 2003b), whereas in the laboratory, trichlorfon-treated netting was insecticidal against *An. stephensi* only at a very high dose of 2 g/m<sup>2</sup> and a pyrethroid-resistant strain was less susceptible to temephos or malathion (Talukdar, 1999). The carbamate carbosulfan killed both susceptible and pyrethroid-resistant strains of *An. stephensi* at lower concentrations (Talukdar, 1999) and was more effective than pyrethroids against *An. gambiae* in sprayed huts in Burkina Faso (Darriet, 1998), where carbosulfan-treated curtains prevented house entry of *An. gambiae* (Fanello *et al.*, 2001, 2003). Field trials in Côte d'Ivoire gave encouraging results on efficacy of carbosulfan-treated net against pyrethroid-resistant *An. gambiae* and *Cx. quinquefasciatus* (Kolaczinski *et al.*, 2000; Guillet *et al.*, 2001; N'Guessan *et al.*, 2003a).

These reports are still not enough to establish whether effective alternatives to pyrethroids are available for net treatment and whether they are safe enough for this use. The present study was carried out to investigate in experimental huts: (i) the protective effect of nets treated with carbosulfan or various pyrethroids in preventing blood-feeding by pyrethroid-resistant mosquitoes; (ii) to assess the insecticidal power of the five net treatments in killing members of wild populations of pyrethroid-resistant *An. gambiae* and *Cx. quinquefasciatus*; (iii) to assess the impact of five washings of these nets.

We collected data on the deterrence of treated nets to hut entry by mosquitoes, and the excito-repellency causing mosquitoes to exit the hut after contact with the net. However,

we consider that the most important parameters are numbers of mosquitoes blood-fed on the sleepers under the various nets (i.e. the personal protection that the treated nets give to their users) and the numbers of mosquitoes killed (i.e. the potential of the nets to suppress the vector population in a community if treated nets were widely used). The deterrence and the excito-repellency effects are relevant only to decisions about the merits of different net treatments in so far as they affect numbers of mosquitoes blood-feeding or being killed.

## Materials and methods

### Study site

This field study was carried out in veranda-trap huts (cases-pièges: Darriet *et al.*, 2002) at the Yaokoffikro station of the Institut Pierre Richet in Bouaké, Côte d'Ivoire, West Africa. In this area, *An. gambiae* s.s. has been reported to carry the *kdr* pyrethroid-resistance gene at very high frequency (94%: Chandre *et al.*, 2000) together with a high proportion of carbamate resistance associated with insensitive acetylcholine-esterase in the S form of *An. gambiae* s.s. (N'Guessan *et al.*, 2003a). *Culex quinquefasciatus* is also pyrethroid resistant and also carries an insensitive acetylcholine-esterase allele (Chandre *et al.*, 1997, 1998).

### Experimental hut and study area

Following procedures described by Darriet (1998), Kolaczinski *et al.* (2000), Guillet *et al.* (2001), N'Guessan *et al.* (2001) and Darriet *et al.* (2002), we used the same six veranda-trap huts spaced ~5 m apart in a north-south row between Yaokoffikro village and ricefields 25 m away. To clear any possible insecticide residue that might remain from previous use, the huts were totally refurbished before implementation of this trial, and cleaned the day after each night's work.

### Sleeper-collectors in the huts

Six adult men, contractually employed by the Institut Pierre Richet and very familiar with catching mosquitoes, slept overnight in the huts. They gave their informed consent to participating in the project. They entered the huts at 20.00 hours and slept on mats under the nets. From 20.00 hours the hut door was closed but window-trap curtains remained rolled up to allow easy entrance of mosquitoes. Inside each hut, the curtain separating the room from the veranda was rolled up to facilitate free-flying of mosquitoes within the hut. At 05.00 hours, the window and veranda curtains were rolled down to inhibit mosquito exit from the hut and movement from one compartment to the other. Each morning after the night of net testing, the sleepers collected all mosquitoes resting inside their net,

inside the hut and inside the veranda-trap. They double-checked that all mosquitoes had been caught. Sleeper-collectors were rotated nightly, from hut to hut, to average out interpersonal variations in attractiveness and collecting skill (Darriet *et al.*, 2000). Prophylaxis against malaria was provided during the period of the trial with chloroquine, as recommended by the national ethical committee and the National Malaria Control Programme (NMCP).

Twice per week, sleepers were asked to respond to a questionnaire on possible side-effects of the insecticide-treated bednets, such as coughing, dizziness, headache, sneezing or itching (Jawara *et al.*, 2001).

#### Collection methods and preliminary observations

Mosquitoes were obtained from inside the nets, huts and exit traps by collectors using torches and aspirators from 05.00 hours to 07.00 hours in the morning. Every individual mosquito was placed in a separate tube, which was stoppered with cotton wool and placed in one of three bags corresponding to collection from the net, the hut or the veranda trap. Specimens were taken from the field station to the laboratory for identification to genus and using the anopheline key of Gillies & De Meillon (1968) and stored for further analysis. After counting each species, those other than *An. gambiae* Giles *sensu lato* and *Culex* spp. were discarded, as was done by Kolaczinski *et al.* (2000), although it would be of interest on another occasion to investigate the large number of *Mansonia* spp.

To assess the relative attractiveness of the six huts, preliminary collections were undertaken for six consecutive nights in September 2000 with sleepers not using nets in the huts.

#### Net treatment installation and washing

Bednets were white 100% polyester 100 denier, mesh 156/inch<sup>2</sup>, dimensions 130 × 200 × 180 cm (width/length/height) manufactured by Vestergaard-Frandsen (Kolding, Denmark). Each net was deliberately holed (80 holes each of 4 cm<sup>2</sup> area: 25 on each side, 15 on each end, none on the top) to simulate badly torn nets allowing access for mosquitoes and to put emphasis on the insecticide treatment (rather than the physical barrier of the net) to protect the sleepers. Except the Permanet<sup>TM</sup>, bednets were treated with insecticide as described by Pleass *et al.* (1993). The following net treatments were tested:

- 1 untreated net (control)
- 2 carbosulfan 200 mg a.i./m<sup>2</sup> (Marshall<sup>®</sup> CS, capsule suspension: FMC, Princeton, NJ, U.S.A.)
- 3 alphacypermethrin 20 mg a.i./m<sup>2</sup> (Fastac<sup>®</sup> WG, water dispersible granules: BASF, Ludwigshafen, Germany)
- 4 lambda-cyhalothrin 18 mg a.i./m<sup>2</sup> (Icon<sup>®</sup> CS, capsule suspension: Zeneca, Fernhurst, Surrey, U.K.)
- 5 permethrin 500 mg a.i./m<sup>2</sup> (Peripel<sup>®</sup> EC, emulsifiable concentrate: Aventis, Frankfurt, Germany)

6 Permanet<sup>TM</sup> with deltamethrin 50 mg a.i./m<sup>2</sup> (Vestergaard-Frandsen, Kolding, Denmark).

Because preliminary results (Table 1) showed there was no apparent hut bias for mosquitoes, net treatments were not rotated between huts for comparisons, i.e. each net was used consistently in the same hut and this avoided any possibility of insecticide contamination between huts.

In phase 1 (implemented by Hutchinson and N'Guessan), the five bednet treatments were tested unwashed for 24 nights in the huts during September to October 2000 (Table 2), then hung in the experimental huts and used by sleepers for eight months. For phase 2 (implemented by Asidi and N'Guessan), the treated nets were washed and dried five times (at intervals of 24 h), then re-tested in the huts for 56 nights during May to July 2001. The washing process was undertaken at the Institut Pierre Richet. To simulate washing customarily done by villagers at Yaokoffikro, nets were vigorously hand-washed for 2 min in a 10 L bucket of tapwater using the locally popular palm-oil based soap Maxi mousse<sup>TM</sup> (Palm industries, Abidjan). Nets were then rinsed with 10 L of tapwater and dried in the shade of a veranda.

#### Statistical analysis

Distributions of data were not normal over different nights of collection. Therefore, non-parametric Kruskal–Wallis tests were used for the statistical analysis of both data sets from September 2000 (before washing) and May 2001 (after washing).

## Results

During preliminary observations without nets, before the washed net treatments were installed, 36 hut-nights (6 days × 6 huts) of collections yielded a total of 628 mosquitoes, comprising *An. gambiae* s.l. (46%), *An. funestus* Giles

**Table 1.** Preliminary results of mosquito collections from each veranda-trap hut: summed data from six huts used on six consecutive nights by six individual sleepers without a bednet, each hut being used once by each sleeper

Hut number	<i>Anopheles gambiae</i>		<i>Culex</i> spp.	
	Mean no. caught per night	Mean no. blood-fed per night	Mean no. caught per night	Mean no. blood-fed per night
1	10.2 <sup>a</sup>	5.5 <sup>a</sup>	3.7 <sup>a</sup>	1.7 <sup>a</sup>
2	9.3 <sup>a</sup>	5.7 <sup>a</sup>	6.2 <sup>a</sup>	5.3 <sup>a</sup>
3	8.5 <sup>a</sup>	5.3 <sup>a</sup>	2.8 <sup>a</sup>	1.5 <sup>a</sup>
4	7.3 <sup>a</sup>	5.8 <sup>a</sup>	4.7 <sup>a</sup>	3.8 <sup>a</sup>
5	7.8 <sup>a</sup>	4.7 <sup>a</sup>	3.0 <sup>a</sup>	1.3 <sup>a</sup>
6	4.8 <sup>a</sup>	2.8 <sup>a</sup>	7.8 <sup>a</sup>	4.5 <sup>a</sup>

Numbers with the same superscript in the same column do not differ significantly by Kruskal–Wallis test

**Table 2.** Mosquitoes collected from six huts over 24 nights testing unwashed treated nets

Hut number and net treatment	Mean no. mosquitoes/night					
	<i>Anopheles gambiae</i>			<i>Culex</i> spp.		
	Caught	Blood-fed	Dead	Caught	Blood-fed	Dead
1. Alphacypermethrin 20 mg/m <sup>2</sup>	3.9 <sup>a</sup>	0.33 <sup>a</sup>	1.61 <sup>ac</sup>	3.6 <sup>a</sup>	0.33 <sup>a</sup>	0.87 <sup>a</sup>
2. Carbosulfan 200 mg/m <sup>2</sup>	5.3 <sup>a</sup>	0.87 <sup>a</sup>	3.54 <sup>b</sup>	3.9 <sup>a</sup>	0.04 <sup>b</sup>	3.08 <sup>b</sup>
3. Lambdacyhalothrin 18 mg/m <sup>2</sup>	2.8 <sup>a</sup>	0.21 <sup>a</sup>	1.25 <sup>ac</sup>	2.8 <sup>a</sup>	0.41 <sup>ac</sup>	0.37 <sup>a</sup>
4. Permethrin 500 mg/m <sup>2</sup>	4.3 <sup>a</sup>	0.95 <sup>ab</sup>	1.45 <sup>a</sup>	3.5 <sup>a</sup>	0.50 <sup>a</sup>	0.66 <sup>a</sup>
5. Deltamethrin 50 mg/m <sup>2</sup> (Permanet)	5.3 <sup>a</sup>	0.54 <sup>a</sup>	3.00 <sup>bc</sup>	3.3 <sup>a</sup>	0.62 <sup>ac</sup>	0.37 <sup>ac</sup>
6. Untreated	5.1 <sup>a</sup>	2.04 <sup>b</sup>	0.50 <sup>d</sup>	5.1 <sup>a</sup>	1.70 <sup>c</sup>	0.37 <sup>c</sup>

Numbers sharing the same superscript in each column do not differ significantly.

(0.8%), *An. pharoensis* Theobald (0.2%), *Culex* spp. (26%), *Mansonia* spp. (20%) and aedines (7%). For the two dominant mosquitoes there was no significant difference in mean numbers collected per night between the six huts without nets (Table 1).

Phase 1 (September 2000) with unwashed treated nets yielded 643 *An. gambiae* s.l. and 530 *Culex* spp. from 144 hut-nights. There were no significant differences in numbers caught in the six huts (Table 2). Four of the treated nets, but not that with permethrin, were associated with significantly reduced mean numbers of blood-fed *An. gambiae* compared to the control, i.e. four of the treatments gave a significant protective effect to sleepers compared with the untreated net (Table 2). In comparison with the untreated net, all five treatments had significant insecticidal activity against *An. gambiae*: the mean of 3.5 dead/hut-night with the carbosulfan net was significantly better than all pyrethroid treatments except the Permanet (mean 3.0 dead/hut-night). Also against *Culex*, carbosulfan gave a significantly better protective effect than the pyrethroid treatments and a significantly higher killing effect, with 3.1 dead/hut-night compared with <1 from all four pyrethroid treatments (Table 2). The Permanet did not have significantly more protective or insecticidal effect than the untreated net against *Culex* mosquitoes. Results with the other pyrethroid treatments did not differ significantly from those with the Permanet but were, in nearly all cases, better than with the untreated net (Table 2).

Phase 2 with the washed nets yielded 6940 mosquitoes from 336 hut-nights (6 huts × 7 days × 8 weeks) comprising 18.5% *An. gambiae* s.l., 0.5% *An. pharoensis*, 0.2% *An. funestus*, 0.3% *An. coustani*, 0.01% *An. welcomei*, 54% *Mansonia* spp., 22.8% *Culex* spp., 4% aedines and 0.1% *Coquillettidia cristata* Theobald.

Significantly fewer *An. gambiae* were caught in huts with washed treated nets (totals of 151–196/treatment) compared to a total of 420 with the untreated net (Table 3), by contrast to results with unwashed nets (Table 2) and the same huts without nets (Table 1). All five treated and washed nets retained significant protective power (i.e. the number of bites was significantly less than with the untreated net) and had insecticidal effects against *An. gambiae* s.l., but results with the different treated nets did not differ significantly from each other (Table 3). The mean numbers of *An. gambiae* blood-fed per night in huts with treated nets ranged from 0.35 to 0.76, significantly fewer than with the untreated net where a mean of 3.35/hut-night succeeded in feeding (Table 3). Significantly fewer *Culex* were caught in huts with treated nets than in the control hut with the untreated net (Table 3). Against *Culex* spp. all treated and washed nets retained significant protective effects as well as insecticidal activity compared with the untreated (Table 3).

Table 3 includes data on the percentages of the catches that were in the veranda, as opposed to the net or the hut. For both mosquito genera these percentages were consistently higher with all the treated nets (including the

**Table 3.** Mosquitoes collected from six huts over 56 nights with washed treated nets

Hut number and net treatment	Mean no. mosquitoes/night							
	<i>Anopheles gambiae</i>				<i>Culex</i> spp.			
	Caught	Blood-fed	Dead	% in veranda-trap	Caught	Blood-fed	Dead	% in veranda-trap
1. Alphacypermethrin 20 mg/m <sup>2</sup>	3.5 <sup>a</sup>	0.76 <sup>a</sup>	1.37 <sup>a</sup>	40.7%	4.3 <sup>a</sup>	1.18 <sup>a</sup>	1.19 <sup>ab</sup>	46.2%
2. Carbosulfan 200 mg/m <sup>2</sup>	2.7 <sup>a</sup>	0.61 <sup>a</sup>	1.25 <sup>a</sup>	46.7%	3.8 <sup>a</sup>	0.57 <sup>b</sup>	1.41 <sup>a</sup>	46.0%
3. Lambdacyhalothrin 18 mg/m <sup>2</sup>	3.0 <sup>a</sup>	0.35 <sup>a</sup>	1.41 <sup>a</sup>	51.8%	4.9 <sup>a</sup>	0.52 <sup>b</sup>	1.80 <sup>a</sup>	55.0%
4. Permethrin 500 mg/m <sup>2</sup>	2.7 <sup>a</sup>	0.35 <sup>a</sup>	1.09 <sup>a</sup>	40.4%	3.8 <sup>a</sup>	0.78 <sup>ab</sup>	1.41 <sup>a</sup>	50.7%
5. Deltamethrin 50 mg/m <sup>2</sup> (Permanet)	3.5 <sup>a</sup>	0.59 <sup>a</sup>	1.53 <sup>a</sup>	54.3%	4.4 <sup>a</sup>	0.69 <sup>b</sup>	1.07 <sup>b</sup>	55.5%
6. Untreated	7.5 <sup>b</sup>	3.35 <sup>b</sup>	0.52 <sup>b</sup>	24.3%	8.1 <sup>b</sup>	3.41 <sup>c</sup>	0.32 <sup>c</sup>	26.5%

Numbers sharing the same superscript in each column do not differ significantly.

carbosulfan treatment) than with the untreated net. This reflects the excito-repellent effects of all the treatments.

No side-effects were reported by the sleepers under any of the nets.

## Discussion

Deterrence of insecticide-treated nets (ITNs) to hut entry by mosquitoes has been recorded from some (but not all) other studies with experimental huts (Darriet *et al.*, 1998, 2000), but was not found to a significant extent in the present series with unwashed ITNs (phase 1, Table 2). Where there is deterrence to hut entry, this enhances the reduction of mosquito bites on sleepers under nets. However, deterrence to hut entry has the disadvantage of reducing the proportion of a village population of vectors that are killed on ITNs, and hence the community-wide ('mass') effect that such nets are likely to have if extensively used. Because of the evidence for significant deterrence in phase 2 with washed ITNs (Table 3), it was considered more meaningful to present data as mean numbers/hut-night of blood-fed and of dead mosquitoes, rather than percentage blood-fed and percentage mortality among those found in the huts.

Comparisons of blood-feeding activity by *Culex* spp. before and after washing (Table 4) showed a significant increase with the alphacypermethrin and carbosulfan nets. Thus there was significant loss of protective power against *Culex* after washing of these two treatments; a similar reduction observed with alphacypermethrin against *Anopheles* (Table 4) did not reach statistical significance. The

mean numbers of dead mosquitoes in the hut with the carbosulfan-treated net decreased significantly from 3.54 *An. gambiae*/night before to 1.25 after washing, and from 3.1 *Culex*/night before to 1.4 after washing (Table 5). Thus five washes significantly reduced the insecticidal power of carbosulfan against both genera. Unexpectedly, however, significantly increased numbers of dead *Culex*/night were observed with three of the pyrethroid-treated nets (lambda-cyhalothrin, permethrin and Permanet) after washing. This result may perhaps be explained by a reduction of irritancy occurring as a result of the washing process, which would allow mosquitoes to stand longer on the treated surfaces, and thus pick up a lethal dose. That would resemble the findings of Hodjati & Curtis, 1997, where a lower dose of permethrin (200 mg a.i./m<sup>2</sup>) gave better killing than the higher one (500 mg a.i./m<sup>2</sup>), and less irritancy of the lower dose was directly observed. The role of irritancy in determining length of exposure to pyrethroids, and hence mortality of *kdr* mosquitoes, has been discussed by Darriet *et al.* (2000).

The experimental hut data on these pyrethroid and carbamate resistant mosquito populations showed that, when unwashed, carbosulfan-treated netting was significantly the most effective against *Culex* spp. and better or equal to pyrethroids against *An. gambiae* s.l. After five washes, all five net treatments retained some protective and insecticidal power compared with an untreated net, but carbosulfan showed significantly reduced power against both genera of mosquitoes. Carbosulfan now appears less promising and less attractive in view of partial cross-resistance of pyrethroid-resistant strains to it and its loss during washing

**Table 4.** Mean numbers of blood-fed mosquitoes/hut/night with treated nets before (24 nights) and after (56 nights) washing of the nets

Hut number and net treatment	<i>Anopheles gambiae</i>		<i>Culex</i> spp.	
	Before washing	After washing	Before washing	After washing
1. Alphacypermethrin	0.33	0.76	0.33	1.18***†
2. Carbosulfan	0.87	0.61	0.04	0.57*†
3. Lambda-cyhalothrin	0.21	0.35	0.41	0.52
4. Permethrin	0.95	0.35	0.50	0.78
5. Deltamethrin (Permanet)	0.54	0.59	0.62	0.69
6. Untreated	2.04	3.35	1.70	3.41

Differences after washing not significant except for two increased values: \* $P < 0.05$ , \*\* $P < 0.01$ .

**Table 5.** Mean numbers of dead mosquitoes/hut/night with treated nets before (24 nights) and after (56 nights) washing of the nets

Hut number and net treatment	<i>Anopheles gambiae</i>		<i>Culex</i> spp.	
	Before washing	After washing	Before washing	After washing
1. Alphacypermethrin	1.61	1.37	0.87	1.19
2. Carbosulfan	3.54	1.25*↓	3.08	1.41***↓
3. Lambda-cyhalothrin	1.25	1.41	0.37	1.80***†
4. Permethrin	1.45	1.09	0.66	1.41*†
5. Deltamethrin (Permanet)	3.00	1.53	0.37	1.07*†
6. Untreated	0.50	0.52	0.37	0.32

Differences after washing not significant except for three increases (†) and two decreases (↓): \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

(Asidi & Curtis, 2001). The sleepers in our trial reported no side-effects with carbosulfan (Asidi *et al.*, 2002), by contrast to the report of Guillet *et al.* (2001). However, we have received information given by the manufacturer to W.H.O. that carbofuran, which has much higher mammalian toxicity than carbosulfan, could be a breakdown product of carbosulfan. This safety question needs more investigation and may further reduce the suitability of carbosulfan for large-scale ITN programmes.

The bednet treated with alphacypermethrin WG significantly lost protective power after washing, but still exerted a high insecticidal effect against mosquitoes. Two other formulations of alphacypermethrin, namely 10% suspension concentrate (SC) and 5% wettable powder (WP) have been approved by the World Health Organization Pesticides Evaluation Scheme (WHOPES, 1998) for bednet treatment. The latter was reported to be more cost-effective in comparison to other pyrethroids: a trial in China (Luo *et al.*, 1994) showed good results for bednets treated with alphacypermethrin WP at 20 mg a.i./m<sup>2</sup> and they quoted a price of only US\$ 0.06/net treated, which is much less than with any other pyrethroid so far tested. Maxwell *et al.* (1999) found similar reductions of infective bites and parasitaemia for Tanzanian villagers using bednets treated with alphacypermethrin SC or lambda-cyhalothrin CS. They reported adverse side-effects (nasal irritation) from users of the latter but not the former ITNs, but neither of these treatments led to reports of side-effects in the present study. Bioassay tests with *An. gambiae* s.l. by Jawara *et al.* (1998) gave similar mortality rates from exposure to alphacypermethrin, lambda-cyhalothrin or permethrin-treated nets, whereas Kolaczinski *et al.* (2000) reported complete failure of alphacypermethrin-treated nets in the experimental huts at Yaokoffikro, which disagrees with the present data. More evidence is needed on wash-fastness of alphacypermethrin SC in comparison with other ITN treatments and the suggestion by Chandre *et al.* (1999b) that alphacypermethrin is particularly strongly resisted by the *kdr* gene. In the experimental conditions of this trial the Permanet, which is sold as a long-lasting (i.e. wash-fast) insecticidal product, performed no better than other nets before or after five washes.

Overall, our results show effectiveness of pyrethroid-treated nets, before and after washing five times, against mosquitoes carrying pyrethroid-resistance genes. As with the findings of Curtis *et al.* (1996) and Darriet *et al.* (1998, 2000), these data provide some corrective to excessive pessimism about deleterious effects of a few washes, and some resistance mechanisms, on the practical effectiveness of ITNs.

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