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Behavioural and insecticidal effects of organophosphate-, carbamate- and pyrethroid-treated mosquito nets against African malaria vectors

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Abstract. Three insecticides – the pyrethroid deltamethrin, the carbamate carbosulfan and the organophosphate chlorpyrifos-methyl – were tested on mosquito nets in experimental huts to determine their potential for introduction as malaria control measures. Their behavioural effects and efficacy were examined in *Anopheles gambiae* Giles *s.s.* (Diptera: Culicidae) and *Anopheles funestus* Giles *s.s.* in Muheza, Tanzania, and in *Anopheles arabiensis* Patton and *Culex quinquefasciatus* Say in Moshi, Tanzania. A standardized dosage of 25 mg/m^2 plus high dosages of carbosulfan (50 mg/m^2, 100 mg/m^2 and 200 mg/m^2) and chlorpyrifos-methyl (100 mg/m^2) were used to compare the three types of insecticide. At 25 mg/m^2, the rank order of the insecticides for insecticide-induced mortality in wild *An. gambiae* and *An. funestus* was, respectively, carbosulfan (88%, 86%) > deltamethrin (79%, 78%) > chlorpyrifos-methyl (35%, 53%). The rank order of the insecticides for blood-feeding inhibition (reduction in the number of blood-fed mosquitoes compared with control) in wild *An. gambiae* and *An. funestus* was deltamethrin > chlorpyrifos-methyl > carbosulfan. Carbosulfan was particularly toxic to endophilic anophelines at 200 mg/m^2, killing 100% of *An. gambiae* and 98% of *An. funestus* that entered the huts. It was less effective against the more exophilic *An. arabiensis* (67% mortality) and carbamate-resistant *Cx quinquefasciatus* (36% mortality). Carbosulfan deterred anophelines from entering huts, but did not deter carbamate-resistant *Cx quinquefasciatus*. Deltamethrin reduced the proportion of insects engaged in blood-feeding, probably as a consequence of contact irritancy, whereas carbosulfan seemed to provide personal protection through deterred entry or perhaps a spatial repellent action. Any deployment of carbosulfan as an individual treatment on nets should be carried out on a large scale to reduce the risk of diverting mosquitoes to unprotected individuals. Chlorpyrifos-methyl was inferior to deltamethrin in terms of mortality and blood-feeding inhibition and would be better deployed on a net in combination with a pyrethroid to control insecticide-resistant mosquitoes.

Key words. Carbamate, carbosulfan, chlorpyrifos-methyl, deltamethrin, insecticide resistance, insecticide-treated net, mosquito, organophosphate, pyrethroid, Tanzania.

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Introduction

Pyrethroids are the only class of insecticide recommended by the World Health Organization Pesticide Evaluation Scheme (WHOPES) for use on mosquito nets (Zaim & Guillet, 2002). Pyrethroids are ideal for net treatment because they have low mammalian toxicity, kill host-seeking mosquitoes and provide personal protection for individual users (Hill *et al*., 2006).

The scaling up of malaria control strategies across Africa, including universal coverage of malaria-endemic communities with long-lasting insecticidal nets (LLINs), brings the inherent risk of accelerating the spread of pyrethroid resistance. Little documentation of resistance following the scaling-up of LLIN coverage exists. Recent alarming observations include a four-fold increase in *kdr-w* resistance over 2 years in Niger following the widescale introduction of LLINs (Czeher *et al*., 2008). Similar findings have been reported in East Africa (Stump *et al*., 2004) and on Bioko Island after indoor residual spraying (IRS) campaigns (Sharp *et al*., 2007). Experimental hut trials in Benin have shown that pyrethroid-treated nets fail to inhibit blood-feeding of *kdr-w* and oxidase-resistant *Anopheles gambiae s.s.* Giles M taxon compared with susceptible mosquitoes. Mortality of resistant *An. gambiae* was similarly compromised for both insecticide-treated nets (ITNs) and IRS (N'Guessan *et al*., 2007).

The danger is that the reduced efficacy evident in hut trials may soon translate to the failure of ITNs in households or communities. This would have severe consequences for the strategy to control malaria through the scaling up of LLIN use and hence it is vital that new classes of insecticide are developed. The example in agriculture of the pollen beetle, an oil-seed rape pest, is a stark warning of the potential consequences of using a single class of insecticide with no resistance management: selection pressure increased pyrethroid resistance across most of Europe, resulting in crop devastation (Insecticide Resistance Action Committee, 2008).

The next trend in the development of LLINs leans towards a combination of pyrethroid and non-pyrethroid insecticides or synergists, administered either as a mixture or as mosaic treatment to improve efficacy and slow the spread of resistance (World Health Organization, 2009). The only available alternative insecticides are from the agricultural sector. An effective insecticide-treated net is one that repels, disables and/or kills mosquitoes coming into contact with insecticide on the netting material. There is no consensus as to which of these properties are most important for the efficacy of an LLIN. The prospect of combination nets raises the question of whether it is necessary for an insecticide component to provide both high mosquito mortality and personal protection. A related issue is user acceptability: one of the main incentives for the acceptance of mosquito nets is the protection they provide against nuisance biting mosquitoes (Binka & Adongo, 1997). Safety is paramount, especially for mosquito nets used in situations where humans are in close contact with insecticide-treated fibres. Permethrin is classed as toxic category 3 (low toxicity) and alternative insecticides should have similarly low mammalian toxicity profiles (Environmental Protection Agency, 2007).

Potential candidate alternatives to pyrethroids include chlorpyrifos-methyl (organophosphate [OP]) and carbosulfan (carbamate). Both of these are broad-spectrum insecticides used to control a variety of agricultural pests.

In the present study we compare members of these two insecticide classes plus a pyrethroid to assess performance in terms of mortality, personal protection and insecticide-induced mosquito behaviour, and discuss how their particular characteristics will determine the most appropriate future use.

Materials and methods

Study areas and experimental huts

This study used experimental huts in two parts of Tanzania. The Muheza huts are located at a field site (5°13′ S, 38°39′ E) of the Amani Medical Research Centre in Zeneti village, Muheza district, in northeast Tanzania. The huts in Moshi are at a field site (3°22′ S, 37°19′ E) of Kilimanjaro Christian Medical College in an area irrigated for rice production. The two sites differ in terms of malaria transmission and mosquito species composition. The area around Muheza has high levels of *Plasmodium falciparum* malaria transmitted by both *An. gambiae s.s.* and *Anopheles funestus s.s.* Giles mosquitoes, with entomological inoculation rates (EIRs) of 34–405 infective bites per person per year (Ellman *et al*., 1998). The vector population fluctuates throughout the year, with *An. funestus* contributing to most of the dry season parasite transmission. The Moshi site has low levels of *P. falciparum* transmission (EIR = 0–10) and *Anopheles arabiensis* Patton is the predominant vector (Oesterholt *et al*., 2006). The experimental huts are identical in design at both sites. The design is slightly modified from that described by Smith (1964). The huts are built in a traditional square design, with burnt brick walls plastered with mud on the inside and a wooden ceiling lined with hessian sackcloth. The roof is made of corrugated iron; there is an eave below the roof measuring approximately 2 cm around the hut. There is a window trap on each wall and a screened veranda on each side. The huts are built on concrete plinths and surrounded by a water-filled moat to prevent entry of scavenging ants.

Mosquito net treatments

Deltamethrin (5% SC [suspension concentrate]; Bayer Crop Sciences, Lyons, France), carbosulfan (8% SC; FMC Corp., Philadelphia, PA) and chlorpyrifos-methyl (24% CS [capsule suspension]; Dow Chemical Co., Midland, MI) were evaluated at a dosage of 25 mg/m^2. Carbosulfan was also tested at dosages of 50 mg/m^2, 100 mg/m^2 and 200 mg/m^2 and chlorpyrifos-methyl at 100 mg/m^2. The insecticides were applied to polyester netting following WHO standards. A total of six holes per net measuring 4 × 4 cm each were added to all nets to simulate torn nets. Two dosages of carbosulfan (25 mg/m^2 and 200 mg/m^2) were evaluated in Muheza, and three dosages (50 mg/m^2, 100 mg/m^2 and 200 mg/m^2) were evaluated in Moshi. Both chlorpyrifos-methyl and deltamethrin

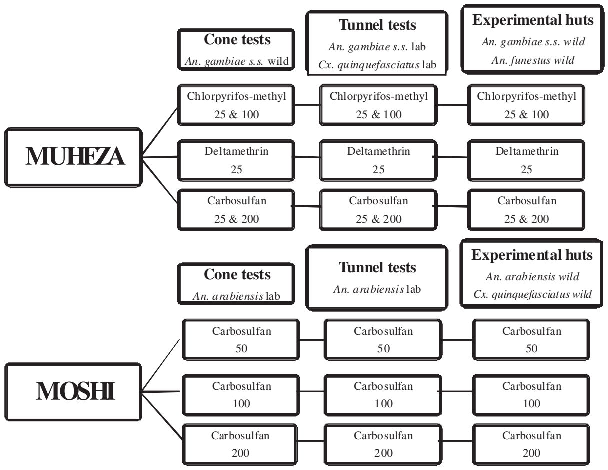

Fig. 1. Array of tests undertaken, species and insecticide dosages (mg/m^2).

were tested at the Muheza site only. The full array of laboratory and field evaluations is summarized in Fig. 1.

Laboratory evaluations

Cone bioassays. Bioassays were carried out on five positions of each of the nets (the top and four sides) at the beginning of the experimental hut evaluations and 3 weeks after the end of the hut evaluations (i.e. allowing ~6–8 weeks between bioassays) to test for the persistence of insecticide efficacy over time. Both wild and laboratory-reared (2–3 days old) mosquitoes were exposed for 3 min in standard WHO plastic cones (World Health Organization, 2006). A total of 100 mosquitoes were used to complete a test for each net. After exposure for 3 min, mosquitoes were transferred to paper cups covered with netting, provided with sugar solution, and scored for mortality 24 h later. Mosquito species used for this purpose were wild pyrethroid-susceptible *An. gambiae* (laboratory reared F1 generation of mosquitoes from the Zeneti region) and laboratory-reared pyrethroid-susceptible *An. arabiensis* (Dondotha strain). To reduce the chance of mosquitoes avoiding contact with netting fibres, plastic cones were attached to a double layer of netting material.

Tunnel tests. The effectiveness of each net treatment was evaluated in a tunnel test according to WHO (2006), with minor modifications. We used double barriers to prevent mosquitoes from contacting the net except after undertaking host orientation flights. The first barrier was a paper screen with a 4-cm diameter hole separating the release chamber from the middle chamber. The second barrier was the test netting, with a total of nine 1-cm diameter holes. Unfed female mosquitoes (two replicates of approximately 100 mosquitoes per test) were released at dusk and left overnight for 12 h in the dark, at $26 \pm 2^{\circ}$C and relative humidity of 70–80%. The following morning the mosquitoes were removed and counted separately for each section of the tunnel and immediate mortality recorded. Live mosquitoes were placed in plastic cups with sugar solution and delayed mortality was recorded after 24 h. All treatments were run simultaneously with a single tunnel containing untreated netting as a negative control. Laboratory-reared mosquitoes tested included pyrethroid-, carbamate- and OP-resistant *Culex quinquefasciatus* Say (Muheza strain), pyrethroid-susceptible *An. gambiae s.s.* (Kisumu strain) and *An. arabiensis* (Dondotha strain).

Resistance tests. WHO resistance tests were conducted using pyrethroid (deltamethrin 0.05%), carbamate (propoxur 0.1%) and OP (malathion 5%) test papers against progeny of field-caught *An. gambiae*, *An. funestus*, *An. arabiensis* and *Cx quinquefasciatus*. Carbosulfan and chlorpyrifos-methyl impregnated papers were not available from WHO and therefore similar carbamate and OP compounds, propoxur and malathion, were used to test for resistance to these insecticide classes. Exposure was for 1 h and mortality was recorded after a 24-h holding period.

Experimental hut evaluations

The evaluations were run for 24 nights in Muheza and 32 nights in Moshi. All treatments and sleepers were rotated using a Latin square design according to a pre-arranged schedule to control for any variation arising from differences in sleepers' attractiveness to mosquitoes. Treatments were rotated every 4 days between huts and one treatment was dropped during each rotation to accommodate the limited number of huts. Open eaves were changed from a north–south to an east–west orientation at each treatment rotation. White sheets were laid over the veranda and room floors to ease the collection of knocked-down mosquitoes. Each morning mosquitoes were collected from the floor, walls, exit traps and inside the nets, and scored for mortality and blood-feeding status. Live mosquitoes were held for 24 h to assess delayed mortality. The huts were washed before each treatment rotation and windows and verandas left open to reduce any risk of insecticide contaminating the next rotation.

Statistical analysis

Significance was determined for cone and tunnel tests using the z-test for proportion at the 95% level.

Experimental hut evaluation primary outcomes were: (a) deterrence (reduction in hut entry relative to entry into control huts fitted with untreated nets); (b) exophily (proportion of mosquitoes found in exit traps relative to control); (c) blood-feeding inhibition (reduction in blood-feeding relative to control), and (d) mortality (proportion of mosquitoes killed).

Differences in outcome variables (mortality, blood-feeding inhibition and induced exophily) between the insecticides relative to control were analysed using logistic regression (STATA 8.0; StataCorp LP, College Station, TX). The comparison of treatments was facilitated using a method described by Asidi *et al*. (2005), whereby treatments were dropped successively from the overall treatments to allow for each treatment to be compared with every other. The number of mosquitoes in the two veranda traps was multiplied by 2 to adjust for unrecorded escapes through the two open verandas, which were left unscreened to allow routes for wild mosquitoes to enter via the gaps under the eaves. The proportions of mosquitoes entering huts were compared using the Wilcoxon rank sum non-parametric test. An estimate for the percentage of personal protection provided by each treatment was derived from the proportions of mosquitoes deterred from hut entry and inhibited from blood-feeding relative to the control using the following formula:

$$\text{personal protection (\%)} = 100(B_u - B_t)/B_u \qquad (1)$$

where B_u = total number of blood-fed mosquitoes in the huts with untreated nets and B_t = total number of blood-fed mosquitoes in the huts with treated nets.

An estimate of the overall killing effect of the treatment as a percentage of the number of mosquitoes entering the untreated control hut was derived from the number dead in treated nets and the number dead in the untreated hut out of the total collection using the following calculation:

$$\text{insecticidal effect (\%)} = 100(K_t - K_u)/(T_u - K_u) \qquad (2)$$

where K_t = number of mosquitoes killed in the huts with treated nets, K_u = number of mosquitoes dead in the huts with untreated nets, and T_u = total collected from the huts with untreated nets.

Ethical clearance

Ethical clearance was obtained from the Medical Research Coordination Committee, the secretariat of which is located at the National Institute for Medical Research, Tanzania, and the London School of Hygiene and Tropical Medicine Ethics Committee. Written informed consent was obtained from all volunteers participating in the study. During the trial all volunteers were monitored each day for signs of fever or possible side-effects of the ITNs.

Results

Cone bioassays

In Moshi, cone bioassays on the carbosulfan-treated nets conducted at the start of the experimental hut evaluations using a laboratory strain of *An. arabiensis* produced 93.5% mortality at 50 mg/m^2 and 100% mortality at 100 mg/m^2 and 200 mg/m^2. Tests performed 3 weeks after the end of the hut evaluation showed significant differences between 50-mg/m^2 (41%), 100-mg/m^2 (75%) and 200-mg/m^2 (90%) dosages ($P <$ 0.007).

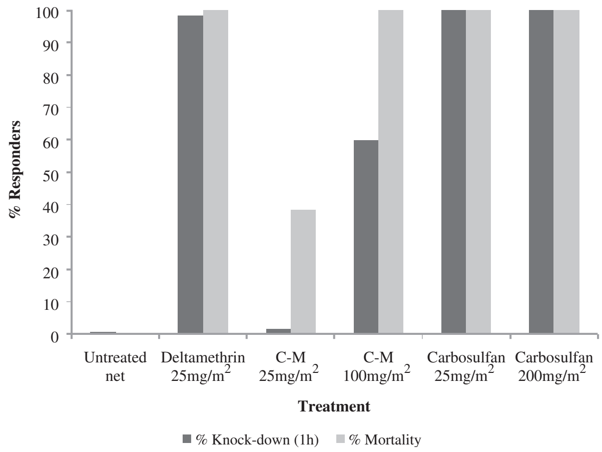

Fig. 2. Cone bioassays using local *Anopheles gambiae* strain exposed for 3 minutes to treated nets.

In Muheza, cone tests conducted before the experimental hut evaluations showed complete knock-down of wild-collected *An. gambiae* after 1 h and 100% mortality after 24 h for deltamethrin and the two dosages of carbosulfan (Fig. 2). Chlorpyrifos-methyl at 25 mg/m^2 produced significantly less knock-down ($P < 0.001$) and mortality ($P < 0.001$) than the 100-mg/m^2 dosage.

Tunnel tests

Tunnel tests were used to assess the percentage of mosquitoes passing through holes in the netting, the percentage killed and the percentage blood-fed. The effects are interrelated because those failing to pass through the holes would have been unable to feed, and those succumbing quickly to insecticide toxicity would have been inhibited from feeding. All types of insecticide inhibited the movement of *Anopheles* through the holed netting. Carbosulfan was most inhibitory, followed by deltamethrin (Fig. 3). Carbosulfan was the only insecticide to inhibit the movement of *Cx quinquefasciatus* (Fig. 4), despite the resistance of the *Culex* strain to carbamates.

Tunnel tests with the lowest dosages of carbosulfan (50 mg/m^2 in *An. arabiensis* and 25 mg/m^2 in *An. gambiae*) produced 100% mortality, whether or not the mosquitoes succeeded in passing though the net. Fewer *Cx quinquefasciatus* were killed; mortality was significantly different ($P <$ 0.001) between dosages of carbosulfan at 25 mg/m^2 (75%) and 200 mg/m^2 (95%) (Fig. 4). Chlorpyrifos-methyl induced much lower mortality, causing 29% and 60% mortality in *An. gambiae* and 37% and 48% mortality in *Cx quinquefasciatus* at dosages of 25 mg/m^2 and 100 mg/m^2, respectively. Deltamethrin (25 mg/m^2) resulted in low mortality in *An. gambiae*, killing significantly less than both dosages of carbosulfan and the 200-mg/m^2 dosage of chlorpyrifos-methyl ($P < 0.001$).

Carbosulfan had the greatest effect on blood-feeding inhibition, with >95% inhibition at dosages of 25 mg/m^2 and 200 mg/m^2 in *An. gambiae.* This effect was probably indirect

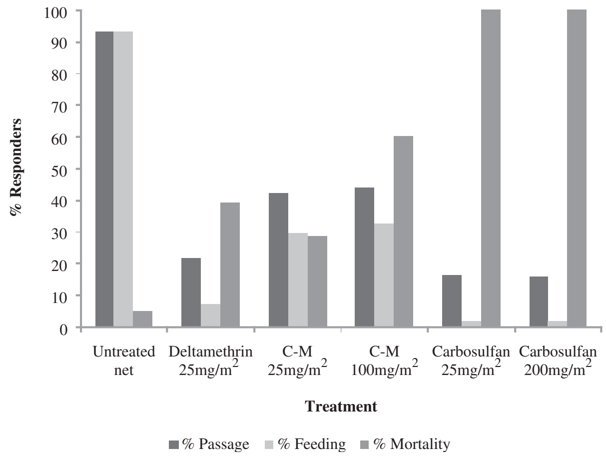

Fig. 3. Tunnel tests with local *Anopheles gambiae* strain on netting treated with 3 types of insecticide.

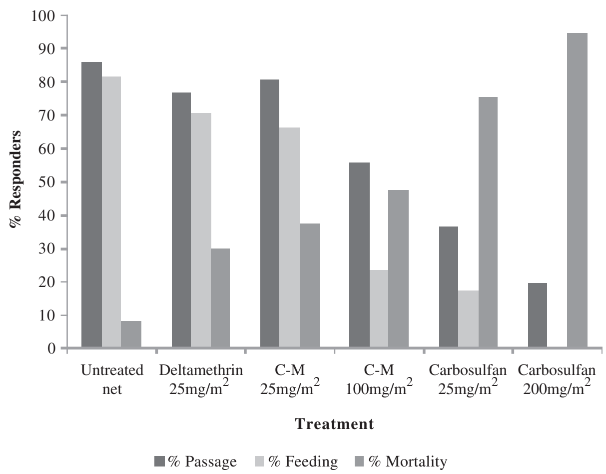

Fig. 4. Tunnel tests with local *Culex quinquefasciatus* strain on netting treated with 3 types of insecticide.

and caused by the low percentage of mosquitoes passing through the net and the high toxicity of carbosulfan. Deltamethrin also caused a large reduction in blood-feeding (92%), similarly to carbosulfan ($P > 0.05$), whereas chlorpyrifos-methyl (25 mg/m^2 and 100 mg/m^2) caused significantly less feeding inhibition (64–68%) than deltamethrin and carbosulfan ($P < 0.001$). In pyrethroid-resistant *Cx quinquefasciatus*, carbosulfan caused 100% feeding inhibition at 200 mg/m^2 and 79% inhibition at 25 mg/m^2, whereas chlorpyrifos-methyl caused only 18% inhibition at 25 mg/m^2 and 71% at 100 mg/m^2, and deltamethrin caused no inhibition. In *An. arabiensis*, all carbosulfan dosages caused an inhibition of blood-feeding of >81%.

Resistance tests

Over 100 mosquitoes were used in each test. *Anopheles gambiae* and *An. funestus* showed 100% susceptibility (100% mortality) on exposure to carbamate, OP and pyrethroid test papers. *Anopheles arabiensis* showed 100% susceptibility to carbamates and *Cx quinquefasciatus* showed 39% mortality on exposure to propoxur test papers and hence were resistant to carbamates.

Experimental hut evaluations

Number of mosquitoes caught. In total, 4524 mosquitoes were recorded from Muheza, of which *An. gambiae*, *An. funestus* and *Cx quinquefasciatus* accounted for 60%, 36% and 4%, respectively. In Moshi 3347 mosquitoes were recorded, of which 81% were *An. arabiensis* and 19% *Cx quinquefasciatus*, respectively. The low numbers of *Cx quinquefasciatus* collected at Muheza meant that data for this species could not be analysed statistically.

Deterrence. The trial design resulted in each treatment being dropped from the rotation at certain points in the trial because there were more treatment options than huts available. In the Moshi trial, deterrence could be estimated reliably, but in the Muheza trial hut entry data showed a high degree of fluctuation over the course of the trial and hence deterrence could not be estimated reliably.

The numbers of *An. arabiensis* captured were significantly lower in huts treated with carbosulfan than in control huts. There was little difference between dosages, with 40–50% deterrence recorded for dosages of 50 mg/m^2, 100 mg/m^2 and 200 mg/m^2 (Table 1). There was no indication that *Cx quinquefasciatus* were deterred from entering huts with carbosulfan-treated nets (Table 2).

Mortality. The mortality of *An. arabiensis* exposed to carbosulfan treatments was clearly dosage-dependent, with statistically significant increases at each increment. The highest mortality was 67% (corrected for control). In Muheza, mortality in *An. gambiae* and *An. funestus* tended to be higher than in *An. arabiensis* and the dosage response less marked (Tables 3 and 4). In Moshi the 50-mg/m^2 dosage produced only 34% mortality in *An. arabiensis*, whereas in Muheza the 25-mg/m^2 dosage caused 88% mortality in *An. gambiae* and 86% mortality in *An. funestus*. The 200-mg/m^2 dosage produced 100% mortality in *An. funestus* and 99% in *An. gambiae*. Lower mortality was observed across all dosages in *Cx quinquefasciatus* in Moshi, with the 200-mg/m^2 dosage causing 36% mortality.

Chlorpyrifos-methyl tested in Muheza caused greater mortality in *An. gambiae* (70%) and *An. funestus* (76%) at 100 mg/m^2 (Fig. 5). This level of mortality was similar to that for deltamethrin, which killed 79% of *An. gambiae* and 78% of *An. funestus*. Both dosages of carbosulfan outperformed deltamethrin and chlorpyrifos-methyl in terms of mortality in *An. gambiae* and *An. funestus*.

All three dosages of carbosulfan produced a small reduction in the overall *An. arabiensis* population through direct

Table 1. Experimental hut results for wild *Anopheles arabiensis*.

	Untreated net*	Carbosulfan 50 mg/m^2*	Carbosulfan 100 mg/m^2*	Carbosulfan 200 mg/m^2*
Total females caught	1035^a	573^b	596^b	506^b
Females caught/night	43	24	25	21
Exophily %	71 ^{a}	74 ^{a}	65 ^{b}	51 ^{c}
	(68–73)	(71–78)	(61–69)	(47–55)
Blood-feeding %	41 ^{a}	32 ^{b}	27 ^{c}	25 ^{c}
	(38–44)	(28–36)	(23–30)	(22–29)
Blood-feeding inhibition %	–	22	36	39
24-h mortality %	19 ^{a}	47 ^{b}	54 ^{c}	73 ^{d}
	(17–22)	(43–51)	(50–58)	(69–77)
Corrected for control	–	34	43	67

*For each treatment, numbers in a row bearing the same superscript do not differ significantly ($P > 0.05$)

Table 2. Experimental hut results for wild *Culex quinquefasciatus*.

	Untreated net*	Carbosulfan 50 mg/m^2*	Carbosulfan 100 mg/m^2*	Carbosulfan 200 mg/m^2*
Total females caught	135^a	190^a	170^a	142^a
Females caught/night	5.6	7.9	7.1	5.9
Exophily %	59 ^{a}	72 ^{a}	87 ^{b}	76 ^{ab}
	(51–67)	(65–78)	(81–91)	(68–82)
Blood-feeding %	41 ^{a}	33 ^{a}	11 ^{b}	23 ^{c}
	(33–49)	(27–40)	(7–17)	(17–31)
Blood-feeding inhibition %	–	18	73	43
24-h mortality %	10 ^{a}	22 ^{b}	16 ^{ab}	43 ^{c}
	(6–17)	(17–29)	(11–22)	(35–51)
Corrected for control	–	13	6	36

*For each treatment, numbers in a row bearing the same superscript do not differ significantly ($P > 0.05$).

Table 3. Experimental hut results for wild *Anopheles gambiae s.s.*

	Untreated net*	Carbosulfan 25 mg/m^2*	Carbosulfan 200 mg/m^2*	Chlorpyrifos methyl 25 mg/m^2*	Chlorpyrifos-methyl 100 mg/m^2*	Deltamethrin 25 mg/m^2*
Total females caught	493	271	244	437	524	740
Females caught/night	31	17	15	27	33	46
Exophily %	88 ^{a}	70 ^{b}	60 ^{c}	82 ^{d}	98 ^{e}	93 ^{f}
	(85–91)	(64–75)	(54–66)	(78–85)	(96–99)	(91–95)
Blood-feeding %	26 ^{a}	28 ^{a}	25 ^{b}	27 ^{ab}	19 ^{c}	14 ^{d}
	(23–30)	(23–34)	(20–31)	(24–33)	(15–22)	(11–16)
BLF inhibition %	–	0	5	0	30	49
24-h mortality						
Corrected for control	–	88	99	35	70	79

*For each treatment, numbers in a row bearing the same superscript do not differ significantly ($P > 0.05$).

Table 4. Experimental hut results for wild *Anopheles funestus*.

	Untreated net*	Carbosulfan 25 mg/m^2*	Carbosulfan 200 mg/m^2*	Chlorpyrifos-methyl 25 mg/m^2*	Chlorpyrifos-methyl 100 mg/m^2*	Deltamethrin 25 mg/m^2*
Total females caught	270	141	240	309	361	327
Females caught/night	17	9	15	19	23	20
Exophily %	81 ^{a}	73 ^{a}	45 ^{b}	81 ^{a}	95 ^{c}	94 ^{c}
	(76–85)	(65–80)	(39–52)	(77–85)	(92–97)	(91–96)
Blood-feeding %	45 ^{a}	28 ^{b}	32 ^{b}	25 ^{bc}	19 ^{c}	10 ^{d}
	(39–51)	(21–36)	(27–38)	(21–30)	(16–24)	(7–13)
BLF inhibition %	–	39	29	44	57	79
24-h mortality						
Corrected for control	–	86	100	53	76	78

*For each treatment, numbers in a row bearing the same superscript do not differ significantly ($P > 0.05$).

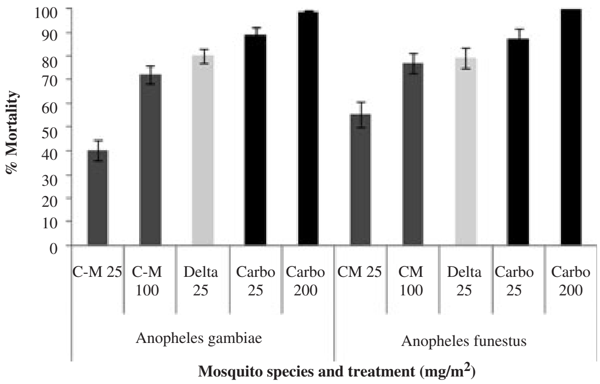

Fig. 5. Experimental hut study of mortality of *An. gambiae* and *An. funestus* when exposed to chlorpyrifos-methyl (C-M), carbosulfan (Carbo) or deltamethrin.

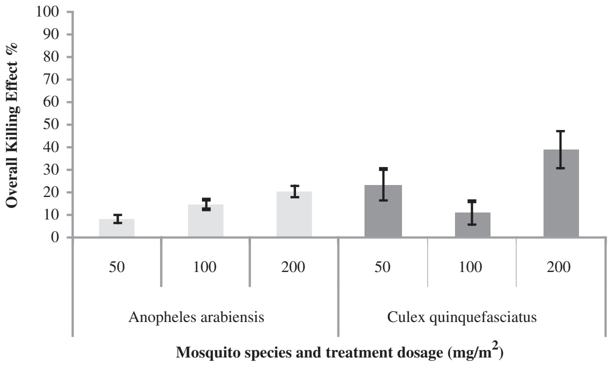

Fig. 6. Overall killing effect of 3 dosages of carbosulfan in experimental huts against *Anopheles arabiensis* and *Culex quinquefasciatus*.

mortality. The 200-mg/m^2 dosage, which caused 67% mortality in all mosquitoes entering the hut, produced only a 20% overall killing effect relative to the untreated control (Fig. 6). This reflected an apparent spatial repellent action of carbosulfan which reduced entry into the huts. The 50-mg/m^2 and 100-mg/m^2 dosages produced killing effects of 7.9% and 14.5%, respectively. In *Cx quinquefasciatus*, the overall killing effect was also low, ranging from 11% to 39%.

Blood-feeding inhibition. Of all the insecticides tested, deltamethrin produced the greatest blood-feeding inhibition in both *An. gambiae* (49%) and *An. funestus* (79%). In Muheza the carbosulfan dosages induced no significant inhibition of blood-feeding in *An. gambiae*, but 29–39% inhibition in *An. funestus*. Carbosulfan produced dosage-dependent feeding inhibition in *An. arabiensis* up to a maximum of 39%. *Culex quinquefasciatus* was most inhibited from blood-feeding at the 100-mg/m^2 dosage (72%). The 200-mg/m^2 dosage inhibited blood-feeding by only 43%, whereas the 50-mg/m^2 dosage gave only 18% inhibition. Chlorpyrifos-methyl caused a 44–57% inhibition of feeding in *An. funestus*, but only 30% inhibition in *An. gambiae* at 200 mg/m^2 and 0% at 25 mg/m^2.

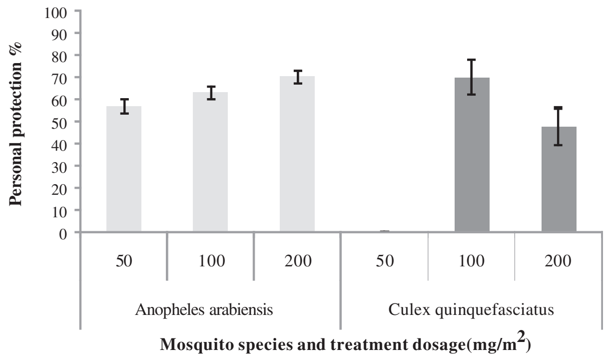

Fig. 7. Personal protection of three dosages of carbosulfan in experimental huts against *Anopheles arabiensis* and *Culex quinquefasciatus*.

All dosages of carbosulfan produced high levels of personal protection against *An. arabiensis*, ranging from 56% to 70% with increasing dosage (Fig. 7). In *Cx quinquefasciatus* the medium and high dosages provided 70% and 48% protection, respectively, and the low dosage showed no protection.

Insecticide-induced exiting into veranda traps. Both *An. gambiae* and *An. funestus* showed similar responses to each of the insecticides in terms of exiting behaviour. The pattern of exiting differed between the anophelines and *Cx quinquefasciatus*. The anophelines were more naturally exophilic and left in greater numbers within 12 h of entering. In the anophelines (including *An. arabiensis*) the toxicity of carbosulfan masked any tendency for this insecticide to stimulate mosquitoes to exit the hut early. Hence, there was an inverse relationship between carbosulfan dosage and the proportion exiting into the veranda traps and the largest reduction was seen in response to the highest dosage in *An. funestus*, in which a 44% decrease in exiting was recorded. A different trend was observed in *Cx quinquefasciatus*. All dosages of carbosulfan induced greater exiting into veranda traps relative to the control, presumably because fewer *Culex* succumbed to toxicity given their resistance to carbamates. The 100-mg/m^2 dosage produced the greatest exiting response (and lowest insecticide-induced mortality).

The 25-mg/m^2 dose of chlorpyrifos-methyl produced no significant change in the percentage of *An. funestus* exiting, but a small but significant increase in exit of *An. gambiae.* The 100-mg/m^2 dosage increased the exiting of both *An. gambiae* and *An. funestus* (98 and 95%, respectively).

Of all the insecticides tested at 25 mg/m^2, the deltamethrin treatment induced the highest exiting rate.

Discussion

Carbosulfan showed remarkably high efficacy in *An. gambiae* and *An. funestus* even at the low dosage of 25 mg/m^2. High mortality for 200-mg/m^2 carbosulfan in *An. gambiae* conforms with other reports on ITNs; Kolaczinski *et al*. (2000) recorded

92% mortality and Guillet *et al*. (2001) recorded 90% mortality for a 300-mg/m^2 treatment on nets. There has been no previous documentation of *An. arabiensis* and *An. funestus* response to carbosulfan. Both *An. funestus* and *An. gambiae* are highly endophilic and mortality rates for both these species were equally high. In Moshi, carbosulfan produced much lower mortality at equivalent dosages in *An. arabiensis*. Similarly, deltamethrin at 25 mg/m^2 produced 80% mortality in both *An. gambiae* and *An. funestus*, but data from earlier trials in Moshi for *An. arabiensis* showed mortality rates of 46% (Mosha *et al*., 2008a) and 50% (Mosha *et al*., 2008b). This is a far lower mortality than in the present study, although the same insecticide and dosage were applied, and it points to behavioural differences between the species. *Anopheles arabiensis* is generally regarded as a more exophilic species than *An. gambiae* and *An. funestus*, but in this instance there were no significant differences in the proportions exiting into veranda traps by morning. Another possibility may be that the amount of time spent actively searching for a bloodmeal differs between species, and thus they differ in the amount of contact they have with treated nets and pick up different doses. *Anopheles arabiensis* in Moshi shows both zoophilic and exophilic characteristics (Mahande *et al*., 2007) and this may mean that mosquitoes initially attracted to human hosts are more likely to abandon searching when confronted with a barrier in the form of a net, and hence pick up a sub-lethal dose of insecticide. At 36% (200 mg/m^2), *Cx quinquefasciatus* mortality in Moshi was lower than seen elsewhere. Kolaczinski *et al*. (2000) and Guillet *et al*. (2001) both observed high mortality in *Cx quinquefasciatus* (96% and 99%, respectively), as did Hougard *et al*. (2003) (90%) and Asidi *et al*. (2004) (79%).

Anopheles arabiensis still showed encouragingly high mortality at the 200-mg/m^2 dosage of carbosulfan compared with deltamethrin (Mosha *et al*., 2008a, 2008b). The inherently high killing effect of carbosulfan on mosquitoes entering huts may be largely negated by the sizeable proportion of *An. arabiensis* that were deterred from entering. Thus, the communitywide benefit is likely to be low in terms of overall population reduction. Personal protection is high at the 200-mg/m^2 dosage and >50% at the two lower dosages. Blood-feeding is significantly reduced by all dosages, but deterrence of mosquitoes from entering the hut is the largest factor in protecting the sleeper. Testing 200-mg/m^2 carbosulfan-treated nets, Asidi *et al*. (2004) found significant deterrence when using washed nets. Using a dosage of 300 mg/m^2, Hougard *et al*. (2003) recorded deterrence rates of >69% in *An. gambiae* at two different sites within Ivory Coast. The deterrence effect may result in diversion to and increased feeding on unprotected individuals or mosquitoes may feed on alternative hosts such as cattle, particularly if carbosulfan-treated nets are used by a large proportion of households.

Some blood-feeding inhibition was evident with carbosulfan in *An. funestus*, *An. arabiensis* and *Cx quinquefasciatus*, but the rate was considerably less than that produced by the contact-irritant deltamethrin. The deterrent effect of carbosulfan may reflect an airborne effect. As the dosage of carbosulfan increases, the percentage of mosquitoes exiting was significantly reduced in all species owing to a dosage-dependent increase in mortality by morning.

The insecticidal effect of chlorpyrifos-methyl was lower than those of deltamethrin and carbosulfan, but at 100 mg/m^2 it still produced high mortality in *An. gambiae* and *An. funestus*. Blood-feeding inhibition caused by chlorpyrifos-methyl was evident in *An. funestus*, but less so in *An. gambiae*. Deltamethrin produced the highest blood-feeding inhibition of all insecticides tested. An interesting species-specific difference is the greater blood-feeding inhibition shown in *An. funestus* over *An. gambiae* across all types of insecticide and dosages tested.

Despite producing high mortality, the relatively low levels of personal protection given to users would make chlorpyrifos-methyl unsuitable as a mono treatment unless community coverage was sufficient to reduce the size of the vector population. Chlorpyrifos-methyl would probably be better used within a combination treatment with a pyrethroid, which provides better personal protection for the individual user.

Comparison of carbosulfan, chlorpyrifos-methyl and deltamethrin show that all have useful qualities for mosquito control. The pyrethroids are considered to represent a reference standard for mosquito net use, but here we show that carbosulfan produces significantly higher mortality in the three main African malaria vectors. Deltamethrin performs better than both carbosulfan and chlorpyrifos-methyl in terms of blood-feeding inhibition, confirming the ascendancy of the pyrethroids for individual user protection.

High levels of deterrence produced by carbosulfan suggest that its use as a mono treatment will be successful only if high coverage levels are achieved. Carbosulfan in combination with another insecticide class is likely to be more beneficial in terms of overall efficacy and delaying resistance. Despite showing good potential for mosquito control, carbosulfan degrades to a more toxic metabolite (carbofuran) and is unlikely to be suitable for longterm use on nets at dosages as high as 200 mg/m^2 (Hougard *et al*., 2003). Carbosulfan nets treated with 25 mg/m^2 show little or no reduction in performance, particularly for endophilic species, compared with the high dosage tested and are less likely to pose a health risk. An alternative approach, which would reduce human contact with the insecticide, is to treat the top of a two-in-one combination net with carbosulfan and to use a less toxic insecticide to treat the sides (Guillet *et al*., 2001). Similarly, application to curtains would limit user contact (Fanello *et al*., 2003).

Chlorpyrifos-methyl is much more suitable in terms of toxicology (1630–2140 mg/kg acute oral toxicity to rats) and its insecticidal effect at 100 mg/m^2 in *An. funestus* and *An. gambiae* is encouraging. Both chlorpyrifos-methyl and carbosulfan provide limited protection against host-searching anopheline mosquitoes and would be beneficial if used in combination with a pyrethroid.

Pyrethroids are ideal for use as mono treatment as they provide high killing and protection levels. Here we show that other insecticides can provide similar (chlorpyrifos-methyl) or better (carbosulfan) killing rates. Given current progress towards combination products, it is not essential for an individual insecticide to have both properties so long as each component has one or the other property. Three assumptions must be met for optimal use of insecticide combinations for resistance management (Tabashnik, 1990): (a) the insect should not be resistant to both components; (b) the combination must maintain its integrity over time and the components should show similar decay rates, and (c) the modes of resistance must be unique. Resistance to all three insecticides evaluated in this paper have been documented in Africa. Combinations of two such insecticides are likely to slow the spread of resistance to both components, particularly as the area of overlap in resistance mechanisms is limited and large areas in Africa are susceptible to both. Until insecticides can be developed with novel mechanisms of action showing no cross-resistance with existing insecticides, the option of combining OPs, carbamates and pyrethroids on mosquito nets is an avenue that should be investigated with urgency.

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