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Comparative efficacies of permethrin-, deltamethrin- and α -cypermethrin-treated nets, against *Anopheles arabiensis* and *Culex quinquefasciatus* in northern Tanzania

F. W. MOSHA^{*}, I. N. LYIMO^{*}, R. M. OXBOROUGH[†], J. MATOWO^{*}, R. MALIMA[‡], E. FESTON^{*}, R. MNDEME^{*}, F. TENU[‡], M. KULKARNI[§], C. A. MAXWELL[†], S. M. MAGESA[‡] and M. W. ROWLAND[†]

^{*}Kilimanjaro Christian Medical Centre, P.O. Box 3010, Moshi, Tanzania

[†]London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, U.K

[‡]National Institute of Medical Research, Amani Research Centre, P.O. Box 81, Muheza, Tanzania

[§]HealthBridge, 1105-1 Nicolas Street, Ottawa, Ontario, K1N 7B7, Canada

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Mosquito nets treated with permethrin, deltamethrin or α -cypermethrin at 25 mg/m² were evaluated in experimental huts in an area of rice irrigation near Moshi, in northern Tanzania. The nets were deliberately holed to resemble worn nets.

The nets treated with permethrin offered the highest personal protection against *Anopheles arabiensis* (61.6% reduction in fed mosquitoes) and *Culex quinquefasciatus* (25.0%). Deltamethrin and α -cypermethrin provided lower personal protection against *An. arabiensis* (46.4% and 45.6%, respectively) and no such protection against *Cx. quinquefasciatus*. Permethrin performed poorly in terms of mosquito mortality, however, killing only 15.2% of the *An. arabiensis* and 9.2% of the *Cx. quinquefasciatus* exposed to the nets treated with this pyrethroid (after correcting for control mortality). The α -cypermethrin and deltamethrin performed marginally better, with respective mortalities of 32.8% and 33.0% for *An. arabiensis* and 19.4% and 18.9% for *Cx. quinquefasciatus*. The poor killing effect of permethrin was confirmed in a second trial where a commercial, long-lasting insecticidal net based on this pyrethroid (Olyset[®]) produced low mortalities in both *An. arabiensis* (11.8%) and *Cx. quinquefasciatus* (3.6%). *Anopheles arabiensis* survivors collected from the verandahs of the experimental huts and tested on 0.75%-permethrin and 0.05%-deltamethrin papers, in World Health Organization susceptibility kits, showed mortalities of 96% and 100%, respectively.

The continued use of permethrin-treated nets is recommended for personal protection against *An. arabiensis*. In control programmes that aim to interrupt transmission of pathogens by mosquitoes and/or manage pyrethroid resistance in such vectors, a combination of a pyrethroid and another insecticide with greater killing effect should be considered.

Pyrethroid-treated nets are an effective tool for protection against mosquitoes, including the vectors of malarial parasites (Magesa *et al.*, 1991; Curtis *et al.*, 1996; Lengeler, 2004). Of the six pyrethroid insecticides currently recommended for mosquito-net

impregnation (WHO, 2005), permethrin and the α -cyano pyrethroids such as deltamethrin and α -cypermethrin are the most widely used in East African countries (Maxwell *et al.*, 1999; Miller *et al.*, 1999; Tami *et al.*, 2004). In Tanzania, the performances of nets treated with various pyrethroids have been investigated and compared, with *Anopheles gambiae* s.s., *An.*

Reprint requests to: M. W. Rowland.

E-mail: mark.rowland@lshtm.ac.uk; fax: +44 (0)207 299 4720.

funestus and *Culex quinquefasciatus* used as the test mosquitoes (Curtis *et al.*, 1992; Jawara *et al.*, 1998). In these trials, more *An. gambiae* s.s. were killed by α -cypermethrin than by permethrin or λ -cyhalothrin (Jawara *et al.*, 1998). There has been no similar evaluation of the performance of nets treated with commonly used insecticides against *An. arabiensis*. This species, which is a member of the *Anopheles gambiae* complex, is predominant in most upland and arid hinterland areas of eastern and southern Africa (White, 1974; Coetzee *et al.*, 2000; Ijumba *et al.*, 2002). The specific feeding and resting behavioural patterns that *An. arabiensis* exhibits may greatly influence its reaction to pyrethroid insecticides. The main objective of the present study was to compare the toxic and behavioural effects of permethrin, deltamethrin and α -cypermethrin, when each was applied to nets at the same concentration and tested against *An. arabiensis*, a vector known for its partial zoophilic and exophilic behaviour. Evaluation of these three pyrethroids was also carried out against *Cx. quinquefasciatus* — a species that is an important vector of filariae and a major biting nuisance in East Africa, especially in urban areas (Lines *et al.*, 1991; Magesa *et al.*, 1991; Ijumba *et al.*, 2002).

MATERIALS AND METHODS

Study Design

In trial 1, rectangular, 100-denier, polyester bednets were treated according to standard procedures (Chavasse *et al.*, 1999; Miller *et al.*, 1999) with permethrin (AmbushTM; Syngenta, Greensboro, NC), deltamethrin (K-Othrine[®]; Bayer Environmental Science, Lyon, France) or α -cypermethrin (Fendona[®]; BASF Agricultural Products, Limburgerhof, Germany), each applied at 25 mg/m². After being tested against laboratory-reared *An. arabiensis* of the Dondotha strain, in contact bio-assays (see below), these nets and untreated control nets were evaluated in

tunnel tests and experimental huts in Moshi, in northern Tanzania, between the June and August of 2005 (see below). Susceptibility tests on the Dondotha strain and on '24-h survivors' of *An. arabiensis*, collected from the verandah traps of huts in which untreated nets and permethrin-treated nets had been tested, were carried out using test papers treated with permethrin (0.75%) or deltamethrin (0.05%), according to the guidelines of the World Health Organization (WHO, 1992, 1998).

Trial 2 involved a commercial, 'long-lasting' permethrin-treated bednet (Olyset[®]; Sumitomo Chemical Company, Tokyo). This net (which is produced with 2% permethrin incorporated into the polyethylene fibre, corresponding to about 1000 mg active ingredient/m²) was compared with an untreated bed sheet, in experimental hut tests that ran between the September and October of 2005, and with a net treated, for the present study, with 500 mg permethrin/m², in contact bio-assays and tunnel tests.

Contact Bio-assays

The contact bio-assays were carried out according to the guidelines of the World Health Organization (WHO, 1998). In brief, the laboratory-reared *An. arabiensis* were exposed, in batches of 10, to treated or untreated netting in World Health Organization test cones for 3 min, and then left in the cones for 24 h before the dead insects were counted.

Tunnel Tests

Tunnel tests were carried out in an apparatus designed to simulate experimental-hut conditions (WHO, 2006). The tunnel used was a glass cuboid measuring 60 cm long, 25 cm high and 25 cm wide, with three chambers ('release', 'middle' and 'baited'). Nine, evenly-spaced, 1-cm-diameter holes were made in each netting sample to be tested before the sample was fixed on a cardboard frame and placed at the

separation between the baited chamber, which held a guinea pig, and the middle chamber. The tunnel was maintained at 26°C and 80% relative humidity. Two replicate tests for each type of treatment were undertaken, each test involving 50–100, non-blood-fed, 5- to 8-day-old, insectary-reared, female *An. arabiensis* (Dondotha strain). The mosquitoes were introduced into the releasing chamber of the tunnel at 18.00 hours. At 08.00 hours on the next day, the blood-fed, dead and living mosquitoes in each chamber were counted so that the percentages entering the bait chamber, blood feeding and dying could be calculated.

Experimental Hut Trials

At Mabogini village, within the Lower Moshi rice irrigation scheme in the Kilimanjaro region of Tanzania, four verandah-trap huts were constructed according to the basic design first described by Smith (1965), with the substitution of concrete for the wooden floors. A 10-cm wide moat filled with water surrounded each hut, to prevent scavenging ants from entering. The working principle of these huts has been described by Smith and Webley, (1969) and Curtis *et al.* (1992). *Anopheles arabiensis* and *Cx. quinquefasciatus* are the predominant mosquito species in the irrigation scheme (Ijumba *et al.*, 2002) and the results of PCR-based tests have shown that *An. arabiensis* is the only member of the *An. gambiae* complex present in this area (Ijumba *et al.*, 2002; Kulkarni *et al.*, 2006a). At the start of the present study, 224 *An. gambiae* s.l. were collected and all 224 were found to be *An. arabiensis* by cytotaxonomy (unpubl. obs.).

For both trial 1 and trial 2, the treatments plus control were rotated in each of the four huts twice, according to a Latin-square design. Before the trials, six, 4-cm-wide, round holes were cut in each net, from the sides and ends, to simulate a worn or torn net. Two volunteers from Mabogini village slept in each hut overnight (between 19.30

and 05.30 hours). These volunteers were rotated between huts on successive nights, in order to reduce the effect of variation in their individual attraction to mosquitoes. Likewise, the direction of the two open verandahs was routinely changed with the treatment rotation, in order to minimise the potentially confounding factor of preferential escape routes.

Mosquitoes were collected in the morning (07.00 hours), from inside the net, the window (exit) traps, the ceiling, walls and floor of the verandah, and inside the hut. The collected mosquitoes from each location were kept separately in paper cups and taken to the field laboratory for species identification and assessment of mortality and blood feeding. All the live mosquitoes were held in a field insectary for 24 h (in paper cups, with access to 10% glucose solution) before the numbers then alive and dead were recorded. The mosquitoes still alive were known as the '24-h survivors'.

Data Analysis

The data were double-entered and analysed to show the effect of each net treatment in terms of deterrence (the number of mosquitoes caught in a treatment hut, as a percentage of the number in the control hut), exophily (the number of mosquitoes caught in the verandah and exit traps, as a percentage of the total number caught), inhibition of blood feeding (the number of unfed mosquitoes in a treated hut, as a percentage of the number of unfed mosquitoes caught in the control hut), and overall mortality (the number of mosquitoes found dead, either at dawn after the exposure or 24 h later). The between-treatment variation in each of these outcome variables was explored by logistic regression, with version 8.0 of the Stata software package (StataCorp, College Station, TX) used for the analyses. A *P*-value of <0.05% was considered indicative of a statistically significant difference.

In order to compare the overall, individual and community protective effect of the

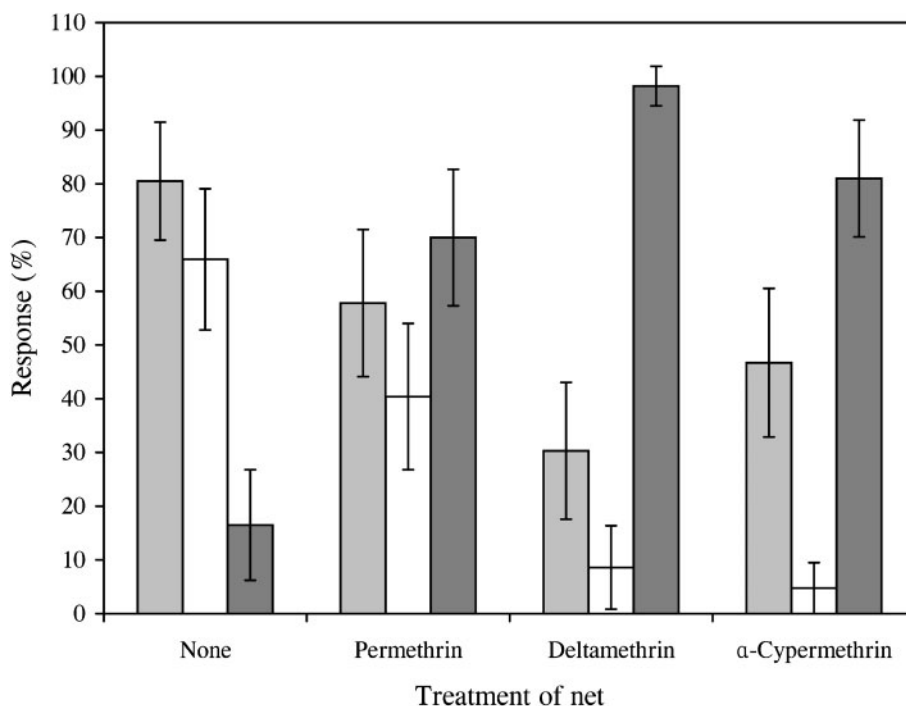


FIG. The results of tunnel tests with the Dondotha strain of *Anopheles arabiensis*, showing the mean values for percentage penetration (■), blood feeding (□) and mortality (■). The vertical lines indicate 95% confidence intervals.

treated nets, levels of personal protection and the overall killing effect were evaluated. Personal protection (%) was calculated as $100 \times (N_C - N_T) / N_C$, where N_C is the number of fed mosquitoes in the control hut and N_T the number of fed mosquitoes in the hut with the treated net. The overall killing effect was evaluated as $100 \times (D_T - D_C) / T_C$, where D_T is the number of dead mosquitoes in the hut with the treated net, D_C the number of dead mosquitoes in the control hut, and T_C the total number of mosquitoes collected from the control hut.

RESULTS

Trial 1

WHO SUSCEPTIBILITY TESTS

Of the wild *An. arabiensis* tested, 96.0% ($N=100$) died when exposed to 0.75%-permethrin papers and 100% ($N=100$) when exposed to 0.05%-deltamethrin

papers. The Dondotha strain of *An. arabiensis* was 100% susceptible to both insecticides (100% mortality; $N=100$ for each).

CONTACT BIO-ASSAYS

In the contact bio-assays, the Dondotha strain of *An. arabiensis* showed 0% mortality ($N=30$) when exposed to the untreated netting but 56.6% ($N=69$), 98.2% ($N=57$) and 92.7% ($N=67$) mortalities when exposed to netting treated, at 25 mg/m², with permethrin, deltamethrin and α -cypermethrin, respectively.

TUNNEL TESTS

The deltamethrin- and α -cypermethrin-treated nets produced lower penetration and blood feeding but higher mortality than nets treated with permethrin (see Figure). The greatest reduction in blood feeding was achieved by α -cypermethrin (4.8%) and the highest mortality with deltamethrin (98.2%).

EXPERIMENTAL HUTS

Of the 1848 mosquitoes collected in the experimental huts over 24 nights, 87.1% were *An. arabiensis* and the rest (12.9%) *Cx. quinquefasciatus* (see Table 1). The mean number caught per night was 77 (67 *An. arabiensis* and 10 *Cx. quinquefasciatus*).

DETERRENCE. Permethrin, deltamethrin and α -cypermethrin achieved deterrences ranging from 35.3% to 25.0% for *An. arabiensis* and 5.7% to 0% for *Cx. quinquefasciatus*. For each species, there was, however, no statistical between-insecticide difference in deterrence.

EXOPHILY. The exophily of *An. arabiensis* showed little variation, ranging from 82.5%

in the control huts to 88.4% in the huts with permethrin-treated nets ($P>0.05$). A similar trend was observed for *Cx. quinquefasciatus*, where exophily (measured in all huts, including the control) only varied between 84.0% and 87.5% ($P>0.05$).

BLOOD FEEDING. The lowest levels of blood feeding were recorded with the permethrin-treated nets: 14.2% for *An. arabiensis* and 6.0% for *Cx. quinquefasciatus*.

MORTALITY. Deltamethrin and α -cypermethrin induced mortalities of around 50% against *An. arabiensis*. These values were statistically different from the control mortality of 25.5% ($P<0.05$ for each). The same trend was observed with *Cx. quinquefasciatus*,

TABLE 1. Comparison of the performance of bednets treated with deltamethrin, α -cypermethrin or permethrin, each at 25 mg/m², against wild *Anopheles arabiensis* and *Culex quinquefasciatus* in experimental huts (Trial 1)

Mosquito species and variable	Value and (95% confidence interval) for:*			
	Untreated nets	Deltamethrin	α Cypermethrin	Permethrin
<i>Anopheles arabiensis</i>				
No. of females caught	521	361	391	337
No. of females caught/night	22 ^a	15 ^a	16 ^a	14 ^a
Deterrence (%)	—	30.7	25.0	35.3
Exophily (%)	82.5 (79.0–85.6) ^a	85.0 (81.0–88.4) ^a	84.4 (80.5–87.7) ^a	88.4 (84.6–91.4) ^a
Blood feeding (%)	24.0 (20.5–27.8) ^a	18.6 (14.9–22.9) ^{ab}	17.4 (13.9–21.5) ^b	14.2 (10.9–18.4) ^b
Inhibition of blood feeding (%)	—	22.5	27.5	40.6
Personal protection (%)	—	46.4	45.6	61.6
Crude mortality (%)	25.5 (22.0–29.4) ^a	50.1 (45.0–55.3) ^b	49.9 (44.9–54.8) ^b	36.8 (31.8–42.1) ^d
Mortality corrected for control value (%)	—	33	32.8	15.2
Overall killing effect (%)	—	9.2	24.6	0
<i>Culex quinquefasciatus</i>				
No. of females caught	53	64	71	50
No. of females caught/night	2 ^a	3 ^a	3 ^a	2 ^a
Deterrence (%)	—	0	0	5.7
Exophily (%)	84.9 (72.6–92.3) ^a	87.5 (76.9–93.6) ^a	85.9 (75.8–92.3) ^a	84.0 (71.1–91.8) ^a
Blood feeding (%)	7.5 (2.9–18.4) ^a	12.5 (6.4–23.1) ^a	14.1 (7.7–24.2) ^a	6.0 (1.9–17.0) ^a
Inhibition of blood feeding (%)	—	0	0	20
Personal protection (%)	—	0	0	25
Crude mortality (%)	7.5 (2.9–18.4) ^a	25.0 (15.9–37.0) ^b	25.4 (16.6–36.7) ^b	16.0 (8.2–28.9) ^{ab}
Mortality corrected for control value (%)	—	18.9	19.4	9.2
Overall killing effect (%)	—	22.6	26.4	7.5

*Within each row, two values with a different supercript letter are significantly different ($P<0.05$).

where deltamethrin and α -cypermethrin caused mortalities, of around 25%, which were significantly higher than the control mortality of 7.5% ($P < 0.05$ for each). With both species, permethrin caused the least mortality of the pyrethroids investigated.

PERSONAL PROTECTION AND OVERALL KILLING EFFECT. Permethrin-treated nets offered the highest personal protection against both *An. arabiensis* (61.6%) and *Cx. quinquefasciatus* (25%). The other insecticides offered personal protections of around 46% against *An. arabiensis* but no such protection against *Cx. quinquefasciatus*.

The highest overall killing effects were achieved by α -cypermethrin against both *An. arabiensis* (24.6%) and *Cx. quinquefasciatus* (26.4%). The corresponding values were lower for deltamethrin (9.2% and 22.6%, respectively) and even lower for permethrin (0% and 7.5%, respectively).

Trial 2

BIO-ASSAYS AND TUNNEL TESTS

In cone bio-assays, similar levels of mortality were achieved for the Olyset net (82.6%; $N=55$) and a net treated with 500 mg permethrin/m² (81.7%; $N=54$). In tunnel

tests, mortality was higher for the holed Olyset net (95.8%) than for this other treated net (66.7%). The net treated with 500 mg permethrin/m² was not tested in the experimental huts, owing to a shortage of experimental huts.

EXPERIMENTAL HUT TRIAL

In the hut trials of the Olyset nets, a total of 2340 mosquitoes (of which 37.6% were *An. arabiensis* and 62.4% *Cx. quinquefasciatus*) was caught (see Table 2).

EXOPHILY. The level of *An. arabiensis* exophily seen with the Olyset net (96.3%) was significantly greater than that seen with the control bed sheet (70.7%). With *Cx. quinquefasciatus* the Olyset net (92.1%) also produced greater levels of exophily than the untreated sheet (33.2%).

BLOOD FEEDING. Use of the Olyset net kept blood feeding below 6% in both *An. arabiensis* and *Cx. quinquefasciatus*. With the untreated control, high levels of blood feeding were observed in both *An. arabiensis* (68.6%) and *Cx. quinquefasciatus* (76.0%).

OVERALL MORTALITY. Use of the control sheet resulted in extremely low mortality of

TABLE 2. Results of the evaluation of Olyset nets against *Anopheles arabiensis* and *Culex quinquefasciatus* in experimental huts (Trial 2)

Mosquito species and variable	Value and (95% confidence interval) for:*	
	Untreated sheet	Olyset nets
<i>Anopheles arabiensis</i>		
No. of females caught	195	196
No. of females caught/night	8.1	8.2
Exophily (%)	70.7 (63.8–76.7) ^a	96.3 (92.0–98.3) ^b
Blood feeding (%)	68.6 (61.7–74.8) ^a	3.7 (1.7–8.0) ^c
Inhibition of blood feeding (%)	–	94.6
Crude mortality (%)	0.0 (0.0–0.0) ^a	11.8 (7.7–17.8) ^b
<i>Culex quinquefasciatus</i>		
No. of females caught	424	298
No. of females caught/night	17.7	12.4
Exophily (%)	33.2 (28.8–37.8) ^a	92.1 (88.0–94.8) ^c
Blood feeding (%)	76.0 (71.6–79.8) ^a	5.2 (3.0–8.7) ^c
Inhibition of blood feeding (%)	–	93.2
Crude mortality (%)	1.2 (0.5–2.9) ^a	3.6 (1.9–6.7) ^{ab}

*Within each row, two values with a different superscript letter are significantly different ($P < 0.05$).

An. arabiensis (0%) and *Cx. quinquefasciatus* (1.2%). The Olyset net produced higher mortality in *An. arabiensis* (11.8%) than in *Cx. quinquefasciatus* (3.6%).

DISCUSSION

In the present study, clear differences in performance were seen between permethrin and the α -cyano pyrethroids deltamethrin and α -cypermethrin when applied to nets at the same level. Although permethrin performed best in terms of personal protection, the two other pyrethroids proved superior in terms of their overall killing effect.

The high protective effect of permethrin, which is linked to its spatial repellent effect, has been reported several times before (Darriet *et al.*, 1984; Lindsay *et al.*, 1991; Miller *et al.*, 1991; N'Guessan *et al.*, 2001; Corbel *et al.*, 2004). The cause is still unclear. Permethrin is known to have low vapour pressure (Wells *et al.*, 1986; Miller *et al.*, 1991), although volatile ingredients present in some permethrin formulations have been shown to be repellent when tested on nets (Lindsay *et al.*, 1991). With certain insecticide formulations, notably DDT [4,4'-(2,2,2-trichloroethane-1,1-diyl)-bis(chlorobenzene)], minute quantities flake off from treated surfaces and become airborne on dust particles, deterring mosquitoes from entering further (Smith and Webley, 1969); the same might happen with permethrin-treated nets.

Higher levels of deterrence than the maximum seen in the present study (35.3%) have been reported for *An. gambiae* exposed to nets treated with relatively high doses of permethrin — 60% with 500 mg permethrin/m² (Lindsay *et al.*, 1991; Miller *et al.*, 1991) and 93% with 1000 mg permethrin/m² (Corbel *et al.*, 2004). Curiously, however, Corbel *et al.* (2004) observed similar levels of deterrence (83%–89%) with nets treated with 50, 100, 250 and 500 mg permethrin/m². The application dosage recommended by the World

Health Organization's Pesticide Evaluation Scheme (WHOPES), for nets treated by dipping, is 200–500 mg/m² (Zaim *et al.*, 2000). In the present study, however, the comparatively high blood-feeding inhibition observed, for both *An. arabiensis* and *Cx. quinquefasciatus*, with nets that had been treated with just 25 mg permethrin/m² demonstrate that much smaller applications can offer high levels of personal protection, despite the associated low levels of mortality.

Permethrin-treated nets killed relatively small numbers of *An. arabiensis* and *Cx. quinquefasciatus* compared with the numbers seen with the other pyrethroid treatments. This low mortality is partly a reflection of the high protective effect of this insecticide: a large proportion of host-seeking mosquitoes are deterred from entering huts with permethrin-treated nets and those that do enter such huts are inhibited from remaining in contact with the treated nets long enough either to probe successfully or to pick up a lethal dose of permethrin (Hossain *et al.*, 1989). Relatively low permethrin-induced mortalities (in comparison with those seen with other pyrethroids) have been observed in several previous studies (Miller *et al.*, 1991; Curtis *et al.*, 1992; N'Guessan *et al.*, 2001; Corbel *et al.*, 2004). In the present study, incipient permethrin-specific resistance among the *An. arabiensis* in Lower Moshi (Kulkarni *et al.*, 2006b) may have made a minor contribution to the low mortalities observed in the experimental huts that contained permethrin-treated nets. The lower-than-recommended permethrin dosage may also have contributed to these low mortalities. In trial 2, however, not even the high concentrations of permethrin incorporated in the Olyset nets (WHO, 1991) led to high mortality in the *An. arabiensis*.

The mortality trends observed in the experimental huts were consistent with the results of the contact bio-assays and tunnel tests, all indicating lower mortality with permethrin than with deltamethrin or α -cypermethrin. The high excito-repellency of

permethrin may explain the low mortalities seen with this insecticide in the tunnel tests and huts and may also account for the comparatively low mortality seen in the contact bio-assays if, in these tests, each mosquito is repelled from the surface of the net and spends proportionately more time flying or resting on the cone (S. Irish and M. Rowland, unpubl. obs.).

Just as the low mosquito mortality associated with permethrin is partly a result of the this compound deterring and repelling mosquitoes, the high mortalities caused by α -cypermethrin and deltamethrin are, to some extent, linked with the relatively poor personal protection offered by these compounds. In failing to deter or repel many mosquitoes, nets treated with α -cypermethrin and deltamethrin are in contact with many host-seeking mosquitoes for relatively long periods of time, during which the insects are likely to pick up a lethal dose of the insecticide from the treated surface. When, in The Gambia, Jawara *et al.* (1998) compared net performances in the field, they found nets treated with α -cypermethrin (at 40 mg/m²) to kill more mosquitoes than nets treated with permethrin (at 500 mg/m²) or λ -cyhalothrin (at 10 mg/m²).

In trial 1 of the present study, the level of *An. arabiensis* mortality recorded in the experimental huts with untreated nets (25.5%) was surprisingly high and much higher than that observed in trial 2 (<2%) or other trials, carried out under the same conditions, in the same experimental huts (unpubl. obs.). Despite the high level of *An. arabiensis* mortality seen in trial 1 with the untreated nets, in both trials the *An. arabiensis* mortality seen with permethrin remained significantly lower than that with α -cypermethrin or deltamethrin, even after adjustment for the control mortality.

At least in the present study area, nets treated with a relatively low dose of permethrin (25 mg/m²) could be effectively used for personal protection. The deterrence effect of this insecticide can be of benefit to households with badly damaged nets or

with insufficient nets to cover all the occupants. In programmes for the control of mosquito-borne disease, however, where coverage with insecticide-treated nets (ITN) is often much less than 100%, the reduction of the vector population remains an important objective. In such settings, the insecticide used to treat nets needs to kill rather than deter or repel mosquitoes, and deltamethrin or α -cypermethrin would probably be good choices. It is not clear, however, whether these less excito-repellent pyrethroids would exert a higher selection pressure for resistance than the more repellent permethrin. Although pyrethroid resistance specific to permethrin is spreading among *An. gambiae* s.s. and *An. arabiensis* in East Africa, it does not appear to constitute a problem for control where permethrin-treated nets continue to be used (Gimnig *et al.*, 2003). The present results indicate that nets with relatively low dosages of permethrin, such as old and much-washed nets originally treated with 200–500 mg/m², probably still offer substantial personal protection in East Africa. This may not be the case in those parts of West Africa where broad-spectrum pyrethroid resistance appears to be reducing the effectiveness of ITN (N'Guessan *et al.*, 2007). To prevent more serious forms of resistance from arising in, or spreading to East Africa, the deployment of new types of ITN, in which a pyrethroid is combined with a non-pyrethroid insecticide, either in a mixture or mosaic, needs to be considered (Hougard *et al.*, 2003; Asidi *et al.*, 2005).

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