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Olyset Net® efficacy against pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus* after 3 years' field use in Côte d'Ivoire

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Abstract. Pyrethroid-impregnated bednets are advocated for personal protection against malaria vectors. To avoid the need for periodic re-treatment, it would be advantageous to have nets that retain insecticidal efficacy for years and withstand repeated washing. Such a type of commercially produced bednet with permethrin 2% incorporated in polyethylene fibres (trademark Olyset Net® supplied by Sumika Life-Tech Co., Osaka, Japan) was evaluated against mosquitoes in veranda-trap huts at Yaokoffikro, near Bouaké, Côte d'Ivoire, by standard WHOPES phase II procedures. Four Olyset Nets were compared with a standard untreated polyester net as control. They comprised three examples previously used in a village for over 3 years (one washed, one dirty, one very dirty) and a previously unused Olyset Net, newly unwrapped, from the same original batch. Bioassays with 3 min exposure of susceptible *Anopheles gambiae* Giles (Diptera: Culicidae) gave >99% mortality of female mosquitoes tested on the 'new' Olyset Net. The used Olyset Nets gave mortality rates averaging 83% for the washed net, 85% for the dirty net and 55% for the very dirty net (within 24-h following 3 min exposure). Thus, Olyset Nets were found to remain remarkably effective against susceptible *An. gambiae* for at least 3 years under field conditions.

Wild pyrethroid-resistant populations of *Culex quinquefasciatus* Say and *An. gambiae* (savanna cytotype with 96% *kdr*) were assessed during June–August 1999 for their responses to sleepers protected by nets in the experimental huts. With regard to hut entry by foraging female mosquitoes, Olyset Nets showed some deterrency against *An. gambiae* (44% reduction by the new net, ~20% by the dirty nets, none by the washed net), but not against *Cx. quinquefasciatus*. Among mosquitoes entering the hut with untreated control net, 30–34% tried to leave (exophily) but were caught in the verandah trap. The permethrin repellency of Olyset Nets increased exophily by 19% for *An. gambiae* and 14% for *Cx. quinquefasciatus*. Blood-feeding rates were 16% *An. gambiae* and 35% *Cx. quinquefasciatus* in the hut with sleeper under the untreated net (showing considerable prevention of biting), 22–26% of both species in huts with washed or dirty used Olyset Nets (not significantly different from control), while the biting success rate of *Cx. quinquefasciatus* (but not kdr *An. gambiae*) was more than halved by the 'new' Olyset Net. Mortality rates of pyrethroid-resistant *An. gambiae* and *Cx. quinquefasciatus* from the huts were, respectively, 3% and 8% with the untreated polyester net, 27.5% and 17% with the 'new' Olyset, 15% and 17.5% with the washed Olyset, 16–25% and 17–20% with dirty old Olyset Nets. Kill differences between nets are significantly different for both *An. gambiae* and *Cx. quinquefasciatus*. Unfortunately the washed used Olyset Net showed least

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activity against resistant mosquitoes, despite its greatest activity against susceptible *An. gambiae*. In each case there was evidence that a high proportion of mosquitoes failed to feed through the net (many of them dying from starvation when they could not leave the closed hut), with indications that dirty Olyset nets enhanced this protective value.

Key words. *Anopheles gambiae, Culex quinquefasciatus, Mansonia,* bednet, bioassay, insecticide resistance, malaria vector, Olyset, permethrin, polyethylene net, pyrethroid, vector control, verandah trap hut, Côte d'Ivoire.

Introduction

During the past decade, pyrethroid-treated mosquito nets have become established as an important defence against malaria transmission (Curtis *et al.*, 1990; Carnevale *et al.*, 1991; Choi *et al.*, 1995; Lengeler *et al.*, 1996a, b; Lengeler, 1998). Large-scale epidemiological field trials involving community use of pyrethroid-impregnated bednets and curtains have demonstrated major benefits in reducing malaria morbidity and mortality (Snow *et al.*, 1988; Alonso *et al.*, 1991; d'Alessandro *et al.*, 1995; Binka *et al.*, 1996; Nevill *et al.*, 1996; Habluetzel *et al.*, 1999; Rashed *et al.*, 2000). A practical obstacle to sustainable implementation of this approach is the need for regular re-impregnation of nets in the field, to renew the insecticidal efficacy of pyrethroid treatment (Chavasse *et al.*, 1999). Re-treatment requires technical knowledge and skill, available and affordable products and community participation. Inadequacy of these and other factors may hamper the efficiency and expansion of large-scale antimalaria programmes involving insecticidal nets, so that re-impregnation rates remain low or decline and the nets become ineffective (Kroeger *et al.*, 1997; Kachur *et al.*, 1999). To overcome these obstacles, various pyrethroid sachet and tablet presentations for 'do-it-yourself' kits (Armstrong *et al.*, 1999; Miller *et al.*, 1999a, b; WHO, 1999) have been developed for individual treatment of nets, promoted by social marketing. National authorities may also introduce 'net impregnation centres' at the district level of primary health care (WHO, 1997; Chavasse *et al.*, 1999).

Some manufacturers supply pretreated nets and it would simplify the needs of bednet users if permanently insecticidal nets could be obtained. Miller *et al.* (1995) developed a wash-resistant permethrin EC (emulsifiable concentrate) formulation but this was no more durable than some alpha-cyano-pyrethroid treatments. Several types of long-lasting insecticidal net treatments are under development (WHO, 2000), of which the Olyset Net® (patented by Sumitomo Chemical Co., Osaka, Japan) has been evaluated in a wide range of situations. Lengeler (1998) refers to 'Olyset Net' field trials against malaria vectors in Cambodia, Côte d'Ivoire, Ecuador, Senegal, Tanzania and Vietnam. The Olyset Net (made of polyethylene fibre with 2% permethrin incorporated during manufacture) is designed to withstand washing repeatedly while retaining long-lasting insecticidal efficacy. Results of field trials have shown encouraging impact and prolonged efficacy of Olyset Nets against vectors of dengue (Itoh & Okuno, 1996; Nguyen *et al.*, 1996) as well as malaria (Ikeshoji & Bakotee, 1996, 1997; Njunwa *et al.*, 1996; Vythilingam *et al.*, 1996; Faye *et al.*, 1998). As the manufacturers claim that Olyset Nets should keep their activity for 3 years, and this was borne-out by our village-scale epidemiological trial (Doannio *et al.*, 1999; Henry *et al.*, 1999), we decided to evaluate their entomological impact against wild mosquitoes, by means of standard experimental hut procedures, after Olyset Nets had been used by villagers for 3 years or more.

Previously, in January and June 1996, a total of 752 Olyset Nets had been distributed in Kafiné village (130 km north of Bouaké), sufficient to cover every bed and mattress for that community. *Plasmodium falciparum* malaria is holoendemic at Kafiné in the savanna zone of Côte d'Ivoire, transmitted by *Anopheles funestus* and pyrethroid-resistant *An. gambiae* (Doannio *et al.*, 1999) with high frequency of *kdr* gene conferring knockdown resistance (Martinez-Torres *et al.*, 1997; Chandre *et al.*, 1999). Malaria parasite rates are 85–100% in children aged <5 years (Henry *et al.*, 1999) and Kafiné residents also suffer from abundant culicine mosquitoes coming from a nearby irrigation scheme. Therefore, the inhabitants of Kafiné have made good use of their Olyset Nets and cared for them well. We lack data on how often Olyset Nets were washed at Kafiné, but it seems that this type of fabric is rather too bulky for easy washing and so most nets have become dirty and remain unwashed during several years of daily use in smoky houses.

In June 1999, after 3 1/2 years' continuous use by villagers, three intact Olyset Nets were taken from Kafiné to be tested in IPR experimental huts at Yaokoffikro field station near Bouaké (7°40′ N, 4°55′ W), according to phase II protocol of the World Health Organization Pesticides Evaluation Scheme, WHOPES (www.who.int/ctd/whopes). In addition to contact bioassays of insecticidal efficacy with susceptible *An. gambiae* (WHO, 1998), we investigated the influence of Olyset Nets on behaviour and survival of local pyrethroid-resistant wild mosquito populations, primarily the main local vector of malaria *An. gambiae*, as well as *Culex quinquefasciatus*, which is generally tolerant to insecticides including permethrin (Brown & Pal, 1971; Hossain *et al.*, 1989; Wu Neng *et al.*, 1991; Curtis *et al.*, 1996) and constitutes a widespread tropical domestic nuisance. The need to protect people effectively against pest culicines such as *Mansonia* spp. and multiresistant *Cx. quinquefasciatus* (Chandre *et al.*, 1997, 1998) is an important consideration for gaining compliance with use of insecticidal nets against malaria transmission (Magesa *et al.*, 1991; WHO, 1998; Chavasse *et al.*, 1999).

This investigation of Olyset Nets against field populations of multiresistant mosquitoes in experimental huts complements our phase II trials of bednets hand-treated with permethrin EC and deltamethrin SC (Darriet *et al.*, 1998), deltamethrin WT (WHO, 1999), alpha-cypermethrin WG, carbosulfan CS, etofenprox EW, pirimiphos-methyl EC (Fanello *et al.*, 1999), lambda-cyhalothrin CS (Darriet *et al.*, 1999) and ongoing trials of other insecticidal net treatments.

Methods

Verandah-trap huts at Yaokoffikro village near Bouaké were described by Darriet *et al.* (1998). Each experimental hut consists of a single room with entry windows on three sides and large screened verandah on the fourth side. A row of these huts is situated along the flank of a part-irrigated valley with highly pyrethroid-resistant populations of *Culex quinquefasciatus* (33–46-fold resistance to permethrin according to Chandre *et al.*, 1998a) and *Anopheles gambiae* savanna cytotype with 96% *kdr* (Chandre *et al.*, 1998b, 1999, 2000). The *modus operandi* of verandah-trap huts involves allowing mosquitoes to enter naturally during the evening, to rest within the hut and bite someone sleeping there, being exposed to any experimental treatment inside the huts, to leave the room and become trapped inside the screened verandah, to survive or die and be collected alive or dead from each part of the hut, so that the numbers of mosquitoes entering and leaving, the proportion blood-feeding successfully and the mortality-rate can be assessed and compared, as previously described for experimental huts in several situations with highly endophilic Afrotropical malaria vectors (Smith, 1964; Smith & Webley, 1969; Coz, 1971; Darriet *et al.*, 1984; Miller *et al.*, 1991; Curtis *et al.*, 1996).

Olyset Net® is made of knitted blue polyethylene thread with permethrin 2% (w/w) incorporated during fibre extrusion. The standard Olyset Net weighs ~600 g with surface area 13.4 m^2 (length 1.80 m, width 1.40 m, height 1.7 m). When the old nets were removed from houses at Kafiné, where they had been used since January 1996 (Doannio *et al.*, 1999), the inhabitants were provided with replacement new Olyset Nets. As before, we are most grateful to Kafiné villagers for their willing cooperation. One of the used Olyset Nets was washed thoroughly with cold water and local soap. Two others were classified as 'slightly dirty' and 'very dirty'. These three 'old' nets were compared with a 'new' Olyset Net® from the original batch obtained in 1995, that had been stored in its original bag. As untreated control for comparison, we used a white polyester multifilament bednet (SiamDutch Mosquito Netting Co., Bangkok, Thailand) of similar dimensions. These five bednets were all intact, not perforated for experimental purpose in the manner described by Darriet *et al.* (1998). Each bednet was allocated arbitrarily to an experimental hut, as listed in Table 1, and was used consistently in the same hut during the whole study, in order to avoid risks of contamination between huts (which may occur when insecticide-treated nets are rotated among huts).

Experimental hut procedures and mosquito collections were as per Darriet *et al.* (1984, 1998). Briefly, one person slept on a mat under a bednet in the middle of each hut from 20.00 to 05.00 hours, after cleaning the hut at 18.00 hours to remove mosquitoes and any spiders or other predators. Wild mosquitoes then entered overnight, until the windows were blocked at dawn (05.00 hours) to prevent mosquitoes leaving. To offset any personal differences in attractiveness to mosquitoes, sleepers systematically changed places between huts night by night. Awaking at 05.00 hours, the sleeper closed the windows and lowered a curtain separating the room from verandah, collected mosquitoes dead and alive separately from three parts of the hut: inside the room, under the bednet and from the verandah. When the light was better at 08.00 hours the collector checked for any extra mosquitoes in these three situations. The hut door was kept closed from 20.00 to 08.00 hours.

After being taken to the laboratory, female mosquitoes from each part of the hut were identified by species, counted and recorded as dead or alive, blood-fed or unfed. Results from the hut with the untreated bednet (control) were compared to those from huts with Olyset Nets, with respect to four criteria:

- entry rate: total number of mosquitoes found in hut (room plus verandah); used to assess relative deterrent effect.
- exit rate: number and proportion of mosquitoes found in verandah; used to assess relative repellency.
- feeding rate: number and proportion of female mosquitoes blood-fed; used to assess protection.
- mortality-rate: number and proportion of moribund female mosquitoes per hut (room and verandah-trap); assessed at 08.00 hours and 24-h later (held in humidified paper cups).

Results and discussion

Insecticidal efficacy of nets was evaluated by WHO (1998) bioassay procedure using plastic cones for 3 min exposure of female *An. gambiae* (susceptible Kisumu strain). Each net was tested in June, July and August 1999, by exposing 10 replicates of five female mosquitoes aged 3–5 days (offered 5% sugar solution *ad libitum* before and after test). Bioassay mortality was scored 24-h post-exposure. Control mortality was < 5% in every case, with no knockdown within 3 min exposure to the untreated net. Knockdown rates of susceptible *An. gambiae* within 3 min exposure averaged 25% (range 13–32%) for the 'new' Olyset Net, but only 3–6% for the dirty old unwashed Olyset Nets and 4% for the freshly washed used Olyset Net (Table 2).

Mortality rates 24-h postexposure of susceptible *An. gambiae* were 100% resulting from 3 min exposure to the 'new' Olyset Net during the first two months and 98% after 3 months of use (Table 2). The freshly washed 'old' Olyset Net (previously used for 3 1/2 years) gave mortality-rates of 79–86% during 3 months post-washing. The unwashed old Olyset

Nets gave mortality-rates averaging 84 ± 11% for the slightly dirty net and 55.3 ± 24% for the very dirty net (Table 2). Thus, all the old Olyset Nets that had been used for up to 44 months remained effective against susceptible *An. gambiae*, whether or not the nets had been washed.

During the period of 3 months' evaluation, (June–August 1999), the five nets were tested in verandah-trap huts on 3 nights per week (total 48 nights) and a total of 2789 mosquitoes were collected, comprising: 596 *An. gambiae* (21%) savanna cytotype with *kdr* frequency >95% (Chandre *et al.*, 1999), 1138 *Cx. quinquefasciatus* (41%) and 1055 *Mansonia africana* plus *Ma. uniformis* (38%).

Considering first the mosquito entry rates into huts where various types of net were used, overall numbers of *An. gambiae* were 143 with the untreated net, 110 and 115 with the dirty old Olyset Nets (21% less than control, $P<0.1$), 148 with the washed used Olyset (similar to control), but only 80 with the 'new' Olyset (44% less than control, $P<0.01$). Evidently the well-known deterrent effect of permethrin on nets (Darriet *et al.*, 1984; Miller *et al.*, 1991; Lindsay *et al.*, 1992), previously seen with Olyset Nets (Curtis *et al.*, 1996), remained detectable against *An. gambiae* except for the freshly washed net (Table 3). Against *Cx. quinquefasciatus*, however, the Olyset Nets failed to reduce house entry (Table 4).

With respect to exophily of mosquitoes after entering our experimental hut with untreated control net, overall 30% of *An.*

Table 1. Type of bednet in each hut.

Hut	Type of bednet
1	New Olyset
2	Used Olyset slightly dirty
3	Used Olyset very dirty
4	Used Olyset washed
5	Untreated polyester net (control)

Table 2. *Anopheles gambiae* (susceptible Kisumu strain) rates of knock-down (within 3 min exposure) and mortality (within 24-h post-exposure) from bioassay exposure to a bednet of the type specified. Tests conducted June–August 1999 with nets used 1–3 months (T + 1–3) in experimental huts.

Type of bednet	Month of test	Number tested	Knock-down (3 min)	Mortality (24 h)
Untreated polyester	T+1	52	0	1 (2%)
	T+2	53	0	1 (2%)
	T+3	55	0	0
	Total	160	0	2 (1%)
Olyset Net:				
New and clean	T+1	53	17 (32%)	53 (100%)
	T+2	55	7 (13%)	55 (100%)
	T+3	52	16 (31%)	51 (98%)
	Total	160	40 (25%)	159 (99%)
Used and washed	T+1	57	2 (4%)	48 (84%)
	T+2	48	0	38 (79%)
	T+3	50	4 (8%)	43 (86%)
	Total	155	6 (4%)	129 (83%)
Used and slightly dirty	T+1	64	3 (5%)	59 (92%)
	T+2	48	0	43 (90%)
	T+3	53	2 (4%)	38 (72%)
	Total	165	5 (3%)	140 (85%)
Used and very dirty	T+1	49	4 (8%)	36 (74%)
	T+2	54	4 (7%)	35 (65%)
	T+3	51	1 (2%)	14 (28%)
	Total	154	9 (6%)	85 (55%)

Table 3. *Anopheles gambiae* females collected in the morning from experimental huts where a bednet had been used overnight, showing proportions found blood-fed and dead in different situations (totals for monthly samples, June–August 1999)

	Type of bednet				
		Olyset®			
			Used		
Sample	Untreated polyester	New	Washed	Slightly dirty	Very dirty
Total/hut	143	80	148	110	115
Under bednet	0	0	2 (1.4%)	1 (0.9%)	2 (1.7%)
Inside room	100 (69.9%)	41 (51.2%)	82 (55.4%)	68 (61.8%)	62 (53.9%)
In veranda-trap	43 (30.1%)	39 (48.8%)	64 (43.2%)	41 (37.3%)	51 (44.3%)
Blood-fed	23 (16.1%)	12 (15.0%)	37 (25.0%)	24 (21.8%)	28 (24.3%)
Mortality:					
Overall	4 (2.8%)	22 (27.5%)	22 (14.9%)	18 (16.4%)	29 (25.2%)
Immediate	4	15	21	12	25
Delayed	0	7	1	6	4
Unfed	3	22	19	17	26
Blood-fed	1	0	3	1	3

Table 4. *Culex quinquefasciatus* females collected in the morning from experimental huts where a bednet had been used overnight, showing proportions found blood-fed and dead in different situations (totals for monthly samples, June–August 1999)

Sample	Type of bednet: Untreated Polyester	Olyset®: New	Olyset®: Used: Washed	Olyset®: Used: Slightly dirty	Olyset®: Used: Very dirty
Total/hut	145	272	189	231	301
Under bednet	4 (2.8%)	0	5 (2.6%)	1 (0.4%)	5 (1.7%)
Inside room	92 (63.4%)	147 (54.0%)	93 (49.2%)	119 (51.5%)	178 (59.1%)
In veranda-trap	49 (33.8%)	125 (46.0%)	91 (48.1%)	111 (48.1%)	118 (39.2%)
Blood-fed	51 (35.2%)	39 (14.3%)	47 (25.0%)	61 (26.4%)	77 (25.6%)
Mortality:					
Overall	11 (7.6%)	46 (16.9%)	33 (17.5%)	40 (17.3%)	60 (19.6%)
Immediate	5	22 (47.8%)	17 (51.5%)	20 (50.0%)	43 (71.7%)
Delayed	6	24 (52.2%)	16 (48.5%)	20 (50.0%)	17 (28.3%)
Unfed	9	45 (97.8%)	31 (93.9%)	37 (92.5%)	52 (86.7%)
Blood-fed	2	1 (2.2%)	2 (6.1%)	3 (7.5%)	8 (13.3%)

Table 5. Comparison of entomological impact on wild mosquito populations entering experimental huts at Yaokoffikro: results with use of a new Olyset® net (one hut in 1999), compared with data reported by Darriet *et al.* (1998) for use of polyester bednets impregnated with permethrin 500 mg a.i./m^2 by dipping (two huts in 1997–98). n = mean number of female mosquitoes per hut.

Factor	*Anopheles gambiae* savanna cytotype ~96% kdr: New Olyset® net	*Anopheles gambiae* savanna cytotype ~96% kdr: Polyester permethrin 500 mg/m^2	*Culex quinquefasciatus* Pyrethroid resistance ratio 33–46x: New Olyset® net	*Culex quinquefasciatus* Pyrethroid resistance ratio 33–46x: Polyester permethrin 500 mg/m^2
Entry reduction*	–44% (n=80)	–18% (n=153)	+88% (n=272)	–11% (n=306)
Exit rate	49% (39/80)	43% (66/153)	46% (125/272)	47% (142/306)
Mortality rate	28% (22/80)	40% (61/153)	17% (46/272)	39% (120/306)

*Compared with untreated polyester bednet.

gambiae (Table 3) and 34% of *Cx. quinquefasciatus* (Table 4) were caught in the verandah-trap. Thus, about one-third of the female mosquitoes were intrinsically exophilic, or tried to leave to go foraging elsewhere because they could not bite the sleeper under the net. The degree of exophily was consistently greater from huts with Olyset Nets for both *An. gambiae* and *Cx. quinquefasciatus*, respectively, 49% and 46% with the 'new' net; 43% and 48% with the washed net; 37–44% and 39–48% with the dirty old nets. Thus, only slight repellency (49% minus 30% = 19% for *An. gambiae*; 48% minus 34% = 14% for *Cx. quinquefasciatus*) was exerted by Olyset Nets, less than might have been expected from the excitorepellency of permethrin (Hodjati & Curtis, 1997, 1999; Chandre *et al.*, 2000).

Considering the proportion of mosquitoes successfully engorging within our experimental huts, overall 16% of *An. gambiae* (Table 3) and 35% of *Cx. quinquefasciatus* (Table 4) were found to have blood-fed in the hut (room plus verandah) with untreated control net. By contrast, blood-fed rates in huts with used Olyset Nets were 22–26% of both species (non-significantly more *An. gambiae* and fewer *Cx. quinquefasciatus*), whereas the 'new' nets apparently more than halved the blood-fed rate of *Cx. quinquefasciatus* (to 14% compared with 35% for the control, $P < 0.01$), while the rate for *An. gambiae* was equivalent to the control.

Evidently none of the nets completely protected the sleeper from being bitten (Tables 3 and 4). Mean numbers of mosquitoes blood-fed per man-night were least with use of the 'new' Olyset Net (0.25 *An. gambiae*, 0.81 *Cx. quinquefasciatus*, 1.08 *Mansonia*), significantly less than for the untreated polyester net (0.48 *An. gambiae*, 0.94 *Cx. quinquefasciatus*, 1.33 *Mansonia*), whereas the used Olyset Nets allowed significantly more bites totalling 3.54/man-night with the washed net (0.88 *An. gambiae*, 1.12 *Cx. quinquefasciatus*, 1.54 *Mansonia*) and 3.62–4.27/man-night with the dirty nets (0.57–0.67 *An. gambiae*, 1.45–1.83 *Cx. quinquefasciatus*, 1.60–1.77 *Mansonia*). Using new Olyset Nets in south-eastern Senegal, Faye *et al.* (1998) achieved major reductions of *An. gambiae*: >90% fewer indoors, 69% less biting rate, 76% lower malaria sporozoite rate and 88% reduction of the entomological inoculation rate of malaria during the first season (5 months), but longer-term evaluation of entomological and parasitological impact has not been reported.

With regard to survival of pyrethroid-resistant mosquitoes after entering experimental huts, overall 24-h mortality-rates were 2.8% of *An. gambiae* and 7.6% of *Cx. quinquefasciatus* collected from huts with untreated polyester nets, partly attributed to starvation due to being unable to bite the sleeper (cf. Curtis *et al.*, 1996). In huts with Olyset Nets, mortality rates of *An. gambiae* and *Cx. quinquefasciatus* were, respectively, 27.5% and 17% with the 'new' net, 15% and 17.5% with the washed net, 16–25% and 17–20% with the dirty old nets (Tables 3 and 4), all significantly greater than control mortalities. For *An. gambiae* the difference was also significant for greater insecticidal efficacy of the 'new' than the washed used Olyset Net ($\chi^2 = 5.32$; $P = 0.02$), as well as for greater impact of the very dirty net than the washed net ($\chi^2 = 6.01$, $P < 0.01$) and the slightly dirty net ($\chi^2 = 3.90$, $P < 0.05$). Overall mortality-rates of 17–20% *Cx. quinquefasciatus* showed no significant differences between the four Olyset Nets (Table 4), in contrast to their widely differing insecticidal effects on susceptible *An. gambiae* (Table 2) and on *kdr* pyrethroid-resistant *An. gambiae* (Table 3).

Impact of the very dirty Olyset Net involved the majority of dead mosquitoes being found unfed (26/29 *An. gambiae*, 52/60 *Cx. quinquefasciatus*). Proportions of immediate : delayed mortality were 25 : 4 for *An. gambiae* (Table 3) and 43 : 17 for *Cx. quinquefasciatus* (Table 4). Apparently they mostly died from starvation, implying that limitation of man-vector contact by the Olyset Net material was enhanced by the sooty grease accumulated on it.

WHOPES approved dosage of permethrin is 200–500 mg ai/m^2 for nets treated by dipping (Zaim *et al.*, 2000), whereas we calculate that Olyset Net carries a loading of about 1100 mg ai/m^2 incorporated in polyethylene to ensure persistence of efficacy and wash-fastness. Table 5 compares our entomological findings for the 'new' clean Olyset Net with those reported by Darriet *et al.* (1998) for polyester nets hand-treated with permethrin 500 mg ai/m^2 and tested in the same verandah-trap huts at Yaokoffikro. Deterrency against house-entry by *An. gambiae* was more than two-fold more from Olyset Net than due to the permethrin-treated polyester net, i.e. 44% vs. 18% reduction in the number of *An. gambiae* entering, compared to the hut with untreated polyester net (control). Surprisingly, this superior effect of the 'new' Olyset Net was not observed against *Cx. quinquefasciatus*. Other workers have also reported only weak deterrency of permethrin-impregnated nets against *Cx. quinquefasciatus* (Curtis *et al.*, 1996; Bogh *et al.*, 1998; Darriet *et al.*, 1998). Exit rates were very similar (43–49%) for both *An. gambiae* and *Cx. quinquefasciatus* from huts with permethrin-treated polyester or 'new' Olyset Net (Table 5). Within experimental huts, mortality rates of the wild pyrethroid-resistant mosquitoes were considerably higher with the freshly permethrin-treated polyester net than with the 'new' Olyset Net: 40% vs. 28% for *An. gambiae* ($\chi^2 = 3.5$, $P = 0.06$), 39% vs. 17% for *Cx. quinquefasciatus* ($\chi^2 = 34.1$, $P < 0.0001$), and presumably would be much more with susceptible populations. Unfortunately the washed used Olyset Net showed least activity in general. Repellency (i.e. mosquito exophily measured by verandah-trap catches) of dirty old used Olyset Nets was more appreciable than their deterrency against house-entry by mosquitoes, and this may not have been entirely due to permethrin effects.

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