Research Articles

J Vect Borne Dis 43, March 2006, pp. 1–6

Tests of Olyset nets by bioassay and in experimental huts

C.A. Maxwell[a,b], J. Myamba[b], J. Magoma[a], R.T. Rwegoshora[b], S.M. Magesa[b] & C.F. Curtis[a]

[a]*London School of Hygiene & Tropical Medicine, London WC1E 7HT, U.K.;* [b]*National Institute for Medical Research, Amani Medical Research Centre, Box 81, Muheza, Tanga, Tanzania*

Abstract

Background: Olyset nets are a type of long-lasting insecticidal net made of polyethylene fibre with permethrin incorporated into it and with a 4 mm mesh size.

Bioassays: Olyset netting was wrapped around a wire frame and the mosquitoes were released inside for bioassays. There was significantly faster knockdown and higher percent mortality than bioassays with the netting attached to a WHO cone with a piece of cardboard on the other side of the net to prevent escapes through the large mesh. It is suggested that with the latter method some mosquitoes place their tarsae through the mesh on to the cardboard, thus avoiding insecticide contact.

Trials in experimental huts: Four mm mesh nets were compared with conventional 1.5 mm mesh nets treated with permethrin. In further trials in huts Olyset nets which were either unwashed or five times washed, with or without subsequent heating, and a Olyset net which had been in domestic use for four years or a new Olyset net were compared with a net treated with bifenthrin.

Results & conclusion: In all cases *Anopheles* biting on sleepers under the nets was reduced and *Anopheles* mortality was increased by the use of the insecticidal nets. No significant impact of washing or heating was detected and an Olyset net was as good as new after four years use, but did not cause as much mosquito mortality as bifenthrin treated nets.

Key words *Anopheles gambiae – An. stephensi* – bioassays – experimental huts – Olyset nets

Introduction

Long-lasting insecticidal nets are intended to have the advantage that one can ensure that they continue to kill mosquitoes through years of use without the need to set up a system for re-treatment of the nets. One of the brands recognised by WHOPES as long-lasting is the Olyset™ net made by M/s. Sumitomo Chemical Company[1]. This is made of very durable polyethylene fibre with a broad (4 mm) mesh and with permethrin incorporated into it with the intention that, if the surface layer of insecticide is washed off, more insecticide will diffuse to the surface, especially at high ambient temperature or if the net is deliberately heated. Olyset nets caused high mortality and considerable prevention of biting among *Anopheles gambiae* (Diptera: Culicidae) entering experimental huts in search of blood meals on sleepers in the huts[2]. Olyset nets also performed well in three years field use in villages[3]. However, low mortality was noted in bioassays in which WHO cones were attached to Olyset netting, with a piece of cardboard on the other side of the netting to prevent mosquitoes escaping through the broad mesh[2]. Lindblade *et al*[4] used this method and reported poor mortality in used Olyset nets. However, it seems that such tests could be misleading because mosquitoes may place their tarsae through the broad mesh and stand on the cardboard, thus avoiding exposure to the insecticidal netting fibres.

Here we report bioassays carried out to directly compare the cone plus cardboard method with that of wrapping netting round a frame and placing mosquitoes inside[5,6]. These bioassays also compared observations on percent mortality after three minutes exposure (plus 24 h holding) with continuous exposure and observation of median time for knockdown, which previous work has suggested is a more sensitive testing method[7,8].

Bioassays provides some data easily and quickly. Tami *et al*[9] used the wire frame method on Olyset nets which had been in domestic use for seven years and showed that these nets retained some insecticidal activity, but the conditions of bioassays with restrained mosquitoes are so artificial that it is not possible to make firm predictions as to whether insecticidal activity is sufficient to provide personal protection to sleepers under nets against mosquito biting or to be likely to reduce infective vector populations in villages where such nets are used by the whole community. Much more realistic data on these questions are provided by trials in experimental huts in which people sleep under nets and into which wild mosquitoes can enter in search of blood meals. In these huts counts are made each morning of bloodfed and unfed, and dead and live mosquitoes[2]. Here we report such hut tests of Olyset nets, mostly carried out for the WHO Pesticide Evaluation Scheme to explore the effects of (a) the unusually large mesh of Olyset nets as compared to nets conventionally treated with permethrin, (b) the effect of realistic washing followed, or not followed, by heating to speed insecticide diffusion, and (c) the impact of an Olyset net which had been in domestic use for four years versus a new Olyset net or nets conventionally treated with bifenthrin.

Material & Methods

For bioassays laboratory reared non-bloodfed *Anopheles stephensi* (Beech strain) (Diptera: Culicidae) about three days after eclosion from pupae were used. One set of bioassays was on Olyset netting attached to a WHO plastic bioassay cone, with a piece of cardboard on the other side of the net to prevent any mosquito escaping though the broad mesh of the netting. The alternative set up was two circles of wire (15 cm in diameter) soldered at right angles to make an approximate sphere and with Olyset netting wrapped twice round so as to prevent escapes of mosquitoes placed inside the sphere. With each test kit, exposure was either (a) for three minutes with mortality recorded after 24 h holding (with access to glucose solution), or (b) indefinite exposure, with observation of the time of knockdown of each mosquito (with its immediate removal to avoid confusion by mosquitoes reviving and being knocked down again). Data are presented here on knockdown time of the median mosquito (the 6th out a sample of 11). Bioassays included tests of pieces of Olyset netting which had been washed five times on a shaker as recommended by WHO[10] or washed five times vigorously by hand; some of the washed samples were subsequently placed in a black plastic bags all day in the sun in July in London; during this exposure the temperature inside the bag rose to about 25°C.

The hut trials were in verandah trap experimental huts at Muheza, Tanzania[2]. There are two sleepers under each net and wild mosquitoes can enter the huts through 1 cm slits at the eaves on two sides of the hut. On the other two sides of the huts similar slits open into screened verandahs which are intended to catch half the mosquitoes exiting through the slits during the night; there are also exit traps on windows. Careful collections are made each morning of all mosquitoes in the huts, the window traps and the screened verandahs. Mosquitoes are sorted by species and by whether they are bloodfed or not and whether live or dead. The mosquitoes which died during the night are found on white canvas floor sheets and have not been scavenged by ants as the huts are built on ant traps incorporating water channels. The numbers of fed/unfed and live/dead mosquitoes and the total which entered the hut during the night are estimated from the numbers found in the hut and window trap plus twice the number found in the screened verandahs (which are on two of the four sides of the hut, with alternation of the screening from the north and south sides to the east and west sides weekly throughout the trial).

The different nets involved in a given trial were rotated between the two available huts according to a pre-arranged schedule. The rotation included an untreated net. On the nights that this was used there was little mortality and that which did occur seemed to be due to starvation despite provision of glucose soaked cotton in the huts. We conclude that there was little or no insecticidal contamination of the huts from the nets tested in them[2].

Polyester nets in use in villages commonly become torn and one of the reasons for insecticidal treatment is to make nets effective despite being torn[11]. To simulate tearing of polyester nets we cut six holes measuring 4 x 4 cm in the nets, for the tests[2]. Polyethylene nets are less prone to tearing and we tested Olyset nets without cutting any holes in them. The distribution of numbers collected each night from experimental huts is clearly non-normal. Therefore, for testing statistical significance we used non-parametric Kruskall-Wallis tests.

Results & Discussion

Table 1 shows that the observed percent mortality after three minutes exposure was higher using the frame method than the cone method on all four types of net and this difference was statistically significant on two of the types of netting, especially the unwashed netting where 300 mosquitoes were tested. Five washes on a laboratory shaker had no significant impact on mortalities observed, but vigorous hand washing did have a significant impact. One day of heating in a black bag in the sun did not restore full insecticidal power.

Table 2 shows very similar results on insecticidal power as expressed by time for knockdown—with the frame method the Olyset net showed quicker knockdown than with the cone method. As before, hand, but not shaker, washing significantly reduced insecticidal power and a day of heating did not restore full insecticidal power. We interpret the lower insecticidal power with the cone method as supporting our hypothesis that, with broad mesh netting backed by cardboard, mosquitoes tend to evade contact with insecticidal netting fibres by resting on the

Table 1. Nos. dead/Nos. tested from Olyset nets bioassayed with three minutes exposure + 24 h holding of *An. stephensi* (Beech strain) in a WHO cone backed by cardboard or with the netting wrapped round a wire frame

Type of nets	WHO cone	Wire frame	Cone v/s Frame
Unwashed	278/300^{a}	299/300^{a}	χ^2 =18.1, p < 0.001
5 x washed on shaker	95/100^{a}	98/100^{ab}	Fisher's exact test, p = 0.44
5 x hand washed	79/100^{b}	92/100^{bc}	χ^2 = 5.81, p = 0.015
5 x hand washed + heating	85/100^{b}	93/100^{bc}	χ^2 = 2.50, p = 0.11

Within each column, data sharing the same letter superscript do not differ significantly.

Table 2. Mean median knockdown time in seconds with *An. stephensi* (Beech strain) on the same Olyset nets as in Table 1. Means and standard deviations of median times for knockdown are from 15 replicates (each of 11 mosquitoes) on unwashed netting and five replicates of 11 mosquitoes in other cases

Type of nets	Mean with cone (s.d.)	Mean with frame (s.d.)	Cone v/s Frame
Unwashed	449.7 (26.6)[a]	334.8 (19.2)[a]	t = 13.5, df = 28, p < 0.001
5 x washed on shaker	409.1 (47.4)[a]	358.5 (46.0)[ab]	t = 1.71, df = 8 , p > 0.05
5 x hand washed	450.6 (41.8)[a]	399.2 (33.2)[bc]	t = 2.15, df = 8, p > 0.05
5 x hand washed + heating	452.2 (18.2)[a]	397.6 (19.2)[bc]	t = 4.61, df = 8, p < 0.002

Within each column data sharing the same superscript letter do not differ significantly.

cardboard surface. We consider that this may well explain the low mortalities reported with the cone-cardboard method by Lindblade *et al*[4].

Tables 3, 4 and 5 show three trials, each lasting six weeks, of Olyset nets and other nets for comparison. As in our other trials at Muheza[2] there was no significant reduction in numbers entering the huts with treated nets as compared to untreated, i.e. no sign of any deterrency due to pyrethroid deposits. Also, as usual in our trials, there was very considerable *Anopheles* mortality associated with pyrethroid nets, but much lower *Cx. quinquefasciatus* mortality due to the pyrethroid resistance, which is known to exist in this species near Muheza[12].

Table 3 shows strong tendencies (but not statistically significant in all cases) for Olyset and for both broad or fine mesh permethrin treated polyester nets to reduce blood feeding of *Anopheles* and *Culex* mosquitoes and to kill more *Anopheles* than did untreated broad or fine mesh nets. Among those *Anopheles*

Table 3. Trial in experimental huts to compare netting of different mesh sizes and netting conventionally treated with 200 mg permethrin/m² or Olyset netting in which permethrin is incorporated in the polyester fibre

Nets (mesh size)	No. nights	Mean no. entering hut/night	% bloodfed	% dead	% dead among those in hut
		Anopheles gambiae and a few *An. funestus*			
Olyset (4 mm)	21	15.9[a]	12.8[a]	56.0[a]	83.7[a]
Permethrin (4 mm)	11	10.3[a]	2.5[b]	46.3[ab]	78.4[a]
Permethrin (1.5 mm)*	11	12.0[a]	17.3[abc]	29.8[bc]	75.0[a]
Untreated (4 mm)	13	16.8[a]	16.4[ac]	7.4[d]	6.9[b]
Untreated (1.5 mm)*	10	15.1[a]	18.0[c]	14.7[cd]	26.7[b]
		Culex quinquefasciatus			
Olyset (4 mm)	21	22.7[a]	17.6[a]	8.5[a]	11.6[a]
Permethrin (4 mm)	11	18.8[a]	10.7[a]	4.8[ac]	12.1[ac]
Permethrin (1.5 mm)*	11	19.5[a]	23.2[ac]	4.8[ac]	12.3[ac]
Untreated (4 mm)	13	19.8[a]	42.3[bc]	0.5[bc]	1.0[b]
Untreated (1.5 mm)*	10	27.7[a]	45.6[b]	0.0[b]	0.0[b]

Data in same column and for same mosquito genus do not differ significantly (p > 0.05 by Kruskall Wallis tests) if they share a superscript letter; *Six holes measuring 4 x 4 cm cut in the fine mesh nets.

Table 4. Trial in experimental huts for WHO Pesticide Evaluation Scheme of effects of washing an Olyset net, with or without subsequent heating to 80°C

Nets	No. nights	Mean no. entering hut/night	% bloodfed	% dead	% dead among those in hut
Anopheles gambiae and a few *An. funestus*					
Olyset unwashed	13	17.2[a]	9.7[ab]	55.0[a]	55.4[a]
Olyset washed 5 x	13	13.1[a]	2.8[b]	70.0[a]	91.8[b]
Olyset washed & heated to 80°C 5 x	10	18.2[a]	1.5[b]	64.3[a]	50.0[a]
Untreated (4 mm)	14	12.9[a]	16.1[a]	25.2[b]	34.5[c]
Culex quinquefasciatus					
Olyset unwashed	13	6.5[a]	9.2[a]	7.6[a]	2.6[a]
Olyset washed 5 x	13	5.1[a]	11.9[a]	5.8[a]	1.9[a]
Olyset washed & heated to 80°C 5 x	10	6.6[a]	1.3[a]	2.8[a]	10.0[a]
Untreated (4 mm)	14	8.9[a]	40.0[b]	3.1[a]	3.2[a]

Meaning of letter superscripts as in Table 3.

which remained in the huts and did not escape into the exit traps, few remained alive in the presence of any of the insecticidal nets.

Table 4 again shows both protection of sleepers from biting and also killing of *Anopheles* by Olyset nets. The insecticidal power was not significantly reduced by five vigorous hand washes given before the commencement of the trial. Nor did heating in water at 80°C after each wash (as requested by WHO Pesticide Evaluation Scheme) have any significant impact on the performance of the nets. It should be

Table 5. Trial in experimental huts for WHO Pesticide Evaluation Scheme of a polyester net treated with bifenthrin and of a new Olyset net and one which had been in domestic use for four years (each net tested for 12 nights)

Nets	Mean no. entering hut/night	% bloodfed	% dead	% in hut among those alive	% dead among those in hut
Anopheles gambiae and a few *An. funestus*					
Olyset, 4 years usage	18.6[a]	18.9[a]	57.5[a]	4.4[a]	79.4[a]
Olyset, new	19.0[a]	19.6[a]	49.0[a]	0.7[a]	79.2[a]
25 mg bifenthrin/m^2 (1.5 mm)*	18.2[a]	12.2[a]	87.6[b]	8.3[a]	91.2[a]
Untreated (1.5 mm)*	18.6[a]	51.9[b]	25.9[c]	46.7[b]	5.1[b]
Culex quinquefasciatus					
Olyset, 4 years usage	20.7[a]	22.7[ab]	7.6[a]	20.5[a]	12.5[ab]
Olyset, new	23.1[a]	28.8[ab]	5.9[a]	25.2[a]	9.6[ab]
25 mg bifenthrin/m^2 (1.5 mm)*	14.7[a]	14.9[a]	20.4[b]	4.2[b]	41.7[a]
Untreated (1.5 mm)*	14.9[a]	39.1[b]	2.3[a]	41.5[a]	1.4[b]

Meaning of letter superscripts as in Table 3; * Six holes measuring 4 x 4 cm cut in 1.5 mm mesh polyester netting.

noted that the trial lasted for six weeks which gave plenty of time for any loss of insecticide from the fibre surfaces due to washing to be replenished by normal diffusion at tropical ambient temperatures.

Table 5 shows that both Olyset nets and a bifenthrin treated net reduced blood feeding and caused mortality of *Anopheles*. Mortality among those *Anopheles* which did not escape from the huts was very high. The killing power of the bifenthrin net was significantly greater than that of the Olyset nets, and bifenthrin even caused significant *Culex* mortality. Comparison of the new Olyset net and the one which had been in domestic use for four years showed that the latter was as good as new. This raises the question how long would an Olyset net remains effective. As noted above, Tami *et al*[9] obtained Olyset nets used for seven years and carried out bioassays on them. Recently these authors have kindly provided these nets and experimental hut data with them which will be reported by Magesa *et al* (in preparation).

Acknowledgement

We thank the team at Muheza for their conscientious work. J. Magoma thanks the Tanzanian Ministry of Health for financing his M.Sc. studies in London during which the bioassay study was carried out. The WHO Pesticide Evaluation Scheme supported two of the experimental hut studies. CFC, CAM and J. Myamba thank the British Medical Research Council for financial support through a Programme Grant. The Director General of the Tanzanian National Institute for Medical Research is thanked for giving permision for publication of this paper.

References

1. Review of Olyset net, bifenthrin, 10% WP. V WHOPES working group. WHO/CDS/WHOPES/2001.4.
2. Curtis CF, Myamba J, Wilkes JT. Comparison of different insecticides and fabrics for anti-mosquito bednets and curtains. *Med Vet Ent* 1996; *10:* 1–14.
3. N'Guessan R, Darriet F, Doannio JMC, Chandre F, Carnevale P. Olyset net efficacy against pyrethroid-resistant *Anopheles* and *Culex* after 3-years field use in Côte d'Ivoire. *Med Vet Ent* 2001; *15:* 97–104.
4. Lindblade KA, Dotson E, Hawley WW, Bayoh N, Williamson J, Mount D, Olang G, Vulule J, Slutsker L, Gimnig J. Evaluation of long-lasting insecticidal nets after 2-years of household use. *Trop Med Int Hlth* 2005; *10:* 1141–50.
5. Curtis CF, Maxwell CA, Finch RJ, Njunwa KJ. A comparison of use of a pyrethroid either for house spraying or for bednet treatment against malaria vectors. *Trop Med Int Hlth* 1998; *3:* 619–31.
6. Test procedures for insecticide resistance, bioefficacy and persistence of insecticides on treated surfaces. WHO/CDS/CPC/MAL/98.12.
7. Maxwell CA, Myamba J, Njunwa KJ, Greenwood BM, Curtis CF. Comparison of bednets impregnated with different pyrethroids for their impact on mosquitoes and on re-infection with malaria after clearance of existing infections with chlorproguanil-dapsone. *Trans R Soc Trop Med Hyg* 1999; *95:* 4–11.
8. Yates A, N'Guessan RN, Kaur H, Akogbeto M , Rowland M. Evaluation of KO-Tab 1-2-3: a wash resistant "dip-it-yourself" insecticide formulation for long-lasting treatment of mosquito nets. *Malaria J* 2005; *4:* 52.
9. Tami A, Mubyazi G, Talbert A, Mshinda H, Duchon S, Lengeler C. Evaluation of Olyset insecticide-treated nets distributed seven years ago in Tanzania. *Malaria J* 2004; *3:* 1–9.
10. Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets. WHO/CDS/WHOPES/GCDPP/2005.11.
11. Lines JD, Myamba J, Curtis CF. Experimental hut trials of permethrin impregnated mosquito nets and eave curtains against malaria vectors in Tanzania. *Med Vet Ent* 1987; *1:* 37–51.
12. Khayrandish A, Wood RJ. A multiple basis for insecticide resistance in a strain of *Culex quinquefasciatus* from Muheza, Tanzania, studied as resistance declined. *Bull Ent Res* 1993; *83:* 75–86.

Corresponding author: Prof. C.F. Curtis, London School of Hygiene and Tropical Medicine, London WC1E 7HT, U.K. e-mail: chris.curtis@lshtm.ac.uk