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BIOEFFICACY OF CYFLUTHRIN (SOLFAC EW050) IMPREGNATED BED-NETS AGAINST *ANOPHELES GAMBIAE* IN SOUTHERN CAMEROON

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ABSTRACT. The bioefficacy of cyfluthrin-impregnated bed-nets was evaluated in the agro-industrial town of Mbandjock (southern Cameroon). The objectives were to assess the knockdown and mortality rates, the protection against bloodfeeding mosquitoes, and the irritant effect of cyfluthrin (SOLFAC EW050)–impregnated bed-nets against a susceptible strain of *Anopheles gambiae.* Polyester bed-nets were impregnated and distributed to inhabitants of Mbandjock for use, then nets were retreated after 6 months. The uptake of active ingredient on nets ranged from 30.8 mg/m^2 to 75.1 mg/m^2 at the initial treatment and from 131.0 mg/m^2 to 225.0 mg/m^2 at retreatment. The susceptible Kisumu reference strain of *Anopheles gambiae* reared in our laboratory was used for bioassays. The knockdown rate on freshly treated nets (2 wk after treatment) ranged from 93 to 96% and the mortality rate ranged from 52 to 70%. During the 12-month trial, knockdown and mortality rates showed 2 peaks, respectively, in the 4th month (94–97% knockdown and 61–96% mortality) and 7th month (i.e., soon after retreatment; 89–98% knockdown and 86–100% mortality), separated by breakdowns on the 5th through 6th and 12th months (46–77% knockdown and 8–69% mortality). Knockdown and mortality rates decreased faster on the lower part of nets than on the top, suggesting that some external factors such as dirt and daily hand manipulation could impede the efficacy of treated nets. Exceedingly dirty nets were less effective than clean nets. The protective rate against bloodfeeding mosquitoes ranged from 60 to 100% during the 1st 4 months and decreased at 40–70% during the 5th and 6th months after the initial treatment. After retreatment, the protective rate ranged from 50 to 90% for the 1st 5 months and from 35 to 64% at the 6th month. More than 70% of mosquitoes that attempt to feed through treated or retreated nettings died within 24 h after contact. Cyfluthrin was found to be mildly irritant during the whole evaluation. This trial reveal that cyfluthrin EW050–treated nets were effective against a susceptible strain of *An. gambiae.*

KEY WORDS Malaria, cyfluthrin, impregnated bed-nets, *Anopheles gambiae,* Cameroon

INTRODUCTION

Pyrethroid-impregnated materials currently are mostly used for control and prevention of malaria (Zaim et al. 2000). Large-scale trials of impregnated bed-nets and curtains have demonstrated their efficacy in reducing malaria morbidity and mortality (D'Alessandro et al. 1995) in addition to having a very low operational cost (Brinkmann and Brinkmann 1995). The protection provided by treated nets can remain from 6 months to a few years, depending on insecticide-treated materials and treatment methods (N'Guessan et al. 2001). The implementation of this method might be suitable for malaria control, especially for primary health care in rural populations, which have low access to high-level health-care resources, because of a lack of knowledge of malaria and of poor financial means.

However, the spread of vector resistance to pyrethroids (Chandre et al. 1999), the low incomes of populations in endemic areas, the irregular retreatment of nets, and nonpersistent efficacy of some insecticide formulations (Chavasse et al. 1999) are some of the concerns of vector control programs involving pyrethroid-treated nets. To overcome such obstacles, alternative insecticides and formulations are being developed (Miller et al. 1999) and new approaches such as combined pyrethroid and carbamate 2-in-1 treated nets (Guillet et al. 2001) have been attempted. The BAYER AG firm manufactured cyfluthrin SOLFAC EW050® (Leverkusen, Germany), has been reported as an adequate formulation for impregnation of netting (Hesse and Deobhankar 1995). Results of field trials have shown the efficacy of cyfluthrin-impregnated bed-nets in reducing vector population density and the good persistence of the chemical after washing (Bartlett 1995, Quilala et al. 1996, Zaim et al. 1998).

Considering the ecoepidemiological diversity of malaria, use of each tool should be followed in different areas to precisely determine its efficacy in various contexts. In Cameroon, most of the large-scale trials on impregnated bed-nets have been carried out with permethrin, deltamethrin, and lambda-

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cyhalothrin (Le Goff et al. 1992, Moyou and Enyong 1994). However, no data were available on cyfluthrin-treated nets.

This report summarizes data on cyfluthrin bioefficacy in Mbandjock (a town located in southern Cameroon), a component of a large-scale multidisciplinary (epidemiology, entomology, and parasitology and clinical) trial in phase III of the World Health Organization pesticide scheme (WHOPES). In our group, the main objective was to assess the knockdown and mortality rate of *Anopheles gambiae* Giles s.s. exposed to treated nets used by inhabitants. In addition, we assessed the protective rate against bloodfeeding mosquitoes and the irritant effect of cyfluthrin-treated nets.

MATERIALS AND METHODS

Study site, impregnation, and distribution of nets

The study was carried out in Mbandjock, between August 1997 and August 1998. Mbandjock is an agro-industrial town (3°25′N, 11°25′E), located at 110 km in northeastern Yaoundé, the capital city of Cameroon. This community is 4 km from the Sanaga River, in the equatorial forest and subtropical savannah transition area. The climate is equatorial, characterized by 2 rainy seasons (March–May and August–November, 1,300–1,600 mm) and 2 dry seasons (November–March and June–July), with temperatures ranging between 23 and 29°C (Melingui et al. 1983).

Bed-nets were made of white multifilament polyester fabric (75 denier; 156 meshes, 12 × 13 holes/in.^{2}), manufactured by SiamDutch Mosquito Netting Co. Ltd. (Bangkok, Thailand). Two sizes of bed-nets were used: X-family (16.3 m^2) and Family (13.13 m^2); both were strengthened on the lower part by a 20-cm sheeting border made of more polyester fibers, to prevent tearing while being tucked in.

The initial treatment was carried out from the August 20 to September 5, 1997. Batches of 10 nets were dipped in a suspension prepared for the impregnation of 100 nets. Based on the absorptive capacity of the nets, the treatment solution was prepared with 48.2 liters of water and 1.8 liters of SOLFAC (cyfluthrin oil-in-water emulsion [EW] at 50 g/liter) for 100 bed-nets (0.5 liter/X-family or Family net), to achieve 50 mg active ingredient (AI)/m^2, as recommended by WHO. Dipped nets were then wrung out, dried horizontally, put in the initial wrapping, and stored at room temperature (≈25°C). From September 5, 1997, to the January 5, 1998, 2,454 nets were provided to inhabitants of the study districts (153 at Bilingue, 475 at Plateau, 1,703 at Mambrat, and 123 at Gare). The coverage rates were approximately 40% at Gare, 70% at Mambrat, and >80% at Bilingue and Plateau districts. People were asked not to wash their nets during the trial, except before retreatment.

A retreatment campaign was organized from March 16 to April 18, 1998. People were asked to wash their nets before new treatment. Nets were impregnated by using the same procedure but the suspension was prepared with 47.8 liters of water and 2.2 liters of SOLFAC. We used more chemical to improve pesticide uptake on nets, because the target dosage of 50 mg AI/m^2 was not achieved at the 1st treatment. At the end of the campaign, 1,558 used nets (63.5%) had been washed and retreated (104 at Bilingue, 302 at Plateau, 1,100 at Mambrat, and 52 at Gare); some households did not bring their nets for re-treatment.

Bioassays on cyfluthrin-treated nets

Biological activity of cyfluthrin was tested on netting collected from 9 freshly treated nets (2 wk after treatment) and on 36 used nets collected monthly (3 nets per month) in the study districts during the 12-month trial. Nets collected from the field were systematically replaced by treated unused ones; the collected nets were brought in laboratory and sampled for bioassays and residue analysis.

In the laboratory, 12 netting samples were collected from each net as follows: 4 samples from the upper portion, 4 from the lower portion, and 4 from the sheeting border. The middle portion was sampled and tested only at the 1st treatment, to testify as to the homogeneity of the treatment at different areas of nets. Each sample was packed in aluminum foil and labeled with the code number of the net. Samples were then kept at 4°C until bioassays were performed (less than 1 month). Among the 4 samples collected from each portion of the net, 3 were used in bioassays and 1 was sent to WHO for insecticide residue analysis by gas–liquid chromatography.

Bioassays were performed in the Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale laboratory of medical entomology, Yaoundé, Cameroon. Three types of bioassays, cone, bloodfeeding, and irritability tests were carried out with the susceptible Kisumu reference strain of *An. gambiae* s.s. according to WHO methods (WHO 1960, 1998).

Cone test: Ten batches of 5 unfed females of Kisumu strain mosquitoes (2–5 days old) were exposed under WHO plastic cones to treated netting for 3 min (WHO 1998). After exposure, the mosquitoes were transferred in white cups and knockdown of mosquitoes was recorded at 60 min (KD_{60}). Mosquitoes were kept in a netting-covered cup and provided with glucose solution under laboratory conditions (80% relative humidity [RH] and 27 ± 2°C). The mortality rate was recorded after 24 h. Eight batches (5 mosquitoes each) were exposed to untreated net as control.

Table 1. Chemical residue on netting samples collected from treated nets at the 1st and the 2nd treatment.[1]

Portion of nets	First treatment[2] x̄ ± SD (mg AI/m²)	Second treatment[3] x̄ ± SD (mg AI/m²)
Top	$33.9^a \pm 10.8$	$141.6^a \pm 59.2$
Middle	$30.8^b \pm 14.8$	$132.0^a \pm 20.0$
Bottom	$33.4^a \pm 10.3$	$131.0^a \pm 26.1$
Sheeting border	$75.1^b \pm 15.3$	$225.0^b \pm 42.5$

[1] Thirty-six netting samples were collected at each treatment (4 samples per net from 9 nets).

[2] Values followed by the same lowercase letter are not significantly different (ddl = 16, $t = 0.1$, $P > 0.3$); values followed by different lowercase letters are significantly different (F = 16.5, $P < 0.001$).

[3] Values followed by the same lowercase letter are not significantly different (F = 3.9, $P > 0.05$); values followed by different lowercase letters are significantly different (F = 13.9, $P < 0.001$).

Bloodfeeding test: Bloodfeeding tests were performed by using WHO test tubes, covered on the top by pieces of treated netting. Each tube was filled with 25 unfed female *An. gambiae* (6–10 days old). A rabbit with shaved abdomen was laid on the top of 4 tubes. Mosquitoes could then attempt to take a blood meal through the treated netting. Control mosquitoes (25 in each of 2 tubes) were bloodfed through untreated netting. After 30 min, the rabbit was removed, and mosquitoes were transferred to observation tubes covered with untreated nettings and kept under laboratory conditions (80% RH and 27 ± 2°C). All control and tested mosquitoes were provided with 10% glucose solution. After 24 h, the numbers of dead and bloodfed mosquitoes were recorded. When control mortality was between 5 and 10%, mortality of tested mosquitoes was corrected by Abbott's formula (1925). The blood feeding protective rate was calculated as protective rate $= 100(y - x)/y$, where x is the percentage of bloodfed females in the treated group and y is the percentage of bloodfed females in control group (Abbott 1925).

Irritability test: By using WHO cones, a single female *An. gambiae* (2–5 days old) was placed on a piece of netting collected from the lower part of nets. The time of 1st taking off was registered after a settling period of 60 sec (Chandre et al. 2000). Fifty mosquitoes were tested per treated netting. Control mosquitoes were exposed to untreated netting.

Data analysis

The average amounts of active ingredient in nettings from 9 freshly treated nets and 9 retreated nets were estimated and compared with analysis of variance (ANOVA) to evaluate variations in net-treatment. Knockdown and mortality rates on each portion of freshly treated nets as well as that of clean, slightly dirty, and exceedingly dirty nets were compared with the Mantel–Haenszel chi-square test.

Table 2. Chemical residue on netting samples collected from nets dipped in different baths of the 1st impregnation.[1]

Bath series	Dosage x̄ ± SD (mg AI/m²)
1	$39.4^2 \pm 21.5$
2	$40.1^2 \pm 17.8$
3	$50.4^2 \pm 30.9$

[1] Thirty-six netting samples were used (4 samples per net) from 9 nets (3 nets per bath).

[2] No significant difference (F = 0.6, $P > 0.05$).

The knockdown, mortality and protective rates, and the time of 1st taking off were recorded monthly to monitor variation within the study period.

Irritability data were analyzed by a log-probit software developed by Praxème (Montpellier, France) based on the work of Finney (1971). The times of 1st taking off for 50% (TO_{50}) and 95% (TO_{95}) of mosquitoes were calculated and the irritability ratio (IR) compared to freshly treated netting was obtained by the following relation: $IR_{50} = TO_{50}$ tested sample/TO_{50} freshly treated sample (Raymond et al. 1993). The irritant effect of tested netting was significantly different from that of freshly treated netting when the 95% confidence interval of the IR did not include the value 1; a high IR_{50} suggested a decrease in irritancy.

RESULTS

Residue analysis

On the 1st impregnation, the target dosage of 50 mg AI/m² recommended by WHO was not reached on the upper, middle, and bottom of nets; residual analysis revealed residue levels of 30.8 ± 14.8 to 33.9 ± 10.8 mg AI/m² (Table 1). However, the uptake of insecticide was doubled on the sheeting border (75.1 ± 15.3 mg AI/m²).

No significant difference was observed among the amount of insecticide taken up on nettings from different net batches from the 1st impregnation. Average residues ranged from 39.4 ± 21.5 to 50.4 ± 30.9 mg AI/m² (ANOVA, F = 0.6, $P > 0.05$; Table 2). A decrease in insecticide uptake by netting was observed as dipping progressed from net

Table 3. Chemical residue on netting samples according to net dipping order in baths of the 1st impregnation.[1]

Dipping order	Dosage x̄ ± SD (mg AI/m²)
1st	$46.8^2 \pm 24.3$
50th	$46.5^2 \pm 20.2$
101st	$36.7^2 \pm 26.7$

[1] Thirty-six netting samples were used (4 samples per net) from 9 nets (3 nets per set).

[2] No significant difference (F = 0.7, $P > 0.05$).

Table 4. Knockdown and mortality rate of the Kisumu susceptible strain of *Anopheles gambiae* (120 control and 450 tested) after 3 min of exopsure to netting freshly treated with cyfluthrin.

Net portion	KD_{60} (%)[1]	Mortality (%)[2]
Control (n = 3)	0	4
Top (n = 9)	94.4[3]	56.9[a]
Middle (n = 9)	92.9[3]	52.0[a]
Bottom (n = 9)	96.2[3]	68.9[b]

[1] KD_{60}, percentage of knocked-down mosquitoes 60 min after exposure to treated nettings.
[2] Values followed by the same lowercase letter are not significantly different (df = 1, χ^2 = 2.2, P = 0.1); values followed by different lowercase letters are significantly different (df = 2, χ^2 = 28.1, P < 0.001).
[3] No significant difference (ddl = 2, χ^2 = 4.8, 0.05 < P < 0.1).

number 1 (46.8 ± 24.3 mg AI/m²) to number 101 (36.7 ± 26.7 mg AI/m²). However, the difference between 3 sets of nettings from nets dipped in the 1st, 50th, and 101st rank was not significant (ANOVA, F = 0.7, P > 0.05; Table 3).

This lower dosage than expected brought us to adjust the amount of insecticide during the retreatment process. After the retreatment, the insecticide residues were greatly increased on the 3 portions of nets (ranging from 131.0 ± 26.1 to 141.6 ± 59.2 mg AI/m²) as well as on the sheeting border (225.0 ± 42.5 mg AI/m²; Table 1).

For both treatments, pesticide residue was greater on the sheeting border than on the 3 other portions of nets (ANOVA, 1st treatment: F = 16.5, P < 0.001, 2nd treatment: F = 13.9, P < 0.001; Table 1).

Knockdown effect and mortality rates

Percentages of knockdown of mosquitoes after 3 min of exposure to freshly treated nets and mortality rates are presented in Table 4. No knockdown effect was found with untreated nets, and the mortality rate was always less than 5%.

On the 3 parts (i.e., top, middle, and bottom) of freshly treated net, KD_{60}s ranged from 93 to 96%, and the mortality rate after 24 h ranged from 52 to 70%. No significant difference was observed between knockdown effects on the top, the middle, or the bottom of nets (χ^2 = 4.8, df = 2, 0.05 < P < 0.1). However, the mortality rate was significantly higher on the bottom than on the top and the middle (χ^2 = 28.1, df = 2, 0.05 < P < 0.001; Table 4).

During the 12-month trial, knockdown effect and mortality rates on the 3 parts of the nets were characterized by 2 peaks at the 4th and 7th months, respectively, separated by breakdowns on the 5th through 6th and 12th months (Fig. 1). At the 4th month, KD_{60}s varied between 94 and 97% and mortality varied between 61 and 96%. During the 5th through 6th months, KD_{60}s fell to 46–77% and mortality fell to 8–69%. The greatest efficacy was observed at the 7th month (i.e., after the 2nd treatment); KD_{60}s ranged from 89 to 98% and mortality ranged from 86 to 100%. This level of efficacy did not decline until the 11th month, after which mortality rate fell between 27 and 67%.

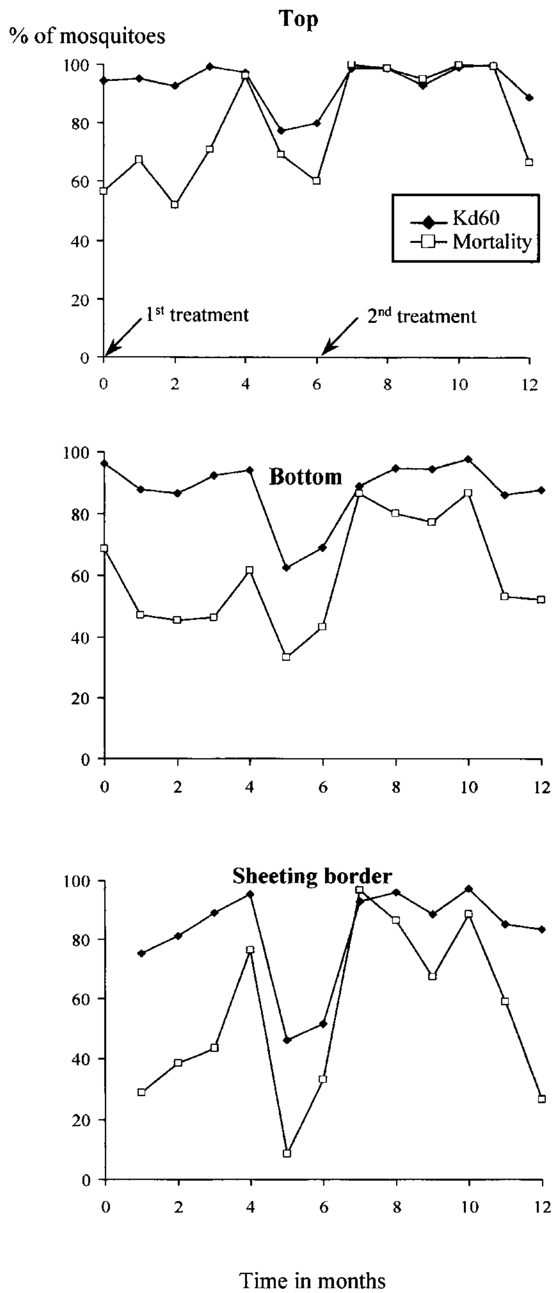

Fig. 1. Knockdown rates within 60 min and mortality rates after 24 h for the susceptible Kisumu strain of *Anopheles gambiae* after 3 min of exposure to the top, the bottom, and the sheeting border of cyfluthrin-treated nets used in households.

We analyzed KD_{60}s and mortality rates on nettings collected from 9 nets used during the 1st 6

Table 5. Knockdown and mortality rate of the Kisumu susceptible strain of *Anopheles gambiae* after 3 min of exposure to domestic-used cyfluthrin-treated nets, taking into account the level of dirtiness of 15 nets.[1]

Net portion	Cleanliness	KD_{60} (%)[2]	Mortality (%)[3]
Top	Clean	95.3[a]	70.5[a]
	Slightly dirty	99.3[b]	79.1[a]
	Exceedingly dirty	83.3[c]	60.6[b]
Bottom	Clean	92.0[a]	70.0[a]
	Slightly dirty	92.7[a]	56.9[b]
	Exceedingly dirty	81.2[b]	45.0[c]
Sheeting border	Clean	80.7[a]	49.3[a]
	Slightly dirty	83.5[a]	35.5[b]
	Exceedingly dirty	72.8[a]	47.1[a]

[1] Thirty-three netting samples were used (6 control and 9 from each portion of treated nets); from 11 nets (2 untreated, 3 clean, 3 slightly dirty, and 3 exceedingly dirty previously treated nets). The number of mosquitoes was 300 control and 1,350 tested (450 on the top, 450 on the bottom, and 450 on the sheeting border).

[2] KD_{60}, percentage of knocked-down mosquitoes 60 min after exposure to treated nettings. Values followed by different lowercase letters are significantly different within the top (df = 2, χ^2 = 31.1, $P < 0.001$); values followed by the same lowercase letter are not significantly different within the bottom (df = 1, χ^2 = 0.05, P = 0.8), values followed by different lowercase letters are significantly different within the bottom (df = 2, χ^2 = 12.3, $P < 0.01$); values followed by the same lowercase letter are not significantly different within the sheeting border (df = 2, χ^2 = 5.3, $P > 0.05$).

[3] Values followed by the same lowercase letter are not significantly different within the top (df = 1, χ^2 = 2.96, P = 0.08); values followed by different lowercase letters are significantly different within the top (df = 2, χ^2 = 11.7, $P < 0.01$); values followed by different lowercase letters are significantly different within the bottom (df = 2, χ^2 = 19.2, $P < 0.001$); values followed by the same lowercase letter are not significantly different within the sheeting border (df = 1, χ^2 = 0.1, P = 0.7); values followed by different lowercase letters are significantly different within the sheeting border (df = 2, χ^2 = 6.5, $P < 0.05$).

months, taking into account the level of their dirtiness (3 clean, 3 slightly dirty, and 3 exceedingly dirty). Percentages of KD_{60} and mortality are presented in Table 5. The overall KD_{60} ranged from 72.8 to 99.3%; the difference between clean and slightly dirty nets was not significant ($0.05 < \chi^2 < 0.4$, df = 1, $0.5 < P < 0.8$), except on nettings from the top, in which the rate was higher for slightly dirty samples than for clean ones (χ^2 = 4.6, df = 1, P = 0.03). However, the KD_{60} was decreased on nettings from exceedingly dirty nets, although this decrease was not significant on the sheeting border (χ^2 = 3.3, df = 2, $P > 0.05$) contrary to the top and the bottom ($12.3 < \chi^2 < 31.1$, df = 2, $P < 0.01$; Table 5).

Mortality rates were significantly decreased on nettings from the top and the bottom of exceedingly dirty nets, on which mortality rates were 45.0–60.6% vs. 56.0–79.1% for slightly dirty and around 70% for nettings from clean nets (χ^2 = 11.7–19.2, df = 2, $P < 0.01$). Within the sheeting border, the lowest mortality rate, 35.5%, was found on nettings from slightly dirty nets vs. 49.3% and 47.1% on nettings from clean and exceedingly dirty nets, respectively (χ^2 = 28.1, df = 2, $P < 0.001$).

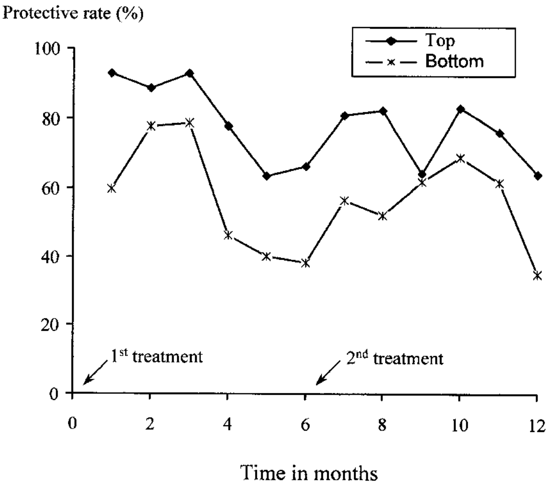

Fig. 2. Bloodfeeding reduction (protective rate) of cyfluthrin-treated nets against the susceptible Kisumu strain of *Anopheles gambiae*.

Bloodfeeding protective rates

The percentage of bloodfeeding in control mosquitoes ranged from 40 to 100%, and the mortality rates ranged from 2 to 14%, meaning that tested mosquitoes actively attempted to take bloodmeals through untreated net. The protective rate on freshly treated nets was not evaluated, because nets were sent to the WHO office for insecticide residue analysis. After 1 month of use, the protective rates against bloodfeeding through treated nets were 93% on the top and 60% on the bottom of nets (Fig. 2). During the whole trial, protection was less on the bottom than on the top. Nets were very effective during the 1st 3 months (>78% protection) and relatively effective at the 6th and 12th months (40–45% protection; Fig. 2).

Among the mosquitoes attempting to take bloodmeals through the treated netting, more than 70% (including fed and nonfed mosquitoes) died 24 h after contact (Fig. 3). For the 1st month, the mortality rate was 93% on nettings from the top and 72% on those from the bottom of nets. Between the

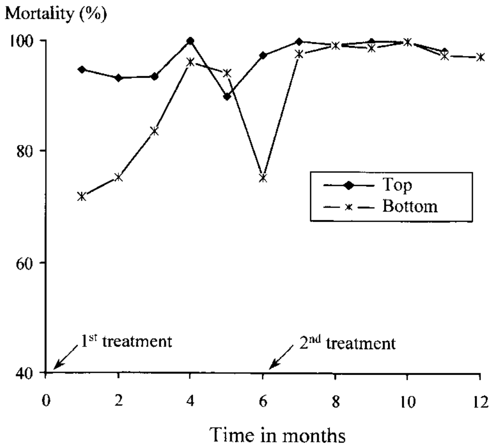

Fig. 3. Mortality rates of the susceptible Kisumu strain of *Anopheles gambiae* after 30 min of exposure to cyfluthrin-treated nets.

1st and the 4th months, the mortality rates ranged from 90 to 100% on the top and increased from 70 to 94% on the bottom nettings. The decrease in the mortality rate appeared at the 5th month for nettings from the top (90.0%) and at the 6th month for nettings from the bottom (75.3%). After retreatment, the mortality rate was maintained between 97.8 and 100%.

Irritant effect

The TO_{50}s and TO_{95}s of the susceptible Kisumu strain of *An. gambiae* in contact with treated nettings are presented in Table 6. On nettings collected from freshly treated nets, the TO_{50} was 7.1 sec and the TO_{95} was 55.6 sec. With untreated nettings, the TOs were increased by more than 50-fold (TO_{50} = 1,120 sec and TO_{95} > 3,600 sec). The IR_{50} was 157.1, meaning that untreated nettings were not irritating.

With nettings collected during the 3rd, 4th, 7th, 8th, 9th, and 10th months, the irritant effect was similar to that of nettings from freshly treated nets (1st treatment); the IR_{50} did not exceed 1.5 ($0.8 < IR_{50} < 1.4$, 0.7 < 95% CI < 1.6; Table 6). The lowest irritant nettings were those collected during the 5th and 6th months as compared to those from freshly treated nets ($10 < IR_{50} < 12$, 8.6 < 95% CI < 13.0). Unexpectedly, nettings sampled during the 1st 2 months (1st and 2nd) and those from the last 2 months (11th and 12th) showed the same level of irritancy ($2.2 < IR_{50} < 2.6$, 2.0 < 95% CI < 3.0).

DISCUSSION

In this trial, nets were impregnated in a bulk mix of insecticide at the target dosage of 50 mg AI/m². Despite the careful and highly supervised impregnation procedure, great variation was observed in the residue analysis of bed-net samples. The uptake of cyfluthrin by treated nets was approximately 33 mg AI/m² for classic netting and approximately 75 mg AI/m² for the sheeting border. The sheeting border absorbed more of the chemical, and this was observed either with the 1st impregnation or after retreatment. This variation was probably due to the difference in mesh size between standard netting and sheeting border. We supposed that more fabric

Table 6. Time of 1st taking off for 50% (TO_{50}) and 95% (TO_{95}) and irritability ratio (IR_{50}) of the Kisumu susceptible strain of *Anopheles gambiae* in contact with netting samples collected from the bottom of treated nets.[1]

Netting	TO_{50}	95% CI	TO_{95}	95% CI	IR_{50}	95% CI
Untreated	1,120	396–9,875	>3,600	—	157.1	111–222[2]
First treatment						
Freshly treated	7.1	6.1–8.2	55.6	43.3–76.1		
Month 1	18.4	14.4–24.0	432.0	226.7–843.3	2.6	2.2–3.0[2]
Month 2	16.5	14.4–19.0	282.4	213.2–393.5	2.3	2.0–2.6[2]
Month 3	5.9	5.2–6.6	61.1	49.4–78.4	0.8	0.7–0.9[3]
Month 4	8.8	6.9–11.4	83.9	55.7–128.5	1.2	1.0–1.5[3]
Month 5	82.5	65.1–108.7	>3,600	—	11.6	10.3–13.0[2]
Month 6	73.1	40.7–133.3	>3,600	—	10.3	8.6–12.2[2]
Second treatment						
Month 7	10.2	8.9–11.5	111.4	88.6–146.1	1.4	1.2–1.6[3]
Month 8	9.5	8.4–10.8	103.5	83.0–133.9	1.3	1.2–1.5[3]
Month 9	9.8	8.8–11.0	82.5	67.5–104.2	1.4	1.2–1.6[3]
Month 10	7.2	6.4–8.2	62.2	50.6–79.4	1.0	0.9–1.2[3]
Month 11	15.6	13.6–17.9	241.1	182.8–335.7	2.2	2.0–2.5[2]
Month 12	15.6	13.6–17.9	235.3	180.6–321.2	2.2	2.0–2.5[2]

[1] Forty samples were used (1 untreated/control and 39 from the bottom of each treated net), from 39 nets (3 freshly treated + 3 nets over 12 months). The number of mosquitoes was 50 control and 150 tested every month.

[2] Significant difference compared with freshly treated nettings.

[3] No significant difference compared with freshly treated nettings.

in a given area could absorb more amount of insecticide than less fabric in the same area. Compared to other fabrics such as cotton, polyethylene, or polypropylene, polyester was reported to retain more insecticide (Curtis et al. 1996). Our study emphasized that in addition to the kind of textile, more attention should also be given to the mesh size, with the 156 mesh (i.e., 1.5-mm space between fibers) being recommended. On the other hand, variation in chemical content also could come from the procedure used for net impregnation, as shown by the difference observed according to rank of impregnation. A single treatment should allow a better target dosage but for mass impregnation, dipping nets in a large amount of insecticide mix was necessary.

The evaluation of cyfluthrin bioactivity was focused on *An. gambiae,* because this species was reported as the main malaria vector in Mbandjock, representing 82.5% of anophelines (Bouchité et al. 1997). Transmission occurred during the rainy season and continued during the dry season around maize and vegetable cultivated swamps, which are suitable breeding sites for *An. gambiae.*

After a contact with freshly treated nets, the KD_{60} rates of the susceptible Kisumu strain of *An. gambiae* reared in our laboratory were higher than 90%, but the mortality rates varied between 50 and 70%. When using polyester netting impregnated with cyfluthrin at 50 mg AI/m^2 under laboratory conditions, the mortality rate of the Kisumu strain of *An. gambiae* was about 98% (our unpublished data). Zaim et al. (1998) also reported 93–100% mortality in a laboratory strain of *Anopheles stephensi* Liston after contact with nylon and light or medium cotton nets impregnated with an EW formulation of cyfluthrin at less than 30 mg AI/m^2. In the same report, heavy cotton appeared to be 2-fold less effective than the other fabrics (40% mortality). In contact with polyethylene netting impregnated with EW cyfluthrin at the target dosage of 50 mg AI/m^2, *Anopheles flavirostris* (Ludlow) displayed 100% mortality in the Philippines (Quilala et al. 1996). The efficacy of cyfluthrin-impregnated bed-nets against *An. stephensi* (93–100% mortality rate to <30 mg AI/m^2) and *An. flavirostris* (100% mortality rate to 50 mg AI/m^2) was similar to that observed against the Kisumu strain of *An. gambiae* in contact with pieces of netting impregnated in our laboratory (96.2% mortality rate to 50 mg AI/m^2) (unpublished data). These results are consistent with those obtained against *An. gambiae* in contact with other pyrethroids such as deltamethrin suspension concentrate (SC) (97% mortality rate to 25 mg AI/m^2), lambda-cyhalothrin emulsifiable concentrate (EC) (100% mortality rate to 10 mg AI/m^2), and permethrin EC (95% mortality rate to 200 mg AI/m^2) (Curtis et al. 1996).

However, bed-nets impregnated in Mbandjock seemed to be less effective, especially at the 1st treatment (50–70% mortality rate to 30–34 mg AI/m^2). Our results should be related to the amount of chemical residue in netting, which also could be associated with the method of impregnation. Curtis et al. (1992) reported that cyfluthrin EW050 at 30 mg AI/m^2 and 50 mg AI/m^2 on polyester netting induced the same mortality rates in *An. gambiae,* which was not significantly different from that recorded with permethrin at 200 mg AI/m^2. Furthermore, Hesse and Deobhankar (1995) indicated that the dosage of 30 mg AI/m^2 was appropriate for control of *Anopheles* but recommended 50 mg/m^2 at an operational level for mixed mosquito populations (*Anopheles* and *Culex*). Hence, cyfluthrin at approximately 35 mg AI/m^2 obtained in Mbandjock was sufficient for the control of the susceptible Kisumu reference strain of *An. gambiae.* Curtis et al. (1992) demonstrated that impregnated bed-nets performed well after 4 months of use in experimental huts, even when bioassays indicated a mortality rate of approximately 60%. Subsequent to high knockdown effect, 50–70% mortality rates were expected to insure a good efficacy at operational level.

During our trial, the decrease in efficacy was faster on the bottom and sheeting border of nets than on the top, probably due to net wear and tear. The residual activity remained for about 4 months, whereas the initial content of cyfluthrin on treated nets was not significantly lost after 6 months of domestic use, suggesting that cyfluthrin persisted on treated nets during domestic use and even after washing. Cyfluthrin is known to persist on nets as well as deltamethrin and lambda-cyhalothrin (Njunwa et al. 1991). Likewise, Curtis et al. (1992) reported that polyester nets with holes, treated with cyfluthrin EW050 at 50 mg AI/m^2, performed well after 15 months of use. The EW formulation was indicated as an ideal formulation for fabric impregnation because it has a synthetic oil type of sticking agent binding it to the fibers, which increases wash-resistance (Hesse and Deobhankar 1995).

This persistence of cyfluthrin residue on the nets contrasted with the decrease in bioefficacy during the trial, especially on the 6th and 12th months. Investigation of the factors involved in the loss of activity of treated nets under natural condition of use was interesting. Bioassays carried out during the 1st 6 months indicated that exceedingly dirty nets were significantly less effective than clean or slightly dirty ones. These results suggested that dirt might impede the availability of cyfluthrin on treated nets. Several authors have underlined the impact of external factors acting on the efficacy of treated nets, as mentioned by Njunwa et al. (1991) and N'Guessan et al. (2001). Washing dirty nets in cold water with mild soap reestablished the knockdown effect and mortality rate of deltamethrin-impregnated nets used for 3–6 months (Etang, unpublished data). Apparently, formulations that favor retention of the insecticide on the fabric even after several washings must be developed to allow cleaning without loss of bioefficacy.

Another important aspect to take into account is insecticide activity against host-seeking mosquitoes. Miller and Gibson (1994) presumed "the response of host-seeking mosquitoes was so characteristic that a signature behavioral pattern could be attributed to each insecticide." In this trial, the role of the cyfluthrin protection was epidemiologically important in reducing host–vector contact and vector density (protective rate>40%, and mortality>70%), which are the main parameters for an efficient vector control program. This useful activity of cyfluthrin was demonstrated in bioassays (Bartlett 1995), experimental huts (Curtis et al. 1996), and field trials (Yadav and Sharma 1994).

Laboratory assays have shown that mortality is closely linked to the time of mosquito contact with treated material (Chandre et al. 2000). The uptake of insecticide by mosquito tarses increases as long as the contact lasts, until the lethal dose is obtained. Unpublished studies carried out in our laboratory showed that cyfluthrin at 50 mg AI/m^2 was a lower irritant than permethrin at 500 mg AI/m^2, but higher than deltamethrin SC at 20 mg AI/m^2. Cyfluthrin also was found to have mild irritancy against host-seeking *An. stephensi* (Zaim et al. 1998). Examination of our data indicated that the decrease in bloodfeeding protection and irritancy of the insecticide at the 5th and 6th months was compensated by the increase of mortality in host-seeking mosquitoes, which demonstrates the efficacy of cyfluthrin-treated nets.

This trial revealed that cyfluthrin EW050 was effective against *An. gambiae* and was suitable for net impregnation in Cameroon. This pyrethroid combined the advantages of high knockdown effect, good mortality, persistence after domestic use, and resistance to washing. In addition, cyfluthrin showed a high protective activity in reducing host–vector contact. The mild irritant activity allowed mosquitoes to remain in contact with the treated net and absorb lethal doses of insecticide. Moreover, no adverse effect was reported by the users of impregnated nets; the acceptability to the residents was clearly perceptible.

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