WORLD HEALTH ORGANIZATION
ORGANIZATION MONDIALE DE LA SANTE

WHO/MAL/99.1088
WHO/VBC/99.1002
Distr.: Limited
Original: French

The impact of permethrin and deltamethrin resistance in *Anopheles gambiae s.s.* on the efficacy of insecticide-treated mosquito nets[1]

F. Darriet,[2] P. Guillet,[3] R.N. N'Guessan,[2] J.M.C. Doannio,[2] A.A. Koffi,[2] L.Y. Konan[2] and P. Carnevale[2]

[1] The World Health Organization has provided financial support for this study.

[2] Institut Pierre Richet, OCCGE, P.O. Box 1500, Bouaké 01, Côte d'Ivoire.

[3]Institut de Recherche pour le Développement (IRD)/LIN, Collaborating Centre for vector control, Montpellier, France.

WHO/MAL/99.1088
WHO/VBC/99.1002

Summary

From October 1997 until April 1998 an experiment was conducted in the experimental huts of Yaokoffikro station (Côte d'Ivoire) to assess the impact of *Anopheles gambiae s.s.* resistance to permethrin and deltamethrin on the efficacy of mosquito nets treated with those products. Capture of *Culex sp.* enabled researchers also to study changes in their behaviour caused by the insecticide treatment of the mosquito nets.

Six mosquito nets of multifilament polyester fibre, with holes deliberately made in them, were tested in the six experimental huts. Two were not treated and were used as controls, two were treated with 500 mg permethrin per m^2, and the last two were treated with 25 mg deltamethrin per m^2.

Analysis of results shows that the presence of treated mosquito nets in houses:

(1) reduced the entry rate of *An. gambiae* s.s. by 18% for the mosquito nets treated with permethrin and by 43% for those treated with deltamethrin. For *Culex sp.*, entry rate to the houses was reduced by 11% and 25% respectively;

(2) reduced by a factor of 2 to 3 (in relation to controls) the number of *An. gambiae s.s.* and *Culex sp.* females found within the treated mosquito nets;

(3) increased by 1.5 to 2 times the exophily of *An. gambiae* s.s. and *Culex sp.*;

(4) reduced by 55% to 65% their blood feeding rate;

(5) led to 40% mortality of *An. gambiae* s.s. females with permethrin and 56% with deltamethrin. For *Culex sp.*, those mortality rates were 39% and 45% respectively. Immediate mortality was always higher (>85%) than delayed mortality (<15%).

Bioassays confirmed the results in the field, i.e., that in an area where *An. gambiae s.s.* and *Culex sp.* are resistant to permethrin and deltamethrin, mosquito nets treated with the two insecticides remain effective, and are therefore, even in these conditions, an excellent means of individual protection.

1. Introduction

At present, insecticide-treated mosquito nets are an essential tool for malaria control, especially in sub-Saharan Africa (Lengeler et al, 1996). Only synthetic pyrethroids can be used because of their effectiveness, speed of action, high excito-repellent effect, and low toxicity for mammals.

Anopheles gambiae s.s. resistance to permethrin has, however, been found in Côte d'Ivoire, first at Bouaké (Elissa et al, 1993) then in the rest of the country (Chandre et al, 1997), associated with with more or less marked resistance to other pyrethroids that can be used for public health purposes (Darriet et al, 1997). This resistance raises a number of questions, such as whether the behaviour of female

mosquitoes in the presence of insecticide is modified, and whether their resistance to pyrethroids is accompanied by reduced effectiveness of treated mosquito nets. Our study aims to answer those questions.

2. Material and methods

2.1 Choice of the study area

As part of a study of *An gambiae s.l.* resistance to synthetic pyrethroids, we conducted a series of sensitivity tests in 1996 and 1997 on many *An. gambiae s.l.* populations in Côte d'Ivoire; this was done in order to find the geographical distribution of that resistance and its level in different agricultural contexts (cultivation of cotton, coffee, cocoa, vegetables, etc.) in the regions surveyed.

A population of *An. gambiae s.l.* that was resistant to permethrin and deltamethrin was found in the immediate vicinity of Bouaké in a low-lying area that is flooded in the rainy season, where the villagers of Yaokoffikro grow rice and vegetables. We have therefore selected the area around the village and its rice paddies to build the six experimental huts, so that we could assess the impact of *An gambiae s.s.* resistance to pyrethroids on the effectiveness of permethrin and deltamethrin treated mosquito nets in standard conditions.

2.2 The sensitivity of the *An gambiae s.s* population of Yaokoffikro to permethrin and deltamethrin

The sensitivity of the *An. gambiae s.s.* population of Yaokoffikro to permethrin and deltamethrin was compared to a susceptible reference strain (Kisumu) from Kenya, and to different generations (F_8, F_{13} and F_{32}) of another strain (Kou) from Burkina Faso. This last strain was found resistant in the field and was then selected with permethrin to increase its resistance. The mosquitos of those two strains plus those of the Yaokoffikro station were identified by PCR (Scott et al, 1993).

The strains were bred at our insectarium in the Institut Pierre Richet.

The tests were conducted with the usual WHO test kits for adult mosquitoes, using control papers and papers treated by our laboratory, at the following concentrations:

- permethrin (25/75) at concentrations of 0.25% (0.091 g a.i./m^2) and 1% (0.364 g a.i./m^2);

- deltamethrin at 0.025% (0.0091 g a.i./m^2) and 0.05% (0.0182 g a.i./m^2).

The concentrations of 0.25% permethrin and 0.025% deltamethrin correspond to the diagnostic concentrations originally recommended by WHO (WHO, 1995). In order to have a more precise idea of the levels of permethrin resistance of the two strains of *An. gambiae s.s* (Yaokoffikro and Kou selected for permethrin) we conducted tests with concentrations of 1% permethrin and 0.05% deltamethrin, which correspond to 4 and 2 times respectively the diagnostic concentrations of those insecticides.

For the control batches and for each concentration of insecticide, five of the WHO tests, each involving 20 *An. gambiae* s.s. females, were conducted. The mosquitos remained in contact with the treated papers for one hour. During that hour of contact, the number of females knocked down at 5, 10, 15, 20, 30, 40, 50 and 60 minutes was noted. The anopheles were then transferred to observation cylinders with a plug of honeyed water, and mortality was noted after 24 hours. KD 95 (the time it took for 95% of mosquitos to be knocked down) for each strain was calculated with the help of Log-Probit software.

2.3 The experimental station

The experimental station consisted of six huts, designed as trap huts, each 3 metres in length, 2.5 metres wide and 2 metres high. The huts were specially built for the study in the most common style of tropical Africa. Each consists of a single living space with breezeblock walls, cement floor, wooden rafters and corrugated iron roof. The materials used were bought in Bouaké and are those generally used for building houses in the region.

The houses are all equipped with controlled openings (the traditional wooden chicanes) that allow mosquitos to go into the hut but prevent them leaving, and a veranda trap of textiglass, the equivalent of an exit for the mosquitos.

The six trap experimental were set parallel to the rice paddy, which is about 50 metres from the huts. The village of Yaokoffikro is on the other side of the rice paddies.

2.4 The insecticides used

The two pyrethroids used for treating the mosquito nets were:

- permethrin, in the form of an emulsifiable concentrate containing 100 g of active ingredient per litre (Peripel ® 10% EC) and

- deltamethrin, in a concentrated suspension containing 25 g of active ingredient per litre (K-Othrine ® SC 25).

Both formulations were supplied to us by the AgrEvo group.

2.5 Mosquito nets and their treatment

The six mosquito nets were single size models measuring 2 m in length, 1 m in width and 1.5 m in height (a surface area of 11 m^2). They were made in Bouaké with multifilament polyester fibre mesh bought locally. Since it is generally found that mosquito nets used in real conditions tend to be in poor condition, we made 225 square holes in them of 4 cm^2 each, accounting for 0.8% of the total surface.

Two mosquito nets were not treated, and they were used as controls.
Two were treated with 500 mg permethrin per m^2.
Two were treated with deltamethrin at 25 mg a.i./m^2.

The mosquito nets were treated on 6 October 1997. They were individually soaked and dried flat for 24 hours in a dark place. They were then wrapped in aluminium foil until they were set up in the trap huts.

No comments were made by the people who treated the mosquito nets, or by the volunteers who slept under them; no sign of irritation was noted.

2.6 Experimental procedure

2.6.1 Distribution of the mosquito nets in the houses

Nets were allocated at random among the huts: first for the number of the hut then for the mosquito net, whether or not it had been treated. The experimental protocol was thus as follows:

Huts 1 and 2: mosquito nets impregnated with 500 mg of permethrin per m^2.

Huts 3 and 5: control mosquito nets.

Huts 4 and 6: mosquito nets impregnated with 25 mg deltamethrin per m^2.

The six mosquito nets were put in the huts one week after they had been treated.

2.6.2 Mosquito collections in the trap huts

Each night, in each hut, one person slept, from 20h00 to 05h00, under the mosquito net. The sleepers systematically changed bed each night to compensate for any particular attraction of individuals. The afternoon prior to the night of collection, all the living mosquitos were removed from each trap hut and the floor of the huts was carefully swept to get rid of any dead mosquitos.

Two mornings per week, the mosquitos were gathered by hand, at 05h00 and at 08h00. The mosquitos captured were identified and then sorted into batches (dead/alive; fed/unfed) and labelled to show the hour and place of capture: within the mosquito net, in the hut (outside the mosquito net, on the walls and ceiling) and in the verandah trap.

The presence of an insecticide or insectifuge in a house can have effects that range from dissuasion to death. In order to evaluate the effect of the treated mosquito nets, we took four entomological criteria into account:

(1) rate of entry = dissuasive effect, obtained by comparing the number of mosquitos captured in the huts with treated mosquito nets to those in the control huts;

(2) exit rate = expulsion effect. This is calculated by comparing the number of specimens found in the huts and in the veranda traps of the control huts (natural exophily) to the numbers in the huts with treated mosquito nets (exophily induced by the presence of insecticide);

(3) blood feeding rate = interference or inhibitory effect, which is calculated by comparing the number of engorged females in the control huts with the number in those with treated mosquito nets;

(4) mortality rate = immediate and delayed mortality. The females found dead (immediate mortality) were identified and counted just after capture. The females captured live were kept for 24 hours in the laboratory in plastic cups with a plug of honeyed water. The mosquitos that died after a day's observation (delayed mortality) were counted. Total mortality was found by adding immediate to delayed mortality.

2.6.3 The bioassays

The purpose of the bioassays (persistency tests) is to check the changes in efficacy of insecticides over time.

The bioassays were conducted once a month with *An. gambiae s.s.* females (Kisumu strain) and with mosquitoes from the local population at Yaokoffikro, using females emerged from larvae gathered in the field and bread at the insectary.

The non-engorged *An. gambiae s.s.* females aged 3-5 days were put in batches of five in plastic cones that were held against the permethrin or deltamethrin impregnated polyester mesh or the control mesh for three minutes. Ten sets of five mosquitos were used for each mosquito net.

After three minutes of contact, the mosquitos knocked down (KD) were counted and kept under observation for 24 hours in the laboratory, to calculate mortality.

3. Results

Identification of mosquitos showed that the Kisumu strain was *An. gambiae s.s.*, the Kou strain was *An. gambiae s.s.*, Mopti form, and the Yaokoffikro strain was *An. gambiae s.s.*, Savanna form.

3.1 Comparing the sensitivity of the Kou and Kisumu strains of *An. gambiae* selected for permethrin and the Yaokoffikro strain

Mortality never exceeded 5% among controls. It was therefore not necessary to correct the mortality rates.

3.1.1 The sensitive Kisumu strain

The Kisumu strain is perfectly susceptible at the diagnostic concentrations of permethrin and deltamethrin, with KD 95 in much less than one hour and systematic 100% mortality (Table 1).

3.1.2 The Kou strain selected for permethrin

– Permethrin (Table 1)

The diagnostic concentration of 0.25% has no more knock down effect and mortality was nil from F_8. At four times the diagnostic concentration (1%), on F_{32}, KD 95 was estimated to more than 24 hours, with 3.9% mortality.

- Deltamethrin (table 1).

At the diagnostic concentration of 0.025%, KD 95 was 5.5 hours on F_{13} with mortality of 13.8%. At twice the diagnostic concentration (0.05%) on F_{32}, KD 95 was 1.5 hours and mortality was 43.3%.

The strain selected therefore was resistant to permethrin and deltamethrin.

3.1.3 The Yaokoffikro wild strain

- Permethrin (table 1).

At concentrations of 0.25% and 1%, the estimated KD 95 was greater than 24 hours and 22.7 hours respectively, with mortality of 15.9% and 29.3%.

- Deltamethrin (table 1).

At the diagnostic concentration of 0.025%, KD 95 was 1.6 hours, with mortality of 67%. At twice the diagnostic concentration (0.05%), KD 95 was one hour with mortality of 79.2%.

This natural population of *An. gambiae s.s.* is therefore clearly resistant to both products.

3.2 General mosquito collection

Over the 26 weeks of the experiment, 2926 female mosquitos were collected:

An gambiae s.s.:	891
An. funestus :	12
An. pharoensis	3
An. coustani :	1
Culex sp. :	1814
Ma. africana + *Ma. uniformis* :	159
Aedes sp. :	46

An. gambiae s.s. accounted for 30.5% and *Culex sp.* for 62.0% of the mosquitos captured in the trap huts, with *C. quinquefasciatus* in the majority.

3.3 The influence of treated mosquito nets on the density and behaviour of *An. gambiae s.s.* and *Culex sp.* in the trap huts.

The results of the captures are shown in table 2 for *An. gambiae s.s.* and in table 3 for *Culex sp.*.

3.3.1 The influence of treated mosquito nets on the density of *An. gambiae s.s. and Culex sp. in the trap huts*

The numbers of *An. gambiae s.s.* caught in the trap huts (table 2) show that entry rates are 18% lower when the mosquitos are treated with permethrin and 43% lower when they are treated with deltamethrin.

For *Culex sp.*, 11% fewer females entered the huts treated with permethrin and 25% fewer entered those treated with deltamethrin (table 3).

3.3.2 The influence of treated mosquito nets on the behaviour of *An. gambiae s.s.* and *Culex sp.* in the trap huts

The numbers of *An. gambiae s.s.* and *Culex sp.* gathered under the mosquito nets, in huts and in the trap huts vary greatly depending on whether the huts have control mosquito nets or treated ones.

3.3.2.1 *An. gambiae s.s.*

(a) Huts with control mosquito nets
In the control huts (table 2), 47% of the *An. gambiae s.s.* females that came in were found within the mosquito nets, 29% in the huts and 24% in the veranda traps (natural exophily).

(b) Huts containing mosquito nets treated with 500 mg permethrin per m^2
After the mosquito nets had been put in place, three times fewer *An. gambiae s.s.* were found within the mosquito nets than in the control huts. However, the numbers of specimens captured in the huts and in the veranda traps were 1.5 and 1.8 times greater respectively than those in the control huts (table 2).

(c) Huts with mosquito nets treated with 25 mg deltamethrin per m^2
The percentage of specimens collected within the mosquito nets was three times lower than in the control huts, but 1.4 times as many females were captured in the huts. In the veranda traps, the percentage of *An. gambiae s.s.* was 1.8 times higher than in the control huts (table 2).

3.3.2.2 *Culex sp.*

(a) Huts with control mosquito nets
The distribution of collected *Culex sp.* was 39% within the mosquito nets, 33% in the huts and 28% in the veranda traps (natural exophily) (table 3).

(b) Huts containing mosquito nets treated with 500 mg of permethrin per m^2
Once those mosquito nets had been installed, the percentage of females collected within the mosquito nets was half that found in the controls, but in the huts it was more or less the same as control. In the veranda traps, we collected 1.7 times more *Culex sp.* than in the veranda traps of the control huts (table 3).

(c) Huts containing mosquito nets impregnated with 25 mg deltamethrin per m^2

The percentage of *Culex* found within the mosquito nets was half that found in the controls; the percentage of mosquitos collected in the huts was similar to control, and 1.6 times more *Culex sp.* were collected in the veranda traps (table 3).

3.4 The influence of treated mosquito nets on the blood feeding of An. gambiae s.s. and *Culex sp.* in the trap huts

In the trap huts the percentage of blood-fed*An. gambiae s.s.* females (table 2) and *Culex sp.* females (table 3) collected depended on whether or not the mosquito nets had been treated.

3.4.1 *An. gambiae s.s.*

In the control huts, 68% of *An. gambiae s.s.* were engorged.

In the huts where the mosquito nets had been treated with permethrin and deltamethrin, the percentages of engorged *An. gambiae s.s.* females were comparable (30%). Thus, in relation to control, the percentage of females engorged was 54% lower for permethrin-treated mosquito nets and 56% lower for deltamethrin-treated nets.

3.4.2 *Culex sp.*

39% of *Culex sp.* in the control huts were engorged, as compared to only 15% in the huts with treated nets, which thus reduced the engorgement rates of *Culex sp.* by some 62% to 65%.

3.5 The influence of treated nets on mortality of *An. gambiae s.s.* and *Culex sp.* in the experimental huts

The percentages of *An. gambiae s.s.* (table 2) and *Culex sp.* (table 3) found dead in the huts with treated mosquito nets was compared to the natural mortality found in the control huts.

3.5.1 *An gambiae s.s.*

(a) Overall mortality

Mortality was low among the *An. gambiae s.s.* females collected in the two control huts (4%; n = 16).

In the huts with treated mosquito nets, overall mortality was 40% (n = 123) for the mosquito nets impregnated with permethrin and 56% (n = 119) for those impregnated with deltamethrin. Overall mortality caused by the mosquito nets impregnated with deltamethrin was therefore significantly higher than that found in huts with permethrin-treated mosquito nets ($X^2 = 13.4$; for 1 ddl, $p<0.05$).

It is worth noting that 83% to 85% of the *An. gambiae s.s.* females found dead in the trap huts with permethrin and deltamethrin-treated mosquito nets were not blood-fed.

(b) Immediate and delayed mortality

For the permethrin-treated mosquito nets, overall mortality was 40%: 88% of that was immediate mortality and 12% delayed.

For the nets treated with deltamethrin, overall mortality was 56%: 92% immediate and 8% delayed.

3.5.2 *Culex sp.*

(a) Overall mortality

In the controls, overall mortality was 6% (n = 44). In the huts with permethrin- and deltamethrin-treated nets, overall mortality was similar, at 39% (n = 239) and 45% (n = 230) respectively.

For permethrin, 96% of the dead females were found unfed, as compared to 94% for deltamethrin.

(b) Immediate and delayed mortality

For permethrin, overall mortality was 39%: 86% immediate and 14% delayed.

For deltamethrin, with 45% of *Culex sp.* found dead, immediate and delayed mortality were 89% and 11% respectively.

3.6 The bioassays

The bioassays conducted on the control mosquito nets with the susceptible Kisumu and Yaokoffikro strains of *An. gambiae s.s.* are shown in table 4. The results of the tests conducted with the mosquito nets impregnated with 500 mg of permethrin per m^2 and 25 mg of deltamethrin per m^2 are shown in table 5 for the Kisumu strain and in table 6 for the Yaokoffikro wild strain.

3.6.1 The susceptible Kisumu strain of *An. gambiae s.s.*

With the control mosquito nets, there was no KD after three minutes contact, and mortality never exceeded 5% (table 4).

For the permethrin-impregnated mosquito nets, KD afetr three minutes was 80% to 100% for the first 14 weeks of the experiment. At week 21, however, KD was only 24%. Mortality was between 90% and 100% until week 14, and was down to 69% on week 21 (table 5).

With deltamethrin, KD at three minutes was much lower than with permethrin. The percentages of mosquitos knock down ranged from 12 to 33, and mortality was 100% (table 5). This low knock down effect can be attributed to the highly irritant effect of this insecticide on the specimens tested.

3.6.2 The Yaokoffikro wild strain of *An. gambiae s.s.*

The tests conducted on the control mosquito nets had no knock down effect after three minutes contact, and mortality did not exceed 6% (table 4).

With the permethrin-impregnated nets, KD at three minutes was 15% on week 2, and never more than 8% to 12% in the weeks that followed. Mortality was 12% in weeks 2 to 6. On week 13, the proportion of dead mosquitos increased (34%), then on week 18 mortality returned to the week 2 level (12%) and dropped to 8% during week 22 (table 6).

For the deltamethrin-impregnated nets, KD at three minutes never exceeded 10% in the first six weeks of the experiment, and mortality was less than 40%. However, on week 13, there was 26% knock down of mosquitos at three minutes, and mortality of 72%. From week 18 onwards, KD at three minutes was once again below 10%, and mortality was between 25% and 29% (table 6).

This temporary increase in mortality rates three months after impregnation of mosquito nets should be noted.

4. Discussion and conclusion

After six months evaluation at the Yaokoffikro experimental station, where *An. gambiae* resistance to pyrethroids is due to the *Kdr* gene (Martinez-Torres et. al. 1997), the results of mosquito collections show that the rates of entry into the trap huts were reduced by 18% and 43% respectively for *An. gambiae s.s.* and by 11% and 25% for *Culex sp.*. Thus the treated mosquito nets always have a certain dissuasive effect, even on populations of *An. gambiae* s.s. that are highly resistant to those products.

In addition to this restricted entry, there is an induced exophilia that increases the exit rate of the mosquitos. Compared to the numbers of mosquitos found under the control nets, there were three times fewer *An. gambiae s.s.* under the treated nets, and half the number of *Culex sp.*. The percentages of *An. gambiae s.s.* and *Culex sp.* collected in the veranda traps of the huts with treated mosquito nets were 1.5 and 2 times higher than in the control huts.

The treated mosquito nets also significantly reduced (by a factor of 2 to 3) blood feeding of *An. gambiae* and *Culex* in the trap huts.

Finally, the treated mosquito nets clearly affected the mortality of mosquitos. In the control huts, mortality was 4% for *An. gambiae s.s.* and 6% for *Culex*. With permethrin and deltamethrin, mortality was 40% and 56% respectively for *An. gambiae s.s.* and 39% and 45% for *Culex sp.*. Examination of the dead mosquitos showed two things: for both *An. gambiae s.s.* and *Culex sp.* immediate mortality from the two insecticides remained very high; it was always much greater (>85%) than delayed mortality (<15%). Although overall mortality was lower in resistant populations of *An. gambiae s.s.*, immediate mortality therefore remained considerable. Furthermore, for both permethrin and deltamethrin, the proportion of mosquitos that died without feeding was always very high: over 80% for *An gambiae s.s.* and over 90% for *Culex sp.*.

Thus in the presence of mosquito nets impregnated with permethrin and deltamethrin, most female mosquitos died without taking their blood meal from humans.

It may be surmised that the irritant effect of the two insecticides prevented the mosquitos from getting under the net and that the females died without being able to feed. The results show that the repellent and killing effects of permethrin and deltamethrin join to limit contact between humans and mosquitos, even if the mosquitos are resistant to the product used for treating the mosquito nets.

The results obtained in 1983 with the susceptible Soumousso strain of *An. gambiae* s.l. (Darriet et al, 1984) can be compared to those obtained in 1998 on the Yaokoffikro resistant strain of *An. gambiae* s.s.:

	An. gambiae s.l. susceptible	*An gambiae* s.s. resistant
	(Soumousso, 1983) holed mosquito nets treated with permethrin 80 mg/m^2	(Yaokoffikro, 1998) holed mosquito nets treated with permethrin 500 mg/m^2
reduction of entry rate into the huts (= dissuasive effect)	70%	18%
percentage *An. gambiae* in veranda traps (= induced exophilia)	97%	43%
reduction of the proportion of engorged *An. gambiae*	18%	54%

The dissuasive effect and the exophily induced by permethrin were greater with the susceptible Soumousso strain than with the resistant Yaokoffikro one. In the area of resistance, however, efficacy in terms of personal protection is maintained, with a significant reduction in the blood feeding rate and an increase in mortality in the huts with treated mosquito nets. Furthermore, in Yaokoffikro, immediate mortality was greater than 85% whereas in Soumousso delayed mortality was 57%. It should be emphasized, however, that the mosquito nets used in the Yaokoffikro experimental station were treated with 500 mg of permethrin per m^2 whereas those in Soumousso had been treated with only 80 mg of permethrin per m^2.

These observations show that even in an area where *An. gambiae s.s.* is resistant to pyrethroids, treating mosquito nets with 500 mg permethrin per m^2 causes a significant reduction in blood feeding of *An. gambiae s.s.* females on human beings, as can be seen from the high percentages of specimens found dead and unfed.

Bioassays show that the Kisumu strain of *An. gambiae s.s.* is perfectly susceptible to permethrin and deltamethrin, and that the mosquito nets treated with those two insecticides remain effective for six months and more.

With the resistant Yaokoffikro strain of *An. gambiae s.s.*, an increase in the effectiveness of the treated mosquito nets was found from week 13 to week 16. This increase in mortality lasted one month. It could be attributed to a fall, over time, of the irritant effect of permethrin and deltamethrin, which would thus increase the time during which the mosquito was in contact with the treated surface, and would therefore increase its lethal effect.

The differences in effect (irritation/deadliness) that depend on insecticide, time and nature of materials treated, should be the subject of a special study that will consider the different levels of susceptibility/resistance of vectors to the products concerned.

One way or another, in an area where *An. gambiae* s.s. is resistant to permethrin and deltamethrin, this study shows that mosquito nets treated with either insecticide remain effective for four months in restricting contact between humans and vectors, and therefore are still an effective means of individual protection. The influence of treated mosquito nets on the incidence of malaria should now be determined in a village in an area where *An. gambiae s.s.* is resistant to pyrethroids. It will also very important to evaluate in an experimental station, insecticides other than pyrethroids, different formulations and different materials to be treated, so as to gain a precise idea of their effectiveness on *An. gambiae s.s.* populations that are resistant to pyrethroids.

References

Chandre F., Darriet F., Manga L., Akogbeto M., Faye O., Mouchet J & Guillet P., 1997. Status of pyrethroid resistance in *Anopheles gambiae* s.l. *Bulletin of the World Health Organization,* in press.

Darriet F., Robert V., Tho. Vien N & Carnevale P., 1984. Evaluation of the effectiveness on malaria vectors of permethrin for the treatment of intact and holed mosquito nets. WHO mimeographed document, WHO/VBC/84.899 and WHO/MAL/84.1008: 20 pages (in French).

Darriet F., Guillet P., Chandre F., N'Guessan R., Doannio J.M.C., Riviére F. & Carnevale P., 1997. Presence and evolution of pyrethroid and DDT resistance in two populations of *Anopheles gambiae* s.s. in West Africa. WHO mimeographed document, WHO/CTD/VBC/97.1001 and WHO/MAL/97.1081: 15 pages (in French).

Elissa Mouchet J., Riviére F., Meunier J-Y & Yao K., 1993. Resistance of *Anopheles gambiae* s.s. to pyrethroids in Côte d'Ivoire. *Ann. Soc. belge de Méd. trop., 73*: 291-294.
Lengeler C., Cattani J & de Savigny D., 1996. Net gain. A new method for preventing malaria deaths. IDRC-WHO pub: 189 pages.

Martinez-Torres D., Chandre F., Williamson M.S., Darriet F., Bergé J.B., Devonshire A.L., Guillet P., Pasteur & Pauron D., 1997. Molecular characterisation of pyrethroid knockdown resistance (Kdr) in the major malaria vector *Anopheles gambiae* s.s. *Insect Molecular Biology, 7 (2)*: 179-184.

Scott J.A., Brogdon W.G. & Collins F.H., 1993. Identification of single specimens of the *Anopheles gambiae* complex by polymerase chain reaction. *Am. J. Trop. Med. Hyg.*, 49: 520-529.

WHO, 1995. Supplies for monitoring insecticide resistance in disease vectors. *WHO Mimeographed document*, WHO/CTD/VBC/95.998 and WHO/MAL/95.1073: 14 pages.

Table 1. KD 95 time (in hours) and percentage mortality of Kisumu and Kou strains of *An. gambiae s.s.* selected for permethrin and Yaokoffikro strain after one hour's contact and 24 hours' observation, with permethrin (0.25% and 1%) and deltamethrin (0.025% and 0.05%)

insecticide (concentration)	permethrin (0.25%)			permethrin (1%)			deltamethrin (0.025%)			deltamethrin (0.05%)		
strain	Kisumu	Kou F_8	Yaokof	Kisumu	Kou F_{32}	Yaokof	Kisumu	Kou F_{13}	Yaokof	Kisumu	Kou F_{32}	Yaokof
numbers	105	60	176	244	102	99	256	58	97	103	97	96
KD 95 (in hours)	0.70	ND	>24	0.28	>24	22.7	0.38	5.5	1.6	0.35	1.5	1.0
mortality after 24 hours	100%	0%	15.9%	100%	3.9%	29.3%	100%	13.8%	67.0%	100%	43.3%	79.2%

ND: not determinable: no KD after 1 hour.

Table 2. Numbers of *An. gambiae s.s.* collected in the huts with control nets, in those with nets treated with 500 mg permethrin/m^2, and in those treated with 25 mg deltamethrin/m^2.

Percentages of *An. gambiae s.s.* found within the mosquito nets, in the huts and in the veranda traps, and percentage of females engorged and dead (females dead without feeding, immediate and delayed mortality).

	controls 2 huts	permethrin 2 huts	deltamethrin 2 huts
Numbers	373	307	211
Percentage within mosquito nets	47%	15%	16%
Percentage in huts	29%	42%	40%
Percentage in veranda traps	24%	43%	44%
Percentage engorged females	68%	31%	30%
Total mortality	4%*	40%	56%
Immediate/total mortality	-	88%	92%
Delayed/total mortality	-	12%	8%
Percentage females dead without feeding	-	85%	83%

* n = 16.

Table 3. Numbers of *Culex sp.* collected in huts with control mosquito nets, nets treated with 500 mg permethrin/m^2 and 25 mg deltamethrin/m^2.

Percentage of *Culex sp.* collected within the mosquito nets, in the huts, and in the veranda traps, and percentage of females engorged and dead (females dead unfed, immediate and delayed mortality).

	controls 2 huts	permethrin 2 huts	deltamethrin 2 huts
Numbers	687	611	516
Percentage within mosquito nets	39%	17%	17%
Percentage in huts	33%	36%	37%
Percentage in veranda traps	28%	47%	46%
Percentage engorged females	39%	15%	14%
Total mortality	6%*	39%	45%
Immediate/total mortality	77%	86%	89%
Delayed/total mortality	23%	14%	11%
Percentage females dead without feeding	79%	96%	94%

* = 44.

Table 4. Results of the bioassays. Percentage KD recorded after 3 minutes contact and percentage mortality of strains of *An. gambiae s.s.* Kisumu and Yaokoffikro after 24 H with control mosquito nets.

Time	Week 1	Week 2	Week 5	Week 6	Week 10	Week 13	Week 14	Week 18	Week 21	Week 22
Strain	Kisumu	Yaokof	Kisumu	Yaokof	Kisumu	Yaokof	Kisumu	Yaokof	Kisumu	Yaokof
Numbers	113	112	114	114	114	99	42	108	112	108
KD 3 minutes in percentage	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Percentage mortality after 24 hours	4.4%	0%	5.3%	6.1%	5.3%	4.0%	4.8%	2.8%	0.9%	3.7%

Table 5. Results of the bioassays. Percentage KD recorded after 3 minutes contact and percentage mortality of the strain of *An. gambiae s.s.* Kisumu (sensitive) after 24 H' with mosquito nets treated with 500 mg/m^2 permethrin and with 25 mg/m^2 deltamethrin

Insecticide	Permethrin					Deltamethrin				
Time	Week 1	Week 5	Week 10	Week 14	Week 21	Week 1	Week 5	Week 10	Week 14	Week 21
Numbers	103	92	98	99	103	113	113	111	51	106
KD 3 minutes in percentage	100%	95.7%	85.7%	79.8%	24.3%	32.7%	27.4%	11.7%	25.5%	25.5%
Percentage mortality after 24 hours	100%	100%	89.8%	100%	68.9%	100%	100%	100%	100%	100%

Table 6. Results of the bioassays. Percentage KD recorded after 3 minutes contact and percentage mortality of the *An. gambiae s.s.* Yaokoffikro strain (resistant) after 24 H with the mosquito nets treated with 500 mg/m^2 permethrin and with the nets treated with 25 mg/m^2 deltamethrin.

Insecticide	Permethrin					Deltamethrin				
Time	Week 2	Week 6	Week 13	Week 18	Week 22	Week 2	Week 6	Week 13	Week 18	Week 22
Numbers	93	96	116	102	102	104	113	105	108	104
KD 3 minutes in percentage	15.0%	8.3%	6.9%	10.8%	11.8%	3.8%	6.2%	25.7%	7.4%	0%
Percentage mortality after 24 hours	11.8%	11.3%	34.5%	11.8%	7.8%	37.5%	33.1%	72.4%	28.7%	25.0%