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# Bifenthrin: A Useful Pyrethroid Insecticide for Treatment of Mosquito Nets

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**ABSTRACT** Bifenthrin, a pyrethroid insecticide already used in agriculture was evaluated in laboratory conditions against susceptible and pyrethroid resistant mosquitoes, as a potential insecticide for treatment of mosquito nets. Two laboratory strains of *Anopheles gambiae* s.s. Giles, the major malaria vector in Africa, and two of *Culex quinquefasciatus* Say, a major pest mosquito in urban areas, were used. Compared with other pyrethroids such as permethrin and deltamethrin, the intrinsic toxicity of bifenthrin, measured by topical application with susceptible strains, was intermediate. By forced tarsal contact on filter papers (cylinder tests) or on netting materials (cone tests), bifenthrin was found slightly more effective against *A. gambiae* than against *C. quinquefasciatus*, in terms of mortality and knock-down effect. With free flying mosquitoes (tunnel tests), bifenthrin was very efficient in killing mosquitoes and inhibiting blood feeding. Against the two pyrethroid resistant strains, bifenthrin was relatively efficient against *A. gambiae* but the impact of resistance was greater with *C. quinquefasciatus*. In tunnel tests, blood feeding remained almost entirely inhibited with the two species despite resistance. The high mortality of susceptible mosquitoes and excellent blood feeding inhibition of susceptible and resistant strains makes bifenthrin a good candidate for treatment of netting materials, particularly in areas where *C. quinquefasciatus*, the main nuisance in urban areas, is resistant to pyrethroids. The slower knock-down and lower irritant effect also makes this insecticide especially attractive when a mass killing effect on mosquito populations is expected.

**KEY WORDS** Bifenthrin, mosquito net, *Anopheles gambiae*, *Culex quinquefasciatus*, malaria, nuisance

INSECTICIDE TREATED NETS using pyrethroid insecticides are effective in reducing malaria morbidity and overall child mortality in a variety of epidemiological conditions (Lengeler 1998). They are now widely used in many tropical countries, particularly in Asia, and have become more popular on the African continent, the area most severely affected by malaria (Lines 1996). To date, six pyrethroid insecticides (alpha-cypermethrin, cyfluthrin, deltamethrin, etofenprox, lambda-cyhalothrin, and permethrin) have been recommended by the World Health Organization (WHO) in the framework of the WHO Pesticide Evaluation Scheme (WHOPES) for the treatment of mosquito nets (Zaim et al. 2000). For most of these products, WHO specifications have been developed for quality control and international trade.

Bifenthrin, a non-alpha cyano pyrethroid insecticide, is used world wide against a range of agricultural pests. It has moderate acute toxicity, as do most of the other pyrethroids recommended for public health. Etofenprox (a non-ester pyrethroid) is classified as unlikely to present acute hazard in normal use. Bifenthrin is classified by WHO as moderately hazardous (WHO 1998a). The WHO/FAO Joint Meeting on Pesticide Residues has allocated an "acceptable daily intake" for humans of 0–0.02 mg/kg body weight on the basis of the "no observed adverse effect level" of 1.5 mg/kg body weight/d from a 1-yr study in dogs using a 100-fold safety factor (FAO 1992). Bifenthrin has a very low vapor pressure (1.81 10–7 mmHg), a low water solubility (<1 $\mu$g/liter), and good stability to hydrolysis and photolysis (2 yr at 50°C under natural daylight). It is non-irritant to skin, virtually non-irritating to eyes on rabbits and presents no skin sensitization on guinea pigs (Tomlin 2000).

Because of these attributes, bifenthrin is potentially a good candidate insecticide for treatment of mosquito nets. Preliminary testing had been made under labo-

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ratory and field conditions, particularly against susceptible and/or pyrethroid resistant strains of *Anopheles gambiae* s.s. Giles, the major malaria vector in Africa, and *Culex quinquefasciatus* Say, a major pest mosquito in urban areas (De Andrade 1990, Finot et al. 1997, Lee et al. 1997, Ali et al. 1999, Curtis et al. 1999, Guillet et al. 2001). The development of pyrethroid resistance in major vector mosquitoes is a very serious concern since insecticide-treated net programs have so far relied entirely on pyrethroids. Resistance is already present in several parts of the world (Malcom 1988, Chandre et al. 1999, Hargreaves et al. 2000). Although all pyrethroids act on the same target site by modifying the gating kinetics of voltage-sensitive sodium channel (Lund and Narahashi 1983), their impact on mosquitoes varies from product to product. Differences in irritantcy, excito-repellency, and knock-down properties may have a significant impact on the overall efficacy of an insecticide (Roberts et al. 2000). In addition, this impact might differ, depending on how insecticides are used e.g., for indoor residual spraying, for treatment of mosquito nets, or for space spraying.

As a new insecticide, bifenthrin has been fully evaluated in the laboratory in the framework of the WHOPES phase I (WHO 1996); and part of those results are presented and discussed in this article.

## Materials and Methods

**Insecticide.** Tests were made with technical grade and formulated product, depending on the type of test. The technical compound used in this study was 91.5% pure and contained at least 97% of the *cis*-isomer. The formulation tested was a Micro-Emulsion containing 0.3% (AI) (Bistar 3 ME, Philadelphia, PA). For tests made with the active ingredient, a comparison was made with permethrin 25 *cis*/75 *trans* and/or deltamethrin using the results obtained and validated in a previous study with the same standard procedure (Finot et al. 1997, Duchon et al. 1998).

**Biological Material.** Two laboratory strains of *A. gambiae* and two of *C. quinquefasciatus* were used. The susceptible strains of *A. gambiae* (Kisumu) originated in Kenya, and *C. quinquefasciatus* (S-Lab) originated in California (Georghiou et al. 1966). They have been colonized for many years and are free of any detectable insecticide resistance mechanism. The resistant strain of *A. gambiae* (VKPR) that originated in Burkina Faso was already strongly resistant to permethrin when collected in the field and has subsequently been maintained under constant permethrin selection at each generation (Darriet et al. 1997). The resistant strain of *C. quinquefasciatus* was collected in Côte d'Ivoire (BKPER) and has also been maintained under continuous selection with permethrin (Chandre et al. 1997). Both strains were homozygous for the knock-down resistance (*kdr*) gene (Martinez-Torres et al. 1998, 1999) with a resistance factor (by topical application) of ≈40-fold (Chandre et al. 2000). The *C. quinquefasciatus* resistant strain also involves monooxygenases. The resistant and susceptible strains were evaluated every 3 mo for resistance status and the R-genotype.

**Substrates and Treatment.** Tarsal contact tests were made using either filter papers treated with technical grade compound or netting material treated with formulated product, as recommended by WHO (1996). Filter papers were treated according to a WHO protocol using acetone solutions of insecticide and silicone oil as the carrier (WHO 1998). The impregnation was done by dripping onto the paper 2 ml of technical grade dissolved in acetone and silicone oil. The paper was then dried for 24 h. The netting material used in this study was a warp knitted multifilament polyester 100 denier, mesh 156 (SiamDutch, Bangkok, Thailand). Pieces of netting (25 by 25 cm) were treated at the recommended dosage for operational use (25 mg/$m^2$) and other samples at one-fourth the recommended dosage (6.25 mg/$m^2$) by using the formulated product diluted with de-ionized water. For treatment, pieces were folded into three equal parts in one direction then the same in the other direction thus creating six layers and then put into a disposable petri dish. A volume of formulation suspension corresponding to specific absorbency of the net was prepared immediately before treatment and was pipetted evenly onto the surface. The pieces of net were then carefully squeezered with fingers (wearing plastic gloves) to ensure a regular distribution of the solution and ensuring that no solution was left over. Once impregnated, nets were kept to dry in the petri dish. Tests were systematically made from 5 to 10 d after treatment to avoid testing deposits of markedly different ages which might have had different impacts on mosquito behavior and affected the results.

**Topical Applications.** The efficacy of an insecticide on a net depends on a number of factors, including the intrinsic toxicity of the insecticide. For adult mosquitoes, activity can be determined by topical application of the insecticide to the adult female mosquito by dropping 0.1 μl of insecticide solution in acetone, with a micro-capillary, onto the upper part of the pronotum of each mosquito that was briefly anesthetized with CO2 and maintained on a cold plate (WHO 1996). Dosages were expressed in nanograms of active ingredient per mg of mosquito body weight. A total of 50 individuals (nonblood fed females, 2–5 d old) was used at each concentration, with at least five concentrations tested. After treatment, the females were maintained at 27 ± 2°C and 80 ± 10% RH in plastic cups with honey solution provided. Mortality was assessed after 24 h in comparison with a control batch of mosquitoes treated with acetone alone. Three replicates with insects from different rearing batches were made on different occasions and the results were pooled. The data were subjected to a computerized log-probit analysis (Raymond et al. 1997) to determine the lethal dosage 50 and 95% (LD50 and LD95) as well as their 95% confidence intervals.

**Tarsal Contact with Treated Filter Papers.** The efficacy of an insecticide on a net also depends upon the behavior of the target when exposed to treated surfaces. This is of particular relevance for insecticides

such as pyrethroids and DDT with irritant and excito-repellent properties. Knock-down and irritant effects resulting from tarsal contact with treated filter paper were measured using WHO susceptibility testing tube for adult mosquitoes (WHO 1998b). Filter paper tests were conducted with a graduated series of dosages using technical grade compounds. Concentrations were expressed in wt:wt percentage of active ingredient in silicone oil. Batches of 25 nonblood fed females, aged 2–5 d, were introduced into holding tubes and maintained for 1 h (adaptation time) at 27 ± 2°C and 80± 10% RH. They were then transferred into the exposure tube, placed vertically for 1 h under subdued light. Since pyrethroids are fast acting insecticides, knocked down mosquitoes at the bottom of the tubes were recorded at regular intervals between 10 and 60 min. The time after which 50 and 95% of mosquitoes are knock down (KD, respectively, KDT50 and KDT95), 95% CL was then determined using log-probit analysis. Mortality was recorded 24 h after exposure. Each concentration was tested four times and each test was repeated four times with different insect batches to take into account inter-batch variability. The minimum concentration that produced 100% mortality with susceptible reference strains was determined. The diagnostic concentration, i.e., double the minimum concentration giving 100% mortality with susceptible strain, was also established.

**Tarsal Contact with Treated Netting Material.** Knock-down and irritant effects resulting from tarsal contact with netting material were measured using WHO cone tests for adult mosquitoes (WHO 1998b). The tests were conducted using the standard WHO plastic cones and a 3-min exposure time. During exposure, the cones were closed with a polyethylene plug so that mosquitoes would not remain on either the cone wall or the plug. Five nonblood fed females 2–5 d old were introduced into cones at a time. Four cones were applied at the same time onto the net sample and tests were made at 25 ± 2°C under subdued light. After exposure, females were grouped into batches of 10 or 20 in 150-ml plastic cups and maintained at 27 ± 2°C and 80 ± 10% RH with honey solution provided. For each sample tested, 50 mosquitoes (10 cones) were used. Knock-down rates were recorded after exposure at fixed intervals of time (every 2–10 min, depending on knock-down rates) up to 60 min after exposure. $KDT_{50}$ and KDT95 with 95% CL were calculated using log-probit analysis. Percentage mortalities were recorded after 24 h and compared by a chi-square test. The Fisher exact test was computed when an expected value was <5. When testing pyrethroids with adult mosquitoes, surviving individuals are commonly found with several legs missing, sometimes up to five. They are alive and able to fly but in the field, they would not be able to survive. To take this phenomenon into account, mortality was recorded in two ways: counting either dead mosquitoes only (real mortality) or including surviving mosquitoes with three legs or fewer (functional mortality). Taking into account "functional" mortality gives additional information and a better estimate of the overall killing effect of a pyrethroid insecticide.

**Irritability Tests.** Nonblood fed 2-to 5-d-old females were introduced individually into plastic cones fitted to treated filter paper or to treated netting material. After an adaptation time of exactly 60 s, the time elapsed between the first landing and the next take off of the mosquito was recorded as the "time for first take off" (Mouchet and Cavalié 1961). Mosquitoes that did not take off at least once during a period of 256 s were discarded. For each test, 50 mosquitoes were tested individually. A simple program using the internal clock of a laptop computer has been developed to run this test and to analyze the results. Mosquitoes are grouped by classes of first take off time and cumulative frequencies are used to calculate the time for 50 and 95% of the mosquitoes to take off (FT50 and FT95). Fairly constant subdued lighting and air temperature (25 ± 2°C) have to be maintained during the test. The number of take offs has also been proposed in the past as a measure of irritability but it is not a reliable indicator, especially for very fast acting insecticides (Mouchet and Cavalié 1961; Hodjati and Curtis 1999; Chandre et al. 2000). Tests in the current study were made using susceptible and resistant mosquito strains at a dosage that killed 100% of susceptible *A. gambiae* on filter paper (0.125%, see Table 2) or netting samples treated at 25 and 6.25 mg/$m^2$ with bifenthrin ME.

**Tunnel Tests.** Tarsal contact tests do not provide a measure of the overall insecticide efficacy under field conditions because of the forced contact which does not allow avoidance behavior of adult mosquitoes in response to the repellent and irritant effects of pyrethroids. To better simulate field conditions, another device has been developed, the tunnel test. This device has given results that were quite comparable to those obtained in the field in experimental huts (Chandre et al. 2000). This was particularly so for mortality and feeding inhibition, which are closely related insecticide properties and the behavioral response of mosquitoes. The basic equipment consisted of a square section glass tunnel (25 by 25 cm), 75 cm long similar to that used earlier by Elissa and Curtis (1995) and described in detail by Chandre et al. (2000). One-third of way along the tunnel, a disposable cardboard frame was placed with the treated netting sample. The surface of netting accessible to the mosquitoes was 400 $cm^2$ (20 by 20 cm) with nine holes (1 cm diameter) precisely positioned. Inside the shorter section of the tunnel, a bait (guinea pig for *A. gambiae* or quail for *C. quinquefasciatus*) was placed, unable to move but available to be bitten by the mosquitoes. At each end of the tunnel, a 30-cm square cage was fitted, covered with polyester netting. In the cage placed at the end of the longer section of the tunnel, ≈100 unfed, 5- to 8-d-old females were introduced at 1800 hours. Females were free to fly in the tunnel but they must contact the treated netting and locate a hole before reaching the bait. After taking a blood meal, females usually fly to the cage at the end of the same section of the tunnel and rest. The fol-

**Table 1. Toxicity of bifenthrin for susceptible *A. gambiae* and *C. quinquefasciatus* adults exposed by topical application (permethrin data are provided as a basis for comparison)**

| Insecticide | $LD_{50}$ (ng/mg mosquito) | | $LD_{95}$ (ng/mg mosquito) | |
|---|---|---|---|---|
| | Value (*N*) | 95% CI | Value (*N*) | 95% CI |
| | *A. gambiae* | | | |
| Bifenthrin | 0.15 (50) | 0.14–0.16 | 0.49 (50) | 0.42–0.59 |
| Permethrin | 1.02 (50) | 0.90–1.18 | 4.45 (50) | 3.40–6.54 |
| | *C. quinquefasciatus* | | | |
| Bifenthrin | 0.16 (50) | 0.13–0.19 | 0.64 (50) | 0.45–0.94 |
| Permethrin | 2.21 (50) | 2.09–2.34 | 3.63 (50) | 3.29–4.23 |

*N*, number tested.

lowing morning, at 0900 hours, females were removed and counted separately from each section of the tunnel and the immediate mortality was recorded. Live females were put in plastic cups with honey solution and delayed mortality was recorded after 24 h. During exposure, cages were maintained in an environmental chamber at 27 ± 2°C and 80 ± 10% RH under subdued light.

Five tunnels were used simultaneously in the same climatic chamber, one serving as the control. Each net sample was used no more than twice, within the same week and then was discarded. Animals used as baits were selected at random. Reduction in blood feeding was assessed by comparing the proportion of blood-fed females, whether they were alive or dead in tunnels with treated and control nets. Irritability was measured as the proportion of mosquitoes that did not pass through the netting, by comparing treated and control tunnels. Overall mortality was measured by combining both immediate and delayed (24 h) mortality of mosquitoes from the two sections of the tunnel. These tests were made with both susceptible and resistant strains at the target operational concentration and one-fourth of this concentration. Percent mortality and blood feeding inhibition were compared using a chi-square test (or Fisher exact test when expected value was <5). All tunnel tests were made in parallel with a control where no insecticide was applied to the net and results were corrected accordingly (Abbott 1925).

## Results

All computerized log-probit analysis have shown no significant heterogeneity in the data (regression lines well fitted by a straight line—$P > 0.05$).

**Topical Applications.** The intrinsic toxicity as measured by LD50 of bifenthrin for adult *A. gambiae* and *C. quinquefasciatus* were similar (Table 1). A small difference was observed in LD95 but it was not significant (overlapping confidence intervals). Depending on the lethal dose parameter and/or mosquito species considered, bifenthrin was 6–14 times more toxic than permethrin.

**Tarsal Contact with Treated Filter Papers.** A minimum concentration of 0.5% was needed to regularly obtain 100% mortality with *C. quinquefasciatus*, but only 0.125% was needed for *A. gambiae* (Table 2). The respective $KDT_{50}$ and KDT95 observed for the two species at these two concentrations were similar, with overlapping confidence intervals. Bifenthrin, at 0.125%, the minimum concentration giving 100% mortality with *A. gambiae*, was five times less toxic than deltamethrin (0.025%) but eight times more toxic than permethrin (1%). The same trend was observed with

**Table 2. Toxicity of bifenthrin for susceptible *A. gambiae* and *C. quinquefasciatus* adults mosquitoes exposed to filter papers (deltamethrin and permethrin data are provided as a basis for comparison)**

| Insecticide | Concn, % | % mortality (*N*) | $KDT_{50}$ (min) | | $KDT_{95}$ (min) | |
|---|---|---|---|---|---|---|
| | | | Value | 95% CI | Value | 95% CI |
| | | *A. gambiae* | | | | |
| Bifenthrin | 0.03125 | 91.0 (400) | 71.7 | 64.4–80.1 | 194.8 | 144.4–267.4 |
| | 0.0625 | 99.5 (400) | 45.7 | 42.7–48.9 | 89.0 | 76.4–103.8 |
| | 0.125 | 100 (400) | 29.1 | 26.8–31.6 | 55.9 | 48.2–64.8 |
| | 0.25 | 100 (400) | 22.0 | 20.1–24.1 | 42.1 | 35.4–50.5 |
| Deltamethrin | 0.025 | 100 (400) | 13.3 | — | 23.5 | — |
| Permethrin | 1 | 100 (400) | 11.3 | — | 17.9 | — |
| | | *C. quinquefasciatus* | | | | |
| Bifenthrin | 0.125 | 92.6 (400) | 54.6 | 44.7–66.7 | 159.0 | 82.2–309.1 |
| | 0.25 | 99.3 (400) | 34.3 | 31.9–36.9 | 84.2 | 70.8–100.4 |
| | 0.5 | 100 (400) | 23.8 | 21.0–27.1 | 42.8 | 32.9–55.8 |
| Deltamethrin | 0.025 | 94.8 (400) | 29.3 | — | 95.5 | — |
| Permethrin | 1 | 98.0 (400) | 14.2 | — | 21.8 | — |

*N*, number tested.

**Table 3. Knock down and mortality of susceptible and resistant *A. gambiae* and *C. quinquefasciatus* exposed under standard WHO cones to netting material treated with bifenthrin ME**

| Concn ($mg/m^2$) | Susceptiblity status | Mortality, % | | | $KDT_{50}$ (min) | | $KDT_{95}$ (min) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Real (*N*) | $\chi^2$ (*P*) | Functional (*N*) | $\chi^2$ (*P*) | Value | 95% CI | Value | 95% CI |
| | | | | *A. gambiae* | | | | | |
| 25 | Suceptible | 41.2 (51) | 16.1 | 60.8 (51) | 32.99 | 6.2 | 5.6–6.7 | 14.0 | 12.0–17.7 |
| | Resistant | 7.6 (53) | (<0.0001) | 7.6 (53) | (<0.0001) | 417.0 | — | 6,286 | — |
| 6.25 | Suceptible | 15.7 (51) | 1.8 | 23.5 (51) | 5.28 | 8.5 | 7.7–9.2 | 21.2 | 18.2–26.3 |
| | Resistant | 7.4 (54) | (>0.05) | 7.4 (54) | (<0.05) | 125.0 | — | 676.0 | — |
| | | | | *C. quinquefasciatus* | | | | | |
| 25 | Suceptible | 2.0 (51) | *F* | 17.6 (51) | 7.1 | 67.0 | 57.0–90.0 | 214.0 | 139–507 |
| | Resistant | 2.0 (51) | (>0.05) | 2.0 (51) | (<0.01) | (NE) | — | NE | — |
| 6.25 | Suceptible | 2.0 (50) | *F* | 2.0 (50) | *F* | 90.0 | 70.0–179 | 250.0 | 141–1,324 |
| | Resistant | 0.0 (50) | (>0.05) | 0.0 (50) | (>0.05) | (NE) | — | NE | — |

*N*, number tested; *F*, Fisher's exact test; NE, no detectable effect.

*C. quinquefasciatus* although the differences were smaller.

At the same concentrations, knock-down time (both KDT50 and KDT95) with bifenthrin was about twice as long as with permethrin and deltamethrin (except with deltamethrin against *C. quinquefasciatus* where similar knock-down treatment values were observed). A suitable diagnostic concentration for bifenthrin would be 0.25% for *A. gambiae* and 1% for *C. quinquefasciatus.*

**Tarsal Contact with Treated Netting Material.** A significant difference in mortality (Table 3) was observed between the susceptible and resistant *A. gambiae* strains, at 25 $mg/m^2$ ($P < 0.0001$) and, to a lesser extent, at 6.25 $mg/m^2$ ($P < 0.05$ when considering functional mortality). No significant difference was noted between the susceptible and resistant *C. quinquefasciatus*, the mortality remaining very low in both strains (except at 25 $mg/m^2$ where $P < 0.01$ for functional mortality). Bifenthrin was far more toxic against susceptible *A. gambiae* than against susceptible *C. quinquefasciatus*, particularly at 25 $mg/m^2$ ($P < 0.0001$ for real and functional mortalities with $\chi^2$ of 47.7 and 63.9, respectively) and, to a lesser extent, at 6.25 $mg/m^2$ (Fisher test, $P < 0.05$ for real mortality and $\chi^2 = 10.44$, $P < 0.005$ for functional mortalities). Knock down times were also ≈10–15 times shorter with susceptible *A. gambiae* than susceptible *C. quinquefasciatus* and 30–500 times longer in resistant than susceptible *A. gambiae.* No knock down was observed with resistant *C. quinquefasciatus.*

**Irritability Tests.** At the minimum concentration giving 100% mortality on filter papers (Table 4), time for the first take off of *An gambiae* was about three times longer with bifenthrin than with permethrin and deltamethrin. It was also 1.5 times longer for susceptible *C. quinquefasciatus* than for susceptible *A. gambiae.* Irritability was greatly reduced (15-fold) in resistant *A. gambiae* and undetectable with resistant *C. quinquefasciatus.*

Results obtained were different with treated netting material (Table 5). *C. quinquefasciatus* was more susceptible than *A. gambiae* to the irritant effect, spending around 40% as long on nets treated at 25 $mg/m^2$ and 60% as long at 6.25 $mg/m^2$. As in filter paper tests, irritability was much less in resistant *A. gambiae* and *C. quinquefasciatus.*

**Tunnel Tests.** Bifenthrin ME at both 25 and 6.25 $mg/m^2$ was very effective in killing between 90 and 100% of the susceptible strains of both mosquito species (Table 6). However, mortality was significantly reduced in resistant *A. gambiae* and was negligible in resistant *C. quinquefasciatus* ($P < 0.0001$ for both species). Interestingly, bifenthrin was highly effective in reducing blood feeding in susceptible as well as resistant mosquitoes of both species (although the effect was significantly less in the resistant than the suscep-

**Table 4. Irritability of susceptible and resistant *A. gambiae* and *C. quinquefasciatus* exposed to filter papers treated with bifenthrin technical grade (deltamathrin and permethrin data are provided as a basis for comparison)**

| Insecticide | Concn, % | Susceptibility status | $FT_{50}$ (seconds) | | $FT_{95}$ (seconds) | |
|---|---|---|---|---|---|---|
| | | | Value (*N*) | 95% CI | Value (*N*) | 95% CI |
| | | | *A. gambiae* | | | |
| Bifenthrin | 0.125 | Suceptible | 18.2 (50) | 15.5–21.5 | 1,237 (50) | — |
| | 0.125 | Resistant | 276.1 (50) | 156–731 | 14,853 (50) | — |
| Deltamethrin | 0.025 | Suceptible | 7.1 (50) | 5.9–8.4 | 34.0 (50) | 25.6–49.8 |
| Permethrin | 1 | Resistant | 6.0 (50) | 5.3–6.9 | 35.9 (50) | 27.7–50.3 |
| | | | *C. quinquefasciatus* | | | |
| Bifenthrin | 0.125 | Suceptible | 29.4 (50) | 25.1–34.8 | 363.5 (50) | 262–542 |
| | 0.125 | Resistant | NE (30) | — | NE (30) | — |

*N*, number tested; NE, no detectable effect.

**Table 5. Irritability of susceptible and resistant *A. gambiae* and *C. quinquefasciatus* exposed to netting material treated with bifenthrin ME**

| Concn (mg/m$^2$) | Suceptibility status | $FT_{50}$ (seconds) | | $FT_{95}$ (seconds) | |
|---|---|---|---|---|---|
| | | Value (*N*) | 95% CI | Value (*N*) | 95% CI |
| | | *A. gambiae* | | | |
| 25 | Susceptible | 26.7 (51) | 21.8–32.7 | 211.0 (51) | 147–345 |
| | Resistant | 66.0 (50) | 44.0–112 | 9,409.0 (50) | — |
| 6.25 | Susceptible | 36.0 (50) | 29.0–45.0 | 320.0 (50) | 209–595 |
| | Resistant | 124.0 (50) | 85.0–214 | 3,664.0 (50) | — |
| | | *C. quinquefasciatus* | | | |
| 25 | Susceptible | 10.9 (50) | 8.9–13.2 | 65.3 (50) | 47.5–102 |
| | Resistant | 172 (50) | 105–359 | 12,136 (50) | — |
| 6.25 | Susceptible | 23.1 (50) | 18.9–28.3 | 177.4 (50) | 124.0–286 |
| | Resistant | 3,148 (31) | — | NE (31) | — |

*N*, number tested; NE, no detectable effect.

tible strain) even at the lowest concentration (79% with *A. gambiae*).

## Discussion

The intrinsic toxicity of bifenthrin, measured by topical application, was almost the same in *A. gambiae* and *C. quinquefasciatus.* However, in bioassays with filter papers or netting materials, bifenthrin was significantly more effective against susceptible *A. gambiae* than against susceptible *C. quinquefasciatus* in terms of mortality and knock-down effect, as observed with other pyrethroids (unpublished data). Nevertheless, in the case of bifenthrin, the difference between the two mosquito species was smaller. Compared with permethrin, the higher intrinsic toxicity of bifenthrin was confirmed at the larval stage by Finot et al. (1997), particularly with *C. quinquefasciatus.* Conversely, the same authors show that bifenthrin is far less toxic than deltamethrin. However, with free flying mosquitoes in tunnel test, bifenthrin was very efficient in killing and inhibition of blood feeding at concentrations as low as 6.25 mg/m$^2$. This emphasizes that the overall effect of pyrethroids on adult mosquitoes is complex, depending upon toxicity, behavioral response, and so on.

The cross-resistance between bifenthrin and permethrin in the tested *kdr* strains is a common feature among all pyrethroids (Chandre et al. 1999). Resistant mosquitoes were less irritated by bifenthrin and were able to stay longer on treated surfaces. Bifenthrin was still killing *kdr A. gambiae* since *kdr* alone induces only a moderate level of pyrethroid resistance (Chandre et al. 2000). The same phenomenon was also observed with pyrethroids such as permethrin, deltamethrin and lambdacyhalothrin (Darriet et al. 2000; Dossou-Yovo et al. 2000). In the *C. quinquefasciatus* strain, having several resistance mechanisms in addition to *kdr* (e.g., mono-oxygenases), resistance impact was greater, except on blood feeding which was almost entirely inhibited. The longer stay of resistant *C. quinquefasciatus* on the treated surface and uptake of insecticide seems to be enough to alter the feeding behavior which may be less affected than mortality by *kdr.*

In view of the high mortality of susceptible mosquitoes and high level of blood feeding inhibition of susceptible and resistant *Anopheles* sp. and *Culex* sp., it is concluded that bifenthrin is a good candidate for treatment of netting materials. The slower knock-down and lower irritant effect, also makes this insecticide especially attractive in a community with high insecticide treated net coverage and where a mass-killing effect on the mosquito population is expected. *C. quinquefasciatus* nuisance in urban areas is the main motivation for most people to use insecticide treated

**Table 6. Efficacy in tunnels (mortality and blood feeding reduction) of netting material treated with bifenthrin ME against susceptible and resistant *A. gambiae* and *C. quinquefasciatus***

| Concn (mg/m$^2$) | Susceptibility status | % mortality (*N*) | $\chi^2$ (*P*) | % blood feeding inhibition (N) | $\chi^2$ or *F* (*P*) |
|---|---|---|---|---|---|
| | | | *A. gambiae* | | |
| 25 | Susceptible | 100 (94) | 92.2 | 97.0 (94) | 5.7 |
| | Resistant | 33.5 (87) | (<0.0001) | 87.2 (87) | (<0.05) |
| 6.25 | Susceptible | 98.5 (84) | 54.3 | 96.7 (84) | 12.2 |
| | Resistant | 48.3 (84) | (<0.0001) | 78.7 (84) | (<0.0005) |
| | | | *C. quinquefasciatus* | | |
| 25 | Susceptible | 90.7 (97) | 145.9 | 100 (97) | *F* |
| | Resistant | 4.1 (97) | (<0.0001) | 93.2 (97) | ($P < 0.05$) |
| 6.25 | Susceptible | 99.0 (96) | 189.0 | 100 (96) | *F* |
| | Resistant | 0.0 (97) | (<0.0001) | 94.4 (97) | (0.059) |

*N*, number tested; *F*, Fisher's exact test.

nets and visible impact of the insecticide on *C. quinquefasciatus*, potentially obtained with bifenthrin, is likely to improve acceptability of treated nets and compliance.

Bifenthrin also has additional potential advantages compared with other pyrethroids. Lower skin irritation when compared with most alpha-cyano pyrethroids and better chemical stability. The latter parameter is of particular relevance when considering the development of "long lasting insecticide-treated mosquito nets."

If the conclusions of this study are confirmed under field conditions, bifenthrin could have comparative advantages over several other pyrethroids. Preliminary studies in experimental huts, at the same concentration (50 mg/$m^2$), has shown bifenthrin to be as effective as deltamethrin in killing resistant *A. gambiae* (81.4 versus 85.5% mortality) but significantly more effective in killing multi-resistant *C. quinquefasciatus* (53 versus 31%). Bifenthrin may also be promising for combined treatment of nets using a pyrethroid and a nonpyrethroid insecticides applied to different parts for the net (Guillet et al. 2001).

Comparative laboratory testing and evaluation of bifenthrin with other WHO recommended insecticides for treatment of mosquito nets as well as comparative field studies are, therefore, recommended.

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