Combining Piperonyl Butoxide and Dinotefuran Restores the Efficacy of Deltamethrin Mosquito Nets Against Resistant *Anopheles gambiae* (Diptera: Culicidae)

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ABSTRACT One strategy suggested for the management of mosquito insecticide resistance consists of combining a pyrethroid with an insecticide that has a different mode of action. To restore the efficacy of deltamethrin (pyrethroid) against pyrethroid-resistant strain of *Anopheles gambiae* Giles (VKPR: homozygous *Kdr*), deltamethrin was combined with the neonicotinoid insecticide dinotefuran and piperonyl butoxide (PBO). Bednets impregnated with deltamethrin, dinotefuran, and PBO alone and in combination were tested in the laboratory. Knockdown (KD) and mortality were measured using WHO cone tests on susceptible and pyrethroid-resistant adult mosquitoes. The combination of deltamethrin and PBO was synergistic against resistant female *An. gambiae* (58.2% mortality). Both mortality and knockdown time ($KDt_{50/95}$ values) of the tricomponent mixture on the VKPR strain were similar to the insecticidal activity of deltamethrin on the pyrethroid-susceptible KIS strain (98.8 and 100% mortality, respectively). The three-compound mixture of deltamethrin + PBO + dinotefuran showed an insecticidal efficacy greater than the deltamethrin + PBO mixture to the extent of completely restoring the efficacy of deltamethrin on pyrethroid-resistant *An. gambiae*.

KEY WORDS deltamethrin, dinotefuran, piperonyl butoxide, resistance management, synergy

Mosquito nets impregnated with insecticides are commonly used for the control of malaria vectors in Sub-Saharan Africa (Lengeler 1998). Only pyrethroid insecticides are used currently because of their speed of action, high repellency and irritation of mosquitoes, and low toxicity for humans. However, *Anopheles gambiae* s.l. Giles, a major malaria vector in Africa, has become resistant to the majority of these insecticides (Darriet 2007) thus representing a threat to the efficacy of vector control programs based on pyrethroids for indoor residual spraying or long-lasting nets. The development of new families of chemicals acting against novel targets requires years of laboratory and field research and no new public health insecticides are likely to be marketed for the foreseeable future. One strategy suggested for the management of resistance in malaria vectors consists of combining a pyrethroid with an insecticide which has a different mode of action. The combination of a pyrethroid (bifenthrin) and a carbamate (carbosulfan) impregnated on mosquito nets had a synergistic effect on mortality of adults of *An. gambiae* susceptible to insecticides (Corbel et al. 2002). The mixture of chlorpyrifos-methyl and bifenthrin also exhibited synergistic toxicity on the adults of *An. gambiae* susceptible to insecticides but not on those resistant to pyrethroids (Darriet et al. 2003). Little additional research has been done on the combinations of two adulticides with different modes of action.

We carried out a laboratory study to examine the effects of a combination of deltamethrin with the neonicotinoid dinotefuran and a synergist and nonspecific esterase inhibitor piperonyl butoxide (PBO) on susceptible and pyrethroid-resistant strains of *An. gambiae*. Deltamethrin (insecticide) is a pyrethroid particularly effective against the malaria vectors. Like all pyrethroids, this compound acts by modifying the gating kinetics of the sodium channels that operate in the transmission of the nerve impulse in insects (Lund and Narahashi 1983). Dinotefuran (insecticide) is an agonist of the nicotinic acetylcholine receptor, affecting the synapses in the central nervous system (Tomizawa and Yamamoto 1993). This neonicotinoid insecticide is used in agriculture for the control of hemipterous and other pests (Tomlin 2000). The absence of cross-resistance of dinotefuran with common insecticides (pyrethroids, carbamates and organophosphates) makes neonicotinoids potential candidates for disease vector control (Corbel et al. 2004). PBO is a well-known inhibitor of oxydases, widely used as a synergist for insecticide formulation (Bernard and Philogène 1993).

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Table 1. Activity of deltamethrin at an operational dose against the insecticide-susceptible strain of *An. gambiae* (KIS)

	Dose (mg/m^2)	Slope of KDt (±SE)	KDt_{50} (min) (95% CI)	KDt_{95} (min) (95% CI)	% mortality
Deltamethrin	25	4.7 (±0.42)	8.1 (7.4–8.9)	18.2 (16.1–21.4)	100

Materials and Methods

Mosquito Strains. Two strains of *An. gambiae* were used in this study. A susceptible strain (KIS) originating from Kisumu in Kenya and a strain highly resistant to pyrethroids (VKPR: homozygous for knockdown [KD]resistance, *Kdr*) originating from Kou Valley in Burkina-Faso have been maintained for >15 yr under laboratory conditions (27 ± 2°C and 80% RH). The VKPR strain was already highly resistant to pyrethroid in the field and has been maintained subsequently under constant permethrin selection (Darriet et al. 1997).

Insecticides and Synergist. A suspension concentrate (SC) at 10 g (AI)/liter (Crackdown, Bayer Crop Science, Monheim am Rhein, Germany) was used. The bioassays were made by using an 89.8% technical grade of dinotefuran (Mitsui Chemicals, Tokyo, Japan). The bioassays were carried out by using a 90% technical grade of PBO (Fluka, Buchs, Switzerland).

Mosquito Net Impregnation. Pieces of netting (multifilament polyester 100 deniers) 25 by 25 cm were treated by a volume of insecticide suspension corresponding to the specific absorbency of the net (60 ml/m^2). Deltamethrin was tested at the recommended dosage for mosquito net operational use: 25 mg/m^2. For dinotefuran and PBO, the dosages selected are those that separately induced a low range of mortality (4–40%) in the VKPR strain, thus allowing a better detection of any synergism effect. Dinotefuran and PBO alone were tested at 367.34 and 222.24 mg/m^2, respectively. The mixtures of deltamethrin + PBO, dinotefuran + PBO, and deltamethrin + dinotefuran + PBO were done using the same concentrations as the insecticides or synergist alone.

Cone Bioassays. Knockdown time and mortality were measured using WHO cone tests for adult mosquitoes. The tests were conducted using WHO plastic cones and a three-min exposure time (WHO 1998). Ten nonblood-fed females (2 to 5 d old) were introduced into the cone. Four cones were applied at the same time onto the net sample, and the tests were carried out at 25 ± 2°C under subdued light. For each sample of netting impregnated with insecticides or synergist, alone or in combination, 16 cones (n = 160 mosquitoes) were used. Knockdown mosquitos were recorded up to 60 min after exposure. The percentages of mortality were recorded after 24 h.

Statistical Analyses. By comparing the results obtained for each mixture with the results theoretically expected in the absence of any interaction (additive effect), it was possible to determine the effect of the different combinations of the three compounds on adult mosquitoes. The expected mortality (Corbel et al. 2002) was calculated by 1 – (adult survival % on deltamethrin × adult survival % on PBO) for the deltamethrin + PBO mixture; 1 – (adult survival % on dinotefuran × adult survival % on PBO) for the dinotefuran + PBO mixture, and 1 – (adult survival % on deltamethrin × adult survival % on dinotefuran × adult survival % on PBO) for the deltamethrin + dinotefuran + PBO mixture. We consider that there is synergy (positive interaction) when the observed mortality is significantly higher (by Fisher exact test) than the expected mortality. There is antagonism (negative interaction) when the observed mortality is significantly lower than the expected results. KDT_{50} and KDT_{95} with a 95% CL were calculated using logprobit analysis (Raymond et al. 1997). The $KDt_{50/95}$ values were tested comparing the overlap of confidence intervals of the mixtures with the individual compounds. $KDt_{50/95}$ values with overlapping 95% CL were not considered to be significantly different.

Results and Discussion

Deltamethrin induced 100% mortality on the KIS strain, and KDt_{50} and KDt_{95} values were 8 and 18 min, respectively. The mortality on the VKPR strain from deltamethrin was only 7.5%, with significantly higher KDt_{50} (31 min) and KDt_{95} (194 min). Mortalities of the VKPR strain to dinotefuran and PBO were 39 and 4%, respectively, and neither compound induced a KD effect (Tables 1 and 2).

The mixture of deltamethrin and PBO produced a synergistic effect on the VKPR strain that was perceptible in the KD effect and mortality. The KDt_{50} and KDt_{95} of 13 and 36 min, respectively, decreased significantly ($P < 0.05$) compared with the KDt recorded with deltamethrin alone (KDt_{50} = 31 min and KDt_{95} = 194 min). In the same way, the mortality observed with the mixture (58%) was almost 5 times higher than expected (12%) [synergistic effect: $P < 0.0001$]. Although knockdown times were reduced, and mortality increased significantly relative to deltamethrin alone for the VKPR strain, the addition of PBO did not completely restore the efficacy of deltamethrin (cf. $KDt_{50/95}$ or mortality for KIS). For the dinotefuran and PBO mixture, no similar effect was observed on the VKPR strain either for KD or mortality. On the contrary the significant difference (P = 0.014) between expected and observed mortalities indicated that adding PBO reduced the efficacy of dinotefuran (antagonism effect, Table 2).

The mixture deltamethrin + dinotefuran + PBO generated a synergistic insecticidal action against VKPR that was stronger than the efficacy of each of the three components alone ($P < 0.0001$). The three-compound mixture was also more effective than the synergistic deltamethrin + PBO mixture. Most importantly, the deltamethrin + dinotefuran + PBO mixture

Table 2. Activity of deltamethrin, PBO, and dinotefuran alone and in combination against a pyrethroid-resistant strain of *An. gambiae* (VKPR)

	Dose (mg/m^2)	Slope of KDt (±SE)	KDt_{50} (mn) (95% CI) observed [expected]	KDt_{95} (mn) (95% CI) observed [expected]	% mortality observed [expected]	P^a (Fisher's exact test)
Deltamethrin	25	2.05 (±0.40)	30.5 (24.7–35.3)	193.5 (120.6–541.9)	7.5	
PBO	222.24	ND[b]	ND	ND	4.1	
Dinotefuran	367.34	ND	ND	ND	39.0	
Deltamethrin + PBO	25 + 222.24	3.6 (±0.81)	**12.6**[c] **(9.6–16.6)** [30.5] [24.7–35.3]	**36.1 (24.9–53.3)** [193.5] [120.6–541.9]	**58.2** [12.0]	**<0.0001**
Dinotefuran + PBO	367.34 + 222.24	ND	ND	ND	**28.1** [42.0]	**0.014**
Deltamethrin + PBO + dinotefuran	25 + 222.24 + 367.34	4.7 (±0.64)	10.4 (8.8–11.6) [12.6] [9.6–16.6]	23.2 (20.0–29.2) [36.1] [24.9–53.3]	**98.8** [46]	**<0.0001**

[a] $P < 0.05$, synergistic or antagonistic effect; >0.05, additive effect of the insecticides.
[b] ND, not detectable.
[c] Bold indicates the KDt_{50} or KDt_{95} or mortality differed statistically from the expected value.

restored the insecticide efficacy of deltamethrin on the females of *An. gambiae* resistant to pyrethroids. The efficacy of the tricomponent mixture on VKPR in terms of mortality and KD effect was similar to the insecticidal activity of deltamethrin on the susceptible KIS strain. As for the KDt, the increased activity of the mixture translates by a slope in the probit regression line on VKPR (4.7) similar to the slope of deltamethrin alone on KIS (4.7).

Deltamethrin (type II pyrethroid, Tables 1 and 2) is very potent at increasing the rate of miniature excitatory postsynaptic potentials, indicating a stimulation of acetylcholine release within the synaptic gap (Salgado et al. 1983). Although the deltamethrin dose of 25 mg/m^2 is not sufficient to cause significant mortality in the resistant VKPR strain, the addition of PBO increased its efficacy against pyrethroid-resistant mosquitoes, suggesting that the acetylcholine concentration within the synaptic gap probably also increased. The concomitant action of enhanced acetylcholine concentration in the synaptic gap and inactivation of nicotinic receptors by dinotefuran probably explains the strong synergy observed after exposure to the three-compound mixture, which caused nearly 100% mortality in a pyrethroid-resistant strain of *An. gambiae.* A field evaluation of this combination should be planned according to the recommendations of WHO for the phase 2 evaluation of bednets (WHO 2005).

The combinations of insecticides with different modes of action could make an important contribution in the fight against mosquitoes, notably in regions where mosquitoes already show high levels of resistance to conventional insecticides. The availability of new families of insecticides has been scarce during the last 10 yr, and relying on the breakthrough of new products is not a realistic option for the control of resistant populations in the short to medium-term future. However, the option of associating insecticides with different modes of action is a possible alternative strategy for resistance management. Nowadays, pyrethroid-impregnated bednets are threatened with inefficacy, and it is urgent to find new insecticide mixtures capable of generating synergistic interactions against vectors of malaria.

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