

Chlorfenapyr: A pyrrole insecticide for the control of pyrethroid or DDT resistant *Anopheles gambiae* (Diptera: Culicidae) mosquitoes

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Abstract

Owing to the development and spread of pyrethroid resistance in *Anopheles gambiae* in Africa there is an urgent need to develop alternative insecticides to supplement the pyrethroids. Chlorfenapyr is a pyrrole insecticide first commercialized for the control of agricultural pests and termites. Performance against *An. gambiae* bearing *kdr* (pyrethroid and DDT resistance) or *Ace-1^R* insensitive acetylcholinesterase (organophosphate and carbamate resistance) mechanisms was studied using a variety of adult bioassay tests including a simulated-experimental hut system (tunnel tests) that allows uninhibited mosquito behaviour/insecticide interactions. Strains resistant to pyrethroids and organophosphates showed no cross resistance to chlorfenapyr. In cone bioassays on treated netting the mortality of adult mosquitoes showed an unexpected curvilinear response, with highest mortality occurring at intermediate dosages. Adults expressed irritability to chlorfenapyr at higher dosages, which might explain the dosage–mortality trend. Toxic activity of chlorfenapyr was slow compared to conventional neurotoxic insecticides and additional mortality occurred between 24 h and 72 h. In tunnel tests, the dosage–mortality trend showed a more typical sigmoid response and most mortality occurred during the first 24 h. Mosquito penetration through the holed, treated netting showed only limited inhibition and blood-feeding was not inhibited. Mortality rates in the *kdr* strain exposed to chlorfenapyr treated netting in tunnel tests were much higher than with permethrin treated netting over the same 100–500 mg/m² dosage range. Chlorfenapyr has potential for malaria control in treated-net or residual spraying applications in areas where mosquitoes are pyrethroid resistant. For treated-net applications chlorfenapyr might be combined with pyrethroid as a mixture to provide personal protection as well as to give control of resistant mosquitoes.

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1. Introduction

Malaria is a major cause of morbidity and mortality in Africa and Asia but is preventable through interventions

of insecticide treated nets (ITN) or indoor residual spraying (IRS) (Curtis et al., 1998; Rowland, 1999; Rowland et al., 2000). Pyrethroids are widely used for control of anophelines and are the only insecticides currently recommended for use on mosquito nets (Zaim et al., 2000). In the last decade, resistance to pyrethroids has become widespread in *Anopheles gambiae* in western and eastern Africa and in *An. funestus* in southern Africa

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(Chandre et al., 1999a; Etang et al., 2003; Hargreaves et al., 2000; Nikou et al., 2003; Vulule et al., 1999). A number of resistance mechanisms appear to be involved: *kdr* site insensitivity is prevalent in *An. gambiae* and enhanced oxidase mechanisms occur in both *An. gambiae* and *An. funestus*. Resistance is a threat to the future of ITNs and IRS but the distribution of resistance is patchy and the seriousness of the problem differs from region to region. For example, *kdr* resistance is present at high frequency in parts of the Ivory Coast but trials of pyrethroid-treated nets in that country continue to show effectiveness against malaria (Darriet et al., 2000; Asidi et al., 2004; Henry et al., 2005; Hougard et al., 2003a). Elsewhere in Ivory Coast or in neighbouring countries (Burkina Faso and Benin) there are instances of *kdr* homozygotes showing survival in the presence of ITN (Kolaczinski et al., 2000; Diabate et al., 2006) or of ITN or IRS failing to control resistant *An. gambiae* (N'Guessan et al., 2007). In South Africa IRS with deltamethrin failed to prevent the reappearance of *An. funestus* in resistant form or to stem the rise in malaria cases (Hargreaves et al., 2000). There is an urgent need to develop alternative insecticides to replace or supplement the pyrethroids before the situation grows worse (Zaim and Guillet, 2002). Whereas a number of insect growth regulators and bacterial insecticides have been registered for larval control in recent years, no new class of chemistry has emerged for adult control since the development of the pyrethroids more than 20 years ago. Malaria control is a small, rather risky market and the main pesticide manufacturers concentrate their efforts on developing pesticide for the agricultural sector where revenues and profits are larger and more reliable. Several new classes of agro-pesticide chemistry have emerged in recent years, but the majority seem unsuitable for adult mosquito control. The spinosads have potential as larvicides rather than adulticides (Romi et al., 2006), some tested neonicotinoids seem insufficiently active (Corbel et al., 2004a) and the fiproles show cross resistance to older classes of insecticide (Kolaczinski and Curtis, 2001). The primary characteristics required in a good adulticide are contact activity, long residual activity and low mammalian toxicity. These criteria are less essential in agro-insecticides. One exception that may meet the vector control criteria is chlorfenapyr, a member of the pyrrole class, used commercially for termite control and crop protection against a variety of insect and mite pests (Lovell et al., 1990; Pimprale et al., 1997; Sheppard and Joyce, 1998). Chlorfenapyr is a pro-insecticide that is activated by cytochrome P450 monooxygenases to its more active metabolite (Black et al., 1994). Unlike pyrethroids that affect nerve function chlorfenapyr is a

mitochondrial electron transport inhibitor (METI) whose mode of action is to disrupt the conversion of ADP to ATP (oxidative phosphorylation) in mitochondria of cells (Anon., 1995). Chlorfenapyr facilitates proton loss from inside to outside the mitochondria. When uncoupled from a proton energy source the mitochondria are unable to generate ATP and the cells cease to function (Anon., 1995). Because chlorfenapyr's mode of action is novel it is unlikely to show cross resistance to standard neurotoxic insecticides. It has a WHO toxicological classification III: an LD50 oral toxicity in rats of >400 mg/kg body weight, acute dermal toxicity of >2000 mg/kg, and inhalation toxicity of 1.9 mg/l. Chlorfenapyr is placed in the category of "slightly hazardous" to humans (Tomlin, 2000), the same category as many insecticides recommended by WHO for public health use. In recent laboratory tests against the mosquito *Aedes aegypti* it showed efficacy against larvae and adults (Paul et al., 2006).

The aim of the present project was to examine the efficacy and personal protection potential of chlorfenapyr against insecticide-resistant and susceptible *An. gambiae* and *An. stephensi* to assess its suitability for use in Africa and Asia as a residual insecticide on netting or other indoor surfaces.

2. Material and methods

2.1. Mosquito strains

Five laboratory colonies were used:

- *An. gambiae* Kisumu; a susceptible reference strain, from Kenya.
- *An. gambiae* Vkpr; pyrethroid resistant, fixed for *kdr*, from the Kou valley in Burkina Faso.
- *An. gambiae* Yao; resistant to organophosphates and carbamates conferred by insensitive acetylcholinesterase, and to pyrethroids conferred by *kdr*, from Yaokoffikro, Bouake, Ivory Coast. The frequency of the *Ace-1^R* allele was 100%.
- *An. stephensi* Beech; a susceptible reference strain.
- *An. stephensi* Dub234; resistant to pyrethroids and OPs, conferred by cytochrome P450 oxygenases, esterases and *kdr*-type target insensitivity, from Dubai.

2.2. Residual activity and cross resistance

The residual activity of chlorfenapyr and potential cross resistance in *An. gambiae* Kisumu, Vkpr and Yao strains was assessed by exposing adult females, 3–5 days of age, in WHO cylinder kits to filter paper impregnated

with 2% chlorfenapyr in silicon oil for periods upto 3 h; mortality was scored 24–72 h later. Test papers were initially treated with chlorfenapyr (BASF Corp, USA) dissolved in acetone (1.3 ml) and silicon oil (0.7 ml) (WHO, 1998). Approximately 100 females were tested at each exposure period. LT50s, LT95s and resistance ratios were estimated using probit analysis (Polo-PC, LeOra Software, Berkeley, CA).

Supplementary assays were performed using papers treated with 0.125–4% chlorfenapyr in silicon oil. Approximately 200 females were exposed to each concentration for 1 h and mortality scored 24–72 h later.

Activity of chlorfenapyr on *An. stephensi* Beech and Dub234 strains was assessed by exposing females to a range of chlorfenapyr concentrations for 1 h. Approximately 100 mosquitoes were tested per concentration.

2.3. Cone and wire ball bioassays

The WHO cone test, in which mosquitoes are exposed to pyrethroid-treated netting for 3 min within a plastic cone is the standard way to measure insecticide efficacy (WHO, 1998). An alternative approach is the wire ball bioassay (WHO, 2006), a metal frame completely covered with netting which has the advantage of presenting no non-insecticidal surfaces for mosquitoes to alight upon or avoid insecticide contact. Formulated chlorfenapyr ('Phantom' 15% SC) from BASF Corp was tested on 100-denier netting in cone and wire ball bioassays against *An. gambiae* Kisumu over a range of concentrations (5–1000 mg/m²) and exposure times (3–24 min). Mortality was scored after 24 h, 48 h and 72 h, and data analysed using logistic regression (STATA 6 software) or probit analysis (Polo-PC, LeOra Software, Berkeley, CA).

2.4. Irritability test

Two chlorfenapyr concentrations (100 mg/m² and 1000 mg/m²) were tested on netting for their irritancy to individual non-blood-fed Vkpr *An. gambiae* females, aged 2–5 days, in WHO plastic cones. After a settling period of 60 s the time elapsing until the next take-off was recorded as the "time to first take-off" (Mouchet and Cavalié, 1961). For each concentration and control, 50 mosquitoes were tested. Mosquitoes were grouped into geometric classes of 'time to first take-off' (0–1 s, >1–2 s, >2–4 s, >4–8 s, ..., >128–256 s). Probit analysis was used to calculate the time for 50% of mosquitoes to take-off (FT₅₀).

2.5. Tunnel tests

The same dosages of chlorfenapyr used in cone bioassays were evaluated in tunnel tests against pyrethroid-resistant *An. gambiae* (Vkpr). The tunnel test is a laboratory system designed to allow mosquitoes to freely express behavioural responses to insecticide that would occur when encountering ITNs under field conditions. Tunnel tests are used as a forerunner to experimental hut trials, and provide useful information on repellency, blood-feeding inhibition and mortality (WHO, 2006). The equipment consists of a 4-sided glass cylinder (25 cm high, 25 cm wide, 60 cm long) which is divided into two chambers by a netting insert which slots across the tunnel. In one of the chambers, a guinea pig is housed unconstrained in an open meshed cage and in the other chamber ca. 100 unfed female mosquitoes aged 2–5 days are released at dusk and left overnight. The netting is deliberately holed with nine 1 cm holes to give mosquitoes opportunity to penetrate into the baited chamber. The following morning, the number of mosquitoes alive or dead, fed or unfed in each chamber are scored. Live mosquitoes are given access to sugar solution and kept up to 72 h to record delayed mortality. Tunnel test data was analysed using analysis of variance of arcsine transformed proportions (STATA 6 software).

3. Results

3.1. Cross resistance

Log-time probit analyses of the mortality of susceptible and resistant strains of *An. gambiae* to 2% chlorfenapyr papers in WHO kits are presented in Table 1. A significant 2.5-fold tolerance to chlorfenapyr was expressed in the Vkpr strain but not in the Yao strain. A tolerance of this magnitude could be due to inter-strain variation, and was not apparent in the Yao strain which also carries *kdr*. With *An. stephensi* the toxicity of chlorfenapyr was similar in pyrethroid susceptible and resistant strains (Table 2).

Mortality of *An. gambiae* Kisumu induced by chlorfenapyr over a 0.125–4% range of concentrations showed a log-linear response. Owing to the delayed activity of chlorfenapyr, the LC₅₀ decreased 3-fold between 24 h and 48 h and 14-fold between 48 h and 72 h (Tables 3A and 3B). The LD₅₀ of 0.04% at 72 h represents the value computed when all insecticide-induced mortality had taken effect. The confidence interval is wide at this point because even the lowest dosage tested was killing more than 50% at 72 h.

Table 1

Log-time mortality probit analysis of 2% chlorfenapyr treated papers against *An. gambiae* susceptible and pyrethroid/OP strains in WHO cylinder kits after 24 h holding period

Strain	Slope (S.E.)	LT50 (mn) (95% CI)	LT95 (mn) (95% CI)	RR50 (95% CI)
Kisumu (S)	3.4 (0.35)	17.4 (11.4–23.7)	53.6 (36.4–126.8)	–
Vkpr (<i>kdr</i>)	2.6 (0.20)	43.7 (29.5–58.4)	184.0 (123.9–400.5)	2.5 (2.2–2.9)
Yao (<i>Ace-I</i>)	2.7 (0.17)	18.9 (5.6–34.9)	137.0 (64.4–1729)	1.1 (0.9–1.3)

RR50 represents the resistance factor for *kdr* or *Ace-I* strains relative to the susceptible strain at LT50 level.

Table 2

Log-dosage–mortality probit analysis of chlorfenapyr against *An. stephensi* pyrethroid susceptible (S) and resistant (R) strains in WHO cylinder kits after 24 h holding periods

Strain	Slope	LD50	LD95	RR50
Beech (S)	2.08 (0.19)	0.87 (0.70–1.08)	5.3 (3.5–10.5)	1.14 (0.85–1.51)
Dub234 (R)	2.30 (0.40)	0.99 (0.77–1.25)	5.1 (2.9–12.2)	

RR50 represents the resistance ratio between the two strains at the LD50 level. LD50 and LD95 are expressed as the % concentration of chlorfenapyr in silicon oil.

Table 3A

Summary of log-dosage–mortality probit analysis of chlorfenapyr against *An. gambiae* Kisumu in WHO cylinder kits after 24 h, 48 h and 72 h holding periods

Holding period (h)	Slope (S.E.)	LD50	LD95	TR50
24	1.9 (0.1)	0.6 (0.5–0.7)	4.6 (3.5–6.7)	–
48	1.6 (0.14)	0.2 (0.1–0.3)	2.3 (1.3–6.6)	2.6 (2.2–3.2)
72	1.2 (0.1)	0.04 (0.01–0.08)	1.0 (0.6–2.3)	14.3 (8.5–24.1)

LD50 and LD95 are expressed as % concentration of chlorfenapyr in silicon oil. TR50 represents the toxicity ratio at LD50 level at 24 h relative to that at 48 h and 72 h.

Table 3B

Mortality (%) of *An. gambiae* females (Kisumu, susceptible strain) after 1 h exposure to chlorfenapyr impregnated papers in WHO cylinder kits

	Chlorfenapyr concentration (no. of tested)						
	0% (205)	0.125% (180)	0.25% (213)	0.5% (200)	1% (206)	2% (167)	4% (227)
24 h	2.4	12.2	22.5	47.0	68.9	85.6	91.6
48 h	3.4	38.3	57.7	66.0	80.6	96.4	100
72 h	3.4	73.3	84.0	89.5	92.2	98.8	100

Mortality was recorded after 24 h, 48 h and 72 h holding periods.

Exposure to 4% chlorfenapyr for 1 h induced *ca.* 90% mortality after 24 h and 100% mortality after 48 h in the Kisumu susceptible, Vkpr and Yao strains, indicating no cross resistance between chlorfenapyr and *kdr* and *Ace-I^R* resistance mechanisms (Table 4).

3.2. Cone and wire ball tests

Mortality of mosquitoes to chlorfenapyr showed delayed expression in cone tests on treated netting; chlorfenapyr took up to 72 h to show full effect ($P < 0.001$).

Table 4

Mortality rates (%) of pyrethroid/OP resistant and susceptible *An. gambiae* on exposure to papers impregnated with 4% chlorfenapyr for 1 h

Post-exposure period (h)	Kisumu susceptible (N=99)	Vkpr <i>kdr</i> (N=102)	Yao <i>Ace-I^R</i> (N=122)
24	91.6	89.2	92.6
48	100	100	100

Mortality was recorded after 24 h and 48 h. Control mortality was <3% after 48 h.

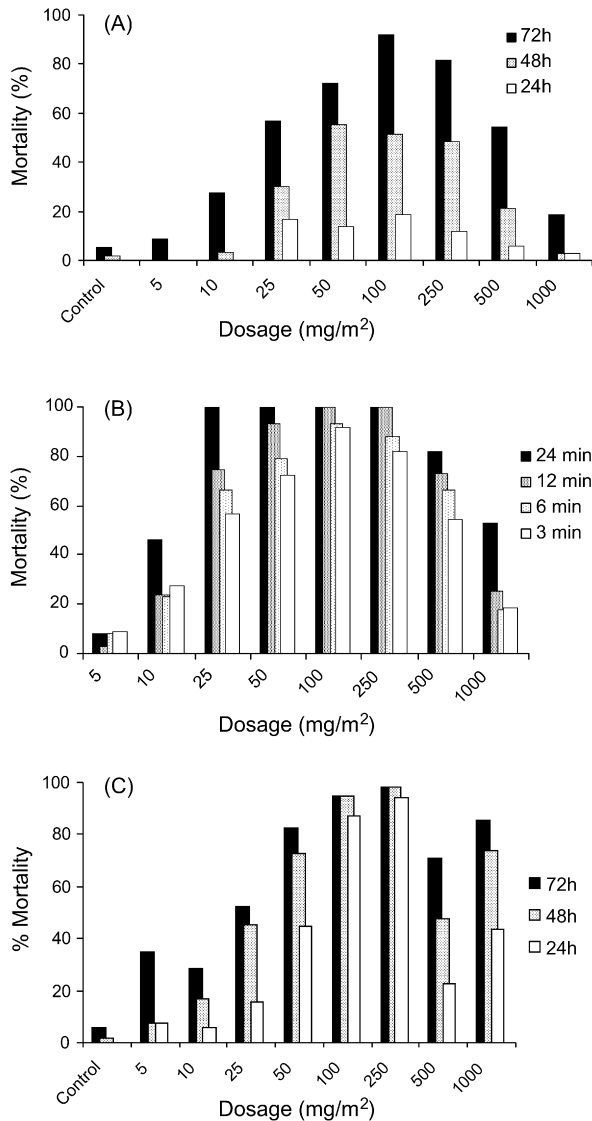


Fig. 1. Efficacy of chlorfenapyr treated netting over a range of concentrations against *An. gambiae* Kisumu. (A) Delayed mortality over 24–72 h holding periods after an initial 3 min exposure in WHO cone assays. (B) Mortality after exposure to a range of concentrations and exposure periods in cones (mortality scored after 72 h holding period). (C) Delayed mortality in wire ball assays after exposure to a range of concentrations for 3 min.

The dosage–mortality response was regular between 5 mg/m² and 100 mg/m² but showed a negative mortality trend at higher dosages (Fig. 1A). Extending the duration of exposure in the cones from 3 min to 24 min had a significant effect on mortality rates ($P < 0.001$) but the curvilinear dosage–mortality response observed with the shorter exposure period was still evident after longer exposures (Fig. 1B).

The value of the wire ball test over cone tests is that it provides only insecticidal surfaces for mosquitoes to rest upon. The ‘ball’ method produced a curvilinear trend but showed higher mortality rates at the higher dosages than did the cone tests (Fig. 1C).

3.3. Irritability tests

The proportion not taking off in the observation period was significantly lower among those exposed to 500 mg/m² treated netting than among those exposed to untreated netting ($\chi^2 = 5.9$, $P = 0.01$) or 100 mg/m² treated netting ($\chi^2 = 12.1$, $P < 0.001$). The proportion not taking off did not differ between those exposed to 100 mg/m² and untreated netting ($\chi^2 = 1.1$, $P = 0.29$) (Table 5). The time to first take-off as indicated by FT₅₀ was faster among those exposed to 500 mg/m² netting than among those exposed to untreated or 100 mg/m² netting; however, the difference was not significant at FT₅₀. The irritability expressed at the higher concentration of chlorfenapyr would be sufficient to explain the negative mortality trend shown in cone tests if contact time with insecticide was indeed reduced at higher concentrations.

3.4. Tunnel tests

The tunnel test results are summarised in Fig. 2A–G. Overall there was 21% reduced penetration of mosquitoes through the chlorfenapyr treated nets compared to the untreated control ($P = 0.007$) (Fig. 2A). The insecticide-induced inhibition did not show a clear dosage-dependent trend ($P = 0.75$). The effect on penetration was more apparent in Kisumu and Yao than in Vkpr strains. Since Yao and Vkpr both carry the *kdr* gene, any between-strain differences seem unlikely to be attributable to *kdr*. The pattern of % blood-feeding (Fig. 2B) showed the same trend as % penetration; this was because a regular proportion (ca. 75%) of individuals that penetrated the chlorfenapyr treated netting went on to feed (Fig. 2C). There were no between-strain differences in blood-feeding rate among those that penetrated the treated netting ($P = 0.755$). Unlike in cone tests, the dosage–mortality trend in tunnel tests was a typical sigmoid curve (Fig. 2D). Dosages of 250 mg/m² or higher induced >80% mortality within 24 h. Delayed mortality was more evident at lower dosages (50–100 mg/m²) than at higher. No cross resistance to chlorfenapyr between Kisumu and *kdr* or *Ace-1^R* bearing strains was evident in the tunnel tests ($P = 0.31$) (Fig. 2E). There was high mortality among all three strains even at the low dosage of 50 mg/m². Dosages of

Table 5

Time to first take-off (FT₅₀) of *An. gambiae* Kisumu exposed to chlorfenapyr treated netting in WHO irritability tests

	Dosage (mg/m ²)	N	% not taking off (95% CI)	FT ₅₀ # (s) (95% CI)	Induced irritancy at FT ₅₀ level relative to control
Control	0	100	77 (68.7–85.2)	181 (74–2019)	–
Chlorfenapyr	100	100	83 (75.6–90.4)	203 (86–1822)	0
Chlorfenapyr	500	100	61 (51.4–70.6)	140 (71–977)	23%

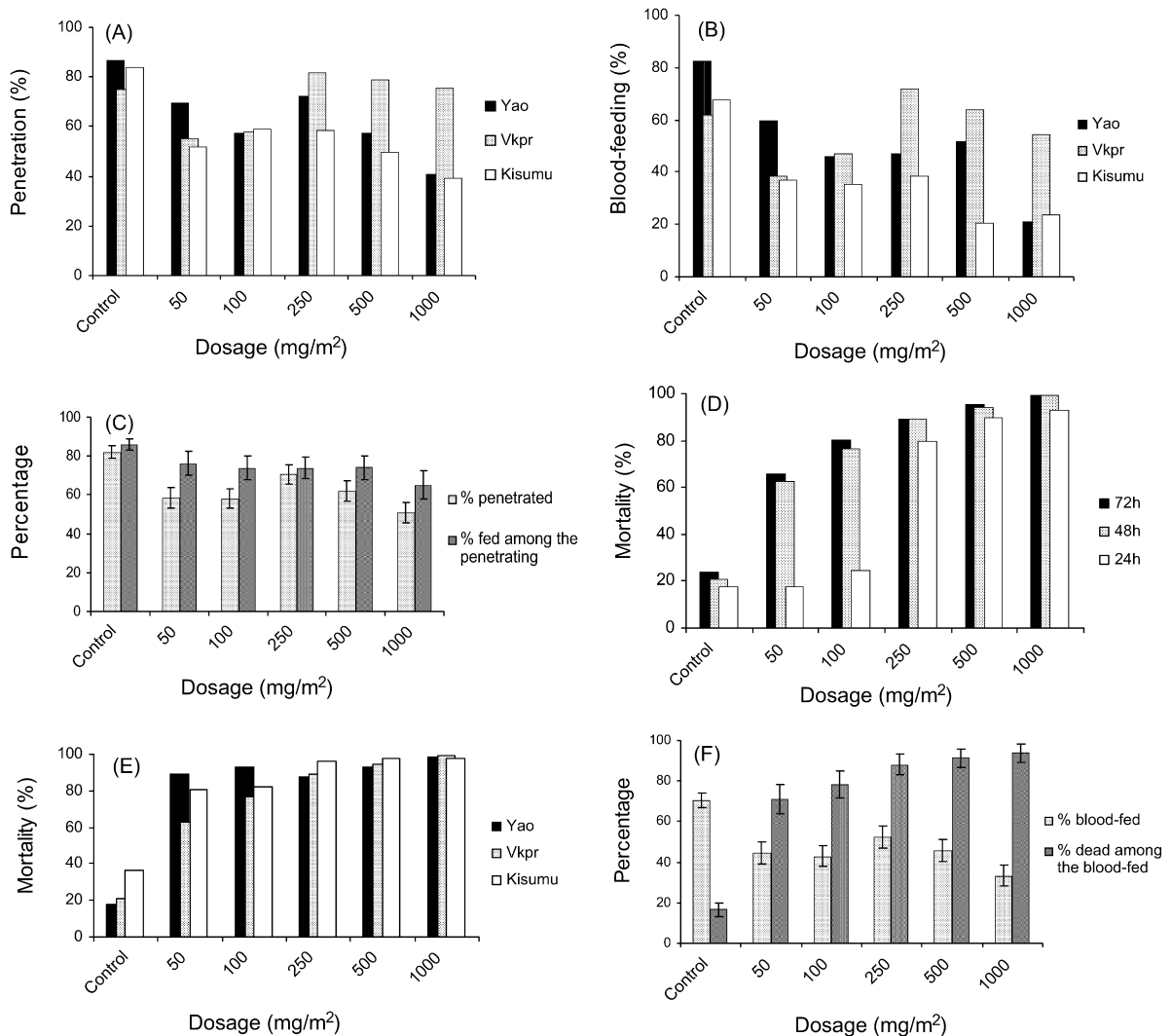


Fig. 2. Efficacy of chlorfenapyr treated netting over a range of concentrations against *An. gambiae* in tunnel tests. (A) Proportion of mosquitoes of Kisumu, Vkpr and Yao strains penetrating the holed netting. (B) Proportion blood-feeding among the three strains. (C) Overall proportion penetrating and the proportion blood-feeding among those that penetrated. (D) Delayed mortality of susceptible *An. gambiae* Kisumu over 24–72 h. (E) Mortality of Kisumu, Vkpr and Yao strains over 48 h. (F) Overall proportion blood-feeding and proportion dead after 48 h among those that blood-fed. (G) Comparison of response of Vkpr to chlorfenapyr and permethrin over a similar range of concentrations (permethrin tunnel data taken from Corbel et al., 2004b). Chlorfenapyr: stippled bars, permethrin: solid black bars.

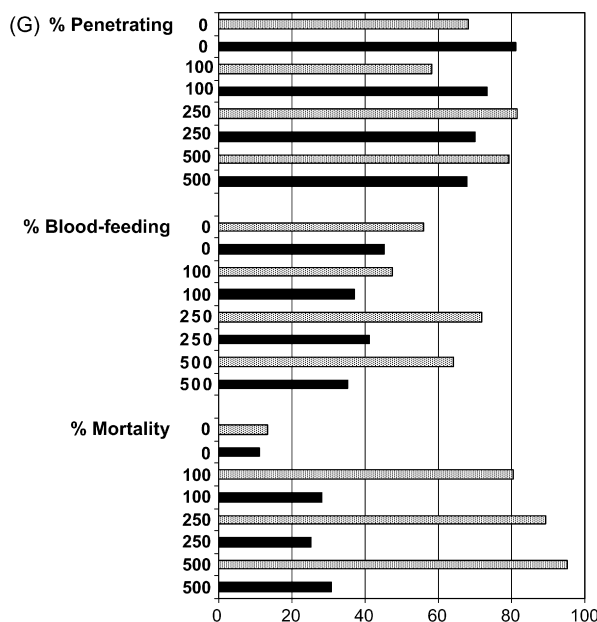


Fig. 2. (Continued).

100 mg/m² or more killed between 77% and 96% of *kdr* and *Ace-1^R* resistant *An. gambiae* within 48 h (Fig. 2E). In the chlorfenapyr tests, the majority (70–90%) of mosquitoes that blood-fed subsequently died (Fig. 2F). There was no significant differences between control and treatments in the proportions that had blood-fed among the dead mosquitoes ($P=0.88$). This indicates that mosquito capacity to blood-feed on the guinea pigs was not reduced in the period immediately after coming into contact with treated netting.

Fig. 2G compares the response of pyrethroid-resistant *Vkpr* in tunnels to chlorfenapyr and permethrin treated netting (cf. Corbel et al., 2004b) across a similar range of concentrations. Mortality is presented at the time at which any insecticide-induced mortality had taken full effect –15 h in the case of permethrin (Corbel et al., 2004b) and 48 h in the case of chlorfenapyr (see Fig. 2D). There was greater blood-feeding inhibition in response to the permethrin treatments but much greater mortality in response to the chlorfenapyr treatments.

4. Discussion

The various bioassay techniques that were used to measure intrinsic toxicity or behavioural responses to insecticide were consistent in showing no cross resistance between chlorfenapyr and the two common mechanisms, *kdr* and *Ace-1^R*, that confer resistance to pyrethroids, organophosphates and carbamates in *An.*

gambiae. Because chlorfenapyr's mode of action is to disrupt oxidative phosphorylation in the mitochondria rather than to target neural receptors, an absence of cross resistance to these mechanisms was anticipated (Anon., 1995; Lovell et al., 1990). Being a pro-insecticide which is metabolised to its active form by mixed function oxidases, chlorfenapyr has shown in some species the property of negative cross resistance. For example, chlorfenapyr is 2–5 times more toxic to pyrethroid resistant pests such as the cattle horn fly, *Haematobia irritans* or the tobacco budworm, *Heliothis virescens*, in which resistance is based on elevation of mixed function oxidase activity, than it is to pyrethroid susceptible strains (Pimprale et al., 1997; Sheppard and Joyce, 1998). In the present series of studies we tested chlorfenapyr against a strain of *An. stephensi* that once had multiple pyrethroid resistance mechanisms (Vatandoost, 1996) but it was never clear whether MFO mediated resistance was still present—certainly there was no evidence of negative cross resistance to chlorfenapyr. Strains of *An. gambiae* or *Culex quinquefasciatus* whose pyrethroid resistance is based on elevated oxidases do exist (Chandre et al., 1999a; Etang et al., 2003; Nikou et al., 2003; Vulule et al., 1999). The prospect of exploiting negative cross resistance to chlorfenapyr as a management strategy to control pyrethroid resistant mosquitoes is an attractive one. Evaluation against pyrethroid resistant *An. funestus* in southern Africa where pyrethroid failure was associated with selection of an MFO resistance mechanism (Hargreaves et al., 2000) is a priority.

The peculiar curvilinear dosage–mortality response expressed in the cone tests appeared to be due to irritability and reduced contact time at higher dosages. Such trends were not apparent in the overnight tunnel tests where contact time of host-seeking mosquitoes was potentially much longer. Some inhibition of penetration was evident with treated netting but the effect was not great (21% inhibition) and those that succeeded in penetrating the holed netting were not affected in their capacity to blood-feed, though many died later on.

The 2–3-fold tolerance to chlorfenapyr in the Vkpr strain was probably due to strain variation since the Yao strain which also carried *kdr* showed no such tolerance. Recently chlorfenapyr resistance has emerged in some agricultural pests (Van Leeuwen et al., 2006; Gunning and Moores, 2002). In the two-spotted spider mite, *Tetranychus urticae*, resistance to chlorfenapyr was associated with an increase in P450 mono-oxygenase activity and to a decrease in TMBZ peroxidation activity that is allegedly responsible for activation of chlorfenapyr (Van Leeuwen et al., 2004). In the housefly, some but not all strains that expressed enhanced mono-oxygenase mediated resistance showed negative cross resistance to chlorfenapyr (Scott et al., 2004; Van Leeuwen et al., 2006). In the cotton bollworm, *Helicoverpa armigera*, the emergence of chlorfenapyr resistance appears to be mediated by an esterase that is also responsible for pyrethroid resistance (Gunning and Moores, 2002). Drawing examples from other pests may provide some insight as to what might happen in mosquitoes but which mechanism of chlorfenapyr resistance will eventually evolve remains unpredictable.

Our 100 mg/m² and 250 mg/m² dosages of chlorfenapyr induced significantly greater mortality in the *kdr* strain than did field rates of permethrin (500 mg/m²), deltamethrin (25 mg/m²) or lambda-cyhalothrin (18 mg/m²) tested in tunnels (Hougard et al., 2003b). Dosages of chlorfenapyr within this range might be appropriate to test on nets in experimental huts against field populations of *kdr* resistant *Anopheles* and *Culex*. A key difference between chlorfenapyr and pyrethroids is the comparatively low personal protection to be gained when the chlorfenapyr-treated netting becomes holed, as shown by high penetration rates through holed netting and by the majority of penetrating mosquitoes then feeding before dying. Because chlorfenapyr treated nets seem more toxic than repellent to pyrethroid resistant mosquitoes, it would be predicted that at high coverage levels (for example, through roll-out programmes of free or subsidized nets), chlorfenapyr treated nets would collectively kill through a mass population effect a substantial proportion of

mosquitoes before they became infective, thereby reducing the force of infection and providing community protection. For individual protection against pyrethroid resistant mosquitoes the best approach might be to combine chlorfenapyr with pyrethroids in a mixture as together they would induce feeding inhibition and mortality of pyrethroid resistant mosquitoes. A mixture might also act to reduce the selection pressure for pyrethroid resistance in areas where mosquitoes are still largely susceptible, as predicted by theoretical models (Curtis, 1985). Chlorfenapyr has a safety profile similar to that of permethrin/deltamethrin and the hazard to users or occupational workers is within acceptable limits (BASF unpublished risk assessment). With its novel mode of action distinct from that of pyrethroids, a mixture of chlorfenapyr with pyrethroids is unlikely to present an enhanced toxicity risk to humans.

The twin characteristics of limited repellency and high toxicity might make chlorfenapyr a strong candidate for indoor residual spraying (IRS) provided good residual activity on interior walls can be achieved. Delayed toxicity is not a major limitation to IRS (DDT and once-effective organochlorines such as dieldrin and γ HCH are slow acting) because the interval between mosquitoes acquiring a malaria infection and being able to transmit it takes several days.

ITNs are used not only as protection against malaria mosquitoes but also as protection against *Cx. quinquefasciatus*, a species which often occurs alongside *Anopheles*. *Culex* is difficult to control with conventional insecticides owing to the presence of multiple resistance mechanisms involving *kdr* and oxidases against pyrethroids and elevated esterases and insensitive acetylcholinesterases against OPs (Khairandish and Wood, 1993; Chandre et al., 1997, 1998) at levels higher than what is observed in *Anopheles* (Curtis et al., 1996; Chandre et al., 1999b; N'Guessan et al., 2003). There is a good prospect for effective control of *Cx. quinquefasciatus* using chlorfenapyr, and this should reduce nuisance biting among net users and might even protect against filariasis transmission.

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concerning protection of animals used for experimental purposes.

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