The effect of the spatial repellent metofluthrin on landing rates of outdoor biting anophelines in Cambodia, S.E. Asia

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Without controlling outdoor transmission, the goal of elimination of malaria is unlikely to be reached. This is particularly the case in places like Cambodia where people spend considerable amounts of time away from houses at night. Metofluthrin is a synthetic pyrethroid insecticide with a high vapor action at ambient temperatures and has been developed as a long lasting insect repellent device that works without the need to apply heat. Emanators of 10% of metofluthrin were therefore tested in landing collections against potential malaria vectors from three areas of the country (Pailin, Pursat and Koh Kong). One to four emanators were hung on wire 1m off the ground on one or four sides of a square 1.5m from collectors. Collections were also undertaken with Furvela tent-traps. 2086 hrs of landing collection were undertaken in Pailin, 528hrs in Veal Veng and 320 in Kroh Salau. Rate ratios were used to determine the significance of the difference between collections. The principal anophelines collected varied between locations. *Anopheles minimus* s.l. was the most common mosquito in Pailin, *An. maculatus* s.l in Veal Veng and *An. sinensis* in Kroh Salau. Among all species collected in Pailin landing rates were reduced by 50% (95% CI 55-44%) when a single emanator was used and by 58% (95% CI 63- 52%) when four were used. The effect was greater in *An.minimus* s.l 51% (95% CI 54-47%) and 70% (72%-66%) respectively. A similar result was obtained in Pursat, where 67% (95% CI 66- 42%) reductions were observed when four emanators were in use, but no significant reduction was observed in Koh Kong. Although the results show promise it is argued that the product needs further development.

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Introduction

An increase in the time to clearance of *Plasmodium falciparum* infections from three to six days following treatment with artemisinin in Cambodia is a major concern for malaria control worldwide (Dondorp et al., 2011, Smith Gueye et al., 2014). Such treatment failure of artemisinin combination therapies (TFACT), may lead to enhanced transmission potential through an excess production of gametocytes (Krishna and Kremsner 2013). Parasites resistant to other drugs, notably chloroquine, have also had their origin in S.E. Asia. The spread of chloroquine resistant parasites had disastrous repercussions when they reached Africa (Trape, 2001). Considerable efforts have, therefore, been undertaken to reduce transmission in Cambodia, including the wide scale distribution of bednets and the establishment of village malaria workers

(Kheang et al, 2011). Many cases, however, may be acquired when people go to the forest for logging activities or when watching television before they go to bed, times and situations when bednets make little difference. Hence the challenge lies in protecting people at these times and in these places. One way might be to use repellents to prevent mosquitoes biting, and a number of products have been developed with this in mind (Chattopadhyay et al., 2013,Kweka et al., 2012, Revay et al., 2013).

The recent development of synthetic pyrethroid insecticides with high vapor action at ambient temperature, has led to the development of devices that work without the need to apply heat (Ogoma et al., 2012, Ujihara et al., 2004. Metofluthrin is a repellent that shows promise as an active ingredient when vaporized indoors, although it is possible that its main effect is as a killing agent (Rapley et al., 2009). When used in a paper-based emanatory, it reduced outdoor biting rates of *Aedes canadensis* and *Ae. vexans* by approximately 90% (Lucas et al., 2007). The reduction is probably due to a disruption of orientation towards the host resulting from neural excitement, which appears at an early stage of pyrethroid toxicity (Kawada et al., 2005). The formulation previously used was, however, effective for only a few days. A formulation of 5% metofluthrin on a plastic lattice, designed to control evaporation rates, reduced resting mosquito densities inside houses in Indonesia for a month (Kawada et al., 2006). When used in houses in Bagamoyo, Tanzania this device, however, failed to reduce densities of *An. gambiae* in light-trap collections but did reduce the numbers caught resting (Kawada et al., 2008). The efficacy of metofluthrin as a repellent may also differ between species or families since against *Culicoides* it apparently has little or no effect (Zoller and Orshan. 2011).

In addition to the principal vector, *Anopheles dirus*, there are a number of secondary or incidental, malaria vectors in Cambodia including *Anopheles minimus*, *Anopheles maculatus*, *Anopheles barbirostris* and *Anopheles sinensis* many of which bite in the early part of the night (Durnez et al., 2013). Recently, emanators with a 10% formulation of metofluthrin were developed, the effect of which should last longer than that of previous emanators. It is not known, however, if metofluthrin repels these mosquitoes. We, therefore, tested such emanators against these secondary vectors from three areas of the country in landing, Furvela tent-trap and CDC light-trap collections.

Methods

Study sites

The study took place in Khum Otavao (N12.789 E102.690), in Pailin Province; Krorhom Krom, (N12.215 E 103.080) in Pursat Province and Kroh Salau (N 11.460 E 103.049) in Koh Kong Province. The study sites have been described by Charlwood et al. (submitted) as have collection methods and descriptions of the species collected. Briefly 12 species or species groups of anopheline were collected in Pailin, 11 in Veal Veng and eight in Kroh Salau. The main anophelines collected varied between locations *An. minimus* s.l. was the most common species group in Khum Otavao (Pailin), *An. maculatus* s.l. in Krorhom Krom (Pursat) and *An. sinensis* in Kroh Salau (Koh Kong). Elsewhere, members of each of these species or species complexes has been found infected with sporozoites at different times, but they are all secondary vectors (Sinka et al., 2011).

Sumitomo Chemical Co. Ltd. (Hyogo, Japan) supplied slow-release emanators made of polyethylene mesh impregnated with 10% (w/w) metofluthrin (2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl (*EZ*)-(1*RS*)-cis-trans-2,2-dimethyl-3-prop-1-enylcyclopropanecarboxylate). The mesh was a dual layer (15 x 8 cm wide) 3–4 mesh held in an open plastic frame. According to the manufacturers the effective life of a single emanator once opened is four weeks.

In order to determine whether they reduced landing rates, in four out of eight sites at least 35 m apart, a collector sat in the middle of a square of thin wire, 1.5 m on a side, 1 m off the ground (Fig 1). In Pailin, one or four emanators were hung on the wire either to the windward side of the collector (when a single emanator was used) or at each side of the square (when four emanators were used). Emanators were not used in the remaining four sites, which acted as the controls. The sites where the emanators were used were alternated on sequential nights, whilst collectors changed locations every other night so that after 16 nights, each collector had worked at each location performing both control and intervention collections. Fresh emanators were used every week and, when not in use experimentally, the emanators were wrapped in aluminum foil.

In addition, in Pailin, eight Furvela tent-traps (Govella et al., 2009) were operated from 22:00 hrs until dawn. An emanator was hung close to the opening of four of the tents whilst the others acted as controls. Intervention and control tents also alternated on alternate nights.

Rate ratios were used to determine if the mean numbers of the most common species or species complex caught when emanators were used were different to those caught in control collections. In Pailin, reductions in anopheline and culicine densities between the metofluthrin and control were also estimated using a negative binomial regression model adjusted for the location and for collection type (see supplementary file 1).

In Pursat and Kroh Kong, tests were only undertaken with four emanators at a time (Figure 1) but with a similar rotation pattern to that used in Pailin.

Ethical statement

The ethical committees of the National Centre of Malariology (CNM) in Phnom Penh, (Cambodia) and of the Liverpool School of Tropical Medicine (UK) approved the study. The mosquito collectors and householders were informed about the objectives, processes and procedures of the study and oral informed consent was sought from them. Collectors were recruited among the adult village population on the understanding that if they wanted to withdraw from the study they could do so at any time without prejudice. Access to malaria diagnosis and treatment was guaranteed throughout the study.

Results

In Khum Otavao, Pailin, 2086 hours of landing collection were undertaken. Densities of all species were generally very low with less than a single specimen being collected per hour of collection for all species other than *An. minimus* s.l. and *An. maculatus* s.l. In general, there was a reduction of approximately one third of the expected numbers biting when a single emanator was used and between 60 to 70% reduction when four emanators were used (Table 1). The supplementary file gives the data by species or species group from Pailin. It also includes the data from the tent-traps.

In Krorhom Krom, Pursat, 528hrs of landing collection were undertaken. Table 2 shows the overall numbers of mosquito collected when four emanators were in use compared to control collections in Krorhom Krom. A similar reduction to that observed in Khum Otavao (Pailin) was seen among species collected in Krorhom Krom (Pursat) (where four emanators were used and where *An. maculatus* s.l. was the principal mosquito collected) ($p < 0.001$)

In Kroh Salau, Koh Khong, 320 hrs of landing collection were undertaken. Table 3 gives landing rates in Kroh Salau.

In Kroh Salau, there was no demonstrable effect of four metofluthrin emanators on landing rates (All rate ratios were not significantly different).

Discussion

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Without controlling outdoor transmission of malaria the goal of elimination is unlikely to be reached. Hence, the search for suitable ways to reduce or control outdoor biting insects in the early evening is an urgent one. A single metofluthrin dispenser reduced biting densities of mosquitoes by approximately one third in Khum Otavao (Pailin) and four dispensers located close to the collectors reduced landing rates by up to two thirds. Reductions of a similar order were obtained when a single emanator was placed close to the opening of Furvela tent-traps. Similarly, numbers of mosquitoes collected in Krorhom Krom (Pursat) were reduced when four emanators were used. However, in Kroh Salau (Koh Kong), landing rates did not appear to be affected by the presence of four emanators close to the collector (Fig 1). The reasons for this are unknown. Despite the difference in species collected, it is unlikely to be a species-specific effect since all of the other species examined from the other sites showed similar reductions in landing rates. Environmental conditions in Kroh Salau were also relatively stable with little wind – indeed it is possible that the lack of wind may have reduced the emanators' efficacy.

The recorded reduction in biting, although significant, may perhaps be insufficient a reduction in annoyance from biting insects by itself to convince the local population of its cost-effectiveness. Whether the use of metofluthrin by individual households diverts mosquitoes to households not using such a product, as apparently do topical repellents (Maia et al, 2013), is unknown. A forest is different to a few isolated trees. Should enough people use the product in a limited area, it is possible that a 'community effect' similar to that observed by Hawley and colleagues (Hawley et al., 2003, Howard et al., 2000) with insecticide treated nets might occur, even though at an individual level, the product is less than perfect.

Due to the small numbers of *An. dirus* collected, we were unable to establish conclusively if metofluthrin reduces outdoor biting in this species. Whether metofluthrin has an effect on indoor biting rates of *An. dirus*, is also not known. In Tanzania, numbers of *An. gambiae* in light-traps were not reduced by metofluthrin but numbers resting were (Kawada et al., 2006). This indicates that part of its effect was through enhanced mortality rather than through repellency, as suggested by Rapley et al. (2009) for their studies with *Stegomyia aegypti* (a.k.a. *Aedes aegypti*) in Australia.

Unlike other Cambodian anophelines, *An. dirus* is highly anthropophilic (whilst the other species are primarily zoophilic) and it enters houses, even those built on stilts, whilst other species are primarily outdoor biting (Sinka et al., 2011). Hence, an effect against this species may be anticipated compared to the effects observed in the present study. The results from such a trial will be reported elsewhere. Using emanators indoors may also reduce transmission of dengue, a common disease in Cambodia, by day biting *Stegomyia aegypti* and *St. albopicta.*

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Figure 1 (on next page)

Waiting for the mosquitoes. Collector with four metofluthrin emanators in situ, Koh Kong Cambodia.

Table 1(on next page)

Mean number of mosquitoes collected landing when one or four metofluthrin emanators were or were not (control) in use Paillin, Cambodia.

Species	Intervention	Mean	RR (95% CI)	P value
An. minimus s.l.	Metofluthrin 4	1.33	0.307 (0.277 – 0.342)	<0.001
	Metofluthrin 1	0.63	0.488 (0.453 – 0.527)	
	Control	1.72	1	
Other *Anopheles*	Metofluthrin 4	0.94	0.420 (0.367 – 0.481)	<0.001
	Metofluthrin 1	0.31	0.504 (0.454 – 0.560)	
	Control	0.88	1	
Culex	Metofluthrin 4	0.79	0.580 (0.507 – 0.665)	<0.001
	Metofluthrin 1	2.07	0.661 (0.618 – 0.707)	
	Control	3.83	1	

Table 2 (on next page)

Mean number of mosquitoes collected landing when four metofluthrin emanators were or were not (control) in use Veal Veng, Pursat, Cambodia. The rate ratio (RR) is adjusted for study location.

Species	Intervention	Mean	RR (95% CI)	P value
An. maculatus s.l.	Metofluthrin 4	0.25	0.427 (0.314 – 0.581)	<0.001
	Control	0.60	1	
Other Anopheles	Metofluthrin 4	0.42	0.432 (0.340 – 0.549)	<0.001
	Control	0.99	1	
Culex	Metofluthrin 4	0.42	0.330 (0.258 – 0.421)	<0.001
	Control	1.28	1	

Table 3 (on next page)

Mean number per hour of mosquitoes collected when metofluthrin was or was not in use in Kroh Salau, Koh Kong, Cambodia. The rate ratio (RR) is adjusted for study location.

Species	Intervention	Mean	RR (95% CI)	P value
An. sinensis	Metofluthrin 4	0.67	0.638 (0.195 – 2.092)	0.458
	Control	0.97	1	
Other *Anopheles*	Metofluthrin 4	0.76	0.864 (0.561 – 1.329)	0.505
	Control	0.81	1	
Culex	Metofluthrin 4	7.22	1.278 (0.985 – 1.657)	0.064
	Control	6.23	1	