Transactions of the Royal Society of Tropical Medicine and Hygiene (2006) **100**, 1091—1097

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevierhealth.com/journals/trst

Evaluation of synthetic repellents on mosquito nets in experimental huts against insecticide-resistant *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes

Raphael N'Guessan[a,b,*], Mark Rowland[a], Traore-Lamizana Moumouni[b], Nestor Bli Kesse[b], Pierre Carnevale[b]

[a] *Gates Malaria Partnership, London School of Hygiene and Tropical Medicine, 50 Bedford Square, London WC1B 3DP, UK*
[b] *Centre Pierre Richet, Bouaké, Côte d'Ivoire*

Received 15 October 2005; received in revised form 11 April 2006; accepted 13 April 2006
Available online 8 September 2006

KEYWORDS
Anopheles gambiae;
Culex quinquefasciatus;
Insecticide-treated nets;
DEET;
Ethyl butylacetyl-aminopropionate;
IR3535;
Côte d'Ivoire

Summary Owing to the development of pyrethroid resistance in *Anopheles gambiae*, there is a need to develop chemical alternatives for use on mosquito nets. Synthetic insect repellents are widely used for personal protection as skin or clothing applications. The efficacy of repellent-treated nets (RTN) was evaluated in experimental huts in Côte d'Ivoire against pyrethroid-resistant populations of *An. gambiae* and *Culex quinquefasciatus*. The repellents tested were DEET (*N*,*N*-diethyl-3-methylbenzamide) at 7.9 g/m^2 and two formulations of ethyl butylacetylaminopropionate (IR3535) at 7.6 g/m^2 and 7.3 g/m^2. Over 45 nights there was a 74—82% reduction in the number of *An. gambiae* entering the huts containing RTNs but no significant reduction in entry of *C. quinquefasciatus*. There was a 63—64% reduction in the proportion of *An. gambiae* blood feeding but no reduction in the proportion of *C. quinquefasciatus* blood feeding in huts with RTNs. An unexpected result was the 69—76% mortality of *An. gambiae* and 51—61% mortality of *C. quinquefasciatus* in huts containing RTNs. Treated filter paper bioassays in WHO test kits confirmed that confined contact with DEET induces mortality. The DEET-based product provided better and longer protection; tunnel test bioassays confirmed that residual activity lasted for up to 6 weeks. Application of repellents to nets warrants further investigation and development.

1. Introduction

Synthetic repellents are widely used for personal protection against insect bites. The CDC estimates that approximately

* Corresponding author. Present address: London School of Hygiene and Tropical Medicine, 50 Bedford Square, London WC1B 3DP, UK. Tel.: +44 20 7299 4719; fax: +44 20 7299 4720.
E-mail address: raphael.n'guessan@lshtm.ac.uk (R. N'Guessan).

0035-9203/$ — see front matter © 2006 Royal Society of Tropical Medicine and Hygiene. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.trstmh.2006.04.005

30% of the US population uses an insect repellent each year, and worldwide use exceeds 200 million applications annually (Barnard, 2000; WHO, 1998). Although repellents are effective when applied topically, their short-term activity on skin necessitates regular replenishment, limiting their usefulness. Skin repellents have not been widely promoted as a public health intervention, partly because of lack of evidence for an impact on vector-borne diseases and partly owing to lack of confidence that recipients would use repellents regularly enough (Curtis et al., 1994; Kroeger et al., 1997; Vittal and Limaye, 1984). Recently, however, a randomised controlled trial carried out in Asia showed a level of protection (56%) from use of DEET (*N*,*N*-diethyl-3-methylbenzamide) against falciparum malaria similar to that of insecticide-treated nets (ITN) or indoor residual spraying trials carried out in the same area (Rowland et al., 2004a), and users of skin repellent and pyrethroid-treated nets attained further protection (Rowland et al., 2004b). Whilst some doubt may remain over the utility of skin repellents, the same cannot be said for ITNs, which in many parts of the tropics remain a highly effective method of malaria prevention (Lengeler, 1998). ITNs work by a combination of exicitorepellency and toxicity and it is unclear which property of pyrethroids on nets is more important to users. It is possible that a conventional, volatile, non-toxic repellent would give equivalent or better protection on nets compared with a contact toxin/repellent as typified by pyrethroids. A non-toxic repellent would also be unlikely to select for resistance. Application of repellent to nets might be more cost effective than application to skin if it could be shown that the repellent has longer residual activity. Early studies showed that when DEET is absorbed onto textiles it forms a reservoir that evaporates slowly and gives long-term repellency. Netting jackets impregnated with 0.25 g of DEET per gram of netting, as used in military or outdoor leisure activities, give several weeks of protection from biting flies (Schreck et al., 1979; Smith and Burnett, 1948). Repellents are more persistent on clothing because adherence is better and because loss through evaporation, absorption or perspiration is reduced (Rozendaal, 1997). Netting curtains treated with DEET inhibited the entry of *Anopheles gambiae* and *An. funestus* by 86% and 51%, respectively, in an early trial in Tanzania, but the work was not taken further because the residual activity of pyrethroids being investigated at the time was clearly much better (Curtis et al., 1987). Interest in repellents waned in the 1980s and 1990s when pyrethroid-treated nets gained ascendancy. In recent years, pyrethroid-resistant *An. gambiae* have spread across West Africa, and pyrethroid-resistant *An. funestus* have caused control failure in southern Africa. The operational significance of pyrethroid resistance in *An. gambiae* is equivocal: Henry et al. (2005) and Asidi et al. (2005) observed continuing effectiveness of pyrethroid-treated nets in the presence of a high frequency of *kdr* resistance, whereas Kolaczinski et al. (2000) and R. N'Guessan and M. Rowland (unpublished observations) observed high survival of *kdr* homozygotes in such situations. The threat of resistance has stimulated the search for alternative toxicants and repellents to supplement or replace the pyrethroids on nets. A number of synthetic repellents have come onto the market in recent years to rival DEET (Barnard et al., 2002). IR3535 (ethyl butylacetylaminopropionate) is a synthetic repellent with a favourable safety profile. It recently obtained interim approval from WHOPES for use on skin and clothing (WHO, 2001). As a skin repellent it is active against *Culex* and *Anopheles* mosquitoes for 5—8 h depending on ambient conditions and the rate of perspiration.

For the present study, we obtained three commercial repellent formulations (one DEET-based and two IR3535-based) for application to polyester netting. We investigated the effect of repellent-treated nets (RTN) on the behaviour and survival of pyrethroid-resistant *An. gambiae* and *Culex quinquefasciatus*, both of which express resistance to a range of insecticide groups at Yaokoffikro, Bouaké (Chandre et al., 1998, 1999). Our study was carried out in experimental huts at Yaokoffikro field station and complements recent phase II trials of nets treated with insecticides (Asidi et al., 2004, 2005; Darriet et al., 1998; Guillet et al., 2001; N'Guessan et al., 2001).

2. Materials and methods

2.1. Repellent products

Three repellent products were evaluated on nets: Prebutix Fort®, produced by the company Pierre Fabre, France, is a lotion containing 20% IR3535; Tropic 5/5®, produced by the company Nicholas Rock, France, is a formulation containing 25% IR3535; and Insect Ecran®, produced by the company Osler, France, is a formulation containing 50% DEET repellent. All three products were pump spray formulations designed for clothing application. No known substances with insecticidal properties were declared in the formulations.

2.2. Nets and repellent treatments

Eight polyester nets of 100 denier netting and 156 mesh size were used in the study. To simulate the badly torn nets often observed in African villages, 80 holes each measuring 2 cm × 2 cm were cut in the sides and ends of the nets. Two nets were treated with each formulation, one of which was used for the hut trial and the other for laboratory tunnel tests. During treatment, the nets were hung in a room and the sides and ends were sprayed evenly. To determine the amount of formulation applied, repellent containers were weighed before and after spraying. There is no previous recommendation regarding the dosage for application to nets. We chose to target doses of approximately 7.5 g/m^2 of netting, and the actual doses were 7.9 g/m^2 DEET (Insect Ecran), 7.3 g/m^2 IR3535 (Prebutix Fort) and 7.6 g/m^2 IR3535 (Tropic 5/5).

2.3. Experimental huts and mosquito collections

The mosquito nets were evaluated in veranda trap huts previously described by Darriet et al. (1998). Each experimental hut consists of a single room with entry slits on three sides and a screened veranda on the fourth side. They were built in a row in an irrigated valley that produces year-round *An. gambiae* Savanna cytotype (S form) with 96% *kdr* frequency (Chandre et al., 1999) and *C. quinquefasciatus* with 33—46-fold resistance to permethrin (Chandre et al., 1998).

Table 1 Mean number (and 95% CI) of mosquitoes collected per night in the four experimental huts over 10 nights prior to installation of the repellent-treated nets

Hut number	*Anopheles gambiae*	*Culex quinquefasciatus*
1	4.2 (3.1—6.8)	3.9 (1.8—5.2)
2	5.3 (4.3—8.5)	2.7 (0.8—4.1)
3	6.5 (5.8—7.4)	1.6 (0.7—4.5)
4	5.1 (3.9—6)	2.9 (1.2—5.6)

Experimental hut procedures and mosquito collections were carried out as per Darriet et al. (1998) and N'Guessan et al. (2001). Briefly, adult male volunteers slept in the huts on mats under the nets from 20:00 hours to 05:00 hours each night after cleaning the hut at 18:00 hours to remove any spiders and other predators. To minimise bias in individual attractiveness, sleepers were rotated between huts on successive nights while treatments remained fixed to avoid any residual carryover. From preliminary observations (Table 1), there were no detectable differences in attractiveness between huts. Sleeper volunteers awoke at 05:00 hours, closed the window slits, lowered the curtain separating the room from the veranda and collected live and dead mosquitoes from the room, bed net and veranda. Female mosquitoes were scored as dead or alive and fed or unfed, and were identified to species level. The trial ran for 45 nights over 8 weeks (7 July to 6 September 2002).

The entomological impact of each treatment was expressed relative to the untreated control in terms of:

- Deterrency: percentage reduction in the number of mosquitoes found in a treated hut compared with the number in the control hut.
- Exophily: proportion of mosquitoes exiting and trapped in the veranda of a treated hut compared with the proportion in the control hut.
- Blood-feeding rate: proportion of mosquitoes that were blood fed.
- Overall mortality rate: proportion of mosquitoes found dead immediately (at time of collection) and after the 24 h holding time.

2.4. Tunnel test design

The tunnel test is a laboratory system designed to simulate many of the behavioural and toxicological interactions that occur with free-living mosquitoes during experimental hut trials. It is used as a forerunner to hut trials and has the advantage that experiments can be performed more quickly and cheaply than hut trials whilst providing comparable information on repellency, mortality and dosage-dependent effects. The system is composed of a square glass cylinder, 25 cm high, 21 cm wide and 60 cm long, with a square of netting with nine 1-cm diameter holes fixed into a frame that slots across the tunnel dividing it into two chambers. A guinea pig is housed in the bait chamber unconstrained in a cage and provided with food and water, and in the other chamber 100 unfed female mosquitoes aged 5—8 days are released at dusk and left overnight in the dark. The following morning, the numbers of mosquitoes found alive or dead and fed or unfed in each compartment are scored. By measuring blood-feeding inhibition and mortality rate every week for 6 weeks after treatment, the residual activities of the RTN treatments were estimated.

2.5. Ethical clearance

The experimental hut trials obtained clearance by the London School of Hygiene and Tropical Medicine Ethics Committee. The volunteer sleepers provided informed consent. The procedure for use of guinea pigs in the laboratory experiments were compliant with criteria laid down in EC Directive 86/609/ECC regarding protection of animals used for experimental purposes.

2.6. DEET-treated filter paper bioassays

Tarsal contact bioassays were conducted using filter papers treated with technical grade DEET. Solutions of DEET and silicon oil (0.7 ml) in acetone (1.3 ml) were spread over filter papers using recommended WHO procedures and dried for 2 h before use. The target concentrations of DEET in silicon oil after evaporation of acetone ranged from 0.125% to 2%. Knockdown and mortality resulting from contact with repellent were measured using WHO test kits. Batches of 25 *An. gambiae* Kisumu females (non-blood-fed, aged 2—3 days) were exposed to the treated papers for 1 h and then transferred to holding chambers. The number knocked down was recorded up to 60 min and mortality was assessed 24 h later. Exposure papers were removed from the test kit during the holding period to avoid any risk of continuing exposure to DEET in the vapour phase. Each concentration was tested four times (i.e. 100 insects per concentration).

2.7. Data analysis

Deterrency was analysed by comparing the number of mosquitoes entering each hut each day using Wilcoxon rank-sum non-parametric test. Proportional data from the hut trial (exophily, blood feeding and mortality) were analysed using a random effects logistic regression model to adjust for the effect of sleeper and intracluster variation. Tunnel test data were analysed using χ^2, and filter paper bioassays were analysed using probit analysis.

3. Results

3.1. Experimental hut trials

Over 180 hut-nights (45 nights $\times$ 4 huts) a total of 1013 mosquitoes were caught, of which 44% were *An. gambiae*, 29% *C. quinquefasciatus*, 15% *Mansonia* spp. and 12% other species (mostly *An. pharoensis* and *Aedes aegypti*). Only *An. gambiae* and *C. quinquefasciatus* data were analysed and the results are summarised in Table 2.

The numbers of *An. gambiae* collected were much lower in huts with RTNs. All types of RTNs deterred entry of *An. gambiae* compared with the hut with the untreated net. For *C. quinquefasciatus* the proportion deterred was not significant, although there was a trend

Table 2 Numbers of *Anopheles gambiae* and *Culex quinquefasciatus* collected from experimental huts over 45 nights at Yaokoffikro[a]

Treatment	Dose (g/m^2)	Total no.	% deterred	% blood fed (95% CI)	% feeding inhibition	% mortality after 24 h (95% CI)	% immediate mortality (95% CI)[b]	% in exit trap (95% CI)
Anopheles gambiae								
Untreated control net	Untreated	264*	–	27.7* (22.6–33.4)	–	4.2* (2.3–7.4)	2.1	19.7* (15.3–24.9)
Insect Ecran (DEET)	7.9	67**	74.6	10.4** (5.1–20.3)	62.5	76.1** (64.5–84.8)	94.6* (80.8–98.6)	26.9*,** (17.6–38.7)
Tropic 5/5 (IR3535)	7.6	70**	73.5	10.0** (4.8–19.5)	63.9	68.6** (56.8–78.3)	83.3* (69.0–91.8)	37.1** (26.7–49.0)
Prebutix Fort (IR3535)	7.3	48**	81.8	10.4** (4.4–22.7)	62.5	75.0** (61.0–85.2)	88.2* (72.5–95.5)	31.2*,** (19.8–45.6)
Culex quinquefasciatus								
Untreated control net	Untreated	88*	–	6.9* (1.6–12.2)	–	8.0* (2.3–13.7)	2/7	38.9* (28.7–49.1)
Insect Ecran (DEET)	7.9	73*	NS	5.5* (0.27–10.7)	20.3	57.5** (46.2–68.8)	95.8* (84.8–99.0)	50.7* (39.2–62.2)
Tropic 5/5 (IR3535)	7.6	72*	NS	5.6* (0.29–10.9)	18.8	51.4** (39.9–62.9)	90.2* (78.5–95.9)	38.9* (27.6–50.2)
Prebutix Fort (IR3535)	7.3	56*	NS	3.6* (1.3–8.5)	47.8	60.7** (47.9–73.5)	91.7* (77.1–97.3)	39.3* (26.5–52.1)

NS: not significantly different from control.
*,** For each species, numbers in a column bearing the same superscript do not differ significantly ($P > 0.05$).
[a] All nets were deliberately holed to simulate damaged nets.
[b] The % immediate mortality refers to the proportion of all deaths that had occurred at the time of collection from the huts.

for more to enter the hut with the untreated net than huts with RTNs.

Approximately 20% of *An. gambiae* and 39% of *C. quinquefasciatus* were trapped and collected in the veranda of the hut with the untreated net. There was evidence for slight but significant induced exophily of *An. gambiae* from the hut with the net treated with Tropic 5/5 (IR3535) but not from the huts with Prebutix Fort (IR3535)- or Insect Ecran (DEET)-treated nets (Table 2). There was no evidence for *C. quinquefasciatus* being repelled by any of the treatments (Table 2).

Untreated nets failed to provide protection when holed: 28% of *An. gambiae* and 7% of *C. quinquefasciatus* succeeded in feeding under the untreated net. Treatment of nets with DEET or IR3535 restored the capacity of the holed nets to prevent feeding of *An. gambiae*. For *Culex*, however, the blood-feeding rates were not reduced relative to the untreated net.

In the huts containing treated nets, there were high rates of mortality among *An. gambiae* and *C. quinquefasciatus* with all types of treatment used. All three treatments performed alike, killing 69–76% of *An. gambiae* that entered the huts, many of which would have been *kdr* resistant from our knowledge of gene frequency in this area (approximately 96% *kdr*), although no genotyping was carried out on this occasion. Percentage mortality among *C. quinquefasciatus* was less than among *An. gambiae* and did not differ significantly between treatments. More than 90% of mosquito mortality occurred before dawn.

No side effects were reported by sleepers under any of the RTNs.

3.2. Tunnel tests

The mortality rate of pyrethroid-resistant *C. quinquefasciatus* in the tunnel apparatus with untreated netting averaged approximately 15%. The high mortality rate and low blood-feeding rate observed with RTNs in the experimental hut trial was also observed in the tunnel tests. Mortality of *C. quinquefasciatus* was >80% with each type of repellent (Figure 1). There was a decline in repellent activity over time. A more rapid decline in toxicity was observed with the IR3535 brands than with DEET (Figure 1).

Figure 1 Mortality of *Culex quinquefasciatus* during overnight exposure to repellent-treated netting in the tunnel test apparatus. Insect Ecran is a DEET-based formulation; Prebutix Fort and Tropic 5/5 are IR3535-based formulations.

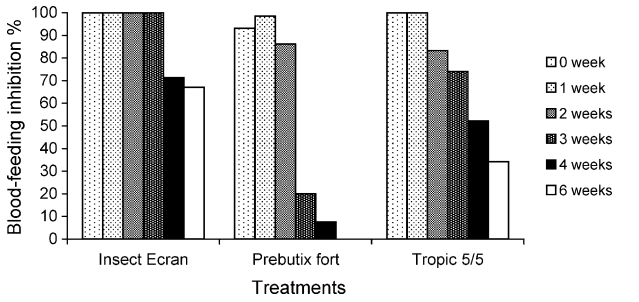

Figure 2 Protection against blood feeding provided by repellent-treated netting against *Culex quinquefasciatus* during overnight tests in the tunnel test apparatus. Insect Ecran is a DEET-based formulation; Prebutix Fort and Tropic 5/5 are IR3535- based formulations.

Blood feeding was inhibited with each of the treatments when newly applied. The IR3535 brand Prebutix Fort remained effective during the second week but no longer gave useful protection by the third week (Figure 2). The other IR3535 brand Tropic 5/5 and the DEET brand Insect Ecran continued to provide significant protection over 3—4 weeks, and the DEET product remained effective after 6 weeks.

3.3. Contact bioassays in WHO test kits

One hour exposure of adult females to filter papers treated with 2% DEET in silicon oil produced 100% mortality after 24 h (Figure 3). The mortality response to a range of concentrations fitted a log-dosage probit response curve (LD_{50} 0.31%, 95% CI 0.21—0.51; LD_{95} 1.1%, 95% CI 0.7—3.0) more normally associated with insecticide response. Knockdown was observed for concentrations of DEET $\geq$1%. First knockdown exposure to 2% DEET was observed within 10 min and the KDT_{50} was 40 min (95% CI 30—53 min). Contact toxicity of DEET on filter paper confirmed the observations of mortality in tunnel tests and field experiments.

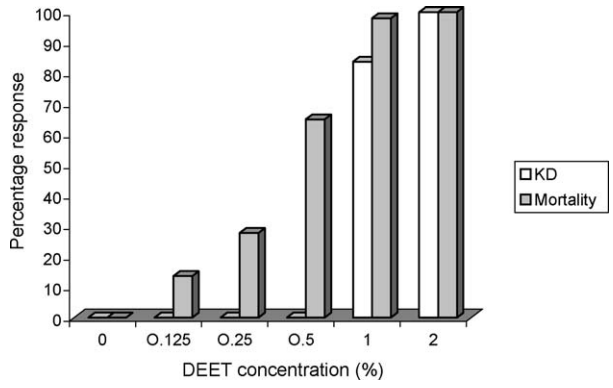

Figure 3 Knockdown (KD) and mortality after 60 min exposure in WHO test kits to filter papers treated with DEET dissolved in silicon oil. Knockdown was recorded 60 min after exposure and mortality was recorded after 24 h.

4. Discussion

Mosquito nets treated with three types of repellent formulation (Prebutix Fort®, Insect Ecran® and Tropic 5/5®) inhibited entry of *An. gambiae* to a greater degree (73.5—81.8%) than that observed with pyrethroid-treated nets (43—64%) earlier at the same site (Darriet et al., 2000; Hougard et al., 2003). The deterrent effect of repellents did not extend to *C. quinquefasciatus*, a species known to be much less responsive to the deterrent effect of pyrethroids (Guillet et al., 2001).

With the exception of the net treated with the brand of IR3535 known as Tropic 5/5, no repellent effect of the RTNs (from the room to veranda trap) was observed once mosquitoes had entered the huts. This finding appears contrary to the observation of deterred entry of mosquitoes into RTN huts, deterrence being a form of repellency. Presumably any repellency within the huts was overshadowed by the toxic effects of the RTNs. The mortality effect was unexpected but was later confirmed in laboratory bioassays. Deterrency, inhibition of blood feeding and mortality together produced a personal protective effect of RTNs equal to or better than that of most pyrethroid-treated nets against pyrethroid-resistant anophelines and culicines (Darriet et al., 2000; Hougard et al., 2003). It has long been thought that repellents simply drive mosquitoes away rather than kill them. This might be true if mosquitoes can orientate and move away from the source of DEET. The mode of action of repellents is, in fact, largely unknown. Repellents disturb the capacity of receptors in the mosquitoes' antennae to respond to host stimuli (Davis, 1985). There is increasing evidence for toxic effects of repellents, as observed in our laboratory bioassays and field experiments. Elsewhere, topical application of DEET to the German cockroach killed 50% (LD_{50}) at a dose of 2.7 mg/g (Moss, 1996). Using electrophysiological techniques, it has been shown that application of 100 micromoles of DEET to Dorsal Umpair Median neurones of the American cockroach induces an increase in Ca^{++} ions and a strong neurotoxic action (Cedric Pennetier, personal communication). It is alleged that bioassay with 5% DEET in silicon oil on filter paper induces knockdown and mortality of mosquitoes (Pennetier et al., 2005). DEET applied as aerosol spray or larvicide also shows toxic activity (Xue et al., 2001, 2003). Repellents might therefore be regarded as toxicants in certain situations and not simply as behaviour-modifying chemicals.

More information is needed to understand the neurotoxic activity of repellents on nets. Repellents have spatial action (through volatility) but also a contact action if mosquitoes alight on the treated surface. Within our style of huts, which are designed to inhibit egress of mosquitoes, mortality might have been induced by a build up of repellent vapour within the room together with an inability to avoid exposure to that vapour. However, the rate of vaporisation is likely to be much less on nets than on skin, as indicated by the much longer residual activity of repellents on nets shown in the present paper. Therefore, a more likely explanation is that mosquitoes may alight for longer periods on RTNs than on treated skin and in so doing may pick up a lethal dose. Distinguishing between the spatial/contact hypotheses might be put to the test by providing more ventilation in huts, in which case a lower mortality would be expected if

spatial repellency was the primary cause of toxicity. This is an important experiment to undertake because although the risks are low, adverse reactions do occasionally occur among users of DEET (Osimitz and Grothaus, 1995). In over 20 000 DEET exposures reported to the US Poison Control Center during the 1990s, 26 individuals reported serious adverse reactions and 2 deaths occurred following dermal exposure (Bell et al., 2002). The exact role of DEET in the toxicity reported was difficult to determine from the reports, and there was no clear relationship between DEET concentration and presence or severity of clinical effect. The authors concluded that the risk of serious adverse effects following the use of DEET was extremely low (Bell et al., 2002; Osimitz and Grothaus, 1995).

Our experiments show that RTNs should provide personal protection for at least 6 weeks. Slow-release formulations of DEET are under development and preliminary experiments indicate long-term efficacy and toxicity on nets (M. Rowland and R. N'Guessan, unpublished data).

The mortality of *kdr* resistant mosquitoes indicates that impregnation of nets with repellents might constitute a useful tool to overcome pyrethroid-resistant mosquitoes and to prevent malaria given that there are few alternatives to pyrethroids currently available. RTNs warrant further development as a malaria control tool.

Conflicts of interest statement
The authors have no conflicts of interest concerning the work reported in this paper.

Acknowledgements

We wish to thank all the staff at the Institut Pierre Richet, Bouaké, Côte d'Ivoire, for their work during the trial, particularly Mr Konan Yao for mosquito rearing. We also thank the company Pierre Fabre, France, for donating commercial formulations of repellents used in this study, and Cedric Pennetier for giving his support. R.N'G. and M.R. are supported by the Gates Malaria Partnership of the London School of Hygiene & Tropical Medicine.

References

Asidi, A.N., N'Guessan, R., Hutchinson, R.A., Traore-Lamizana, M., Carnevale, P., Curtis, C.F., 2004. Experimental hut comparisons of nets treated with carbamate or pyrethroid insecticides, washed or unwashed, against pyrethroid-resistant mosquitoes. Med. Vet. Entomol. 18, 134—140.

Asidi, A.N., N'Guessan, R., Koffi, A.A., Curtis, C.F., Hougard, J.M., Chandre, F., Corbel, V., Darriet, F., Zaim, M., Rowland, M., 2005. Experimental hut evaluation of bednets treated with an organophosphate (chlorpyrifos-methyl) or a pyrethroid (lambdacyhalothrin) alone and in combination against insecticide-resistant *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes. Malaria J. 4, 25.

Barnard, D.R., 2000. Repellents and Toxicants for Personal Protection. Global Collaboration for Development of Pesticides for Public Health. World Health Organization, Geneva, WHO/CDS/WHOPES/GCDPP/2000.5.

Barnard, D.R., Bernier, U.R., Posey, K.H., Xue, R., 2002. Repellency of IR3535, KBR3023, para-menthane-3,8-diol, and deet to back salt marsh mosquitoes (Diptera: Culicidae) in the Everglades National Park. J. Med. Entomol. 39, 895—899.

Bell, J.W., Veltri, J.C., Page, B.C., 2002. Human exposures to *N,N*-diethyl-*m*-toluamide insect repellents reported to the American Association of Poison Control Centers 1993—1997. Int. J. Toxicol. 21, 341—352.

Chandre, F., Darriet, F., Darder, M., Cuany, A., Doannio, J.M.C., Pasteur, N., Guillet, P., 1998. Pyrethroid resistance in *Culex quinquefasciatus* from West Africa. Med. Vet. Entomol. 12, 359—366.

Chandre, F., Darriet, F., Manguin, S., Brengues, C., Carnevale, P., Guillet, P., 1999. Pyrethroid cross resistance spectrum among populations of *Anopheles gambiae* s.s. from Côte d'Ivoire. J. Am. Mosq. Control Assoc. 15, 53—59.

Curtis, C.F., Lines, J.D., Ijumba, J., Callaghan, A., Hill, N., Karimzad, M.A., 1987. The relative efficacy of repellents against mosquito vectors of disease. Med. Vet. Entomol. 1, 109—119.

Curtis, C.F., Wilkes, T.J., Mbwana, H., Chambika, C., Aina, Y., 1994. Comparison of the effectiveness and persistence of mosquito repellency due to *quwenling* and deet. Trans. R. Soc. Trop. Med. Hyg. 88, 372—373 [abstract].

Darriet, F., Guillet, P., N'Guessan, R., Doannio, J.M.C., Koffi, A.A., Konan, L.Y., Carnevale, P., 1998. [Impact of resistance of *Anopheles gambiae* s.s. to permethrin and deltamethrin on the efficacy of impregnated mosquito nets]. Med. Trop. (Mars.) 58, 349—354 [in French].

Darriet, F., N'Guessan, R., Koffi, A.A., Konan, L., Doannio, J.M.C., Chandre, F., Carnevale, P., 2000. [Impact of pyrethrin resistance on the efficacity of impregnated mosquito nets in the prevention of malaria: results of tests in experimental cases with deltamethrin SC]. Bull. Soc. Path. Exot. 93, 131—134 [in French].

Davis, E.E., 1985. Insect repellents: concepts of their mode of action relative to potential sensory mechanisms in mosquitoes (Diptera: Culicidae). J. Med. Entomol. 30, 179—183.

Guillet, P., N'Guessan, R., Darriet, F., Traore-Lamizana, M., Chandre, F., Carnevale, P., 2001. Combined pyrethroid and carbamate 'two-in-one' treated mosquito nets: field efficacy against pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus*. Med. Vet. Entomol. 15, 105—112.

Henry, M.C., Assi, S.B., Rogier, C., Dossou-Yovo, J., Chandre, F., Guillet, P., Carnevale, P., 2005. Protective efficacy of lambda-cyhalothrin treated nets in *Anopheles gambiae* pyrethroid resistance areas of Côte d'Ivoire. Am. J. Trop. Med. Hyg. 73, 859—864.

Hougard, J.M., Corbel, V., N'Guessan, R., Darriet, F., Chandre, F., Akogbeto, M., Baldet, T., Guillet, P., 2003. Efficacy of mosquito nets treated with insecticide mixtures against insecticide resistant *Anopheles gambiae* and *Culex quinquefasciatus* (Diptera: Culicidae) in Côte d'Ivoire. Bull. Entomol. Res. 93, 491—498.

Kolaczinski, J.H., Fanello, C., Herve, J.P., Conway, D.J., Carnevale, P., Curtis, C.F., 2000. Experimental and molecular genetic analysis of the impact of pyrethroid and non-pyrethroid insecticide impregnated bednets for mosquito control in an area of pyrethroid resistance. Bull. Entomol. Res. 90, 125—132.

Kroeger, A., Gerhadus, A., Kruger, G., Mancheno, M., Pesse, K., 1997. The contribution of repellent soap to malaria control. Am. J. Trop. Med. Hyg. 56, 580—584.

Lengeler, C., 1998. Insecticide treated nets and curtains for malaria control. The Cochrane Library, Issue 3. Update Software, Oxford.

Moss, J.I., 1996. Synergism of toxicity of *N,N*-diethyl-*m*-toluamide to German cockroaches (Orthoptera: Blattellidae) by hydrolytic enzyme inhibitors. J. Econ. Entomol. 89, 1151—1155.

N'Guessan, R., Darriet, F., Doannio, J.M.C., Chandre, F., Carnevale, P., 2001. Olyset Net efficacy against pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus* after 3 years' field use in Côte d'Ivoire. Med. Vet. Entomol. 15, 97—104.

Osimitz, T.G., Grothaus, R.H., 1995. The present safety assessment of deet. J. Am. Mosq. Control Assoc. 11, 274—278.

Pennetier, C., Corbel, V., Hougard, J.M., 2005. Combination of a non-pyrethroid insecticide and a repellent: a new approach for

controlling knockdown-resistant mosquitoes. Am. J. Trop. Med. Hyg. 72, 739—744.

Rowland, M., Downey, G., Rab, A., Freeman, T., Mohammad, N., Rehman, H., Durrani, N., Reyburn, H., Curtis, C., Lines, J., Fayaz, M., 2004a. DEET mosquito repellent provides personal protection against malaria: a household randomized trial in an Afghan refugee camp in Pakistan. Trop. Med. Int. Health 9, 335—342.

Rowland, M., Freeman, T., Downey, G., Hadi, A., Saeed, M., 2004b. DEET mosquito repellent sold through social marketing provides protection against malaria in an area of all-night mosquito biting and partial coverage of insecticide-treated nets: a case—control study of effectiveness. Trop. Med. Int. Health 9, 343—350.

Rozendaal, J.A., 1997. Vector Control. Methods for use by individuals and communities. World Health Organization, Geneva.

Schreck, C.E., Kline, D.L., Smith, D., 1979. Protection afforded by the insect repellent jacket against four species of biting midge (Diptera: Ceratopogonidae). Mosq. News 39, 739—742.

Smith, C.N., Burnett, D., 1948. Laboratory evaluation of repellents and toxicants as clothing treatments for personal protection from flies and ticks. Am. J. Trop. Med. Hyg. 28, 599—607.

Vittal, M., Limaye, L.S., 1984. Field village scale trial of use of repellent in malaria control. Indian J. Med. Sci. 38, 201—203.

WHO, 1998. Guideline Specifications for Household Insecticide Product. Report of the WHO consultation 3—6 February. World Health Organization, Geneva, WHO/CTD/WHOPES.98.3.

WHO, 2001. Report of the 4th WHOPES Working Group meeting — IR3535, KBR3023, (RS)-methoprene 20%EC, pyriproxyfen 0.5%GR and lambda-cyhalothrin 2.5%CS. 4—5 December 2000. World Health Organization, Geneva, WHO/CDS/WHOPES/2001.2.

Xue, R.D., Barnard, D.R., Ali, A., 2001. Laboratory and field evaluation of insect repellents as larvicides against the mosquitoes *Aedes albopictus* and *Anopheles albimanus*. Med. Vet. Entomol. 15, 374—380.

Xue, R.D., Ali, A., Barnard, D.R., 2003. Laboratory evaluation of toxicity of 16 insect repellents in aerosol sprays to adult mosquitoes. J. Am. Mosq. Control Assoc. 19, 271—274.