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Field evaluation of the efficacy and persistence of insect repellents DEET, IR3535, and KBR 3023 against *Anopheles gambiae* complex and other Afrotropical vector mosquitoes

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Summary Synthetic insect repellents, IR3535 and KBR 3023 (also known as picaridin, or by the trade name Bayrepel®), were tested in Burkina Faso against mosquito vectors of disease to compare their relative efficacy and persistence profiles to those of the 'gold standard' DEET. Collection of >49 000 mosquitoes (~95% belonging to the *Anopheles gambiae* complex) showed that after an exposure of 10 h, KBR 3023 produced the highest protection against anophelines, followed by DEET, then IR3535. The response of aedines was more variable. By fitting a logistic plane model we estimated 95% effective dosages (ED_{95}) for *An. gambiae* s.l., as well as a decay constant characterizing the exponential loss of repellent from the skin, with time. The ED_{95} values for DEET, IR3535, and KBR 3023 were 94.3, 212.4, and 81.8 $\mu g/cm^2$ respectively. The decay constants were estimated at −0.241, −0.240, and −0.170 h^{-1} respectively. The corresponding estimates of half-life were 2.9, 2.9, and 4.1 h. Immunoenzymatic detection of the circumsporozoite protein (CSP) of *Plasmodium falciparum* in 842 *An. gambiae* s.l. showed that CSP-positive mosquitoes were equally frequent in treated and control subjects, indicating that the repellents could produce a reduction in the number of malaria infectious bites.

1. Introduction

Synthetic repellents are a common means of personal protection against biting arthropods. N,N-Diethyl-3-methyl-benzamide (DEET) is the main or sole active ingredient of most commercial repellent formulations, and because of its efficacy and low toxicity proven over many decades of widespread consumer use is arguably the standard ingredient against which the performance of other compounds is generally evaluated.

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In spite of the extensive use of traditional self-protective measures (e.g. mosquito coils or repellent plant remedies) adopted by individuals in the tropics against biting pests, synthetic repellents are rarely used to protect communities from malaria and other vector-borne diseases. Cost and, sometimes, safety constraints are the main reasons for this situation (Gupta and Rutledge, 1994); moreover, the lower efficacy of DEET against several anophelines (Curtis et al., 1987; Frances et al., 1993), and its relative short persistence contribute to this status quo. Impregnation of bednets or garments with DEET can prolong its persistence (Curtis et al., 1987; Gouck and Moussa, 1969), but must be repeated more frequently than when using pyrethroids, such as permethrin, in order to achieve comparable levels of protection (Curtis et al., 1991). Pyrethroids are generally more cost-effective, providing the additional benefit of killing a proportion of mosquitoes that come in contact with the treated surface (Gupta et al., 1990). However, exposure to mosquito bites early in the evening, when people usually stay outdoors, offers a window for disease transmission. Even for species whose biting cycle is delayed until later at night, as in the case of the Afrotropical malaria vectors *Anopheles funestus* Giles, *An. gambiae* Giles, and *An. arabiensis* Patton, human–vector contact during this period may be non-negligible whenever high mosquito densities are experienced. Although there is as yet no conclusive formal evidence, it is likely that efficient, long-lasting, and cheap repellents in suitable formulations may contribute to lower entomological inoculation rates, especially in target groups suffering increased risks from disease. Moreover, given the appearance of insecticide resistance in vector populations (e.g. that provided by the *kdr* allele in West African populations of *An. gambiae*) (Chandre et al., 1999), repellents might profitably be used in resistance management strategies aimed at prolonging the useful life of mainstay active ingredients.

We assessed the relative efficacy and persistence properties afforded by topical application of two synthetic repellents under field conditions, namely 3-(N-n-butyl-N-acetyl)-aminopropionic acid ethyl ester (Insect-Repellent 3535 or IR3535), and the recently-developed 1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-, 1-methylpropylester (known as KBR 3023), and compare them to the 'gold standard' DEET. KBR 3023 is also known as Picaridin and marketed by Bayer AG under the trade name Bayrepel® in the Autan® brand of repellent formulations; here we employ the original name as this is found in some earlier reports about this compound.

2. Materials and methods

2.1. Study area

Field tests were performed in the rural village of Goden (12°25'N, 1°20'W), in the Oubritenga Province of Burkina Faso, about 30 km east of the capital Ouagadougou. A description of this area and its malaria vectors can be found in Costantini et al. (1996). Three taxa of the *An. gambiae* sensu lato complex are present in the area, namely *An. arabiensis*, and *An. gambiae* sensu stricto molecular forms M and S.

2.2. Collection procedures

Collections were performed by four teams of two human volunteers each (20 to 30-year-old males) sitting side-by-side and using modified pooters to aspirate all mosquitoes landing on their exposed limbs (i.e. the feet and legs from the knees to the ankles) into paper cups, before they had a chance to bite and engorge. In the entomological literature, this collection technique is often called the human landing catch. Cups were changed every half hour, and mosquitoes were provided with a 5% sugar solution and stored in a cool box until processing. At this time, the two collectors of each team swapped their relative positions. Teams worked inside four compounds in the village separated by at least 20 m from each other. Collections were carried out outdoors during the early part of the evening, starting at dusk (18:00–22:00 hours), and later in the night indoors, during the peak biting time of the main malaria vectors *An. gambiae* s.l. and *An. funestus* (00:00–04:00 hours). This allowed collectors to rest between the two collection periods. Moreover, this procedure simulated more closely the real conditions under which repellents may be used by local people.

2.3. Application procedures

Repellents were applied as uniformly as possible on the feet and lower limbs just before the start of the first collection period. Four target doses were tested: 0.1 mg/cm^2, 0.3 mg/cm^2, 0.6 mg/cm^2, and 0.8 mg/cm^2. We estimated the area of skin to be treated for every collector. Then, we assessed the volume of technical grade repellent needed to treat both legs of each collector, and diluted it in 20 ml of 90% ethanol (two glass vials of 10 ml each, one for each leg). The concentration of the solutions ranged from 2 to 13% v/v. Control collectors were given 20 ml of 90% ethanol as a placebo. On each test

night, both collectors in a team received the same treatment, but all were unaware of what it was.

2.4. Experimental protocol

By performing a series of preliminary human landing catches before the start of the tests, teams of collectors were matched for numbers of collected mosquitoes, so that relative differences among teams in skills/attractiveness could be minimized. Then, each team was permanently assigned to a given compound, and the composition of teams remained the same throughout the experiment. Thus, spatial variability in mosquito densities within the village was confounded with differences in collectors' skills/attractiveness. Four compounds were identified, and a randomixed 4 × 4 (night × compound) Latin Square was implemented with the three repellents and the placebo rotating among teams on subsequent nights. In this way, we could not separate the contribution to the total variance accounted for by collectors' skills/attractiveness from that accounted for by different compounds (this would have required a more complex design such as a Graeco–Latin Square; however this was deemed unnecessary in relation to the objectives of the experiment, given that we ensured treatments were randomly and homogenously exposed to these two combined sources of variance). Collectors completed each Latin Square in one week. This constituted one experimental replicate. During each replicate, the same application dose was tested for all repellents. A series of independently randomized Latin Squares was completed in six months. Different doses were tested on successive replicates according to a 0.1 – 0.8 – 0.3 – 0.6 cycle.

2.5. Processing of specimens

Collected mosquitoes were identified morphologically using the keys of Edwards (1941) for culicines, and Gillies and Coetzee (1987) for anophelines. Mosquitoes were stored in test tubes containing a desiccant according to treatment, species and gonotrophic stage. Subsamples of specimens belonging to the *An. gambiae* complex were tested with an ELISA to detect the presence of the *Plasmodium falciparum* circumsporozoite protein (CSP) in the thorax of individual corpses (Wirtz et al., 1985).

2.6. Assessment of microclimatic conditions

During mosquito collections, the temperature (resolution ±0.1 °C, accuracy ±0.1 °C) and relative humidity (resolution ±0.1%, accuracy ±0.5%) were measured, in loco, out of doors during the first catching phase, and indoors during the second catching phase, with an electronic probe (Delta-T Devices Ltd, Cambridge, UK). Minimum temperatures ranged from 18.2 to 27.7 °C, and maximum temperatures from 25.8 to 33.0 °C. Relative humidity (RH) was high throughout the wet season (July to October), with median values often reaching saturation point, whereas values dropped steadily after the end of the rains; minimum RH values ranged from 30.3 to 94.4%.

2.7. Statistical analysis

The total number of mosquitoes collected every hour was pooled over all replicates according to treatment and dose. The probability of mosquito response to the treated skin (q) was expressed as a function of the proportion (p) of the number of mosquitoes caught by those collectors who applied the repellents over the numbers caught by the control collectors; that is, $q = 1 - p$. We estimated the effective dosages and persistence afforded by each repellent by fitting a logistic version of the probit plane model of Rutledge et al. (1985) using maximum likelihood procedures with the GLIM software (Numerical Algorithms Group Ltd, Oxford, UK):

$$\ln\left[\frac{q}{(1-q)}\right] = a + b_1 X_0 + b_2 t \qquad (1)$$

where X_0 is the natural logarithm of the dose of repellent in mg/cm^2 applied at the start of the test, t is the time in hours from application, and a, b_1, and b_2 are coefficients which depend on the repellent, mosquito and testing conditions. The model assumes that there is a logistic increase of q with the logarithm of the dose of repellent present at any time on the skin, and that the loss with time of repellent from the skin approximates an exponential decay process according to the equation:

$$X_t = X_0 \exp(\lambda t) \qquad (\lambda < 0) \qquad (2)$$

where X_t is the amount of repellent remaining on the skin at time t, and λ is a decay constant. The model allows the estimation of effective dosages (ED) by holding the test period constant and equal to zero ($t = 0$ in Eq. (1)). The decay parameter λ was estimated as b_2/b_1, whereas the repellent's half-life, i.e. the time from application corresponding to a residual deposit of repellent half of that applied at time zero, was estimated as $\ln(1/2)/\lambda$. GLIM uses the binomial denominator (here the control sample size) as a weight in regression, and it allows for the non-constant binomial variance.

Formally, such a procedure is not correct because our binomial denominator, i.e. the number of mosquitoes attempting to bite the collectors, was strictly unknown. We estimated the denominator from the total number of mosquitoes landing on the control collectors. We believe that our protocol and sampling effort reduced the error to a level accurate enough for our analytical purposes. The extra-binomial variation in the data, i.e. overdispersion, was allowed for by applying Williams' algorithm (Collett, 1991). Statistical inference for differences among the model coefficients was tested first by likelihood ratio tests removing the interaction terms and main effects, in turn, from the full model containing all parameters, and comparing the resulting changes in deviance to an *F*-distribution for the corresponding number of degrees of freedom (Crawley, 1993). Confidence limits for EDs and decay constants were calculated using Fieller's theorem (Collett, 1991).

2.8. Ethical considerations

The volunteers were recruited from the study village after having obtained their informed consent. Before the start of the experiments, they received vaccination against yellow fever. During the tests, they followed a prophylactic regime against malaria, and were under strict clinical and parasitological surveillance for drug-resistant disease. No such case was detected during the course of the study. The protocol received formal ethical approval from the Ministry of Health of Burkina Faso.

3. Results

More than 49 000 mosquitoes, stratified by treatment and species (Table 1) were caught during the tests. Anophelines formed 98.5% of the total catch, with mosquitoes of the genus *Aedes* making up the remaining 1.5%. Among anopheline mosquitoes, 95% belonged to the *An. gambiae* complex, *An. nili* was the second most abundant species, followed by *An. funestus*, and then *An. pharoensis*. Among aedines, mosquitoes of the *Aedes taylori* group (*Ae. taylori* and *Ae. furcifer*) were the most frequently caught, followed by *Ae. vittatus* and *Ae. hirsutus*. The frequency of mosquitoes landing on the treated collectors, compared with the control subjects, varied according to the repellent used (Table 1). At the end of the 10 h exposure period, KBR 3023 always produced the highest protection against anophelines, followed by DEET, and then IR3535. Conversely, the response of aedine mosquitoes was more variable, as reflected by the lower sample sizes obtained.

Table 1 Total number of the most common mosquitoes collected landing on humans in repellent field trials carried out in Burkina Faso during 96 test nights

Species	Repellent					Control	Total
	DEET		IR3535		KBR 3023		
Anopheles funestus	133	48.9% (43.0–54.8%)	162	59.6% (52.3–68.4%)	60	22.1% (16.9–30.0%)	272	627
Anopheles gambiae s.l.	6454	23.7% (20.4–29.2%)	8891	32.7% (28.5–39.2%)	3485	12.8% (10.8–16.3%)	27231	46061
Anopheles nili	217	17.2% (14.1–22.3%)	274	21.8% (18.1–27.6%)	117	9.3% (7.3–12.7%)	1259	1867
Anopheles pharoensis	13	20.0% (12.0–33.4%)	18	27.7% (18.1–42.0%)	8	12.3% (6.3–24.2%)	65	104
Aedes hirsutus	4	2.8% (1.1–7.7%)	2	1.4% (0.4–5.7%)	0	0.0%	144	150
Aedes taylori gr.	12	3.1% (1.7–5.8%)	18	4.6% (2.9–7.9%)	17	4.3% (2.7–7.5%)	393	440
Aedes vittatus	3	2.1% (0.7–6.6%)	8	5.5% (2.8–11.5%)	11	7.6% (4.2–14.2%)	145	167
Total	6836		9373		3698		29509	49416

The percent frequency of mosquitoes collected by treated collectors relative to the total number caught by the control is shown for any given species and repellent; 95% confidence intervals are shown in parentheses.

DEET was the most efficient repellent for *Ae. vittatus* and mosquitoes of the *Ae. taylori* group. KBR 3023 produced the highest protection against *Ae. hirsutus*. It is important to note, however, that the efficacy of repellents in Table 1 should not be compared across species as the efficacy is affected by differences in their biting cycle in relation to the repellents' loss rates (see below).

The decay with time in the repellents' efficacy against *An. gambiae* s.l. depended on the application dose as shown in Figure 1. As expected, for any given level of protection, the repellent's effect lasted longer when applied at a higher dose. Within the time frame of our tests, KBR 3023 showed the longest-lasting effect at all application doses, followed by DEET and then IR3535. Both interaction terms of the logit plane model accounted for a significant portion of the total deviance ($F_{2,89} = 6.2$; $P < 0.01$ for the TIME*REPELLENT term and $F_{2,89} = 14.6$; $P < 0.001$ for the DOSE*REPELLENT term) indicating that the model coefficients were significantly different across repellents. Summary statistics in Table 2 show that according to the logit plane model, KBR 3023 had the lowest and IR3535 the highest ED estimates at both the 50% and 95% endpoints. The ED estimates of KBR 3023 and DEET, however, were similar and their 95% confidence limits overlapped extensively, suggesting that the functional dose-response relationship and sensitivity of *An. gambiae* s.l. for these two compounds was not substantially different. DEET and IR3535 had similar estimates of the loss rate λ, whereas KBR 3023 estimates were lower, suggesting that, at any application dose, the longer-lasting protective efficacy observed with KBR 3023, exemplified by its higher half-life, was presumably not due to higher sensitivity of *An. gambiae* s.l. to this compound, but rather to a longer degree of persistence on the skin, compared with the other two repellents tested.

The immunoenzymatic detection of infectious mosquitoes in 842 specimens showed that the proportion of CSP-positive *An. gambiae* s.l. varied considerably with time (range 1.8–12.1% according to replicate). Accordingly, the proportion of positive mosquitoes in each sample was stratified by replicate and compared across treatments. No statistically significant difference ($G = 0.27$; d.f. $= 3$; $P > 0.96$) was found between the CSP rates of mosquito pools collected by treated (5.3%) and control (4.8%) subjects.

4. Discussion

Two synthetic insect repellents, IR3535 and the recently-introduced KBR 3023, were tested in the

Table 2 Summary statistics for the three repellents tested against *Anopheles gambiae* s.l. Parameter estimates (±SE) a, b_1, b_2 of the logistic plane model (Eq. (1) in text), estimated median effective dosage (ED_{50} in mg/cm^2) and ED_{95}, decay parameter λ (h^{-1}), and half-life (in hours), characterizing each repellent's loss from the skin with time

Repellent	a	b_1	b_2	ED_{50}	ED_{95}	Slope λ	Half-life
DEET	8.160 (±0.3472)	2.209 (±0.1064)	−0.532 (±0.0326)	0.0249 (0.0197–0.0308)	0.0943 (0.0776–0.1149)	2.21	−0.241 (−0.270–−0.214) 2.9 (2.6–3.2)
IR3535	5.406 (±0.4113)	1.589 (±0.0770)	−0.382 (±0.0226)	0.0333 (0.0223–0.0470)	0.2124 (0.1515–0.3115)	1.59	−0.240 (−0.292–−0.195) 2.9 (2.4–3.6)
KBR 3023	9.413 (±0.5910)	2.583 (±0.1632)	−0.439 (±0.0381)	0.0262 (0.0183–0.0354)	0.0818 (0.0620–0.1073)	2.58	−0.170 (−0.212–−0.132) 4.1 (3.3–5.3)

95% confidence intervals are shown in parentheses.

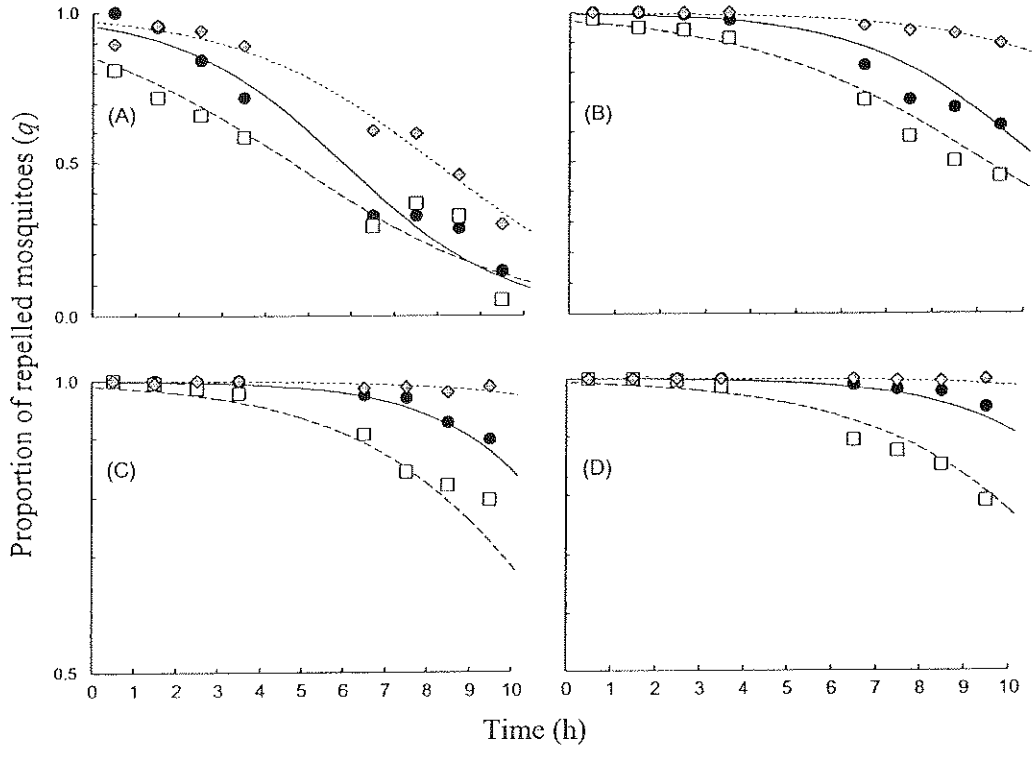

Figure 1 Decline with time in repellents' efficacy. Mean proportion of *Anopheles gambiae* s.l. responding to the repellents (q, on the ordinate) in relation to time from application. DEET, closed circles; IR3535 open squares; KBR 3023 shaded diamonds. Application target dose (A) 0.1 mg/cm^2; (B) 0.3 mg/cm^2; (C) 0.6 mg/cm^2; (D) 0.8 mg/cm^2. Superimposed curves drawn according to the logistic plane model of Eq. (1) whose parameters are shown in Table 2. Note the difference in scale of the ordinate between the upper and lower diagrams.

field against Afrotropical mosquitoes vectors of disease, and their relative efficacy and persistence profiles compared with that of the standard repellent DEET. The sensitivity of *An. gambiae* s.l. to KBR 3023 was similar to that of DEET, confirming observations from laboratory tests showing the same relative potency between these two compounds (Badolo et al., 2004). Conversely, *An. gambiae* s.l. was more tolerant to IR3535, as indicated by ED_{95} estimates about two-fold higher than those of DEET or KBR 3023. The persistence profile of DEET and KBR 3023, however, was different. The coefficient λ of an exponential decay model describing the rate of loss of repellent from the skin was significantly lower in the case of KBR 3023, leading to an estimated half-life of 4.1 h for this compound, about an hour longer than the corresponding figures calculated for DEET and IR3535. These results may explain why KBR 3023 consistently showed the longest persistence at all application doses tested, and retained more than 99% protection after 10 h at an application dose of 0.8 mg/cm^2 under climatic conditions unfavourable to the repellents persistence on the skin (high temperatures and relative humidity). It must be noted that the decay model of Eq. (2) does not discriminate between different mechanisms of repellent loss from the skin, i.e. evaporation, dermal absorption and rubbing, so it is not known what their relative contribution was to the decay process.

KBR 3023 is an active compound developed using molecular modelling techniques, which has good cosmetic properties, a favourable toxicological profile (reviewed in WHO, 2001), and low aggressiveness against plastic materials (Nentwig et al., 2002). Few studies have been published on its efficacy and persistence against mosquitoes, particularly anophelines. In field tests in Malaysia it was found to be as effective as DEET in protecting against *Ae. albopictus* and *Culex quinquefasciatus* (Yap et al., 1998). Similar field results were obtained in the Czech Republic with *Ae. cantans*, *Ae. annulipes* and *Ae. sticticus* (Rettich, 1999). In the Florida Everglades, KBR 3023 afforded the same protection as DEET against high biting densities of *Ae. taeniorhynchus*, and both repellents provided >89% repellence at the end of 6 h tests (Barnard, unpublished report in WHO, 2001).

As found in similar studies with *An. funestus* (Copeland et al., 1995), no significant difference

was found among treated and control subjects in the proportion of landing *An. gambiae* s.l. harbouring *P. falciparum* sporozoites, demonstrating that the response to the repellents did not change depending on the infectious state of the mosquito, i.e. a reduction in the number of bites afforded by the repellents caused a reduction in the number of infectious bites as well.

After the 10 h exposure period, KBR 3023 always ranked as the most efficient repellent against the anopheline mosquitoes captured during our tests, including all the other important savanna malaria vectors *An. funestus* and *An. nili*, and the secondary vector *An. pharoensis*. Conversely, IR3535 was consistently the least efficient among the three compounds tested. DEET ranked as the most efficient in repelling *Ae. vittatus* and species of the *Ae. taylori* group; these species are implicated in the circulation of several arboviruses such as dengue, yellow fever, and chikungunyia in wild populations of reservoir hosts (Diallo et al., 1999). In laboratory tests, Badolo et al. (2004) demonstrated a higher relative potency of KBR 3023 with respect to DEET for a West African strain of *Ae. aegypti*. The difference in efficacy among anophelines and aedines observed in our field tests, therefore, was probably due to the earlier, crepuscular activity at dusk of the latter species which concealed the superior persistence profile of KBR 3023. Thus, regardless of considerations of cost and safety, KBR 3023 appears on the basis of these results as the synthetic repellent having the most favourable efficacy/persistence profile for personal protection against malaria in Burkina Faso and, presumably, in the Afrotropical region.

At the community level, however, the main constraint to the use of repellents as public health tools against vector-borne diseases lie in their cost, compounded by the short persistence they usually afford. On the basis of its patent-free status, and the experience accumulated over many decades, DEET is likely to remain one of the most cost-efficient and safe options for repellent-based vector control campaigns. Because of the more favourable efficacy/persistence profile of KBR 3023, however, at least two areas of application could be envisaged to reduce transmission in target human groups. First, the higher protective efficacy of KBR 3023 could be exploited whenever exceptional vector densities are encountered, as is often the case in wetlands, for example in large-scale rice plots. Second, to reduce cost to affordable levels, lower doses could be applied in areas or at times of reduced transmission intensity. As an example it is possible to calculate the estimated doses of KBR 3023 and DEET to be applied to provide 95% and 99.99% protection for 5 h, a reasonable interval covering the period from the start of the active phase of a night-biting vector at dusk and the time people generally move indoors where they can afford the protection of a mosquito net. A dose of about 0.2 mg/cm^2 of KBR 3023, which is 33% less than the manufacturer's recommended dose or that required for DEET, can provide $\geq$95% protection up to 5 h. This may prove sufficient and affordable, especially to protect the reduced skin surface of infants, in some epidemiological facies where transmission is not particularly intense. Because of insufficient data, however, the application of KBR 3023 in children less than 2 years of age is as yet to be recommended (Nentwig et al., 2002). In fact, despite its good toxicological profile, KBR 3023 has not yet received the extensive testing that DEET has gained by the many decades of its widespread commercial use (reviewed in Qiu et al., 1998). Reproductive and developmental toxicity studies did not demonstrate any significant effect of KBR 3023 for doses ranging from 50 to 400 mg/kg of bodyweight in both rats and rabbits, but in the rabbit, dermal effects (slight erythema, squamous and cracked skin) were noted at the dose site of virtually all doses administered (Astroff et al., 1999, 2000).

Whenever biting densities are exceptionally high, even 99% protection may not be enough. A dose of about 2 mg/cm^2 of KBR 3023 is estimated to provide $\geq$99.99% protection for up to 5 h. This is roughly the dose that most people will apply when putting a liquid repellent ad libitum (W.G. Reifenrath, personal communication in Rutledge, 1988). Conversely, the corresponding figure for DEET exceeds the empirical limit of 4 mg/cm^2 before the inception of repellent runoff from the skin (W.G. Reifenrath, loc. cit.). In both these examples, comparable levels of protection within a given time frame are afforded using lower application doses of active ingredient, because of a slower decay profile. Depending on the local cost of alternative active ingredients and other circumstances, such reductions may prove cost-effective. In this respect, our logistic plane model provides a rational quantitative framework over which such informed decisions can be taken on a case-by-case basis. The inherent lower persistence of repellents due to their mode of action makes it unlikely that topical application will ever replace insecticidal treatments in vector control programmes. However, in accordance with the spirit of locally-adapted integrated ways of vector and disease control (WHO, 1995), repellents can usefully complement existing control strategies and provide an additional tool in the management of insecticide resistance.

For practical reasons, in our tests we evaluated an alcoholic formulation of the repellents. Formulations adapted to local habits and hence more acceptable, such as petroleum jelly or shea-butter mixtures, might provide further incentive to the use of repellents, especially where the cosmetic use of such creams is already common practice. As Curtis et al. (1991) commented, use of traditional self-protective measures using repellent plants and plant extracts are widespread among different cultures, and may be very common within communities. Such practices could be enhanced by adapting appropriate active ingredients to acceptable formulations. Thus, it is desirable that the performance properties of these repellents are validated using more appropriate formulations.

Conflicts of interest statement

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

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