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Evaluation of the sensitivity of *Aedes aegypti* and *Anopheles gambiae* complex mosquitoes to two insect repellents: DEET and KBR 3023

Athanase Badolo[1,2], Edith Ilboudo-Sanogo[2], Albert Patoin Ouédraogo[1] and Carlo Costantini[2,3]

1 *Département d'Entomologie Appliquée, Université de Ouagadougou, Burkina Faso*
2 *Centre National de Recherche et Formation sur le Paludisme, Ouagadougou, Burkina Faso*
3 *Fondazione Istituto Pasteur-Cenci Bolognetti & Sezione di Parassitologia, Dipartimento di Sanità Pubblica, Università di Roma "La Sapienza", Italy*

Summary

We conducted laboratory tests to assess the sensitivity to the insect repellent 1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-, 1-methylpropylester (known as KBR 3023 or Picaridin, trade name Bayrepel®) of West African strains of the yellow fever mosquito *Aedes aegypti* and of malaria vectors of the *Anopheles gambiae* complex, in comparison with the standard repellent N,N-diethyl-3-methyl-benzamide (DEET). Test mosquitoes were exposed according to a 'separate arms' protocol to logarithmic dose increments applied on one arm of human subjects to evaluate the relative potency, and the median effective dosages (ED_{50} and ED_{90}). According to a logistic regression model fitted to the experimental data, the dose–response relationship for the two repellents was the same within each species, thus pooled ED values were assessed for each mosquito separately. The median ED of KBR 3023 and DEET was estimated at 0.78 (95% confidence limits (CI): 0.57–1.04) and at 0.018 μg/cm^2 (0.004–0.052) for mosquitoes of the *An. gambiae* complex and *Ae. aegypti*, respectively. ED_{90} values were 125.6 (81.4–201.3) and 24.0 μg/cm^2 (5.7–208.5) for *An. gambiae s.l.* and *Ae. aegypti*, respectively. The relative potency of KBR 3023 was not significantly different from that of DEET for *An. gambiae s.l.* (95% confidence limits 0.7–1.0), whereas in the case of *Ae. aegypti* it was with 95% probability 1.1–2.0 times more potent than DEET. On the basis of available evidence, KBR 3023 represents a promising alternative to DEET for personal protection against bites of these important vectors of disease in the Afrotropical region.

keywords *Anopheles gambiae* complex, *Aedes aegypti*, insect repellents, DEET, KBR 3023, Picaridin, relative potency, effective dosage, laboratory assays, West Africa

Introduction

N,N-diethyl-3-methyl-benzamide (DEET) is an effective broad-spectrum repellent used as the main or sole active ingredient in many commercial formulations available to protect humans from arthropod bites (Gupta & Rutledge 1989). The response to this compound, however, varies according to the pest species considered (Rutledge *et al.* 1983; Curtis *et al.* 1987). Some anopheline vectors of malaria, such as *Anopheles pulcherrimus* and *An. albimanus*, are more tolerant to DEET than *Aedes* mosquitoes, particularly *Ae. aegypti* (Rutledge *et al.* 1978; Schreck 1985; Curtis *et al.* 1987). The behavioural response of mosquitoes to DEET is variable also within species, in relation to the geographical origin of the test strains (Rutledge *et al.* 1978). Other well-known sources of variability in the response to repellents include, among others, the quantity of active ingredient applied, the density of biting insects present in any given area, the environmental conditions, and the level of activity of the user (Gupta & Rutledge 1989). The assessment in the laboratory of repellents properties has the advantage of controlling at least some of these factors, which can affect estimates of the effective dosages (ED).

The relatively short protection time usually afforded by DEET, its unpleasant smell and feeling on the skin, and especially its solvent properties for plastics (Smith 1970), combined with a moderate risk of adverse reactions (Qiu *et al.* 1998), may make this repellent unattractive. Hence novel compounds without these shortcomings and with repellent properties at least comparable with those of DEET would undoubtedly be useful. An active compound

developed recently using molecular modelling techniques is 1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-, 1-methylpropylester (KBR 3023, also known as Icaridin or Picaridin, marketed by Bayer AG under the trade name Bayrepel®); it has good cosmetic properties, a favourable toxicological profile and low aggressiveness against plastic materials (Nentwig *et al.* 2002). Few studies have been published on its efficacy and protection time against mosquitoes, particularly anophelines. Moreover, despite its extensive use for over half a century, there are as yet few reports on the sensitivity to DEET of member species of the *An. gambiae* complex (Curtis *et al.* 1987), comprising the main vectors of malaria in Africa south of the Sahara. The objectives of this study were to determine for two important mosquitoes vectors of disease in tropical Africa, the yellow fever and dengue vector *Ae. aegypti* (Linn.), and the malaria vectors of the *An. gambiae* Giles complex, the performance characteristics of these repellents based on laboratory tests to establish their ED, and to determine the relative potency of KBR 3023 with respect to the golden standard DEET.

Materials and methods

Mosquitoes

The experiments used the F1 progeny of wild indoor-resting *An. gambiae s.l.* females collected in the rural village of Goden, 30 km east of Ouagadougou, Burkina Faso. In the case of *Ae. aegypti*, tests were performed with a strain established from larvae collected from container-breeding habitats found in Ouagadougou. Rearing and testing conditions in the insectary were 32 ± 1 °C temperature, 70 ± 10% relative humidity and a 12:12 photoperiod.

Repellents

Ethanolic formulations of technical grade DEET and KBR 3023 were used during the tests. The repellents were tested at increasing logarithmic doses from 10^{-5} to 1 mg/cm^2 expressed in mass of active ingredient (a.i.) per surface unit of skin. The required quantity of repellent was diluted in 5 ml of ethanol, which was applied on one arm of the test volunteer. The other arm was applied with 5 ml of ethanol as a control.

Test procedures

The tests began at 06:00 PM for *Ae. aegypti* and at 01:00AM for *An. gambiae s.l.* to take into account differences in the biting cycle of each species. The test was based on the 'separate arms' protocol described in Curtis *et al.* (1987). The test began by exposing first the control arm treated with ethanol only. The hand was protected with a glove, and the number of mosquitoes attempting to bite the arm during 30 s was recorded by two observers. The arm of the volunteer was then removed from the cage before mosquitoes had the chance to engorge. The other arm treated with the lowest dose of repellent was then introduced into the test cage and the same procedure repeated. After counting the number of mosquitoes, the arm was retrieved from the cage, rinsed with ethanol, and the next dose was applied. This procedure was repeated until the highest dose was tested, after which the control arm was reintroduced and the number of mosquitoes attempting to bite was counted again to account for any change in the propensity to bite of the test mosquitoes. This protocol was repeated on a different night with another repellent compound using the same batch of mosquitoes. Each repellent was tested six times randomizing every time the presentation sequence.

Statistical analysis

The protective properties of each repellent were expressed as a coefficient of protection $q = 1 - T/C$, where T represents the total number of mosquitoes landing on the treated arm and C the mean number of mosquitoes landing on the control arm at the beginning and at the end of the tests. The coefficient of protection q was related to the logarithm of the dose of repellent applied, the species of mosquito and the tested compound in a logistic regression model with the software GLIM v. 3.77 (Payne 1987). The model assumed a binomial error distribution with regression parameters calculated by maximum likelihood. The statistical significance of main effects and interaction terms in the model was tested by F-tests in an analysis of deviance (ANODEV) by looking at the change in deviance caused by the removal of each term from the maximal model after having allowed for overdispersion in the data by calculating an heterogeneity coefficients with the Williams algorithm (Collett 1991; Crawley 1993). Confidence limits for the median ED were calculated using Fieller's theorem (Collett 1991; Crawley 1993). Confidence limits for the ED_{90} were estimated by plotting the model profile deviance at $q = 0.9$ according to the procedures outlined in Aitkin *et al.* (1989). The relative potency of the repellents, defined as the ratio of equally ED was calculated as ED^S_{50}/ED^N_{50}, where the N and S superscripts indicate the new and standard ingredient (in our case the latter was represented by DEET), respectively, after verification in the preceding ANODEV that the slope of the two regression lines relative to the S and N ingredients

Table 1 Median and 90% effective dosages (95% confidence limits) expressed in μg/cm^2 for the repellents DEET and KBR 3023 against *Anopheles gambiae s.l.* and *Aedes aegypti* mosquitoes

	Species			
	Ae. aegypti		*An. gambiae s.l.*	
Repellent	ED_{50}	ED_{90}	ED_{50}	ED_{90}
DEET	0.05 (0.01–0.14)	20.8 (5.2–142.6)	0.60 (0.41–0.86)	89.4 (54.9–154.5)
KBR 3023	0.002 (0.0007–0.005)	63.8 (21.8–282.9)	0.99 (0.67–1.43)	180.0 (100.0–345.5)

ED, effective dosage; DEET, N,N-diethyl-3-methyl-benzamide; KBR 3023, 1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-, 1-methylpropylester.

was the same (Collett 1991). Confidence limits of relative potency were determined with the same procedures as for the ED_{90}.

Ethical approval

This study received formal ethical approval from the Ministry of Health of Burkina Faso.

Results

Effective dosage estimates of DEET were lower both at the median and 90% endpoints than the corresponding figures for KBR 3023 in the case of *An. gambiae s.l.*, but their 95% confidence limits overlapped indicating that the sensitivity to these two repellents was similar (Table 1). For *Ae. aegypti*, the ranking was the same at the 90% endpoint, but it was the reverse at the 50% endpoint. The analysis of deviance of the logistic regression model, however, showed that the only statistically significant terms were the log-dose covariate, the mosquito species main effect and their interaction term (Table 2), indicating that on the basis of these data the functional dose–response relationship was the same between repellents within each species, and it could be described by two regression lines, one for each mosquito. The model was therefore simplified to take account of non-significant differences; the regression parameter estimates of the minimal adequate model are shown in Table 3. It can be assumed, therefore, that the best estimates of ED that can be inferred from our data are the same for DEET and KBR 3023, and they depend only on the mosquito considered (Table 3). The relative potency of KBR 3023 was not statistically different from unity for *An. gambiae s.l.* (mean: 0.84; 95% confidence limits: 0.69–1.02), whereas it was 1.50 (1.11–2.04) more potent than DEET for *Ae. aegypti*.

Table 2 Analysis of deviance of the logistic binomial regression model relating the response of *Anopheles gambiae s.l.* and *Aedes aegypti* (species) to the log-dose (dose) of the two tested repellent active ingredients (repellent)

Source	Deviance	d.f.	*F*	*P*
Dose	592.00	1	18.67	<0.001
Repellent	0.65	1	0.30	0.589
Species	97.50	1	13.97	0.001
Dose × repellent	3.13	1	2.35	0.144
Dose × species	10.03	1	5.77	0.028
Repellent × species	4.25	1	3.04	0.099
Residual	19.53	16		

Discussion

Our results show that KBR 3023 and DEET had similar potencies against West African strains of *Ae. aegypti* and *An. gambiae s.l.* malaria vectors, both in terms of their ED and relative potency. Just after application, subjects treated with KBR 3023 and exposed to the bites of a laboratory colony of *Ae. aegypti*, received on average a greater or smaller number of bites than subjects treated with DEET depending on the application dose (Rettich 2000). Few studies have assessed under standardized laboratory conditions the sensitivity of *An. gambiae* to repellents expressed in terms of ED. Our ED_{50} estimates for DEET are lower than the figures reported by Curtis *et al.* (1987) and Robert *et al.* (1991) with laboratory strains of *An. gambiae*. The ED_{90} values we observed, however, are comparable with those found by Curtis *et al.* (1987), but higher than the ED_{95} values estimated by Robert *et al.* (1991); it is important to note that the latter authors used *in vitro* methods and rabbits as baits for *An. gambiae*. Our results might be due to an increase in the propensity of *An. gambiae* to bite the human baits. In addition, our test mosquitoes were the F1 progeny of a wild *An. gambiae s.l.* population, and the response may reflect an inherent higher variability of the strain used. The ED of DEET we observed for *Ae. aegypti* are comparable with those found with a choice box protocol by Curtis *et al.* (1987), but lower than

Table 3 Regression parameters (SE) and fitted median and 90% effective dosages (95% confidence limits) expressed in $\mu g/cm^2$ of the minimal adequate logistic regression model fitted to the repellents tests data

Species	Intercept	Slope	ED_{50}	ED_{90}
Aedes aegypti	1.224 (0.158)	0.306 (0.049)	0.018 (0.004–0.052)	24.0 (5.7–208.5)
Anopheles gambiae s.l.	0.108 (0.064)	0.432 (0.020)	0.78 (0.57–1.04)	125.6 (81.4–201.3)

ED, effective dosage.

those reported by Rutledge *et al.* (1983) for a North-American strain of this species. This could be again due to differing environmental conditions, including temperature and relative humidity, experimental protocols, and to the test strains. This reiterates the question of how results obtained under particular conditions can be generalized to other circumstances, and the need to standardize experimental protocols, environmental testing conditions and materials to be able to compare results from different studies.

Based on our laboratory assays, it appears that *Ae. aegypti* was more sensitive to the two repellents tested than *An. gambiae s.l.* Curtis *et al.* (1987) tested several repellents using the same experimental protocol, and obtained median ED for *An. gambiae* three to 20 times higher than the corresponding values for *Ae. aegypti*. Several studies have shown that *Ae. aegypti* is more sensitive to repellents than *Anopheles* mosquitoes (Travis 1947; Smith *et al.* 1963; Rutledge *et al.* 1983; Curtis *et al.* 1987) and our results are consistent with these observations. As a result of its convenience as a laboratory mosquito, *Ae. aegypti* is extensively used world-wide for repellents screening, and its sensitivity to active ingredients can be an indicator of repellent activity, but care must be taken when attempting to extrapolate such results to other species.

As a result of its efficacy and low toxicity proven over many decades of widespread consumer use, DEET is the repellent of reference against which new ingredients or formulations are tested to evaluate their properties. Our results show that KBR 3023 was at least as effective as DEET against *An. gambiae s.l.* and our West African urban strain of *Ae. aegypti*. Similar results were obtained in field studies in Malaysia with *Ae. albopictus* and *Culex quinquefasciatus* (Yap *et al.* 1998), and in the Czech Republic with *Ae. cantans*, *Ae. annulipes* and *Ae. sticticus* (Rettich 2000). KBR 3023 generally shows equal or longer protection times than those afforded by DEET for equal levels of protection (reviewed in World Health Organization 2001). On the basis of the as yet limited experience available concerning this compound, therefore, it appears that KBR 3023 offers a promising valid alternative to DEET, in particular against Afrotropical vector mosquitoes (Costantini *et al.* in press). More data on the sensitivity to KBR 3023 of other mosquito species, however, are awaited to extend these observations. Moreover, whenever repellents are exploited in community vector control applications, efficacy cannot be the sole criterion used to judge about the usefulness of different compounds, as the relationship between efficacy and protection time, and other properties such long-term toxicity and cost should be taken into account.

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Authors

Athanase Badolo and **Albert Patoin Ouédraogo**, Faculté des Sciences et Techniques, Université de Ouagadougou, 01 B.P. 7021, Ouagadougou 01, Burkina Faso. Fax: +226 307242; E-mail: athanase_badolo@univ-ouaga.bf

Carlo Costantini (corresponding author) and **Edith Ilboudo-Sanogo**, Centre National de Recherche et Formation sur le Paludisme, 01 B.P. 2208 Ouagadougou 01, Burkina Faso. Tel.: +226 324696; Fax: +226 310477; E-mail: costantini.cnlp@fasonet.bf, carlo.costantini@uniroma1.it, edith.cnlp@fasonet.bf