Field Evaluation of Repellent Formulations Containing Deet and Picaridin Against Mosquitoes in Northern Territory, Australia

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ABSTRACT Field efficacy of repellent formulations containing picaridin (1-methyl-propyl 2-(2-hydroxyethyl)-1-piperidinecarboxylate) or deet (*N,N*,-diethyl-3-methylbenzamide) against mosquitoes in Northern Territory, Australia, was evaluated. The following repellent treatments were evaluated: 19.2% picaridin (Autan Repel Army 20), a solution of 20% deet in ethanol, and 35% deet in a gel (Australian Defense Force [ADF]). The predominant mosquito species were *Culex annulirostris* Skuse (57.8%), *Anopheles meraukensis* Venhuis (15.4%), and *Anopheles bancroftii* Giles (13.2%). The protection provided by repellents against *Anopheles* spp. was relatively poor, with 19.2% picaridin and ADF deet providing >95% protection for only 1 h, whereas 20% deet provided <95% protection at 1 h after repellent application. In contrast, the repellents provided good protection against *Cx. annulirostris*, with 19.2% picaridin providing >95% protection for 5 h and both deet formulations providing >95% protection for 7 h when collections ceased. This study provides additional field data showing tolerance of *Anopheles* spp. for repellents. The response of field populations of *Cx. annulirostris*, an important vector of arboviruses in Australia, to repellents containing deet and picaridin is reported for the first time.

KEY WORDS *N,N*-diethyl-3-methylbenzamide, deet, picaridin, repellents, Australia, *Culex annulirostris*

PICARIDIN (1-methyl-propyl 2-(2-hydroxyethyl)-1-piperidinecarboxylate), also known as KBR 3023 and bayrepel, is a relatively new mosquito repellent currently available in at least 50 countries and was recently registered for use in the United States (Klun et al. 2003). Field studies have shown picaridin to be an effective repellent against mosquitoes in Malaysia (Yap et al. 1998, 2000) and Florida (Barnard et al. 2002). In a study in Australia, a formulation containing 19.2% picaridin was as good or better that *N, N*,-diethyl-*3*-methylbenzamide (deet) against *Verrallina lineata* (Taylor) in a rainforest habitat (Frances et al. 2002). The purpose of this field study was to evaluate and compare repellent formulations containing picaridin with deet against *Anopheles* spp. and *Culex annulirostris* Skuse mosquitoes in the Northern Territory, Australia.

Mention of a commercial product does not constitute an endorsement of the product by the ADF. The volunteers gave informed consent to participate in the study.

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Materials and Methods

Study Site. The study was conducted at the edge of a forest at Mount Bundey Training Area, Northern Territory (131°50′ E, 12°52′ S), in March 2003. This site was located ≈145 km east of Darwin, Northern Territory, and was primarily native woodland forest. This area was characterized by a wet tropical climate with a distinct wet (November–April) and dry season (May–October). This study was timed to coincide with the occurrence of a high density of adult mosquitoes (at the end of the wet season).

Test Repellents. The following repellent formulations were used: (1) Bayrepel Army, containing 19.2% picaridin (1-methyl-propyl 2-(2-hydroxyethyl)-1-piperidinecarboxylate) in ethanol, formulated by Bayer (Australia), for the Australian Defense Force (ADF); (2) 20% deet (*N, N*,-diethyl-3-methylbenzamide) (Fluka, Switzerland) in ethanol; (3) ADF Repellent, containing 35% deet in a gel consisting of propylene glycol, hydroxypropyl cellulose, and Laureth-3, produced by Colbar (Melbourne, Australia) for the ADF. The Bayrepel and 20% deet formulations were applied using a pump action spray container. Although both picaridin and deet are available in commercial formulations, none of the repellent formulations used in this study were commercially available.

Test Procedures. Four volunteer males (mean age, 42 ± 7 yr) participated in the study, and each wore a long-sleeved shirt, buttoned at the wrist, long trousers, and open foot wear (sandals). A mesh jacket (Bugout, Wautosa, WI) was worn over the head and arms, and the legs of the trousers were rolled to the knee to expose only the lower legs and feet to biting mosquitoes.

The repellents or 100% ethanol (control) were spread evenly by the volunteers over each lower leg from the base of the knee and the foot. The amount of repellent applied was determined by weighing the repellent container before and after application. The amount of repellent active ingredient per centimeter squared varied among volunteers because of leg size differences. The approximate application area [A = one-third (a + b + c) × h + one-half (d × f)] was calculated from measurements of leg length (h, knee to the ankle), leg circumference (a, just below the knee; b, the calf; c, the ankle), and foot (d, circumference of foot at junction with leg; f, length).

The repellent formulations were evaluated against nighttime biting mosquitoes on each of eight nights and were applied under supervision at 1900 h on each night, 1 h before the start of each test at 2000 h. The treatments were randomized among each of the four volunteers and were rotated so that each individual tested each repellent and control over four nights; this was repeated for an additional four nights. Participants entered the test area, sat in predetermined positions ≈5 m apart, and collected all mosquitoes biting in the next 20 min, followed by a 40-min break, which was taken 500 m to 1 km from the test area. Collectors sat in the same position during each night of testing, but positions were rotated so that each volunteer sat in a different position on each of four nights. Mosquitoes were captured using mouth aspirators and placed into plastic cups covered with netting that also had a piece of dental dam in the side to allow mosquitoes to be easily aspirated into them. This procedure was repeated hourly for 7 h to obtain seven biting collections by each volunteer. The cups were returned to a field laboratory, where they were placed on dry ice to immobilize the mosquitoes, which were counted and identified using the keys of Lee et al. (1980).

The totals at each of the hourly time points were determined for the controls and for each repellent group. Percentage protection was calculated at each time point by comparing the number of bites for controls against the number of bites for repellent-treated test participants using Abbott's formula (Abbott 1925). Percentage protection, defined as the number of bites received by an individual in a treatment group relative to that of the control, was calculated as (control − treatment)/control × 100. Comparison of repellent efficacy was made among the three treatment groups using a two-way analysis of variance (ANOVA) method for two factors. The treatment factor, type of repellent, was studied at three levels with repeated measures made during the second factor time. Time was measured in hours after repellent application and studied at seven levels. The restriction on randomization of the treatments within a block (i.e., day) resulted in two error terms for this design. Because the data were expressed as percentages (percentage protection), an arcsine transformation was performed on values before statistical analysis.

Table 1. Mean ± SE application rate of repellents to the lower legs of volunteers at Mt. Bundey Training Area, Northern Territory, Australia

Repellent formulation	Amount applied (mg/cm^2)
19.2% picaridin	1.74 ± 0.33
20% deet	1.33 ± 0.14
ADF deet (35% deet)	1.20 ± 0.15

Results

The average area of the volunteers' legs and feet that was protected was 3,287.5 cm^2 (range, 2,947.8–3,618 cm^2), and the amount of repellent applied to their legs is shown in Table 1. A total of 2,609 mosquitoes was collected during the tests, and the main species collected were *Cx. annulirostris* (57.8%), *Anopheles meraukensis* Venhuis (15.4%), *Anopheles bancroftii* Giles (13.2%), and *Ochlerotatus normanensis* (Skuse) (8.9%) (Table 2). An overall total of 746 *Anopheles* spp. (*An. meraukensis* and *An. bancroftii*) mosquitoes and 1,507 *Cx. annulirostris* were collected, and statistical analysis to determine the effectiveness of repellents against both was conducted. The overall mean biting rate of mosquitoes on ethanol-treated (control) volunteers was 9.6 bites/20 min per person for *Anopheles* spp. and 25.9 bites/20 min per person for *Cx. annulirostris.* The mean number of mosquitoes collected throughout the collection period was fairly uniform, and there were few differences in the mean number of mosquitoes collected each hour (Table 3).

The percentage protection provided by the three repellents against *Anopheles* spp. was not significantly different ($F = 1.5$, df = 2,14, $P = 0.26$). There were

Table 2. Mosquito species and overall number collected at Mt. Bundey Training Area, Northern Territory, Australia, March 2003

Mosquito species	Number collected
Anopheles amictus	7
Anopheles annulipes	1
Anopheles bancroftii	344
Anopheles farauti s.l.	4
Anopheles hilli	4
Anopheles meraukensis	402
Anopheles novaguinesis	16
Coquillettidia xanthogaster	14
Culex annulirostris	1,507
Culex gelidus	1
Culex palpalis	3
Culex pullus	3
Mansonia uniformis	53
Ochlerotatus elchoensis	2
Ochlerotatus lineatopennis	2
Ochlerotatus normanensis	232
Ochlerotatus purpureus	2
Ochlerotatus stenoreum	1
Ochlerotatus tremulus	9
Ochlerotatus vigilax	2
Total	2,609

Table 3. Mean ± SE number of mosquitoes biting per 20 min. on untreated (control) volunteers during field repellent tests at Mt. Bundey Training Area, Northern Territory, Australia, March 2003

Time after repellent application (h)	*Anopheles* spp. (n = 536)	*Cx. annulirostris* (n = 1,413)
1	6.5 ± 2.0 a	9.7 ± 3.0 a
2	10.5 ± 3.2 a	23.9 ± 7.8 a, b
3	12.6 ± 2.5 a	22.4 ± 7.5 a, b
4	9.0 ± 2.2 a	26.7 ± 10.0 a, b
5	9.8 ± 2.6 a	30.0 ± 8.8 a, b
6	10.4 ± 3.7 a	38.4 ± 9.9 b
7	8.3 ± 2.6 a	30.3 ± 11.8 a, b

Within-column means followed by same letter are not significantly different using the Student-Newman-Kuels method ($P < 0.05$).

highly significant differences in the percentage protection provided over time ($F = 3.6$, df = 6,42, $P = 0.006$), but there was no significant effect on percentage protection because of the interaction of repellent type and time ($F = 0.8$, df = 12,84, $P = 0.6$). The protection provided by all repellents against *Anopheles* spp. was relatively poor. Both 19.2% picaridin and ADF deet provided >95% protection for only 1 h, whereas 20% deet did not provide >95% protection against *Anopheles* spp. (Table 4). In contrast, all repellents provided good protection against *Cx. annulirostris.* The percentage protection provided by the three repellents against *Cx. annulirostris* was not significantly different ($F = 3.6$, df = 2,14, $P = 0.06$). There were significant differences in the percentage protection provided over time ($F = 2.5$, df = 6,42, $P = 0.04$), but there was no significant effect on percentage protection caused by the interaction of repellent type and time ($F = 1.4$, df = 12,84, $P = 0.18$). The 19.2% picaridin provided >95% protection for 5 h, and both formulations of deet provided >95% protection for at least 7 h when collections ceased (Table 4).

Discussion

The purpose of this study was to compare the efficacy of a repellent containing picaridin with deet repellents against *Anopheles* spp. mosquitoes. In an earlier study, Yap et al. (2000) found that 12% picaridin (as a Bayrepel formulation) provided >96% protection for 8 h, and 5% picaridin provided >95% protection for 4 h against *Anopheles* spp., primarily *Anopheles sinensis* (Weid.). In the current study, all repellents provided relatively poor protection against *Anopheles* spp. (*An. bancroftii* and *An. meraukensis*), with 19.2% picaridin providing >95% protection for only 1 h after repellent application. Previous studies have shown *Anopheles albimanus* Weid. (Schreck 1985) and *Anopheles dirus* Peyton and Harrison (Frances et al. 1993) to be tolerant of lower (≤35%) concentrations of deet in the laboratory. Subsequent field trials have shown the response of four *Anopheles* spp. to 25% deet in ethanol to be variable, with 95% protection provided for <1 h against *Anopheles koliensis* Owen, 2 h against *An. dirus*, 4 h against *An. farauti* no. 4, and 5 h against *An. farauti* s.s. (Frances et al. 2001). In this study, 20% deet provided only 87% protection against *Anopheles* spp. 1 h after repellent application.

Culex annulirostris is an important disease vector of arboviruses, including Japanese Encephalitis virus (Hanna et al. 1996), Ross River virus (Russell 2002), and Barmah Forest virus (Russell 1995) in Australia. Despite the importance of this species as a vector of disease, its response to repellent treatments has not previously been reported. The results of this study show that 19.2% picaridin provided >95% protection for 5 h, and both deet formulations provided at least 7 h of protection when collections ceased. Use of deet repellents in Australia is widespread (Larson et al. 2000), and their use as protection against vectors of arboviruses, including *Cx. annulirostris,* is widely recommended. In addition, this study showed that repellents containing deet and picaridin provide good protection against this important vector.

The formulation type and packaging of repellents is also an important consideration in the evaluation of repellents. Recent surveys in the ADF have shown the preference of soldiers for repellents formulated in pressurized aerosols (Frances et al. 2003). Because it is not possible to carry pressurized cans on military aircraft, Australian soldiers prefer a nonpressurized pump action spray applicator (Frances and Cooper 2002).

Previous studies have shown that picaridin provided similar protection against mosquitoes as formulations of deet (Yap et al. 1998, Barnard et al. 2002, Frances et al. 2002). This study showed that 19.2% picaridin was as effective as deet formulations against *Anopheles* spp.

Table 4. Mean ± SE percentage protection provided by three repellents against *Anopheles* spp. and *Cx. annulirostris* at Mt. Bundey Training Area, Northern Territory, Australia, March 2003

Hours after repellent application	Percentage protection					
	Anopheles spp.			*Cx. annulirostris*		
	19.2% picaridin	20% deet	ADF deet (35% deet)	19.2% picaridin	20% deet	ADF deet (35% deet)
1	98.0 ± 2.0	86.9 ± 12.4	96.9 ± 3.1	100	100	100
2	78.4 ± 12.0	88.5 ± 5.5	85.9 ± 9.2	99.5 ± 0.3	100	100
3	94.0 ± 3.2	87.9 ± 3.8	95.9 ± 4.1	96.9 ± 3.1	100	100
4	84.5 ± 10.1	88.9 ± 3.5	89.4 ± 7.6	99.0 ± 0.5	97.4 ± 2.1	99.6 ± 0.3
5	86.7 ± 6.5	74.5 ± 12.7	76.9 ± 12.9	99.2 ± 0.8	99.1 ± 0.9	99.1 ± 0.9
6	65.7 ± 14.9	37.5 ± 14.5	73.3 ± 12.2	85.0 ± 12.2	99.4 ± 0.4	99.5 ± 0.4
7	71.5 ± 12.6	72.1 ± 12.2	75.4 ± 13.4	97.0 ± 1.3	96.3 ± 1.6	98.3 ± 1.3

and less effective against *Cx. annulirostris* in the Northern Territory, Australia. However, both picaridin and deet are effective repellents against *Cx. annulirostris,* an important vector of arboviruses in Australia.

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