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FIELD EVALUATION OF THE REPELLENTS DEET, CIC-4, AND AI3-37220 AGAINST *ANOPHELES* IN LAE, PAPUA NEW GUINEA

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ABSTRACT. The repellents diethylmethylbenzamide (deet), (2-hydroxymethylcyclohexyl) acetic acid lactone (CIC-4), and 1-(3-cyclohexen-1-yl-carbonyl)-2-methylpiperidine (AI3-37220) were compared for their effectiveness in protecting 5 soldiers against the bites of *Anopheles* spp. at a village in Papua New Guinea. All 3 repellents, applied as 25% ethanol concentrations, provided ≥95% protection against primarily *An. farauti* 4 for at least 3 h after application.

KEY WORDS *Anopheles farauti*, diethylmethylbenzamide, deet, (2-hydroxymethylcyclohexyl) acetic acid lactone (CIC-4), 1-(3-cyclohexen-1-yl-carbonyl)-2-methylpiperidine (AI3-37220), repellents, Papua New Guinea

INTRODUCTION

Malaria is widespread in the coastal lowlands of Papua New Guinea (PNG), and the main vectors are members of the *Anopheles punctulatus* group. In PNG, this group has been found to contain up to 9 species including *Anopheles punctulatus* s.s. Donitz, *Anopheles* species near *punctulatus, Anopheles koliensis* Owen, and 6 sibling species of *Anopheles farauti* s.l. (*An. farauti* s.s. Laveran and *An. farauti* 2, 3, 4, 5, and 6) (Foley et al. 1993, Cooper et al. 1997). Species of the *An. punctulatus* group cannot be reliably separated on their external morphology, and DNA methods have been developed for their identification (Beebe et al. 1994).

The use of personal protection measures such as the application of repellents to the exposed skin have long been advocated to minimize human contact with vector and nuisance mosquitoes (Gupta and Rutledge 1994). However, few studies have examined the response of malaria vectors in PNG to repellent chemicals. During World War II, field tests were conducted in Lalapipi village, in Central Province, PNG, which investigated the response of *An. farauti* and culicine mosquitoes to ethylhexanediol, dimethylphthalate, diethylphthalate, citronella oil, and pyrethrum. Dimethylphthalate was found to be superior, providing 40–60 min of protection, whereas ethylhexanediol and diethylphthalate provided 20–40 min of protection and citronella and pyrethrum provided <20 min of protection (McCulloch and Waterhouse 1947). These tests were conducted before the development and release of diethylmethylbenzamide (deet) in 1954, and this chemical is now the active ingredient in most commercially available repellent formulations (Gupta and Rutledge 1994). Charlwood and Dagaro (1987) tested the effect of a repellent containing 20% deet and 1.0% permethrin formulated in a bar of soap, against primarily *An. farauti* and *An. koliensis* in a village near Madang, PNG. They found that fewer mosquitoes were collected on repellent-treated humans compared with untreated controls. The protection provided during a 4-h collecting period was 51.0% when the repellent soap was applied 4.5 and 2.5 h before collection began, and 69.9% when soap was applied just before the commencement of collections (Charlwood and Dagaro 1987).

In an effort to improve protection against mosquitoes in PNG, we compared the effectiveness of deet with that of 2 experimental chemicals, (2-hydroxymethylcyclohexyl) acetic acid lactone (CIC-4) and 1-(3-cyclohexen-1-yl-carbonyl)-2-methylpiperidine (AI3-37220). These repellents, developed by the U.S. Army (Coleman et al. 1993), have been shown to be effective against anopheline vectors of malaria in other parts of the world (Frances et al. 1996, Walker at al. 1996). The results of our findings are reported in this article.

MATERIALS AND METHODS

Chemicals tested: Three repellents were evaluated: deet, 95% active isomers (Colbar, Melbourne, Australia); CIC-4, 98% (synthesized by Angus Chemical, Northbrook, IL); and AI3-37220, 99% (synthesized by the late T. P. McGovern, Insect Chemical Ecology Laboratory, U.S. Department of Agriculture, Agriculture Research Service, Beltsville, MD).

Field procedures: The study was conducted at the village of Ngasuwampum (6°34′S, 146°49′E), approximately 26 km northwest of Lae Township, Morobe Province, PNG, in May 1996. The village is surrounded by forest, and the area is endemic for malaria. Collections were made at the edge of the village, between the forest and approximately 20 m from the nearest house.

Two Australian (Caucasian) entomologists and 3 PNG (Melanesian) preventive medicine technicians (aged >21 years) were involved in this study. The 5 soldiers wore a military uniform consisting of a shirt, buttoned at the wrist, long trousers, and open sandals. The legs of the trousers were rolled to the knee to expose the lower leg to biting mosquitoes.

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All 5 men were on doxycycline prophylaxis to protect them against malaria, and gave informed consent to participate.

The test repellents and 100% ethanol (control) were applied as 2-ml aliquots of ethanol solutions and were spread evenly over each lower leg from the base of the knee to the foot. All solutions were formulated on a volume–volume (v/v) basis at a concentration of 25%. The amount of repellent active ingredient per cm^2 varied due to leg size differences. The application area ($A = \frac{1}{3}(a + b + c) \times h$) was calculated from measurements of leg length (h, knee to the ankle), and circumference (a, just below the knee; b, the calf; and c, the ankle).

Repellent formulations were applied under supervision at 1700 h on each of 4 nights, 2 h before the start of each test at 1900 h. Treatments were assigned by rotation to each soldier so that all individuals tested a different treatment on each of 4 nights. The 5 soldiers entered the test area, sat in predetermined positions approximately 2 m apart, and collected all mosquitoes biting in the next 30 min, followed by a 30-min break. Mosquitoes were captured using aspirators and placed into cups. This procedure was repeated hourly for 6 h, so that 6 biting collections were made by each soldier. At the end of each 30-min test period, the cups were placed in a large cooler, until the conclusion of each nights collecting, when the mosquitoes were returned to a field laboratory, placed into a freezer, then identified morphologically using the keys of Lee and Woodhill (1944), and stored in liquid nitrogen. The specimens were subsequently returned to our laboratory in Australia and identified using species-specific DNA probes (Beebe et al. 1994).

The daily totals at each of the 6 hourly time points were determined for the controls and for each repellent group. These daily totals then were summed and percent protection 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 3 treatment groups using 2-way analysis of variance. Because the data were based on counts expressed as percentages (percentage protection), the analysis was performed on arcsine-transformed observations.

RESULTS

The average area of the collectors' legs that was protected was 1,171 cm^2 (range, 936–1,333 cm^2), and the average amount of repellent applied to them was 0.43 mg/cm^2 (range, 0.38–0.53 mg/cm^2). A total of 388 *Anopheles* spp. mosquitoes were collected, and morphologically identified as *An. punctulatus* group (97.7%) and *Anopheles longirostris* Brug (2.3%). Species-specific DNA probes showed that the *An. punctulatus* group were *An. farauti* 4 (96.8%), *An. koliensis* (1.3%), and *An. punctulatus* (0.5%), and 1.3% were unidentified. The mean biting rate of all mosquitoes on ethanol-treated (control) volunteers was 11.3 ± 1.4 bites/human/30 min and was relatively uniform throughout the tests (Table 1).

Table 1. *Anopheles* spp. collected hourly on ethanol (control)-treated volunteers at Ngasuwapum, Morobe Province, Papua New Guinea, May 5–8, 1996.

Time (h)	Mean (±SE) bites/human/30 min[1]
1900	9.6 ± 1.7 A
2000	17.4 ± 4.6 A
2100	14.2 ± 5.6 A
2200	10.6 ± 2.8 A
2300	9.4 ± 2.4 A
2400	7.0 ± 1.1 A

[1] Means followed by the same letter are not significantly different using the Student–Newman–Keuls method ($P < 0.05$) on log(x + 1)-transformed data.

The percentage protection provided by the 3 repellents was not significantly different ($F = 0.49$, df = 2,72, $P = 0.61$). All 3 repellents provided >95% protection against primarily *An. farauti* 4 for at least 3 h after repellent application (Table 2).

DISCUSSION

Several recent field studies have shown that ethanol formulations of the piperidine AI3-37220 provided better protection against *Anopheles* spp. than deet, although the duration of protection provided was variable. A study in Chanthaburi Province, southeastern Thailand, showed that 25% ethanol solutions of AI3-37220 provided better protection than deet or CIC-4 against *Anopheles dirus* Peyton and Harrison (Frances et al. 1996). The protection provided by deet and CIC-4 fell to below 95% 2 h after repellent application, whereas AI3-37220 provided greater than 95% protection for 4 h. In a study conducted on the shores of Lake Victoria, Kenya, a 5% ethanol solution of AI3-37220 provided significantly better protection than 5% deet against *Anopheles funestus* Giles and *Anopheles arabiensis* Patton (Walker et al. 1996). In their study, low concentrations of both chemicals provided <95% protection against both species 2 h after repellent application. Both chemicals provided better protection 7–9 h after repellent application against *An. arabiensis* compared to *An. funestus,* and this was important because *An. funestus* is the primary malaria vector in the study area (Walker et al. 1996).

In a recent field study conducted in northern Australia, 25% concentrations of deet, CIC-4, and

Table 2. Total number of mosquitoes collected and percentage protection provided by 3 repellents against *Anopheles* spp. at Ngasuwampum, Morobe Province, Papua New Guinea, May 5–8, 1996.[1]

Hours after repellent application	Repellent treatment and percentage protection compared to control			
	Ethanol (control)	deet (% protection)	CIC-4 (% protection)	AI3-37220 (% protection)
2	48	0 (100)	0 (100)	2 (95.8)
3	87	0 (100)	0 (100)	0 (100)
4	71	1 (98.6)	1 (98.6)	8 (88.7)
5	53	5 (90.6)	2 (96.2)	4 (92.5)
6	47	6 (87.2)	6 (87.2)	1 (97.9)
7	35	3 (91.4)	5 (85.7)	3 (91.4)
Total	341	15	14	18

[1] deet, diethylmethylbenzamide; CIC-4, C2-hydroxymethylcyclohexyl) acetic acid lactone; AI3-37220, 1-(3-cyclohexen-1-yl-carbonyl)-2-methylpiperidine.

AI3-37220 provided >95% protection against *An. farauti* s.s. for 5 h. The protection provided by AI3-37220 was ≥94% for 9 h and was significantly better than either deet or CIC-4, which provided ≤50% protection 9 h after repellent application (Frances et al. 1998).

The results of the current study have shown that all 3 of the repellent chemicals tested provided >95% protection for at least 3 h after repellent application. However, unlike earlier studies, the protection provided by AI3-37220 was not significantly different from that provided by deet or CIC-4. All 3 of these repellents are effective adjuncts to other measures, such as appropriate chemoprophylactic drugs and protective clothing, to reduce the risk of malaria infection.

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