

## FIELD EVALUATION OF REPELLENTS CONTAINING DEET AND AI3-37220 AGAINST *ANOPHELES KOLIENSIS* IN PAPUA NEW GUINEA

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**ABSTRACT.** The repellent 1-(3-cyclohexen-1-yl-carbonyl)-2-methylpiperidine (AI3-37220) was compared with 2 formulations of diethylmethylbenzamide (deet) for its effectiveness in protecting 4 humans against the bites of *Anopheles koliensis* mosquitoes at a village in Central Province, Papua New Guinea. A mean of  $77.2 \pm 10.5$  bites/human/10 min of *An. koliensis* was collected on ethanol-treated (control) volunteers, a much higher density than most previous studies with *Anopheles* sp. mosquitoes. The protection provided by all repellents did not last long. Both 25% AI3-37220 in ethanol and a formulation containing 35% deet in a gel provided >95% protection for only 2 h. A formulation of 25% deet in ethanol provided only 93% protection 1 h after repellent application and 39% protection 5 h after application.

**KEY WORDS** *Anopheles koliensis*, diethylmethylbenzamide, deet, 1-(3-cyclohexen-1-yl-carbonyl)-2-methylpiperidine (AI3-37220), repellents, Papua New Guinea

### INTRODUCTION

The use of personal protection measures such as the application of repellents to exposed skin has 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 Papua New Guinea (PNG) to repellent chemicals.

In an effort to improve protection against mosquitoes in PNG, we compared the effectiveness of 2 formulations of diethylmethylbenzamide (deet) with that of a newer repellent, the piperidine 1-(3-cyclohexen-1-yl-carbonyl)-2-methylpiperidine (AI3-37220). This repellent, developed by the U.S. Army (Coleman et al. 1993), has been shown to be effective against anopheline vectors of malaria in other parts of the world (Frances et al. 1996, Walker et al. 1996, Debboun et al. 1999). In an earlier study, 25% ethanol solutions of deet, AI3-37220, and the lactone CIC-4 were shown to provide at least 3 h protection against primarily *Anopheles farauti* 4, in a village near Lae, PNG (Frances et al. 1999). This report describes the effectiveness of AI3-37220 and deet as protectants against high densities of *Anopheles koliensis* Owen.

### MATERIALS AND METHODS

**Chemicals tested:** Three repellents were evaluated: deet, 95% active isomers, Colbar, Melbourne, Australia; AI3-37220, 99%, synthesized by the late T. P. McGovern, Insect Chemical Ecology Laboratory, USDA-ARS, Beltsville, MD; and 35% deet in

a gel containing propylene glycol, hydroxypropyl cellulose, and laureth-3, produced by Colbar, Melbourne, Australia, for the Australian Defence Force (ADF).

**Field procedures:** The study was conducted at the village of Dasiana, Central Province (9°18'S, 147°13'E), located approximately 30 km north of Port Moresby, PNG, in April 1998. The village is located near a large freshwater swamp, and the area is endemic for malaria. Collections were made at the edge of the village, within 500 m from the edge of the swamp.

Four men (aged >21 years) participated in the study, and each wore a long-sleeved shirt, buttoned at the wrist, long trousers, and open 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 leg and feet to biting mosquitoes. All 4 men were on doxycycline prophylaxis to protect them against malaria.

The liquid repellents and 100% ethanol (control) were applied as 2-ml aliquots of ethanol solutions and were spread evenly by the volunteers over each lower leg from the base of the knee to the foot. The gel formulation was applied evenly to the lower leg according to label instructions. The amount of repellent applied was determined by weighing the container before and after application. Both liquid solutions were formulated on a volume-volume (v/v) basis at a concentration of 25%. The amount of repellent active ingredient per square centimeter varied among volunteers because of leg size differences. The application area [ $A = 1/3(a + b + c) \times h$ ] 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 1800 h on each of 3 nights, and 1700 h on 1 night, 1 and 2 h before the start of each test at 1900 h. Participants entered the test area, sat in predetermined positions approximately 2 m apart, and collected all mosquitoes biting in the next 10 min, followed by a 50-min break, which was taken

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Table 1. *Anopheles koliensis* collected hourly on ethanol-treated (control) volunteers at Dasiamia, Central Province, Papua New Guinea, April 19–22, 1998.

Time (h)	Mean ( $\pm$ SE) bites/human/10 min <sup>1</sup>
1900	23.0 $\pm$ 9.6 A
2000	84.5 $\pm$ 27.0 A
2100	78.0 $\pm$ 17.2 A
2200	92.5 $\pm$ 23.2 A
2300	92.0 $\pm$ 26.2 A

<sup>1</sup> Mean followed by the same letter is not significantly different using the Student–Newman–Keuls method ( $P < 0.05$ ) on  $\log(x + 1)$ -transformed data.

about 2 km from the test area. Mosquitoes were captured using aspirators and placed into cups. This procedure was repeated hourly for 5 h, so that 5 biting collections were made by each volunteer. At the end of each 10-min test period, the cups were placed into a large cooler, until the conclusion of each night's collecting, when the mosquitoes were returned to a field laboratory, placed into a freezer to immobilize them, 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 a sample of 41 adults was identified by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis (Beebe and Saul 1995).

The daily totals at each of the 5 hourly time points were determined for the controls and for each repellent group. These daily totals then were summed and percentage 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  $(\text{control} - \text{treatment})/\text{control} \times 100$ . Comparison of repellent efficacy was made among the 3 treatment groups with a 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 AND DISCUSSION

The average area of the collectors' legs that was protected was 1,134 cm<sup>2</sup> (range, 968–1,290 cm<sup>2</sup>), and the average amount of liquid repellent applied to them was 0.44 mg/cm<sup>2</sup> (range, 0.39–0.52 mg/cm<sup>2</sup>). A mean of 3.8 g (0.17 mg/cm<sup>2</sup>) of the ADF gel formulation was applied to the collectors. A total of 2,454 *Anopheles* sp. were collected, and 2,446 were identified morphologically as *An. koliensis* and 8 were *An. farauti* s.l. A sample of 41

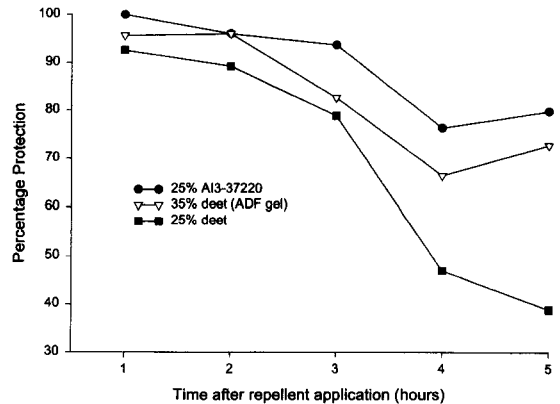


Fig. 1. Percentage protection provided by 3 repellents against *Anopheles koliensis* at Dasiamia, Papua New Guinea, April 1998.

specimens was identified using PCR-RFLP, including the 8 *An. farauti* s.l. and an additional 33 *An. koliensis*, of which 4 were *An. farauti* s.s., and 37 were *An. koliensis*. The overall mean biting rate of all mosquitoes on ethanol-treated (control) volunteers was  $77.2 \pm 10.5$  bites/human/10 min and no significant differences were found in the mean number of mosquitoes collected at different times ( $F = 2.59$ ,  $df = 4$ ,  $P = 0.08$ ; Table 1).

The percentage protection provided by the 3 repellents was significantly different ( $F = 7.26$ ;  $df = 2,42$ ;  $P = 0.002$ ; Fig. 1). The protection provided by AI3-37220 and ADF deet (35% deet in gel) was significantly greater than that provided by 25% deet, but no difference was found between AI3-37220 and ADF deet.

Analysis of the results of this trial shows that all 3 repellent formulations provided relatively poor protection against high biting densities of *An. koliensis*. Laboratory studies have shown the protection provided by repellents against mosquitoes is dependent on mosquito density (Khan et al. 1975, Barnard et al. 1998). The duration of protection provided by 25% ethanol concentrations of deet and AI3-37220 has been assessed against several species of *Anopheles* in different habitats (Frances et al. 1996, 1998, 1999), and the comparative protection provided against 4 species is shown in Table 2. The natural populations of *Anopheles* sp. in these studies were variable, but sufficient mosquitoes were present to assess the effectiveness of the test repellents against these species. In the current tests with *An. koliensis*, the biting density was unusually high for *Anopheles* sp. mosquitoes. No strong correlation between mosquito density and protection provided was observed in these studies (Table 2), and this is probably due to the variable response of different mosquito species to repellents (Travis 1950, Schreck 1977).

Although the densities were high and the percentage protection provided by all repellents fell

Table 2. Comparison of mosquito biting density and hours of greater than 95% protection against *Anopheles* spp. in the field to 25% deet and AI3-37220.

Species	Mean biting/ human/30 min	Time (h) for >95% protection	
		Deet	AI3-37220
<i>An. dirus</i>	0.9	2	4
<i>An. farauti</i> 4	11.3	4	3
<i>An. farauti</i> s.s	19.4	5	6
<i>An. koliensis</i>	231.6	<1	2

below 95% after 2 h, both 35% deet in a gel and 25% AI3-37220 provided >68% protection against *An. koliensis* for at least 5 h, when the tests ceased. Although the 25% deet solution provided less protection, it still provided 39% protection 5 h after repellent application.

This study provides additional information showing that AI3-37220 is at least as effective as deet against another malaria vector in PNG. Laboratory tests have shown that protection can be increased or decreased using various combinations of deet, AI3-37220, the piperidine AI3-35675, and the lactone CIC-4 (Debboun et al. 1999). These findings indicate the need for further studies with AI3-37220 in combination with other repellents to determine whether any synergistic activity might exist between these compounds.

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