

## FIELD EVALUATION OF ARTHROPOD REPELLENTS, DEET AND A PIPERIDINE COMPOUND, AI3-37220, AGAINST *ANOPHELES FUNESTUS* AND *ANOPHELES ARABIENSIS* IN WESTERN KENYA<sup>1</sup>

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**ABSTRACT.** A field evaluation of the repellents *N,N*-diethyl-3-methylbenzamide (deet) and 1-(3-cyclohexen-1-yl-carbonyl)-2-methylpiperidine (AI3-37220, a piperidine compound) was conducted against *Anopheles funestus* and *An. arabiensis* in Kenya. Both repellents provided significantly more protection ( $P < 0.001$ ) than the ethanol control. AI3-37220 was significantly more effective ( $P < 0.001$ ) than deet in repelling both species of mosquitoes. After 9 h, 0.1 mg/cm<sup>2</sup> of AI3-37220 provided 89.8% and 71.1% protection against *An. arabiensis* and *An. funestus*, respectively. Deet provided >80% protection for only 3 h, and protection rapidly decreased after this time to 60.2% and 35.1% for *An. arabiensis* and *An. funestus*, respectively, after 9 h. *Anopheles funestus* was significantly less sensitive ( $P < 0.001$ ) to both repellents than *An. arabiensis*. The results of this study indicate that AI3-37220 is more effective than deet in repelling anopheline mosquitoes in western Kenya.

### INTRODUCTION

The vectors of human malaria parasites in western Kenya are *Anopheles gambiae* Giles, *An. arabiensis* Patton, and *An. funestus* Giles (Githeko et al. 1993). *Anopheles gambiae s.s.* and *An. arabiensis* are morphologically indistinguishable members of the *An. gambiae* complex (Gillies and de Meillon 1968, Taylor et al. 1993).

Githeko et al. (1993) reported that the intense perennial transmission of malaria at Ahero, a village in western Kenya, was primarily due to *An. funestus*, not members of the *An. gambiae* complex. *Anopheles arabiensis*, although present in this area, appears to play a minor role in malaria transmission. No field data are available

concerning the susceptibility of these mosquito species to arthropod repellent compounds.

The United States Army is currently evaluating a novel piperidine compound (AI3-37220) that has been shown to be effective in the laboratory against several anopheline species, including laboratory-reared *An. gambiae* (Coleman et al. 1993, Frances et al. 1993). In a recent field test, this compound was shown to be effective against wild populations of *Anopheles dirus* Peyton and Harrison in Thailand (S. P. Frances, unpublished data) AI3-37220 is currently under consideration, either alone or in combination with deet, as a U.S. Army repellent formulation.

The objective of this study was to determine if AI3-37220 provides more effective and longer lasting protection than deet against *An. funestus* and *An. arabiensis* mosquitoes in the field. Deet is the standard against which the efficacies of candidate skin repellents are evaluated. Field evaluation of new repellent compounds is necessary because behavioral responses to repellents differ between feral arthropod populations and laboratory-reared populations (Frances et al. 1993).

### MATERIAL AND METHODS

Two repellents, *N,N*-diethyl-3-methylbenzamide (deet), 100% (Morflex Inc., Greensboro, NC) and 1-(3-cyclohexen-1-yl-carbonyl)-2-methylpiperidine (AI3-37220), 99% (synthesized by T. P. McGovern, Insect Chemical Ecology Laboratory, USDA-ARS, Beltsville, MD) were evaluated. The study was conducted in Nyanza Province, western Kenya, adjacent to the Ahero rice irrigation scheme. Ahero is located

<sup>1</sup> The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense. Mention of a commercial product does not constitute an endorsement of the product by the Department of Defense. The volunteers gave informed consent to participate in the study.

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23 km SE of Kisumu, located on the shore of Lake Victoria.

Twelve adult male residents (>21 years old) of the area volunteered to participate in this study. Repellents were evaluated on the lower legs, using the area between the knee and ankle. Both trouser legs were rolled up to just above the knees and a line was drawn at the knee with an ink marker to provide a line of demarcation for repellent application and mosquito collection. Low-top canvas shoes, all of the same brand, provided a demarcation line at the ankle. Volunteers wore hooded screened jackets (Bug Out Outdoor Wear® Ltd., Wauwatosa, WI) to restrict mosquito feeding to the lower legs.

Repellents were prepared as 5% solutions in absolute ethanol (50 mg/ml) and applied at a rate of 0.1 mg/cm<sup>2</sup>. The amount of repellent varied among volunteers and was based on the surface area of application,

$$A = [(a + b + c)/3]h,$$

calculated from the averaged measurements of leg height ( $h$  = length between the crease of the knee and the ankle bone) and circumference ( $[a + b + c]/3$ , where  $a$  = circumference just below the knee,  $b$  = circumference at mid-calf, and  $c$  = circumference just above the ankle bone). Prior to treatment, the lower legs of each volunteer were washed with 70% ethanol to ensure that the treatments were applied directly onto the skin. The ethanol was then allowed to evaporate before the treatments were applied to the volunteers' legs. The legs of the control volunteers were treated with absolute ethanol.

The repellent tests were conducted inside local mud-walled houses, because *An. arabiensis* and *An. funestus* primarily bloodfeed indoors (Githeko et al. 1993). Due to the design of houses in western Kenya, mosquitoes have free access to the interior through the open eaves. Mosquitoes can enter and exit freely even though all doors and windows may be closed. Bednets were provided to the house occupants so that the volunteers were the only accessible blood-meal source.

The study was arranged in a 3-way factorial design (2 repellent treatments plus a control  $\times$  8 time intervals  $\times$  6 collection nights). The 12 volunteers were selected at random for treatment and house so that for each collection night there were 4 participants per treatment and 3 treatment participants per house (deet, AI3-37220, and control). Once assigned to a house, volunteer treatment groups were evaluated in that house throughout the study. Treatments were changed nightly so by the end of the study each volunteer had received each treatment twice.

The treatments were applied at approximately

2100 h, 1 h prior to the start of each repellent test. At 2200 h, the volunteers entered their designated houses, sat on chairs, and collected all mosquitoes biting them on their lower legs in 20-mm scintillation vials for 45 min during each hour of the night. A 15-min break followed each collection period. The time, collector, and house were recorded for each captured mosquito. This collection procedure was repeated each hour until approximately 0600 h the next morning so that 8 biting collections were made by each volunteer. Ambient temperature and humidity were recorded hourly throughout the test nights. This 9-h repellent efficacy test was replicated for 6 test nights.

The collected mosquitoes were transported to the Kenya Medical Research Institute (KEMRI), Vector Biology and Control Research Centre, at Kisian for processing and identification. In a previous study at this site, Githeko et al. (1993) reported that *An. arabiensis* comprised 100% of the mosquitoes captured from the *An. gambiae* complex. The ribosomal DNA polymerase chain reaction (PCR) amplification method described by Taylor et al. (1993) was used to confirm the identification of mosquitoes identified morphologically as *An. gambiae s.l.*

Nightly collection totals were determined for each of the 8 collection times. Totals for each 45-min period were then summed and the percent protection at each collection time, defined as the number of bites received by an individual in the treatment group relative to that of the control, was calculated [ $100(\text{control} - \text{treatment})/\text{control}$ ]. Results of preliminary repellent tests indicated that the data did not meet the distributional assumptions of an analysis of variance and that transformations would not normalize the data. Statistical comparisons of repellent efficacy were made among the treatment groups using Kruskal-Wallis nonparametric analysis of variance (Statistix 1987).

## RESULTS AND DISCUSSION

Nightly weather conditions throughout the study were relatively constant. The mean temperature was 20.6°C (range: 16.7–23.4°C) and the mean percent relative humidity was 81% (range: 63–100% RH). There was little or no wind.

Three genera of mosquitoes (*Anopheles*, *Aedes*, and *Culex*) were collected. Two species of *Anopheles* (*An. funestus* and *An. gambiae s.l.*), representing 72% of the total number, were collected, providing sufficient numbers (5,029) to evaluate repellent efficacy. Initially, 3,668 *An. funestus* and 1,361 *An. gambiae s.l.* were identified. Twenty percent (272) of the *An. gambiae*

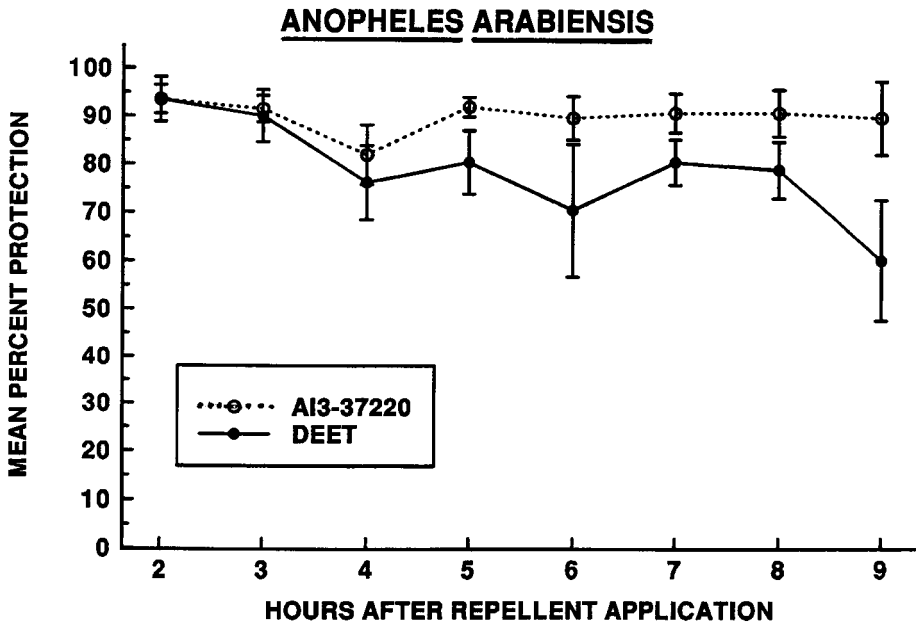


Fig. 1. Mean percent protection ( $\pm$  SE) provided by AI3-37220 and deet against *Anopheles arabiensis*.

*s.l.* were analyzed by using ribosomal DNA; all were subsequently identified as *An. arabiensis*. Based upon these results, we therefore assumed that all *An. gambiae s.l.* were *An. arabiensis*. This agrees with earlier work by Githeko et al. (1993) who reported that *An. gambiae s.s.* rarely occurs in the Ahero area.

The incidence of *Plasmodium falciparum* infection of the collected *Anopheles* in the study and the biting behavior of infected versus uninfected *Anopheles* on humans using mosquito repellents is discussed by Copeland et al. (1995).

Deet and AI3-37220 were significantly more effective (Kruskal-Wallis statistic [KWS] =

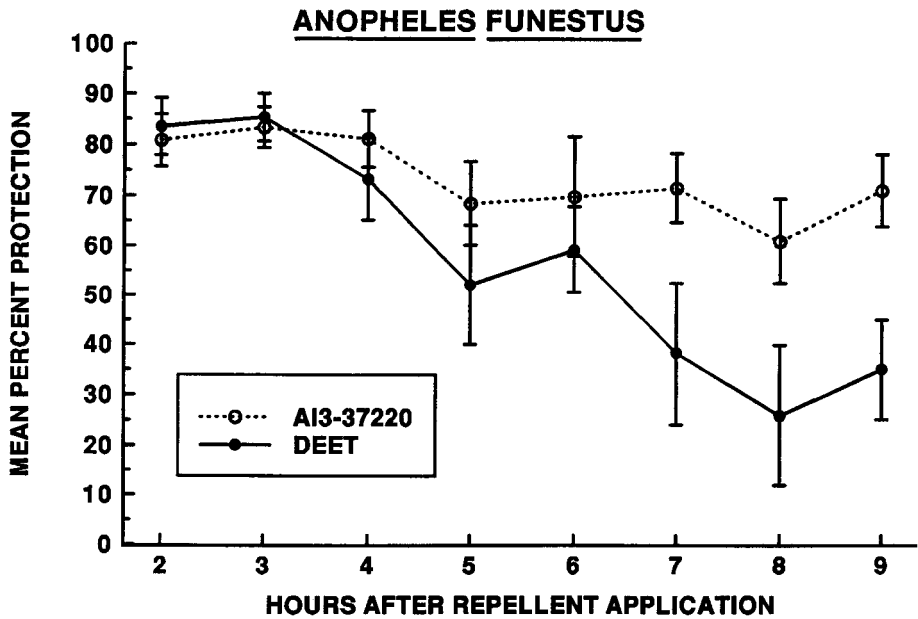


Fig. 2. Mean percent protection ( $\pm$  SE) provided by AI3-37220 and deet against *Anopheles funestus*.

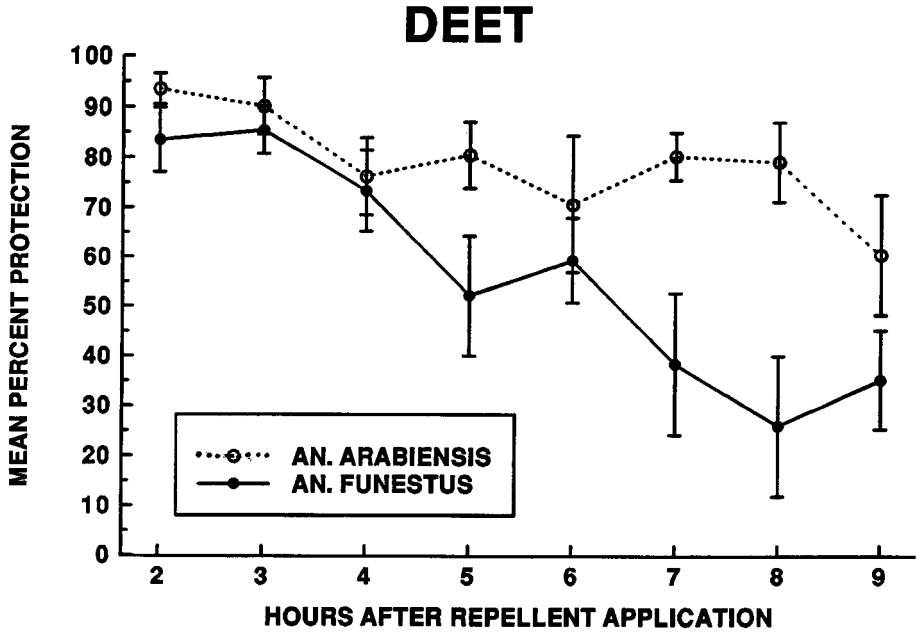


Fig. 3. Mean percent protection ( $\pm$  SE) provided by deet against *Anopheles arabiensis* and *An. funestus*.

17.1,  $P < 0.001$ ) than the ethanol control in repelling *An. arabiensis* and *An. funestus*. Over the 9-h test period, AI3-37220 was significantly more effective (KWS = 9.0,  $P < 0.003$ ) than deet in repelling both *An. arabiensis* and *An. fu-*

*nestus*. There was no significant difference in the repellency of AI3-37220 and deet during the first 4 h after repellent application. However, at 7 h postapplication, AI3-37220 was significantly more effective at repelling *An. arabiensis* (Fig.

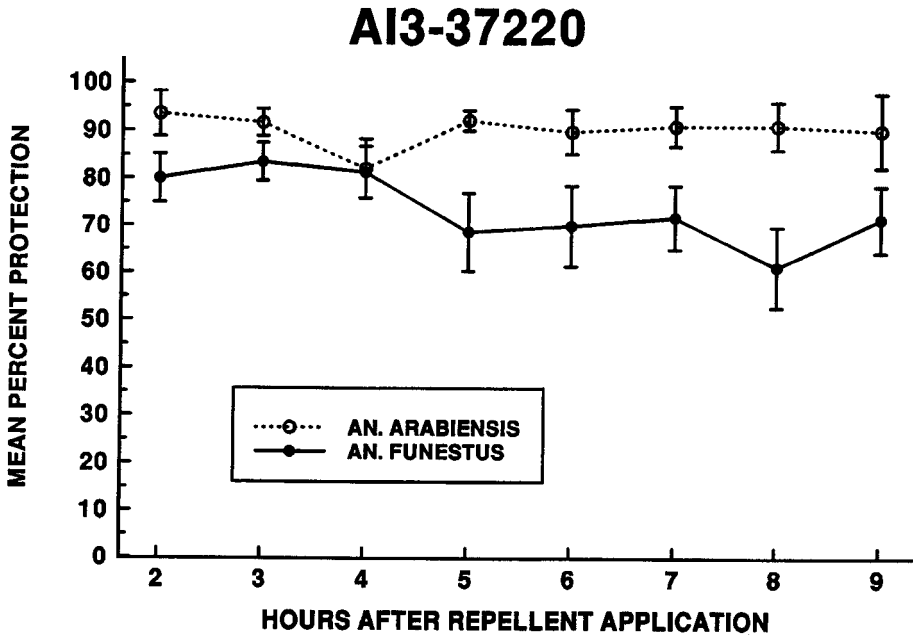


Fig. 4. Mean percent protection ( $\pm$  SE) provided by AI3-37220 against *Anopheles arabiensis* and *An. funestus*.

1) and *An. funestus* (Fig. 2) (KWS = 8.4,  $P < 0.004$ ) compared to deet. From 5 to 9 h, AI3-37220 continued to provide the volunteer with >90% and >60% protection against biting of *An. arabiensis* and *An. funestus*, respectively. In contrast, 9 h after repellent application, the percent protection provided by deet was only 60.2% and 35.1% for *An. arabiensis* and *An. funestus*, respectively.

From 7 to 9 h after repellent application, both deet (Fig. 3) and AI3-37220 (Fig. 4) provided significantly better protection (KWS = 11.6,  $P < 0.002$ ) against *An. arabiensis* (60.2% and 89.8% at 9 h, respectively) compared to *An. funestus* (35.1% and 71.1% at 9 h, respectively). These data have serious implications concerning repellent efficacy relating to protection against vectors of malaria parasites in the Ahero area. *Anopheles funestus* is the primary vector of malaria in this area, with *An. arabiensis* playing a secondary role. This study indicates that AI3-37220 is clearly a better repellent for use in this area, as compared to deet. Further studies are warranted to evaluate the effectiveness of AI3-37220 against other species of the *An. gambiae* complex in western Kenya.

### CONCLUSIONS

In initial studies using laboratory-reared mosquitoes, AI3-37220 was as effective as deet against *An. gambiae* and other anopheline species (Coleman et al. 1993). We also found that AI3-37220 provided as good or better protection than deet in duration tests. In a field study, S. P. Frances (unpublished data) found that AI3-37220 provided significantly better protection than deet against wild populations of *An. dirus* in Thailand. Coleman et al. (1994) reported that AI3-37220 provided better protection than deet from biting of *Culex pipiens* Linn. in Saudi Arabia. Our data and previous findings indicate that AI3-37220 provides significantly better protection from biting of several mosquito species from different genera.

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