RELATIVE REPELLENCY OF TWO FORMULATIONS OF N,N-DIETHYL-3-METHYLBENZAMIDE (DEET) AND PERMETHRIN-TREATED CLOTHING AGAINST *CULEX SITIENS AND AEDES VIGILAX* IN THAILAND[1]

RALPH E. HARBACH,[2] DOUGLAS B. TANG,[3] ROBERT A. WIRTZ[2] AND JOHN B. GINGRICH[4]

ABSTRACT. Field tests were conducted to compare the effectiveness of 2 repellent formulations of N,N-diethyl-3-methylbenzamide (deet) in combination with permethrin-impregnated military uniforms against *Culex sitiens* and *Aedes vigilax* in Thailand. Repellency was determined during a 2 h crepuscular period using volunteers who had been treated with repellents 6, 8, 10, and 12 h prior to the end of each test period. An extended-duration repellent formulation (EDRF) containing 35% deet repelled significantly more *Ae. vigilax* than 75% deet in ethanol. Although not statistically significant, the EDRF also resulted in fewer biting attempts by *Cx. sitiens*. Neither formulation provided complete protection against either species 4–12 h post-application, but both provided greater overall protection against *Ae. vigilax*. Volunteers who wore treated uniforms without repellents were attacked by significantly fewer mosquitoes than controls.

INTRODUCTION

The use of protective clothing and repellent is an inexpensive means of reducing arthropod-man contact and the incidence of arthropod-borne diseases. For several years, the Department of Defense and the U.S. Department of Agriculture have been evaluating the effectiveness of a new personal protection system for use among military personnel (Gupta et al. 1987, Sholdt et al. 1988, Lillie et al. 1988, Schreck and Kline 1989). The purpose of this study was to test the new system against mosquitoes in Thailand. These tests were conducted to compare the effectiveness of 75% deet (N,N-diethyl-3-methylbenzamide) in an ethanol solution to that of an extended-duration repellent formulation (EDRF) of 35% deet when these topical repellents are used in combination with permethrin-treated battle-dress uniforms (BDU). The tests were designed to determine whether the 35% deet in the EDRF would provide greater protection (or more effectively reduce mosquito-man contact) than 75% deet in duration studies.

This paper reports the results of field tests conducted against *Culex sitiens* Wied. and *Aedes vigilax* (Skuse). Both species are potential vectors of human pathogens. *Culex sitiens* has been found naturally infected with larvae of *Brugia malayi* in Thailand (Iyengar 1953), and *Ae. vigilax* appears to be the principal vector of non-periodic filariasis in New Caledonia (Iyengar 1954). Experimental transmission of Japanese encephalitis virus has been demonstrated for *Cx. sitiens* (Hodes 1946, as *Cx. jepsoni* Theobald), and *Ae. vigilax* has been found naturally infected with Murray Valley encephalitis virus in Australia (Doherty et al. 1963).

MATERIALS AND METHODS

Field tests were conducted at the margin of a brackish water *Nipa* palm swamp located near the southern edge of Bangkok (Tombol Bang Phla Kod, Amphur Phra Samut Jadee), Thailand. Preliminary human bait collections made at the site showed that peak mosquito biting activity occurred during the evening twilight period beginning shortly after 1800 h. Consequently, this study was designed to expose test subjects to biting mosquitoes between 1800 and 2000 h.

Ten male college biology students volunteered as test subjects. Two-hour collections (1800–2000 h) were made by each volunteer under each of the following 10 exposure conditions: (1) untreated BDU (100% cotton fabric dyed with a camouflage pattern), no repellent (control); (2) permethrin-impregnated BDU (impregnated, using the individual dynamic absorption procedure, at a concentration of 0.125 mg/cm^2 by the U.S. Army Natick Research Development and Engineering Center, Natick, MA), no repellent; (3–6) permethrin-impregnated BDU, 75% deet in ethanol applied at 0800, 1000, 1200 and 1400 h; and (7–10) permethrin-impregnated BDU, EDRF (35% deet in a cream base) applied at 0800, 1000, 1200 and 1400 h. The application times correspond to wearing the repellents 12, 10, 8 and 6 h by the end of the collection period. A light even coating of repellent was applied on the forearms, lower legs, and the face and neck according to instructions printed on the respec-

[1] The views of the authors do not purport to reflect the views of the Department of the Army or the Department of Defense. Human subjects participating in this study gave free and informed voluntary consent.

[2] Department of Entomology, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

[3] Department of Mathematics and Biostatistics, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

[4] AFMIC, Fort Detrick, Frederick, MD 21701-5004.

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tive containers. The amount of repellent used by each volunteer was calculated by weighing the containers before and after each application. The volunteers received an average of 1.37 g of 75% deet (1.03 g AI) and 4.03 g of EDRF of deet (1.41 g AI). Volunteers were instructed not to wash, scratch, or rub treated areas and asked to bathe with soap and water to remove repellent residues at the end of each test day.

Collections were made on 10 days, 5 with moonless evenings (March 29–April 2, 1988) and 5 when a full moon was out during the collection period (April 16–20, 1988). A Latin square design was used to assign each of the 10 exposure conditions to the 10 volunteers over the 10 days (Box et al. 1978). During the tests, uniforms were worn with the trousers rolled up to the knee, socks rolled down to the ankle (shoes were worn instead of boots), shirt sleeves rolled up to the elbow, and caps on. Volunteers sat in identical portable folding chairs stationed at 5 m intervals along a small dike at the margin of the swamp. Flashlights and aspirators were used to capture mosquitoes that were biting or attempting to bite through exposed skin. Mosquitoes collected while attempting to bite were considered to be capable of completing a successful bite. However, the volunteers were relatively inexperienced and some mosquitoes may have been captured before they actually attempted to bite. Captured mosquitoes were immediately transferred into prelabeled cups with screen tops. The cups were gathered at the end of the collection period, taken to the laboratory, and placed in a freezer. The mosquitoes were identified the next morning at the Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok.

The F test from the analysis of variance (ANOVA) for a Latin square design was used to test for overall differences between the 10 exposure conditions (treatments), adjusting for differences between volunteers, and collection periods (Box et al. 1978). Based on the factorial structure of the 8 repellent groups (2 repellent types × 4 application times), ANOVA was used to test for differences between repellent type (75% deet vs. EDRF), effects of time the repellent was worn (12, 10, 8, 6 h), and the interaction of the 2 factors. The model for this analysis included (eliminated effects of) volunteers and collection times, but excluded data from the 2 exposure conditions that did not involve repellent use. All analyses used the square root transformation (of the number of mosquitoes) to help stabilize variance and reduce skewness. Box plots (Velleman and Hoaglin 1981) were used to summarize data and provide a graphical interpretation of the results of the above analyses. In this paper, percent of repellency is defined as the difference between the number of mosquitoes captured by control and treated volunteers expressed as a percentage.

RESULTS

A total of 4,399 mosquitoes representing 7 species were captured during the tests, with *Cx. sitiens* and *Ae. vigilax* accounting for 97.9% (*Cx. sitiens* 80.3% and *Ae. vigilax* 17.6%). Other species included *Ae. lugubris* Barraud (1.0%), *Cx. quinquefasciatus* Say (0.4%), *Ae. amesii* (Ludlow) (0.3%), *Anopheles subpictus* Grassi (0.3%) and *Cx. gelidus* Theobald (0.1%).

Figures 1A and 1B summarize the collection data for *Ae. vigilax* and *Cx. sitiens* for each of the 10 exposure conditions. For *Ae. vigilax* (Fig. 1A) the overall difference in numbers of collected mosquitoes between the 2 repellents was significant ($F(1,54) = 15.67$, $P < 0.0001$) and was consistently lower for the EDRF for each of the 4 application times (no interaction). Although for *Cx. sitiens* (Fig. 1B) the overall difference between repellents was not significant ($F(1,54) = 3.23$, $P = 0.078$), the mean number collected by volunteers wearing the EDRF was again consistently lower for each of the 4 application times. The absence of interaction between repellent type and application time implies that the difference in effectiveness between the 2 formulations does not depend on application time. For both species: 1) there was a significant time-related decline in repellency for both repellent formulations (min $F(3,54) = 7.79$, $P < 0.0001$), 2) the number of collected mosquitoes for the 8 repellent exposure conditions (2 types × 4 application times) was significantly less than either the treated BDU alone or the control, and 3) the number of mosquitoes captured while volunteers wore the permethrin-treated BDU without repellent was significantly less than the control.

The relative effectiveness (percent repellency) of the protective treatments is contrasted for the 2 species in Table 1. The average protection against *Cx. sitiens* during the collective exposure period between 4–12 h post-application was 72.9% for 75% deet and 78.8% for the EDRF. Both repellents provided greater protection against *Ae. vigilax*, 83.4% for 75% deet and 93.5% for the EDRF over the 8-h period. Wearing the treated uniform without repellents afforded 37.1% protection against *Cx. sitiens* and 43.1% against *Ae. vigilax*.

Substantially more mosquitoes (both species) were captured on bright (moonlit) evenings (3,530 specimens) than on dark (moonless) evenings (775 specimens), perhaps because mosquito populations were larger but more likely because the presence of moonlight extended the

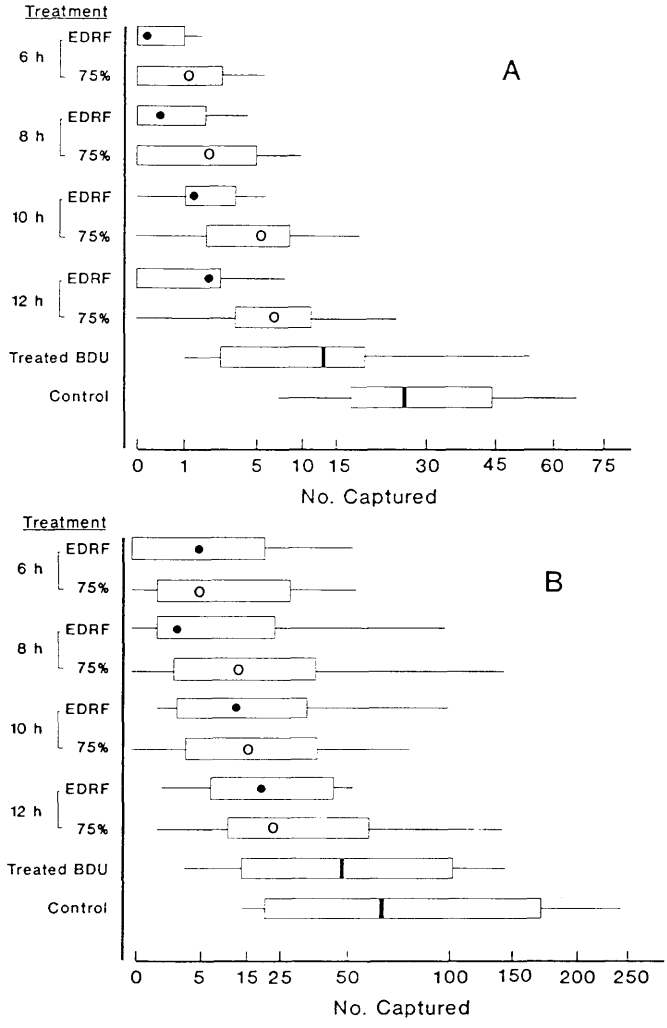

Fig. 1. Distribution of numbers of *Aedes vigilax* (A) and *Culex sitiens* (B) captured while biting or attempting to bite during ten 2-hour collection periods by treatment (exposure) conditions. Box plot shows extremes (ends of extended line), quartiles (ends of box), and median number captured (symbol within box). Number of mosquitoes captured (horizontal axis) is shown as a square root scale. EDRF = extended-duration repellent formulation (35% deet + treated BDU); 75% = 75% deet in ethanol + treated BDU; treated BDU = permethrin-impregnated uniform (0.125 mg/cm^2) without repellents; control = untreated BDU without repellents.

crepuscular period of biting activity. An analysis of variance using a model that included the effect of moon (moon vs. no moon) indicated a significant effect with no interaction between the repellent type or the time of application.

DISCUSSION

If the 2 repellent formulations of deet tested in this study are capable of providing complete protection against the bites of *Cx. sitiens* and *Ae. vigilax*, then the complete protection time is obviously less than 4 h. Tests were not initiated earlier than 4 h post-application because significantly longer periods of complete protection were anticipated for both repellents. For this reason, the results achieved were completely unexpected.

From the results it is apparent that the EDRF containing 35% deet may be more effective than 75% deet in ethanol against some mosquito species, but neither formulation will provide complete protection over long periods of time. The actual degree of repellency for each formulation probably depends on a complexity of factors, including mosquito density, host attractiveness

Table 1. Relative effectiveness (percent repellency) of 75% deet and an EDRF (extended-duration repellent formulation of 35% deet) against *Culex sitiens* and *Aedes vigilax* when worn with permethrin-treated battle-dress uniforms (BDU).

		75% deet		EDRF	
Species	Treatment*	No. captured	Repellency (%)	No. captured	Repellency (%)
Cx. sitiens	6 h	144	85.5	136	86.3
	8 h	291	70.6	195	80.3
	10 h	245	75.3	280	71.7
	12 h	396	60.0	229	76.9
	Treated BDU	623	37.1	623	37.1
	Control	991	—	991	—
Ae. vigilax	6 h	17	94.5	4	98.7
	8 h	36	88.4	12	96.1
	10 h	64	79.4	37	88.1
	12 h	89	71.4	28	91.0
	Treated BDU	177	43.1	177	43.1
	Control	311	—	311	—

* h indicates length of time repellents worn by volunteers wearing treated BDUs (mosquitoes captured while biting or attempting to bite during last 2 h); treated BDU indicates permethrin-impregnated uniforms (0.125 mg/cm^2) worn without repellents; control indicates untreated uniforms worn without repellents.

and various environmental parameters. Apart from this, it must be realized that the average application of the EDRF used in this study was 3 times greater by weight and contained nearly 1.4 times more active ingredient than the average application of 75% deet. This, coupled with the fact that most of the Thai volunteers expressed a pronounced preference for the ethanol solution of 75% deet because the EDRF felt sticky when first applied, may outweigh any practical advantage derived from using the EDRF against natural populations of mosquitoes. Furthermore, the results presented here were achieved by testing the repellents in combination with permethrin-treated uniforms. Considering that volunteers who wore the treated uniform and no repellent were attacked by substantially fewer mosquitoes than the control group (Table 1), it seems likely that a significant portion of the repellency observed among treated volunteers was attributable to the permethrin-impregnated uniform.

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