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AGAINST *ANOPHELES FLAVIROSTRIS*

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COMPARISON OF THE EFFECTIVENESS OF TWO FORMULATIONS OF DEET AGAINST *ANOPHELES FLAVIROSTRIS*¹

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ABSTRACT. The effectiveness of 2 formulations of deet (diethylmethylbenzamide) was tested against the principal vector of malaria in the Philippines, *Anopheles flavirostris*. A new extended duration repellent formulation (EDRF) was compared with the standard military-issue liquid formulation. The EDRF was significantly more effective than the liquid formulation between 6 and 12 h post-application. There was no difference between the 2 formulations in the first 6 h following application, or between 12 and 15 h post-application. The continued usefulness of the liquid formulation is discussed in light of these results.

INTRODUCTION

Personal protection measures such as the use of topical repellents play an important role in the protection of military personnel against vector-borne diseases such as malaria. In an operational environment, the use of vector control measures may be severely constrained. In addition, effective human malaria vaccines do not yet exist and the effectiveness of chemoprophylaxis can be reduced by poor troop compliance or the presence of drug-resistant parasite strains (Hooper and Wirtz 1983).

The current standard military issue insect repellent (NSN 6840-00-753-4963), hereafter referred to as the liquid formulation, has been judged unacceptable by a large proportion of users due to its smell, feel or duration of effectiveness (Hooper and Wirtz 1983). It may quickly lose potency due to exposure to water or perspiration, is harmful to plastics and provides less than satisfactory protection against some species of vectors (Sholdt et al. 1988).

To overcome some of these problems, a newly developed controlled-release formulation of deet has recently been adopted by the Department of Defense. The extended duration repellent formulation (EDRF) has been shown to provide 99% protection for up to 9 h under heavy biting pressure by *Culiseta impatiens* (Walker) (Lillie et al. 1988). However, Gupta et al. (1987) reported that a controlled-release cream formulation of 33% deet was no more effective than the standard military liquid formulation against

several species of Australian mosquitoes, including *Anopheles farauti* Laveran, a malaria vector.

Anopheles flavirostris (Ludlow) is the principal vector of malaria in the Philippines, which is endemic in 72 of 75 provinces (Salazar et al. 1988). Large numbers of military personnel are permanently assigned to the Philippines, and thousands more come each year for varying lengths of time to participate in training exercises. The effectiveness of personal protection measures is of particular concern to such personnel, who are often exposed to natural vector populations under operational conditions. This study compares the efficacy of the new EDRF and the liquid formulation against *An. flavirostris*.

MATERIALS AND METHODS

Four tests were conducted in the village of Santo Nino on the west coast of the island of Palawan (approximately 9°43'N, 118°30'E). Test subjects were Filipino male volunteers between the ages of 19 and 40. In each test, replicates consisted of 3 volunteers; an untreated control and 2 treated subjects. One subject was treated with EDRF (31.58% diethylmethylbenzamide isomers in a cream base) and the other with liquid formulation (71.25% diethylmethylbenzamide, 3.75% other isomers of deet and 25% ethanol). Each night, subjects rotated treatments. Each test consisted of 27 replicates. Every effort was made to use the same volunteers for the duration of a test, but occasional substitutions were necessary.

Repellents were applied in a manner simulating actual use under field conditions. A given amount of repellent was placed in the palm of the hand. The hands were rubbed together and then the repellent applied to the leg below the knee and the top of the foot. The diagram on the side of the tube was used to estimate 2.5 ml of EDRF, which was applied to each leg. A measured amount of liquid formulation (1.1 ml) containing the same amount of active ingredient

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was applied to each leg of the other treated subject. Due to the fundamental difference in the nature of the inert ingredients of each formulation, control subjects did not receive a placebo treatment. Subjects wore long-sleeve jackets with hoods, but no attempt was made to mask the face or hands, nor was repellent applied to other body surfaces.

Subjects were seated 10 m apart in a large (approximately 100 × 100 m) grassy field in the center of the village. Landing mosquitoes were aspirated from lower legs and transferred to paper cups with net screen fastened to the top. Cups were changed every hour and the mosquitoes taken to a nearby field laboratory for identification.

Tests were conducted between 2100 and 0300 h, peak biting time for *An. flavirostris*. In the first experiment, repellents were applied immediately prior to the beginning of the test. Treatment was applied at 1800 h in the second experiment, 1500 h in the third, and 1200 h in the fourth test. Following treatment, the subjects were told to go about their normal activities until 2100 h, although they were asked to avoid washing or immersion of their legs in water.

The tests were conducted during July, August and September of 1989. The mean temperature, mean minimum temperature, total rainfall and mean wind speed for the 3 months were: July, 27.0°C, 21.8°C, 140 mm, 2 mph; August, 27.0°C, 21.5°C, 66 mm, 2 mph; September, 27.1°C, 21.8°C, 136 mm, 2 mph.

Because of the relatively low biting rates, treatments were compared using the mean number of bites received per man during the 6-h period. Results were analyzed by analysis of variance (ANOVA) and the least significant difference (LSD) was used to compare means. Analyses were performed using Statgraphics (Statistical Graphics Corporation 1987).

RESULTS AND DISCUSSION

Untreated subjects received an average of 37.7 bites per night by *An. flavirostris* during the four 6-h periods. This biting rate is typical of periods of moderate malaria transmission in the area (Oberst et al. 1988).

There was no significant difference between formulations in either the test begun immediately following treatment or the test begun 3 h after treatment (Table 1). Between 6 and 12 h post-treatment, significantly fewer bites were received by subjects treated with EDRF (8.2 per night) than those treated with the liquid formulation (15.6 per night).

Repellent compounds can also be evaluated by calculating percent protection (Lillie et al.

Table 1. Mean numbers of bites received by treated and untreated subjects during 6-hour tests begun at various times following application of repellents.

Hours post-application	Mean no. of bites per person				
	Control	Liquid	EDRF	F	P
0-6	44.3 a*	0.9 b	0.1 b	116.11	0.000
3-9	35.2 a	6.2 b	1.3 b	65.07	0.000
6-12	34.0 a	15.6 b	8.2 c	25.95	0.000
9-15	37.2 a	25.5 b	24.6 b	5.68	0.005

* Means in the same row followed by the same letter are not significantly different by LSD, $P < 0.05$.

1988). As shown graphically in Fig. 1, both formulations provided a high degree of protection for 6 h. After 6 h, the effectiveness of the liquid formulation began to decline, while the EDRF provided greater than 90% protection for up to 8 h. The greatest difference in effectiveness between the 2 formulations occurred between 6 and 12 h post-treatment. After 12 h, biting rates were again similar for both formulations, but the treatments were still significantly different from controls.

Where the separate tests overlapped, the degree of protection was generally slightly lower during the initial 3 h of a test than the terminal 3 h of the test begun earlier. Activities of the treated subjects in the pre-collection period may have degraded the effectiveness of the repellents somewhat.

While the EDRF provided significantly greater protection than the liquid formulation between 6 and 12 h post-application, the degree of protection it provided began to decline after 8 h. To provide protection against disease transmission, reapplication would be necessary before significant degradation in protection occurred. Thus, reapplication of the EDRF would be required at about 8 h while the liquid formulation would require reapplication at about 6 h. Both formulations come in a 2-fl. oz. container. At the recommended rate of 2.5 ml per application, the EDRF provides 22.7 applications per container. Using the same amount of active ingredient per application (1.1 ml), a 2 oz. bottle of liquid formulation provides 51.6 applications. The need to apply EDRF less frequently offsets this disparity somewhat. Assuming 8- and 6-h protection per application, respectively, 2 oz. of EDRF provides 182 "protection hours," while a bottle of liquid provides 310, or 70% more. Thus, a greater bulk of EDRF would have to be carried to provide the same protection. The packaging of the EDRF is also less efficient since not all of the contents can be removed from the tube (Gupta et al. 1987).

It should be noted that these tests were per-

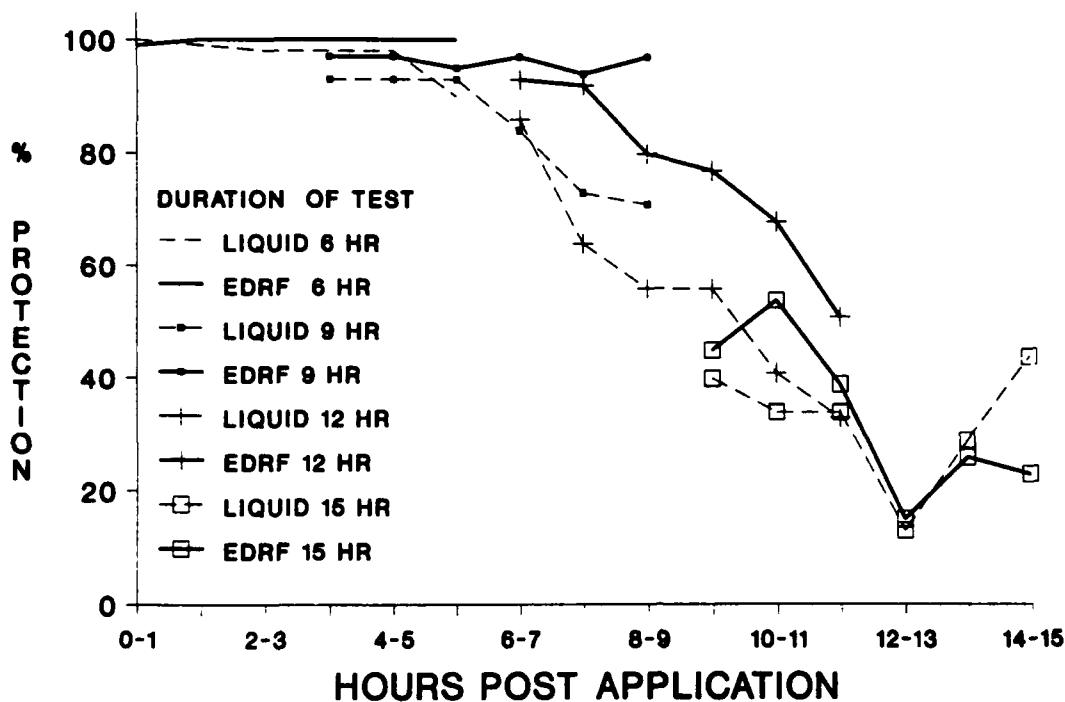


Fig. 1. Percent protection provided by 2 formulations of deet in four 6-h tests begun 0, 3, 6 and 9 h post-application.

formed using sedentary subjects. The ability of the 2 formulations to withstand perspiration or exposure to water was not tested. The comparative advantage provided by the EDRF may be greater under conditions more closely resembling the activities of troops in the field.

Even with the adoption of the EDRF, the liquid formulation will remain in the military supply system as a necessary treatment for the deet repellent jacket. Liquid deet may continue to have its uses as a topical repellent among troops in the field as well. At least some users find the EDRF uncomfortably "greasy" or "sticky" (Sholdt et al. 1988). In addition to being less bulky, the 2-ounce liquid-deet bottle fits conveniently under the helmet strap. In light of these factors, it may remain the formulation of choice for some individuals.

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