PERSONAL PROTECTION AFFORDED BY CONTROLLED-RELEASE TOPICAL REPELLENTS AND PERMETHRIN-TREATED CLOTHING AGAINST NATURAL POPULATIONS OF *AEDES TAENIORHYNCHUS*[1]

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ABSTRACT. Field tests evaluated repellent formulations containing deet in combination with permethrin-impregnated or untreated military uniforms against *Aedes taeniorhynchus*. No significant difference was determined between repellents in duration of protection whether or not permethrin-treated clothing was worn, but there were differences in efficacy relative to site of application. The head was the site of shorter duration of protection regardless of repellent tested. On repellent-treated skin, 12–30% of the bites were on arms, whereas 70–88% were on the head. When military repellent was used, the head was bitten 35% more often than with experimental repellents. On untreated clothing 80% of bites were through pants and 20% through shirts. Mean bites through untreated clothing were 0.7 bites/min/person (42/hr), whereas mean bites through permethrin-impregnated clothing were 0.0004/min/person (0.02/hr). Protection by permethrin-treated clothing relative to untreated clothing was 99.9%.

INTRODUCTION

Since 1974, the personal protection unit of the Insects Affecting Man and Animals Research Laboratory and other cooperators have been developing a database for the U.S. Department of Defense on permethrin as a clothing treatment to protect military personnel against hematophagous arthropods, particularly mosquitoes (Schreck et al. 1978, 1980, 1982; Breeden et al. 1982, Gupta et al. 1987, Lillie et al. 1988, Sholdt et al. 1988). The ultimate objective is to provide the armed services with a system that effectively protects against blood-sucking pests and disease vectors anywhere in the world. The system combines an effective repellent, deet (*N,N*-diethyl-3-methylbenzamide, formerly *N,N*-diethyl-meta-toluamide and other isomers), on exposed skin and a clothing impregnation of permethrin at the rate of 0.125 mg/cm^2. Earlier studies (Schreck et al. 1984) indicated that the system provided 99.9% protection against bites in a 9-hr exposure period and that bites occurred only on the repellent-treated skin.

This paper reports the results of a field study comparing 3 formulations when they were applied as part of the protection system in conjunction with the permethrin-treated military battle dress uniform (Schreck et al. 1984).

MATERIALS AND METHODS

Field bioassays of the deet-permethrin protective system included a comparison of 3 formulations containing deet applied to all exposed skin in combination with a permethrin-impregnated uniform tested against *Aedes taeniorhynchus* Wiedemann at Everglades National Park, Flamingo, Florida.

The formulations were 1) a controlled-release formulation containing 35% deet produced by Personal Care Products, 3M Center, St. Paul, MN; 2) a controlled-release formulation containing 44% deet produced by Biotek, Inc., Woburn, MA; and 3) the current U.S. military all-purpose repellent containing 75% deet in ethanol as a standard.

Clothing tested was the U.S. Army Woodland Camouflage battle dress tropical weight uniform 100% cotton ripstop fabric (MIL-C-43468D) which included trousers, coat and hat. Two sets of the tropical weight uniforms, each of the 3 repellent formulations and a treatment schedule were issued to each of 8 test volunteers. One set of uniforms was treated with permethrin (Permanone® emulsifiable concentrate of 40% permethrin produced by Fairfield American Corp., Newark, NJ) at the rate of 0.125 mg/cm^2 by the U.S. Army Natick Research Development and Engineering Center, Natick, MA, and the second set was untreated.

The repellent bioassays followed the ASTM standard methodology (Anonymous 1983) but with some modifications comparing the 2 experimental formulations and the deet standard. Repellents were carefully applied at 0600 hr at the

[1] This paper reports the results of research only. Mention of a pesticide does not constitute a recommendation for use by the U.S. Department of Agriculture, nor does it imply registration under FIFRA as amended. Human volunteers who participated in this study gave their free and informed voluntary consent.

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rate of 1 ml or gm to each arm and 1.5 ml or gm to the head (including face, ears and neck to the hairline). Tests were initiated in the field at 0900 hr and terminated between 1830 and 1900 hr.

The bioassays were designed to determine protection from bites when a person wore 1) the permethrin-impregnated uniform without repellent on exposed skin, 2) the impregnated uniform with repellent on exposed skin, 3) an untreated uniform with repellent on exposed skin and 4) an untreated uniform without repellent on exposed skin (check).

Volunteers were paired to aid in determining bite locations on the body. The number and time bites occurred on the repellent-treated skin of the arms, hands and face, and through the pants and shirt were recorded separately. Tests were arranged so that each person wore the treated and untreated uniforms an equal number of times. Biting rates and species biting were determined at hourly intervals throughout the day on all treatment combinations. This was accomplished by lifting the right pants leg to the knee each hour and counting the number of mosquitoes biting in 60 sec. At times, because of very high densities of mosquitoes biting, the biting count observation was made in 15 sec and calculated to bites/min. Species observed biting were identified by trained participating volunteers.

Tests with the treated and untreated uniforms were performed concurrently but in separate areas to avoid any interaction resulting from the knockdown effect of permethrin on the mosquitoes. During the tests the uniform was worn with the trouser cuffs bloused, sleeves rolled up above the elbows (except for volunteers with no repellent) and cap on. Volunteers made it a point to move further into new areas during each subsequent exposure to assure that repellents and, in particular, the permethrin-treated uniforms were tested on untried mosquitoes each time. This was to prevent a fatigue or knockdown effect on the mosquitoes because of repeated contacts with the repellents and/or permethrin in the same areas.

Because of the hardship encountered from high densities of biting mosquitoes, persons without repellents wore head nets and kept their sleeves rolled down except at 15- or 30-min intervals when 15- to 60-sec bite counts were taken. Temperature and relative humidity were recorded with a hygrothermograph throughout the study.

Data gathered from these tests were analyzed to determine 1) duration of complete protection from bites, 2) overall percent protection and 3) the total number of bites on treated skin for all repellents tested.

Duration of protection is defined in ASTM document E939-83 as "complete protection time (CPT)—the time from application of the repellent to the time of the first confirmed bite (a second bite by the same species within 30 min of the first)." The data were also broken down into body areas, i.e., arms, head and hands, to determine if all sites were equally protected by the repellents. In addition, we compared locations of biting sites on treated and untreated clothing.

The CPT and treatment site data were compared separately using an ANOVA with a Waller-Duncan multiple range test for differences between means at the 0.05% level of significance.

RESULTS

The duration of protection data (CPT) are summarized in Table 1. There were no significant differences between repellent formulations regardless of whether the uniform was treated with permethrin or untreated. However, there were some differences in the efficacy of the repellents depending upon site of application. Significantly shorter CPT was observed when the deet standard and the Biotek repellent were applied to the head than when they were applied

Table 1. Comparison of the CPT (complete protection time) of 3 repellent formulations containing deet when applied to the head and arms of volunteers wearing permethrin-treated or untreated uniforms and tested against field populations of *Aedes taeniorhynchus* (means of 4 tests).

Repellent formulation	% deet	Mean CPT in hours[a]			
		Treated uniform		Untreated uniform	
		Arms	Head	Arms	Head
Deet standard	75	12.5 Aa	6.5 Ab	11.3 Aa	8.7 Aa
3M	35	11.7 Aa	9.3 Aa	12.0 Aa	6.7 Ab
Biotek	44	12.3 Aa	9.2 Ab	11.9 Aa	10.1 Aa

[a] Means with the same capital letters are not significantly different for vertical data, whereas means with the same lower case letter are not significantly different for the horizontal data (0.05% level of confidence; ANOVA).

to arms of volunteers wearing the treated uniform. When volunteers wore the untreated uniform, the CPT was significantly shorter for head treatments with the 3M repellent only. It appears unlikely that these differences were related to whether the uniforms were treated or untreated. Rather, the inconsistency in significant differences may be a function of the inherent variability between volunteers and the low biting rate observed with treated subjects, resulting in an insufficient sample size to show a significant difference between treatment sites.

Thus, when sites of repellent application (arms and head) were compared with an ANOVA using all CPT means (8 for each formulation tested), it was found that the head had a significantly lower CPT than the arms regardless of the repellent used. Further, when the total number of bites on treated skin for all tests is compared, 12–30% of the bites were on arms, whereas 70–88% were on the head (Table 2). It is interesting to note that although there were no significant differences in the CPT of the 3 formulations (Table 1), the deet standard-treated skin was bitten 35% more than the 3M and Biotek treatments combined.

Table 3 summarizes data on the numbers and location of bites through the uniform fabric. Volunteers wearing untreated uniforms recorded a total of 3,497 bites, of which 2,806 (80%) were through pants and 691 (20%) through shirts. This difference was significant on 3 of the 4 test days. Bites were mainly on the buttocks, upper thighs and calves through a single layer of fabric when volunteers were at rest.

The mean number of bites through the untreated uniform/person/hr was 42 or ca. 0.7 bites/min/person during exposure and was considered uncomfortably high, although the mean bite count on untreated skin was 60/min (bite counts were not significantly different in the separate test areas).

The mean number of bites through permethrin-treated clothing/person/hr was 0.02 or 0.0004/min. These figures actually represent 2 bites—1 on pants and one on the shirt (a bite felt but not visibly confirmed). The calculated percentage of total protection for the entire study afforded by the permethrin-impregnated uniform relative to bites through the untreated uniform was 99.9%. Average daily temperature and relative humidity during these tests (October 1986) was 25.2°C and 80.9 RH.

DISCUSSION

The purpose of these assays was to determine how effective the deet-permethrin protective system would be against high density biting populations of naturally occurring mosquitoes and which of the repellent formulations would be best suited for this purpose.

Most interesting was the matter of failure of all the repellents to provide long-lasting protection on the head. It was determined that if effective protection from bites is desired, reapplication of the repellent to the skin of the head must be made more often than to the skin of the arms and legs. Although the deet standard con-

Table 2. Total number of bites on repellent-treated skin of 8 test volunteers in field studies with *Aedes taeniorhynchus* (five 1-hr exposures of each volunteer on each of 4 days).

	Treatment site			
	Arms		Head	
Repellent formulation	No. bites	% of total	No. bites	% of total
MR	235 A[a]	30	536 A	70
3M	36 A	12	270 A	88
Biotek	67 A	25	196 A	75

[a] Means with the same letter are not significantly different (0.05% level of confidence; ANOVA).

Table 3. Number and location of bites through permethrin treated or untreated 100% cotton tropical weight Battle Dress Uniform (BDU) during daily field exposures to *Aedes taeniorhynchus* (4 volunteers for each treatment each day).

	No. bites through BDU				
	Untreated[a]		Treated[a]		% total protection relative to bites through untreated BDU
Day	Pants	Shirt	Pants	Shirt	
1	998	19	0	1[b]	99.9
2	575	453	0	0	100.0
3	762	140	0	0	100.0
4	471	79	1	0	99.8
Mean/day	701.5	172.8	0.25	0.25	99.9

[a] Exposed to mosquitoes for 1 hr at 0900 and 1100 hr and 1300, 1500 and 1700 hr at 1 of 2 locations by 4 volunteers who wore a different treatment each day.

[b] Bite not confirmed.

tained 75% deet, more bites were recorded on the head when it was applied than with 3M or Biotek. This occurrence may have been due in part to more rapid absorption of the deet standard into facial skin because it was not in a controlled-release formulation.

Because it was difficult to conclude from most of the data which formulation provided the most satisfactory all-round protection, the comparisons of total bites on treated skin and location of bites (Table 2) were analyzed. This data appeared to show that the deet standard with up to 2 times the amount of deet far exceeded the candidate repellent formulations in total bites recorded, particularly on the head. However, there was wide variation in the results among test volunteers, and an ANOVA (0.05% level of confidence) of the data presented in Table 2 showed there was no significant difference between formulations or bite locations. Although the Biotek repellent had an apparent advantage, suggesting the additional 9% deet may have resulted in fewer bites than with the 3M product, it could not be justification for first choice when cosmetic acceptability was considered. The order of preference by 6 of 8 test volunteers was 3M, deet standard and Biotek. Furthermore, the 3M formulation contained only 47% the amount of deet in the standard, yet provided about the same all-round protection. It is likely that this reduction would coincidentally reduce complaints of irritation, oiliness and melting of plastics and paint finishes commonly associated with high concentrations of deet.

The large numbers of bites through the pants of the untreated uniforms each day probably can be attributed to tightness of the clothes over the skin when persons are sitting, squatting or bending and were sufficiently numerous to cause considerable discomfort even for the volunteers who were accustomed to intense mosquito attack. Rarely did bites occur when persons were moving because the pants were loose fitting, thus making it difficult for mosquitoes to penetrate and reach the skin beneath. Bites on shirts were on the upper back, shoulders and upper arms and occurred when volunteers were at rest as well as when moving. Volunteers were instructed to wear T-shirts during tests, but on one occasion a shirt was not worn and the number of bites through the untreated shirt increased on that individual. Thus, the likelihood of bites appears to be greater when an undershirt is not worn. Similarly, the uniform is double-layered on knees, elbows and seat, and bites were not noted in these areas even when clothing was tight. On the other hand, the permethrin-treated uniform provided nearly total protection and was unanimously preferred by the test participants.

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REFERENCES CITED

Anonymous. 1983. Standard method of field testing topical applications of compounds as repellents for medically important and pest arthropods (including insects, ticks, and mites). I. Mosquitoes. Am. Society for Testing and Materials, ASTM-Standard E939-83, pp. 1–5.

Breeden, G. C., C. E. Schreck and A. L. Sorensen. 1982. Permethrin as a clothing treatment for personal protection against chigger mites (Acarina: Trombiculidae). Am. J. Trop. Med. Hyg. 31:589–592.

Gupta, S. K., A. W. Sweeney, L. C. Rutledge, R. D. Cooper, S. P. Frances and D. R. Westrom. 1987. Effectiveness of controlled-release personal-use arthropod repellents and permethrin-impregnated clothing in the field. J. Am. Mosq. Control Assoc. 3:556–560.

Lillie, T. H., C. E. Schreck and A. J. Rahe. 1988. Effectiveness of personal protection against mosquitoes in Alaska. J. Med. Entomol. 25:475–478.

Schreck, C. E., D. G. Haile and D. L. Kline. 1984. The effectiveness of permethrin and deet, alone or in combination, for protection against *Aedes taeniorhynchus*. Am. J. Trop. Med. Hyg. 33:725–730.

Schreck, C. E., G. A. Mount and D. A. Carlson. 1982. Pressurized sprays of permethrin on clothing for personal protection against the lone star tick (Acari: Ixodidae). J. Econ. Entomol., 75:1059–1061.

Schreck, C. E., K. Posey and D. Smith. 1978. Durability of permethrin as a potential clothing treatment to protect against blood-feeding arthropods. J. Econ. Entomol. 71:397–400.

Schreck, C. E., E. L. Snoddy and G. A. Mount. 1980. Permethrin and repellents as clothing impregnants for protection from the lone star tick. J. Econ. Entomol. 73:436–439.

Sholdt, L. L., C. E. Schreck and A. Iqbal. 1988. Evaluation of a personal protection system against mosquitoes in Pakistan. J. Am. Mosq. Control Assoc. 4:233–236.