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FIELD BIOASSAYS OF PERMETHRIN-TREATED UNIFORMS AND A NEW EXTENDED DURATION REPELLENT AGAINST MOSQUITOES IN PAKISTAN[1]

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ABSTRACT. Field bioassays were conducted to evaluate the effectiveness of different treatment combinations of permethrin-impregnated clothing and a new extended duration repellent formulation of deet against natural populations of mosquitoes in Islamabad, Pakistan. Tests were initiated 2–2½ hours prior to sunset and volunteers who wore the topical repellent applied it 8 hours earlier. The most effective treatment was a combination of wearing both the permethrin-impregnated clothing and the repellent on exposed skin. This combination provided 100% protection from bites whereas volunteers wearing only treated clothing received 57% protection. Those wearing only repellent received 89% protection, but, under the biting pressure observed, this was not significantly different from the treated clothing-repellent combination.

INTRODUCTION

Protective measures for personal use against blood-feeding arthropods, including repellents and clothing impregnants, have a vital role in the prevention of vector-borne disease transmission, especially among military troops. The current military standard topical repellent, however, does not provide satisfactory protection against some important disease vectors, may lose effectiveness rapidly when exposed to rain, washing or perspiration, dissolves plastics and is often not cosmetically acceptable. The military all-purpose clothing impregnant, M-1960,[5] has poor user acceptance and is no longer manufactured. A new "systems" approach under development by the U.S. Department of Agriculture (USDA) and U.S. Department of Defense (DoD) includes permethrin-treated clothing and a new extended duration repellent formulation (EDRF) of deet (N,N-diethyl-3-methylbenzamide) for skin application.

Permethrin, a contact toxicant, is quick-acting, nearly odorless, non-staining and is resistant to degradation when exposed to heat, sunlight, wear, laundering, rinsings and immersion in water. Permethrin protects the wearer by preventing bites through the treated clothing and also reduces the biting population in the immediate area (Schreck et al. 1984). As a clothing impregnant, it has been found effective against a number of biting arthropods including mosquitoes, stable flies and tabanids (Schreck et al. 1978a, 1978b), ticks (Schreck et al. 1980, 1982a, 1982b, 1984), chiggers (Breeden et al. 1982) and black flies (Lindsay and McAndless 1978). Permethrin has been widely sold for tick protection for several years as "Permanone®[6]," a spray-on application for clothing. Likewise, a 1% permethrin cream rinse ("Nix") is marketed by Burroughs Wellcome Co. for the control of head lice (*Pediculus capitis*) in the U.S.A. The relative safety of permethrin as a clothing impregnant for human use was reviewed by Schreck et al. (1984). Earlier studies have shown that a combination of deet repellent and permethrin-treated clothing offers potentially more effective protection to military personnel from biting arthropods and vector-borne diseases than either method used alone (Schreck et al. 1984, Gupta et al. 1987). Although the military repellent formulation of 75% deet in ethanol

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[5] A mixture containing 30% of 2-butyl-2-ethyl-1,3 propanediol as a mosquito repellent, 30% of N-butylacetanilide as a tick and flea repellent, 30% of benzyl benzoate for protection against chigger and fleas and 10% of an emulsifier such as Triton X-100.

[6] Manufactured and registered by Fairfield American Corporation, New Jersey.

effectively repels a number of arthropod pests, it is quickly diluted with perspiration, rubbed off mechanically and rather rapidly absorbed into the skin. A formulation of deet applied to the user's skin which would extend the duration of protection to 12 hours or longer would be desirable because many species of arthropods are active after dark when troops are involved in nighttime operations or are sleeping and unable to protect themselves from bites. Candidate controlled-release formulations have been developed and field tested under contract with the U.S. Army, and one has been selected recently for final testing and adoption as the new DoD standard topical repellent. It contains 35% deet in a cream base of film-forming polymers.

The purpose of this study was to determine by field bioassays, the effectiveness of permethrin-treated clothing and the new deet EDRF against natural populations of mosquitoes in Pakistan.

MATERIALS AND METHODS

Field studies were conducted on the grounds of a government nursery located on the eastern edge of Islamabad and 2.5 km south of the Rawal Dam in Pakistan. Test sites were in heavy stands of deciduous trees adjacent to open fields of grass and near a government farm where truck crops and rice were grown.

Four treatment combinations were used: 1) wearing permethrin impregnated clothing only (TO); 2) wearing impregnated clothing supplemented with the EDRF on exposed skin (TR); 3) wearing untreated clothing but with the EDRF on exposed skin (OR) and 4) wearing untreated clothing without the EDRF (OO or check). Treatment combinations were replicated 8 times using 7 test personnel: Three of the volunteers wore each combination twice; combinations varied for the other volunteers because of limited availability of test personnel. Tests were performed concurrently, but in separate areas to avoid any influence on the results due to possible interaction caused by the knockdown effect of permethrin. Treatments were also rotated between areas on different nights in the test site.

Two sets of U.S. Army tropical weight Battle Dress Uniforms (BDU) of 100% cotton were issued to each volunteer. The BDUs are field uniforms which have been dyed and overprinted with a 4-color camouflage print pattern. One set (including cap) was impregnated at the rate of 0.125 mg permethrin active ingredient/sq cm of fabric six weeks prior to the study and in a manner described by Schreck et al. (1984). The second set was left untreated. During the tests, the BDUs were worn with the trousers bloused, coat sleeves rolled to above the elbow and the cap on.

Volunteers applied the repellent in the morning under supervision, and 8 hours prior to exposure in the field. Using the fingertips, EDRF was applied at the rate of 1 ml to each forearm (wrist to elbow), and to the head and neck, including face and ears to the hair line. Volunteers were all involved in laboratory and field work requiring extensive use of the hands and unavoidable washing, therefore, a small amount of the EDRF was applied to the hands only, one hour prior to the initiation of each day's test.

The permethrin-treated clothing was not washed during the study to determine the persistence of permethrin when worn under the climatic conditions that existed in the area. Between tests, the clothing was stored in plastic bags. At the end of each test day, all test subjects washed with soap and water to remove any repellent residues that remained on the skin.

All field exposures were made from 2–2½ hours before sunset to ½ hour after sunset when mosquito activity was greatest. Collections were terminated between 1830 and 1900 hr. Volunteers were positioned on folding chairs approximately 15 m apart with their backs to the prevailing wind. Each person carried 100 containerized collection vials (15 ml) and data sheets.

Mosquitoes were captured in individual vials while in the act of biting. Vials containing mosquitoes were then inverted and placed sequentially in their respective compartmented box and later matched with the indicated treatment and the time of the bite recorded by each individual. This procedure permitted us to determine the species, time of bite, total number of bites during the exposure period, the protection provided by the EDRF on skin and by permethrin in clothing when compared with the controls. Biting data on cloth and skin collected each night by the untreated check individual were used to calculate the percentage of protection each treatment provided. Volunteers made counts each hour of the number of mosquitoes biting a bare leg (knee to ankle) for 1 minute or more as determined by the density of biting mosquitoes. The data were then extrapolated into bites per hour as a standard unit of time. This provided information on numbers of mosquitoes biting untreated skin regardless of treatment during the time of testing.

The mean temperature and percent relative humidity during the study were 26.6°C (range 24.4–28.9) and 77.9% (range 56–91), respectively. An analysis of variance (ANOVA) was performed on the bite collection data for each volunteer. The data were summarized for analysis as total bites on skin, through the clothing and total for both.

RESULTS AND DISCUSSION

Table 1 gives the results of the field bioassays and demonstrates that all of the three treatments gave significantly greater protection from bites than did the checks (OO). The most effective treatment combination, permethrin-treated BDU and EDRF (TR) received no bites during the tests. It was followed by EDRF only (OR) with a mean of 3.9 bites/test and treated BDU only (TO) with a mean of 14.8 bites/test. The untreated check had a mean of 34.4 bites/test. Under the biting pressure observed, the difference in percent protection between the TR and OR combinations was not significant ($P > 0.05$).

There were no significant differences among the treatment combinations for the number of bites recorded through the BDU cloth only. Though the untreated fabric appeared to act as a mechanical barrier to biting mosquitoes, no bites occurred through the permethrin-treated fabric whereas 16 bites were recorded through the untreated fabric. Biting through untreated clothing may have species behavioral or density dependent restrictions.

The mean number of bites received on the skin of volunteers wearing the repellent with or without treated uniforms were not significantly different. However, both of these two treatments (TR, OR) provided significantly greater protection ($P < 0.05$) than treated uniforms without repellent (TO) or the checks (OO).

Volunteers wearing the treated BDU without the repellent had significantly fewer bites ($P < 0.05$) than the untreated checks. The treated BDU apparently reduced the number of bites through its toxic action. However, without the repellent, significantly more bites occurred on the skin than on the TR and OR treatments. Bites recorded by those wearing the TO treatment occurred on the skin only. This agrees with earlier observations by Schreck et al. (1984) who found that 99% of the bites on volunteers wearing permethrin-treated clothing were on exposed skin. Volunteers wearing the repellent with the untreated BDU had a greater number of total bites on the exposed skin (77%), than through the clothing. This is the reverse of that found by Schreck et al. (1984) who reported that subjects protected with deet only, experienced bites from *Aedes taeniorhynchus* (Wied.) primarily through untreated clothing (88%). Although those treated with the repellent alone received nearly 90% protection, this was somewhat less than our anticipated protection of 95% or more.

Table 1. Relative effectiveness of protective treatments against natural populations of mosquitoes in Pakistan (mean number of mosquitoes biting during 8 tests).

Treatment*	Mean number of bites**			Percent protection
	Skin	Clothing	Both	
TR	0.0a	0.0a	0.0a	100
OR	3.0a	0.9a	3.9a	89
TO	14.8b	0.0a	14.8b	57
OO	33.3c	1.1a	34.4c	0.0

* T = permethrin-treated military clothing, R = repellent, O = no treatment.

** Column means with the same letters are not significantly different (P 0.05 level of confidence; ANOVA with a Waller-Duncan Multiple Range Test).

Reasonable care was taken to prevent accidental removal of the repellents after application except for the hands which, as explained earlier, were treated shortly before each test. Because of previous reports (Letterman Army Institute of Research and C. E. Schreck, unpublished data) on the effectiveness of the repellent, bites to the hands within 2–3 hours after reapplication and to other exposed skin areas at 6–8 hours after postapplication were unexpected. Although only 6% of the bites recorded (424) were on skin treated with repellent, further evaluations, especially with higher densities of mosquitoes, may be justified to determine if the repellent will provide satisfactory protection. The EDRF used in this study may be more effective against some mosquito species and on certain individuals than on others, depending on mosquito density, geographical location, climate and other factors.

Seven mosquito species were identified from the collections. The most common were *Aedes albopictus* (Skuse) (84%), *Ae. culicinus* Edwards (6%) and *Ae. w-albus* (Theobald) (6%). Those taken in small numbers included *Ae. vittatus* (Bigot), *Ae. thomsoni* (Theobald), *Armigeres subalbatus* (Coquillett) and *Culex bitaeniorhynchus* Giles (4%). Most are tree hole breeders which readily attack man during the daytime. Of public health interest is *Ae. albopictus*, a known vector of dengue and possibly several other viruses.

The overall biting rate was 47.2 bites/hour (range 24–85) based on periodic bite counts on the untreated skin of the leg made by all volunteers during each test day. Population pressure, while adequate to show significant differences between treatments, was unexpectedly low because of an exceptionally dry monsoon covering a significant land area of Pakistan and India.

The volunteers did not complain of skin irritation, odor or plasticizing from wearing the permethrin-treated clothing; however, a few commented that the new EDRF felt "sticky" and somewhat "greasy" upon initial application.

We believe that this new EDRF promises to

be a significant improvement over the current standard military repellent. Obtaining compliance for adequate repellent usage, however, will continue to be difficult especially during emergency situations including combat. The passive protection provided by the permethrin-impregnated uniform itself is, therefore, very important. The 57% protection from bites on TO volunteers recorded during this study might be adequate to significantly reduce transmission of vector-borne diseases like malaria, dengue or filiariasis. Furthermore, the toxicity of permethrin is sufficient to knock-down or interrupt the biting behavior of mosquitoes in the immediate area of the human host. Thus, a reduction in vector potential brought about by a reduction in population and biting rate may also result in a significant decrease in disease risk.

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