CONTROLLED RELEASE REPELLENT FORMULATIONS ON HUMAN VOLUNTEERS UNDER THREE CLIMATIC REGIMENS[1,2]

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ABSTRACT. Two controlled-release repellent formulations containing 33% (3M) and 42% (Biotek) deet and an Army repellent containing 75% deet were evaluated in 3 different climatic regimens (tropical forested, tropical open and basic hot environments). The 3 repellents provided similar protection for different time periods after application under all 3 climates against *Aedes aegypti*, *Ae. taeniorhynchus* and *Anopheles stephensi* whereas there was no difference in protection period against *An. albimanus*.

A long-lasting repellent formulation along with a clothing impregnant, permethrin (0.125 mg/cm^2), are the 2 parts of a protection system currently being developed to protect soldiers from blood-sucking arthropods around the world (Gupta et al. 1990). This protection system provides greater protection (Gupta et al. 1987) than either the topical repellent or permethrin-treated clothing separately. Lillie et al. (1988) and Sholdt et al. (1988) also observed a similar trend in protection from mosquito bites when they evaluated the protection system in field studies.

Two experimental Extended Duration Topical Insect/Arthropod Repellent (EDTIAR) formulations of diethylmethylbenzamide (deet) were selected for further development and testing (Gupta and Rutledge 1989). During the selection process, the 3M repellent formulation was selected for further development and testing based on the results of field and laboratory efficacy testing, toxicological evaluation, cosmetic acceptability, material compatibility and infrared signature of the repellent formulation. The selected repellent formulation was modified to improve its shelf life and ease of application. The modified repellent formulation after subsequent laboratory testing was standardized within the Department of Defense.

This paper reports on laboratory studies conducted in an environmental chamber for effectiveness of EDTIAR formulations against laboratory-reared mosquitoes under 3 climatic regimens: tropical open, tropical forested and basic hot environments.

The mosquito species tested were: *Aedes aegypti* (Linn.), obtained from the University of California at San Francisco, CA; *Anopheles stephensi* Liston, from the Walter Reed Army Institute of Research, Washington DC; and *An. albimanus* Wiedemann and *Ae. taeniorhynchus* (Wiedemann) from the Insects Affecting Man and Animals Research Laboratory, U.S. Department of Agriculture, Gainesville, FL. Mosquitoes were reared and maintained as described by Gupta and Rutledge (1989). The mosquitoes used were nulliparous females between 5 and 15 days old.

The active ingredient in all EDTIAR formulations was diethylmethylbenzamide. All other components of the formulations, including excipients, and additives were inert ingredients. The repellents were: a polymer cream formulation "Controlled-Release Personal Use Arthropod Repellent Formulation" (33% deet) (3M) and a subsequent modification with improved shelf life (33% deet) (3M plus), both produced by Personal Care Products, 3M Company, St. Paul, MN; and a microparticulate formulation "Sustained Action Arthropod Repellent" (41.8% deet) (Biotek) produced by Biotek Corp., Woburn, MA. These EDTIAR formulations were tested along with the U.S. military repellent containing 75% deet in ethanol (Army). The composition of each experimental EDTIAR formulation is proprietary.

The test method used was similar to those of Gupta and Rutledge (1989). A short summary of this test method is as follows. The 3 repellent formulations, 3M, Biotek and Army, were applied at random to the flexor region of the forearms of 3 volunteers according to the label instructions. A fourth volunteer served as the control. The weight of repellent applied by each volunteer was obtained with a platform balance. Control subjects were rotated daily in random order. A test of each species of mosquito was conducted on each repellent formulation at 0, 2,

[1] Opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the U.S. Department of the Army. Use of trade names does not constitute an official endorsement or approval of the products mentioned.

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4, 6, 8, 10 and 12 h after application on the skin. At the start of the test, a 4 × 5 × 18 cm plastic cage containing 15 mosquitoes was bound to the forearm with Velcro® tape, and a slide was withdrawn to expose the repellent-treated skin. The number of mosquitoes biting was recorded at the end of 90 sec after which the cages were removed. The test cages with mosquitoes were brought into the environmental chamber an hour before the test for acclimation. This procedure was repeated 6 times on 6 successive days, 2 days in each climatic regimen. This provided 4 replications of each repellent formulation (2 forearms × 2 days) in each climatic regimen against each species of mosquito.

The 3M and 3M plus (with improved shelf life) were compared for their efficacy against mosquitoes. In this case the 2 formulations were applied at random to one of the forearms of 4 volunteers. Each volunteer's untreated arm served as a control.

The test volunteers stayed in the environmental chamber for the entire 12 hours. Each formulation was tested under the 3 regimens using average values of the 24-h test cycles (U.S. Department of the Army 1979). The average temperature and relative humidity (RH) recorded for each regimen was: 1) basic constant high humidity (tropical forested), 24°C with 98% RH; 2) basic variable high humidity (tropical open), 30°C for entire 12 h with 78% RH for first 6 h and 98% RH for the last 6 h; and 3) basic hot, 37°C with 31% RH for the entire 12 hours.

A three-way analysis of variance was done on the number of bites recorded using the Biomedical Data Program (BMDP2V) statistical package (Dixon et al. 1983) to check for significant differences between the repellent formulations (Biotek, 3M and Army), climatic regimens (tropical open, tropical forested and basic hot) and time (hours after application of repellent). Differences were considered to be significant at $P \leq 0.05$. Also, the percentage repellency was determined from the total number of bites on control and repellent treated test volunteers by Abbott's formula (Abbott 1925).

The average weight ± SE of repellent applied by the volunteers was 0.9 ± 0.1 mg/cm^2 for the Biotek, 1.1 ± 0.2 mg/cm^2 for the 3M and 0.6 ± 0.2 mg/cm^2 for the Army formulation, respectively. Analysis of variance indicated that the amount of Biotek and 3M repellent used was significantly greater than the amount of Army repellent used. When 3M and 3M plus were compared, volunteers used 1.7 ± 0.4 mg/cm^2 of 3M and 1.6 ± 0.3 mg/cm^2 of 3M plus repellent formulation, respectively. The difference between the amount of 3M and 3M plus repellent used was not statistically significant.

The differences in amount of repellent formulation used by volunteers may be due to differences in individual skin surface areas and/or individual preferences. The tested formulations contained different amounts of active ingredient, employed unique formulation techniques and were of different consistency. In addition, a repellent with a thin (lotion) consistency will cover more area than the same amount of another with a thick (cream) consistency, and a person will therefore use a greater amount of a repellent with a thick or cream-like consistency. In this study, if each volunteer had used an equal amount of formulation, they would have applied more of the Army repellent (75% deet) and less of the others (35 and 42% deet).

All repellent formulations provided extended protection from mosquito bites. The duration of protection against mosquitoes with the Biotek, 3M and Army formulation is summarized in Table 1. Under tropical forested climatic regimen all formulations provided 100% protection against all species immediately after application except for the Army formulation (95% with *An. albimanus*). Against *Ae. aegypti*, 3M provided 95% or better protection for the entire duration of the test, Biotek for 10 h and Army for 8 hours. The 3M and Biotek repelled 100% *Ae. taeniorhynchus* for 12 h except at 8 h (87.5%) and Army repellent prevented bites for 6 h before losing its effectiveness. In the case of *An. stephensi*, 3M and Biotek provided 100% protection for 12 h except for Biotek at 10 h (87.5%) whereas Army repellent lasted for 6 hours. Biotek and 3M exhibited 100% protection against *An. albimanus* for 8 and 6 h whereas Army repellent was effective for 4 h except 90% at 2 h. The 3M formulation provided the best protection under this climatic regimen.

Under tropical open environmental conditions (Table 1), 3M provided 95% or better protection for 10 h against *Ae. aegypti*, Biotek was 100% effective for 8 h, and the Army repellent lasted for only 4 hours. Biotek provided 100% protection from *Ae. taeniorhynchus* bites for the entire duration of the test, whereas 3M and Army provided 100% protection for 10 h and 4 h, respectively. Both Biotek and 3M provided 95% or better protection from *An. stephensi* bites for 10 h as compared with only 4 h for the Army repellent. All repellent formulations were 100% effective for 4 h against *An. albimanus* bites. Biotek formulation provided the best overall protection from mosquito bites in this climatic regimen.

In the basic hot environment (Table 1), Biotek, 3M and Army repellent were 95% or more effective against *Ae. aegypti* bites for 10, 6 and 4 h, respectively. In the case of *Ae. taeniorhyn-*

Table 1. Percent protection from bites on volunteers using controlled-release repellent formulations against 4 mosquito species under 3 climatic conditions ($n = 4$).

	Percent protection											
	Ae. aegypti			*Ae. taeniorhynchus*			*An. stephensi*			*An. albimanus*		
Hour	Biotek	3M	Army	Biotek	3M	Army	Biotek	3M	Army	Biotek	3M	Army
						Tropical forested[1]						
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	95.0
2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	90.0
4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
6	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	87.5
8	100.0	100.0	100.0	87.5	87.5	87.5	100.0	100.0	87.5	100.0	75.0	−75.0
10	96.9	96.9	71.9	100.0	100.0	0.0	87.5	100.0	70.8	71.4	100.0	28.6
12	84.1	97.7	63.6	100.0	100.0	62.5	100.0	100.0	57.9	81.8	63.6	95.5
						Tropical open[2]						
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
4	100.0	100.0	97.1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
6	100.0	100.0	76.7	100.0	100.0	90.0	100.0	100.0	92.9	85.0	90.0	50.0
8	100.0	96.4	71.4	100.0	100.0	70.0	100.0	97.1	94.1	100.0	91.7	75.0
10	72.7	97.7	45.5	100.0	100.0	50.0	96.2	96.2	65.4	100.0	83.3	50.0
12	50.0	68.2	−13.6	100.0	50.0	50.0	93.8	87.5	77.1	75.0	37.5	87.5
						Basic hot[3]						
0	100.0	100.0	100.0	100.0	100.0	100.0	97.5	100.0	100.0	100.0	100.0	100.0
2	100.0	100.0	100.0	100.0	100.0	100.0	96.7	100.0	100.0	100.0	100.0	100.0
4	100.0	100.0	95.7	100.0	100.0	85.7	100.0	100.0	100.0	100.0	100.0	100.0
6	100.0	95.8	91.7	100.0	100.0	91.7	100.0	80.0	100.0	100.0	100.0	100.0
8	95.8	83.3	62.5	100.0	83.3	50.0	95.0	97.5	92.5	87.5	87.5	100.0
10	100.0	94.4	55.6	90.0	90.0	70.0	100.0	100.0	83.3	50.0	100.0	100.0
12	−5.0	60.0	15.0	100.0	50.0	50.0	75.0	100.0	75.0	100.0	100.0	100.0

[1] Average temperature = 24°C, average RH = 98%.
[2] Average temperature = 30°C, average RH = 78% for the first 6 h and 98% for the last 6 hours.
[3] Average temperature = 37°C, average RH = 31%.

chus, Biotek provided 100% protection for 12 h except 90% at 10 h; 3M was 100% effective for 6 h and Army repellent was 100% effective for 2 hours. The Biotek repellent provided 95% or better protection from *An. stephensi* bites for 10 h, 3M for 12 h except at 6 h (80%) and Army repellent for 6 hours. Surprisingly, Army repellent was 100% effective for 12 h against *An. albimanus* as compared with 6 h for Biotek and 3M. Overall, 3M formulation provided the best protection in this environment.

Against *Ae. aegypti*, *Ae. taeniorhynchus* and *An. stephensi*, the analysis of variance indicated significant differences in time of protection from bites provided by the 3 repellent formulations but no differences in protection under the 3 climatic regimens, implying that each formulation provided similar protection under all 3 climatic regimens. In the case of *An. albimanus*, there were no significant differences, indicating all repellent formulations provided similar protection under all 3 climatic regimens. The Biotek and 3M provided similar overall protection (94.9 and 94.8%) from bites of all mosquito species under all climatic regimens as compared with the Army repellent which provided only 81.8% protection.

The analysis of variance of the test data for various species indicated that there was no difference in protection provided by the 3M and 3M plus repellent formulations. The results obtained for the 2 formulations were so similar that statistical analysis was not required. However, the 3M plus formulation felt smoother and was applied more easily than the 3M formulation.

Even though the Biotek formulation and Army repellent had more deet as compared with 3M, the overall protection against various mosquitoes was at best similar or less than that provided by the 3M formulation. In addition, demonstrable improvement over the Army repellent with regard to evaporation rate, 24-h skin penetration, resistance to washing and material compatibility were achieved (W. G. Reifenrath and G. S. Hawkins, unpublished data) with the controlled-release formulations. Because of its longer protection time, 55% less

active ingredient, improved user comfort, reduced odor and plasticizer effects as compared with the current U.S. military repellent, the new 3M plus controlled-release repellent formulation can be expected to reduce common complaints about insect repellents and increase repellent acceptance and usage by the soldiers in the field. Furthermore, smaller amounts of deet used in controlled-release formulations may reduce allergic and toxic effects that may be associated with repeated applications of high concentrations of deet (MMWR 1989).

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