EFFECTIVENESS OF CONTROLLED-RELEASE PERSONAL-USE ARTHROPOD REPELLENTS AND PERMETHRIN-IMPREGNATED CLOTHING IN THE FIELD[1,2]

RAJ K. GUPTA,[3] A. W. SWEENEY,[4] L. C. RUTLEDGE,[3] R. D. COOPER,[4] S. P. FRANCES[4] AND D. R. WESTROM[3]

ABSTRACT. Two topical controlled-release personal-use arthropod repellent formulations of diethyl methylbenzamide (deet) and permethrin-impregnated clothing were tested on human volunteers in a tropical rain forest near Innisfail, Queensland, Australia. The field trials were arranged in a four-way factorial design which compared fabric types, permethrin treatment and repellent treatments over a 14-hour test period. An analysis of variance with each factor treated as a fixed effect indicated that treatment of the clothing with permethrin and use of topical repellents were effective in preventing bites. The controlled release formulations were not significantly better than the current U.S. Army formulation of deet under field conditions. The repellent formulations and the permethrin-treated clothing used as one system provided better protection than the repellent formulations or permethrin-treated clothing used separately.

INTRODUCTION

Personal protection is an inexpensive and practical means of reducing the biting activity of blood-sucking arthropods and for the prevention of arthropod-borne disease transmission. Previous work has concentrated mainly on simple solutions of topical repellents and the chemical treatment of clothing to prevent the bites of blood-sucking arthropods (Rutledge et al. 1978). Current studies of topical controlled-release personal-use arthropod repellent formulations (Mehr et al. 1985) and the impregnation of fabrics with permethrin (Schreck et al. 1977, 1978) are an attempt to exploit modern materials and technology to reduce or eliminate annoyance and disease due to arthropods.

This study was designed to evaluate three topical formulations of diethyl-3-methylbenzamide (deet) and two types of permethrin-impregnated military field uniforms for protection against mosquitoes in tropical areas. The results obtained will be used in conjunction with other data on the same materials in the direction and management of current U.S. Army programs for development of new and improved arthropod repellents.

The study was conducted in a tropical rain forest at the Joint Tropical Trials Research Establishment of the Australian Department of Defense near Innisfail, Queensland, Australia. The climate in the test area is one of high humidity. The mean daily relative humidity is 80% and the relative humidity exceeds 70% during 80% of the year. The average yearly rainfall is 2.9 m, and it occurs predominantly from December to March. There are an average of 186 rain days in the year.

MATERIALS AND METHODS

Three topical repellent formulations were tested: (1) 3M Insect Repellent Lotion, a controlled-release formulation provided by Personal Care Products Department, 3M Center, St. Paul, MN 55144, (2) Biotek Long-Acting Insect Repellent, a controlled-release formulation prepared by Biotek Inc. Woburn, MA 01801, and (3) U.S. Army Insect Repellent, Type IIA, a simple solution (FSN 6840-00-753-4963). The active ingredient in the topical repellent formulations was N,N-diethyl-3-methylbenzamide (deet). All other components of the topical formulations, including excipients, additives, and structural elements, were inert ingredients. The composition of the Biotek (42% deet) and 3M (33% deet) repellent formulations is confidential. The U.S. Army insect repellent formulation consists of 75% deet (71.3% N,N-Diethyl-3-methylbenzamide and 3.7% other N,N-Diethyl-3-methylbenzamides) in ethanol. Permethrin was tested at 0.125 mg/cm^2 in cotton and nylon/cotton U.S. Army battle dress uniforms (BDU). The BDU's are field uniforms made of 100% cotton fabric and blend of 50% cotton and 50% nylon fabric which is dyed and overprinted with a 4-color camouflage print. Both treated and untreated uniforms were tested.

At the beginning of each test day, the test participants put on their assigned uniforms and rolled the sleeves above the elbows. The partic-

[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. or Australian Departments of Defense. Use of trade names does not constitute an official endorsement or approval of the use of the products mentioned.

[2] Human subjects participating in this study gave free and informed voluntary consent.

[3] Letterman Army Institute of Research, Presidio of San Francisco, CA 94129.

[4] Army Malaria Research Unit, MILPO, Ingleburn, New South Wales 2174, Australia.

ipants then formed into pairs; each pair remained together throughout the test day to assist one another in the test procedures. Test repellents were then applied according to the directions and precautions printed on their respective labels to the face, neck, forearms, and hands. The weight of repellent applied by each participant was obtained with a platform balance. Participants were instructed not to rub, scratch or wash the treated areas for the duration of the test period. Thirty volunteers participated in the tests.

This field study was arranged in a four-way factorial design (2 fabric types × 2 permethrin treatments × 4 repellent treatments × 8 time intervals over the 14 hour test period). Each volunteer was assigned one of the following treatment combinations at the beginning of each test day:

(1) Untreated cotton uniform and no repellent
(2) Untreated cotton/nylon uniform and no repellent
(3) Permethrin-treated cotton uniform and no repellent
(4) Permethrin-treated cotton/nylon uniform and no repellent
(5) Untreated cotton uniform and 3M, Biotek, or U.S. Army repellent on exposed skin
(6) Untreated cotton/nylon uniform and 3M, Biotek, or U.S. Army repellent on exposed skin
(7) Permethrin-treated cotton uniform and 3M, Biotek, or U.S. Army repellent on exposed skin
(8) Permethrin-treated cotton/nylon uniform and 3M, Biotek, or U.S. Army repellent on exposed skin.

The treatments were assigned to test volunteers at random by a computer-generated list and were replicated five times on five different days; no volunteer was assigned the same treatment more than once.

The repellent formulations were applied 30 minutes before the start of the test. The test participants then entered the test area and collected all mosquitoes biting (either directly on the skin or through the uniforms) in the next 20 minutes using individual pre-labeled capture vials. This procedure was repeated every two hours for 14 hours. Thus eight biting collections were made by each participant at 0, 2, 4, 6, 8, 10, 12 and 14 hours after the start of the test. The test participants moved as a group between the base camp and the test area (1 km) by vehicle. The starting times on the five test days were staggered over the 24 hours of the day to incorporate all peaks of biting activity of the mosquito species present in the test area.

The mean temperature and percent humidity recorded during the study were 25.1 ± 0.7°C SE (range 18.5–33.5°C) and 83.4 ± 2.4% SE (range 60–100%), respectively.

The collection data were analyzed as: (1) The four-way analysis of variance with each factor treated as a fixed effect and the times of the collections (0200, 0400, 0600 ..... 2400 hours) as covariables to adjust the data for the diel cycle of biting activity. The analyses were performed on a Data General MV/8000 computer using the BMDP (Biomedical Programs) computer software package (Dixon et al. 1983). (2) Percent repellency of the different treatments against all species of mosquitoes and against *Anopheles farauti* Laveran, *Aedes kochi* (Doenitz), *Aedes carmenti* Edwards and *Culex annulirostris* Skuse separately was determined from the adjusted cell means of the analysis of variance. Percent repellency was calculated from the adjusted total number of bites on the control and repellent-treated test participants by Abbott's formula (Abbott 1925). For purposes of this analysis the control was considered to be the individual wearing the untreated BDU with no topical repellent. Since calculations were based on adjusted cell means from analysis of variance, values of more than 100% repellency were sometimes obtained. These are reported in the tables as 100% repellency.

RESULTS AND DISCUSSION

Fourteen species were collected during the field trials (Table 1). *Anopheles farauti, Aedes kochi, Aedes carmenti* and *Culex annulirostris* were the predominant species collected; the most abundant species were *Anopheles farauti* (the major malaria vector in the southwest Pa-

Table 1. Mosquito species collected during the field study.

Species	Number[1] collected	Percent of total
Culex annulirostris	174	25.7
Anopheles farauti	155	22.9
Aedes carmenti	132	19.5
Aedes kochi	97	14.3
Aedes vigilax	41	6.1
Mansonia septempunctata	27	3.9
Coquillettidia xanthogaster	17	2.5
Aedes funereus	11	1.6
Hodgesia quasisanguinea	6	0.9
Tripteroides sp.	6	0.9
Aedes notoscriptus	5	0.7
Aedes lineatus	4	0.6
Tripteroides magnesiana	2	0.3
Aedes tremulus	1	0.2
	678	100.0

[1] Three mosquitoes were damaged on collection and could not be identified.

Table 2. Average amount of topical repellent used for one application to cover exposed skin (face, neck, forearms and hands).

Repellent formulation	Amount applied (g)[1]	Average number of applications per container[1]
	Product	
U.S. Army deet formulation	1.84 ± 0.24	31.57 ± 0.58
Biotek formulation	2.44 ± 0.29	21.79 ± 0.10
3M formulation	3.39 ± 0.31	12.69 ± 0.14
	Active ingredient	
U.S. Army deet formulation	1.38 ± 0.18	—
Biotek formulation	1.03 ± 0.12	—
3M formulation	1.12 ± 0.10	—

[1] Mean ± SE.

cific region) and *Culex annulirostris* (an important arbovirus vector).

The mean weight of repellent applied by the participants to the face, neck, forearms, and hands is shown in Table 2. Analysis of variance indicated that the differences in amount of the three repellents used were highly significant. The Tukey Studentized range test indicated that the amount of 3M formulation used was significantly greater than that of either the Biotek or the U.S. Army formulation at the 1% level of significance. However, there was no significant difference in the amount of active ingredient used by the participants. The U.S. Army formulation provided 2.5 times more applications per 2-fluid-ounce container than the 3M formulation, and 1.7 times more applications per 2-fluid-ounce container than the Biotek formulation. (Not all of the contents can be removed from the containers.)

The percent repellency against all species of mosquitoes is shown in Tables 3 and 4. In some instances the observed percent repellency did not decline evenly with time (i.e. from 0 through 14 hours), but in most cases a decreasing trend was evident. The time factor was statistically significant in the analyses of variance for all species (Table 5) and for most of the individual species.

The results recorded in Tables 3 and 4 reflect in part the variability in environmental, biotic and human factors that is present in all field studies and in part the relatively low number of mosquitoes present in the area at the time of the study. The mean mosquito-biting rate for persons using no repellent and wearing untreated clothing was 8.8 mosquito bites/man/hour with a standard deviation of 4.5. While this rate is lower than those of most other repellent studies, it is more representative of the actual epidemiological situation in many areas of the world than a higher rate would be.

In some instances the repellent treatment was apparently attractant to mosquitoes. This effect occurred most often in the late hours of testing when the repellent residues were presumably low. It was observed only in the analyses of variance for the separate species and is not reflected in Tables 3 and 4. The attractancy of such residues has also been observed in other studies (Dubitskii 1966, Kost et al. 1971, Potapov et al. 1977, Mehr and Rutledge 1985).

The analysis of variance of the data for all species showed that three of the main effects (permethrin treatment, repellent treatment and time intervals after application of repellent) and two interactions (between the permethrin treatment and fabric type and between permethrin treatment and repellent treatments) were significant at the 5% level (Table 5). The significant interactions imply that the effectiveness of the permethrin treatment varies with the fabric type and the repellent formulation used. Permethrin-treated cotton/nylon fabric provided

Table 3. Effectiveness (percent repellency) of repellent formulations and permethrin treatment of cotton uniforms against all species of mosquitoes.

Hours after application	100% cotton uniform					
	Untreated			Permethrin treated		
	U.S.	Biotek	3M	U.S.	Biotek	3M
0	67.6	40.5	13.5	81.1	95.3	81.1
2	92.3	76.9	61.5	100.0	100.0	100.0
4	46.5	27.9	46.5	55.8	83.7	74.4
6	69.4	62.5	62.5	90.3	83.3	76.4
8	82.0	65.6	16.4	100.0	100.0	49.2
10	62.3	51.0	68.0	73.7	62.3	79.3
12	6.8	40.8	68.0	54.4	40.8	47.6
14	36.4	72.7	27.3	18.2	27.3	0.0

Table 4. Effectiveness (percent repellency) of repellent formulations and permethrin treatment of nylon/cotton uniforms against all species of mosquitoes.

Hours after application	50% cotton-50% nylon uniform					
	Untreated			Permethrin treated		
	U.S.	Biotek	3M	U.S.	Biotek	3M
0	40.3	80.7	64.7	96.8	97.2	96.8
2	54.6	72.7	63.6	90.9	91.0	100.0
4	41.8	83.6	83.6	89.6	89.6	77.6
6	87.7	43.9	61.4	96.5	87.7	70.2
8	69.2	100.0	69.2	100.0	100.0	100.0
10	41.5	31.1	51.8	72.5	62.2	41.5
12	19.3	72.5	77.3	82.1	91.8	96.6
14	42.1	57.9	68.4	79.0	94.7	100.0

Table 5. Four-way analysis of variance for all mosquito species.

Source	Degrees of freedom	Mean square	F
Fabric type (G)	1	8.10	3.59
Permethrin treatment (H)	1	102.40	45.37[2]
Repellent formulations (I)[1]	3	44.64	19.78[2]
Test period (time interval after repellent application (J)	7	10.98	4.86[2]
GH interaction[3]	1	15.01	6.65[2]
GI interaction	3	1.58	0.70
HI interaction	3	8.94	3.96[2]
GJ interaction	7	0.55	0.25
HJ interaction	7	1.04	0.46
IJ interaction	21	1.79	0.79
All covariates[4]	11	13.05	5.78[2]
Error	501	2.26	

[1] Includes the "no repellent" treatment.
[2] Significant at 5% level.
[3] The three factor and four factor interactions (not shown) were not statistically significant.
[4] Main effect and interaction sums of squares were adjusted by including the times of collection (0200, 0400....2400 hr) as covariables in the analysis of variance.

greater protection than permethrin-treated cotton fabric. The average protection from biting mosquitoes over the 14-hour test period was 80% for the U.S. Army formulation, 82% for the Biotek formulation and 74% for the 3M formulation when the formulations were used in conjunction with permethrin-treated uniforms, but it was only 54% for the U.S. Army formulation, 61% for the Biotek formulation and 56% for the 3M formulation when the formulations were used with untreated uniforms. There were no significant differences in the protection provided by the U.S. Army, Biotek, or 3M formulations.

In separate analysis of variance for *Anopheles farauti* (not shown), the effects of permethrin treatment, repellent treatment, and time interval after application were significant at the 5% level of significance. The interaction between the fabric type and permethrin treatment was also significant. In this case, however, the permethrin treatment provided significantly greater protection when used with the 100% cotton fabric.

In the case of *Aedes kochi*, the effects of permethrin treatment and repellent treatment were significant at the 5% level of significance, but there were no significant interactions. The analysis for *Aedes carmenti* indicated a significant effect with respect to the permethrin treatment only. In the case of *Culex annulirostris*, the effects of permethrin treatment, repellent treatment and time interval after application were all significant.

It has been shown that permethrin in clothing is resistant to removal by both wearing and washing (Schreck et al. 1982). Since permethrin does not act in the vapor state, mosquitoes can land and remain on the permethrin-treated clothing long enough to acquire a toxic dose. The study of Schreck et al. (1978) indicated that topical repellents drive the mosquitoes to the treated clothing, thereby exposing them to the toxic effects of permethrin. This is confirmed by our finding (unpublished data, Letterman Army Institute of Research) that mosquitoes are knocked down within 15 minutes after exposure to permethrin-treated clothing. The consequent reduction of the attacking mosquito population could be particularly important in situations where the attacking mosquitoes are vectors of diseases. However, this potential for reducing the mosquito population needs to be evaluated in the field environment with a substantially large number of human subjects.

In conclusion, the repellent formulations and the permethrin-treated clothing provided greater protection against mosquitoes when used together as compared to when either was used separately. However, fabric composition (cotton or nylon/cotton) and repellent formulation (3M, Biotek or U.S. Army) can significantly affect the overall efficiency of the permethrin treatment. There were no significant differences in the effects of the three topical repellent formulations tested.

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