FIELD TESTING OF REPELLENTS AGAINST ANOPHELINE MOSQUITOES[1]

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ABSTRACT. The duration of repellency of 4 different mosquito repellents was determined against heavy field populations of *Anopheles freeborni* Aitken (88 bites per hr). Four volunteers participated in the study. A dose of 0.4 mg/cm^2 of each repellent was applied to 1 of 4 sites on the forearms. The criterion for failure of a repellent was 5 bites per site. Ranges of protection time obtained during the 4 day study were as follow: Cyclohexamethylene carbamide (carbamide) 10 to 16+ hr, triethylene glycol monohexyl ether (SRI-6) 4–12 hr, 1-(butylsulfonyl)hexahydro-1*H*-azepine (sulfonamide) 4–10 hr, and N,N-diethyl-m-toluamide (m-deet) less than 4 to 10 hr. The 3 experimental repellents provided equal or greater protection than m-deet for each volunteer.

INTRODUCTION

As part of a Letterman Army Institute of Research (LAIR) program to find a mosquito repellent superior to N,N-diethyl-m-toluamide (m-deet), the most widely used insect repellent, field tests of candidate repellents have been conducted. In this paper we report the efficacy of several experimental mosquito repellents, relative to m-deet, against natural populations of anopheline mosquitoes. This field test was staged in an area bordering rice fields near Colusa, California during a 4-day period. In addition to m-deet, carbamide (cyclohexamethylene-carbamide), sulfonamide (1-(butylsulfonyl)-hexahydro-1*H*-azepine), and SRI-6 (triethylene glycol monohexyl ether) were tested. We are unaware of any previous studies where these experimental compounds were tested in the field against anopheline mosquitoes.

MATERIALS AND METHODS

Repellents used were N,N-diethyl-m-toluamide, Eastman Chemical Co.; triethylene glycol monohexyl ether, SRI International (Johnson et al. 1975), 1-(butylsulfonyl) hexahydro-1*H*-azepine, (Pervomaiskii et al. 1967), and cyclohexamethylene-carbamide (Stepanov et al. 1969).

Repellents were applied using the LAIR four-site field technique (Shimmin et al. 1974), which involves the skin surface area from the wrist to the elbow of each forearm divided into 2 equal sections separated by an adhesive-back foam strip to prevent mixing of repellents via diffusion across the skin. Four different repellents, including a standard repellent (m-deet) were applied in doses of 0.4 mg/cm^2 to the 4 sites on each of 4 individuals at time intervals ranging from 4 to 16 hours before the evening test period. The time intervals were sought to detect the threshold at which m-deet would protect each volunteer. Tests were conducted over a 4-day period. The test subjects took 4 mile hikes each afternoon after applying the repellents earlier. Test subjects were then exposed to a natural population of anopheline mosquitoes during the evening hours (1915–2130).

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[1] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Department of the Army or the Department of Defense.

Human subjects participated in these studies after giving their free and informed consent. Investigators adhered to (Army Regulation) 70–25 and (U.S. Army Medical Research and Development Command Regulations) 70–25 on use of volunteers in research.

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Sunset occurred at 1930–1940 during those days. The time was recorded when each individual received a bite. When 5 bites were received on a particular site, the repellent on that site was defined as "failed" and the site was covered with a heavy dose of m-deet (approximately 1 mg/cm²) to prevent further bites. This dose of m-deet should not affect the adjacent test area, since laboratory tests on man have shown that the duration of protection of m-deet at a dose of 0.16 mg/cm² against *Aedes aegypti* was not affected by an adjacent m-deet dose of 2.3 mg/cm² (unpublished results, T.S. Spencer).

Additionally, in vitro repellency tests of m-deet against *Anopheles stephensi* showed that feeding counts obtained on untreated areas adjacent to a treated one (0.16 mg/cm²) did not differ significantly from those obtained at any of the untreated areas more distant (Rutledge et al. 1976). The biting rate during each day's test period for *Anopheles freeborni* (between 1915–2045 for each test day) was determined from the untreated arm of a control subject. Thirteen 5-min biting collections were made during the 4 day test period. Biting rate per hour was calculated by extrapolation from the 5 min rate. Headnets, fatigue uniforms, and gloves were worn by the volunteers to protect other areas of the body from mosquito bites.

RESULTS

The number of bites per treatment site for the various pretreatment intervals is shown in Table 1. The durations for each repellent were calculated and are presented in Table 2. Table 3 presents the species and biting rate of mosquitoes collected from the untreated forearm of the control subject. Meteorological conditions during the 4 days are shown in Table 4.

DISCUSSION

These 4 repellents had been previously field tested by LAIR at Camp Lejeune, North Carolina (1973) against predominantly *Aedes taeniorhynchus* (Wiedemann) species (Shimmin et al. 1974), and at Turtle Mound, Florida (1973), Fort Wainwright, Alaska (1974), and Colusa, California (1974) where the predominant species were *Aedes taeniorhynchus, Ae. communis* (De Geer) and *Ae. dorsalis* (Meigen) respectively (Spencer and Akers 1976). In these field tests, the repellents carbamide and sulfonamide protected as long as or longer than m-deet under all test conditions at equal concentrations. SRI-6 was statistically no different from m-deet in the tests.

Field tests comparing carbamide to m-deet, principally against *Ae. taeniorhynchus,* were also conducted at New Smyrna Beach, Florida (1975, 1976) by the U.S. Department of Agriculture (Schreck and Smith 1977). In those field tests, m-deet provided greater duration of protection than carbamide in contrast to the studies cited above.

In the present study, carbamide and SRI-6 provided longer protection than m-deet for all 4 volunteers, and sulfonamide's duration was equal to or greater than that of m-deet for all volunteers tested. Additional test days were not available for examining shorter pretreatment intervals for m-deet to detect the threshold at which protection was achieved for all subjects. In laboratory studies (Spencer et al. 1974), subjects were reported to have consistently short, medium, or long protection times. This effect may be responsible for the variation in protection times observed among the subjects in this study (Tables 1 and 2), as subject 1 has protection times equal to or greater than those for all other subjects and subject 4 has protection times equal to or less than those for all other subjects.

Differences in test technique and the type of mosquito tested against do not permit direct comparison to past field tests. However, the variable test results reported for carbamide deserve comment.

In recent laboratory tests with volunteers, carbamide was found to have a

Table 1. Number of bites during observation period (1915–2130) for repellents at the given pretreatment intervals.

Volunteer	Pretreatment interval (hours)	Test date	Number of bites per observation period			
			Carbamide	m-Deet	Sulfonamide	SRI-6
1	16	30 Aug 77	1	5	1	5
1	14	31 Aug 77	0	5	1	5
1	12	1 Sept	1	5	5	1
1	10	29 Aug 77	0	3	0	0
2	16	29 Aug 77	0	5	3	5
2	14	30 Aug 77	2	5	5	5
2	12	31 Aug 77	3	5	5	3
2	8	1 Sept 77	3	5	1	1
3	14	29 Aug 77	5	5	5	5
3	12	30 Aug 77	5	5	5	3
3	10	31 Aug 77	4	5	5	1
3	6	1 Sept 77	0	5	0	1
4	12	29 Aug 77	5	5	5	5
4	10	30 Aug 77	4	5	5	5
4	8	31 Aug 77	4	5	5	5
4	4	1 Sept 77	0	5	0	0

Table 2. Duration (hours) of repellency.

Volunteer	Carbamide[a]	Deet[b]	Sulfonamide	SRI-6
1	>16	10	10	12
2	>16	<8	8	12
3	10	<6	6	12
4	10	<4	4	4
Range	10 to 16+	<4 to 10	4 to 10	4 to 12

[a] In two out of four individuals carbamide remained protective at the longest duration tested.
[b] In three out of four individuals m-deet did not protect at the shortest duration tested.

Table 3. Species and biting rate of mosquitoes collected from untreated arm of a control subject at Colusa, California (August 29–September 1, 1977).

Species	Mosquitoes collected[a]		Mean bites/hr[b] (between 1915–2045 hrs)
	Total number	% of total	
Anopheline			
Anopheles freeborni	95	78	88
Culicine			
Aedes dorsalis	5	4	5
Aedes vexans	18	15	17
Culex tarsalis	4	3	4

[a] Four day total.
[b] Based on average of thirteen (5 min.) biting collections over four days. Daily mean bites/hr from *Anopheles freeborni*, obtained by extrapolation from 3 or 4 five-minute checks, are as follows: 29 Aug 77, 102; 30 Aug 77, 108; 31 Aug 77, 69; 1 Sept 77, 72.

Table 4. Meteorological conditions, August 29–September 1, 1977[a] during mosquito repellent test.

Day	Temperature (deg C)		Relative humidity (%)		Wind (mph)	
	1300[b]	1900[b]	1300[b]	1900[b]	1300[b]	1900[b]
29 Aug 77	35	30	23	25	21	5
30 Aug 77	34	28	22	31	14	4
31 Aug 77	30	20	32	61	9	13
1 Sept 77	26	21	39	51	7	9

[a] Sacramento, CA Executive Airport. Sacramento is approximately 40 miles southeast of Colusa.

[b] Observation time.

minimum effective dose (MED) of 0.16 mg/cm^2 and a duration of 2.1 hr (at 0.32 mg/cm^2) against *Ae. aegypti* (Linnaeus) mosquitoes (Hill et al. 1979). Duration for m-deet at 0.32 mg/cm^2 was 5.7 hr. In similar earlier laboratory studies conducted with volunteers at LAIR (Gabel et al. 1976), carbamide had a duration of 17.4 hr vs 6.8 hr for m-deet (both chemicals were applied at a dose of 0.32 mg/cm^2). Carbamide is considerably less volatile than m-deet; that is, the evaporation rate for carbamide from an aluminum planchet at 30°C is 10 times lower than that of m-deet (22.6 vs. 2.23 $ug/cm^2/hr$) (Gabel et al. 1976). In vitro measurements of the evaporation rate of carbamide from skin have shown that the evaporation rate measured as a function of time for carbamide during a 12 hr period following application of 0.32 mg/cm^2 can be approximated as a straight line of shallow slope (-0.032 $ug/cm^2/hr^2$) with a value of 0.99 $ug/cm^2/hr$ at time zero. This initial value is close in magnitude to the evaporation rate (1.1 ± 0.1 $\mu g/cm^2/hr$) arising from a MED dose of carbamide (0.16 mg/cm^2) in a 1 hr period following application (W. G. Reifenrath, unpublished data). If the concept of a minimal effective evaporation rate (MEER) is accepted as necessary for protection, then doses of carbamide of 0.32 mg/cm^2 in the laboratory would result in an evaporation rate close to the MEER. The performance (protection time) of carbamide is likely to be sensitive to small changes in biting rate by the mosquitoes or to ambient conditions which would affect the evaporation of the material. Although one cannot make direct comparisons to field tests, the volatility factor may play a role in the variable results. Similar considerations may apply to other low volatility repellents or formulations of repellents in which the evaporation rate of the active ingredient has been reduced to levels near the MEER.

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