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FOUR-SITE METHOD FOR MOSQUITO REPELLENT FIELD TRIALS.(U)
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Four repellents were field tested at Camp Lejeune, North Carolina using a newly developed four-site test method. Hexamethylene-carbamide and n-butane-hexamethyleneimime-sulfonamide were found to be significantly better than deet, while triethylene-glycol ether was found to be only slightly less effective than deet. The four-site field test method was found to be an efficient screening method, affording economical use of subjects.

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FOUR-SITE METHOD FOR MOSQUITO REPELLENT FIELD TRIALS

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INTRODUCTION.—As part of a Letterman Army Institute of Research (LAIR) program to find a mosquito repellent better than the repellent most frequently used, N,N-diethyl-m-toluamide (DEET), new repellents and repellent formulations have been evaluated by comparison to DEET in laboratory screening tests using a four-site testing method. To determine the efficacy of these compounds under field conditions and to evaluate laboratory screening techniques, a series of laboratory trial results were compared to field test results. This paper is a report on the four-site method used in the field trials.

MATERIALS AND TEST DESIGN.—The compounds which were evaluated in the field trials are as follows:

cyclohexamethylene-carbamide (carbamide), 99% pure, (Stepanor 1967 and Dremova 1970)

n-butane-hexamethylene-sulfonamide (sulfonamide), 99% pure, (Maslii 1966; Zhogolev 1970; and Prevomaiskii 1967)

3,6,9-trioxapentadecan-1-ol (SRI-6), 96% pure, (Skinner 1974)

N,N-diethyl-m-toluamide (DEET), 95% pure, (Eastman Chemicals, Practical Grade)

The standard LAIR testing procedure is a four-site design (Brodel 1974) using two sites on each forearm, with defined areas of 7 x 10 cm each. This design permits testing three compounds simultaneously in addition to a control repellent. This capability is of importance when performing field trials because of day to day changes in climate, test subject, and mosquito populations. Moreover, the data can be analyzed by a paired comparison of a test formula to DEET (Ostle, 1963).

In the laboratory, repellent solutions are applied to two 7 x 10 cm test sites on each forearm, and are spread with a clean glass rod (Brodel, 1974). The arm is then inserted into a plastic sleeve measuring 20 cm x 70 cm (Khan, 1968). This sleeve has two 5 x 8 cm holes, exposing the repellent application sites to the mosquitoes.

Testing takes place for three minutes in two 1.25 cubic foot cages each containing approximately 250 avid female *Aedes aegypti* mosquitoes. The volunteers[3] are tested each hour in alternate cages. Failure of a site is determined by two bites during a testing period or two consecutive bites over two periods (Brodel 1974).

For field trial testing, the LAIR method (Brodel, 1974) was modified to include the total circumference on each forearm. The field trials were accomplished in the following manner:

1. Adhesive backed foam strips about ¼" wide were wrapped around the wrist, upper forearm, and mid-forearm. The foam strips served as boundaries and as protective devices against abrasion for the given sites. The foam strips outlined two sites on each forearm of every volunteer; four sites per individual.
2. One of the four repellents, DEET, SRI-6, carbamide, or sulfonamide, was applied in ethanol solution to each site at a constant concentration per unit of forearm area. The volume applied varied according to the surface area of the site on the various individuals. To determine the surface area, the circumference of the arm was measured at points 3 cm, 12 cm, and 21 cm from the wrist. The surface area of each test site was calculated as the area of a frustum of a cone: $A = s(R_1 + R_2) \times \pi$ (Selby, 1968) where A = area, s = 9 cm, and R_1, R_2 are the radii at the upper and lower boundaries of a site.
3. The total circumference of the area marked off by the foam strips was covered with the repellent solution for that site. The repellent was applied so that no repellent appeared on a given site more than once in any group of four individuals. Each subject had his own DEET control.

   All results were analyzed using a paired comparison with DEET and having statistical significance at the 95% level as determined by the Student's t-distribution. Six and 7 August were replicates and the data for the two days were combined for analysis.
4. The twelve individuals used in the test were divided into three equal groups each containing four volunteers and the application times for the volunteers were staggered to obtain differentiation between repellent protection. Testing occurred from one-half hour before to one-half after sundown, except on 10 July 1973. A concentration of 0.48 mg/cm^2 was used in repellent tests except as stated elsewhere. This concentration was designed to last approximately from an initial morning application to testing at sunset. The application times are as follows:

	25 Jun 73	6, 7 Aug 73	8 Aug 73
Group I	0800	0700	1000
Group II	1000	0930	1000
Group III	1200	1200	1000

5. During the actual field trials the volunteers were issued the following items: tropical fatigues and boots, plastic gloves for the hands, head nets, canvas square to sit on,

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[3]Informed consent was received from each volunteer prior to participation in the tests.

pen and notebook for recording their own results, and pen size flashlight for verifying bites after sunset. Only the actual repellent sites were exposed to mosquitoes.

RESULTS AND DISCUSSION.–On 10 July 1973 the first of two preliminary experiments was conducted. The first experiment was designed to compare the four-site field method using the total forearm circumference to the four-site technique designed at LAIR (Brodel, 1974). Two of four individuals had field test sites on the right forearm and two had the field test sites on the left forearm. The other forearm of the individuals had the standard laboratory test sites. Repellents were applied at 0900 hours. For this test two stock repellent solutions, carbamide and DEET, were applied at 0.48 mg/cm^2 and testing was done by the usual LAIR laboratory method (Brodel, 1974).

The results show the field test values were exactly two hours longer in protection on each individual than in the lab method. This situation is probably an experimental artifact resulting from the small population at risk. The DEET field test method gave a longer dry protection time than the DEET four-site method, while with the carbamide the four-site method gave a longer time than the field test method. This difference was not statistically significant probably because carbamide at 0.48 mg/cm^2 gives protection between 20-25 hours such that abrasion is a substantial factor. In the field method, the total circumference of the arm was covered by repellent while a smaller rectangular site on the ventral forearm is used in the laboratory design. Abrasion would be more likely to occur when using the field design.

On 25 July 1973, twelve volunteers were divided into three groups of four volunteers with each group having its repellents applied at LAIR at different times as described above. After busing to Hamilton Air Force Base, about twenty miles north of San Francisco where the climate is much warmer, the men did twenty minutes of calisthenics, followed by a 1.5 mile run. Then the test subjects played softball for one hour, creating an abrasion factor. At sundown, upon returning to LAIR, test sites were exposed in the laboratory for one minute in one of two test cages. The total number of bites received in one minute was recorded.

It was found that the 0800 group (Table 1) shows carbamide as having significantly fewer bites than DEET. Under similar circumstances without abrasive factors, one would expect to find that sulfonamide offers protection equivalent to that of carbamide. The sulfonamide data have a greater standard deviation attributed to abrasion. In a small population group, the abrasion factor disturbs the normal distribution and becomes the dominant influence in statistical analysis. As a result of this, the softball game was excluded from planned activities in field trials.

The 1000 hours group showed no compound significantly better than DEET due to the absence of bites in one individual which prevented any statistical differentiation between the repellent compounds (Table 1). Moreover, abrasion in at least one other site gives a large standard deviation for the group data. Carbamide and sulfonamide showed significantly better results than DEET in the 1200 group where abrasion factors did not predominate.

On 5 August 1973, a group of 12 volunteers went to Camp Lejeune, North Carolina. On 6 and 7 August the applications were made and volunteers engaged in moderate activity such as jogging and marching. During the test the individuals were distributed randomly around the testing area. Each volunteer sat on a canvas square and held his arms away from his body. One volunteer was chosen randomly each night from the group as a treated control while one individual acted as an untreated control. Mosquitoes collected from the treated control showed what species bit each repellent site. Mosquitoes collected from the untreated control were a measure of species population and density of the mosquitoes. Mosquito population screening during all tests showed that *Aedes taeniorhynchus* was the most prevalent species along with *Aedes sollicitans* and *Anopheles crucians* which were present in small numbers. Mosquito density was estimated at 200 man bites/hour. The *Aedes taeniorhynchus* whole body count from the untreated control was 193, while only 10 were collected from the treated test site used as a control on 6 August. Repellency results were analyzed by paired comparison of the number of bites received on a test repellent site to the control DEET site.

Table 1.–Preliminary field test, Hamilton Air Force Base, California.

	Repellent Efficacy (Bites/Minute/Test Site)[a]			
	SRI-6	Carbamide	DEET	Sulfonamide
	5	3[b]	4	3
	3	3	6	0
0800	10	0	2	0
	1	0	2	0
	1	0	0	0
	0	0	1	0
1000	4	0	14	0
	4	0	8	0
	2	0[b]	2	1[b]
	1	0	3	0
1200	0	1	2	0
	1	0	2	1

[a]Test 25 July 73 on 12 volunteers at a dose rate of 0.48 mg/cm^2. Each number represents an individual test site.

[b]Significantly different than DEET at a 95% level.

On 8 August the test area was changed. All applications were made at a dose rate of 0.31 mg/cm^2 at 1000 hours. Otherwise the testing procedures followed that of the two previous days.

The purpose of the field trial was to relate laboratory results to field results by comparing the test repellents to the standard Army repellent, DEET. Results on 6 and 7 August (Table 2) are reported in bites per one hour of exposure to mosquitoes at Camp Lejeune. In the 0700 group, carbamide and sulfonamide show significantly better protection time than DEET. The two other repellent site groups, 0940 and 1200 groups, did not receive enough bites to differentiate between repellents. The three application times in the field trials allowed one to detect differences in repellents regardless of high or low mosquito density and/or avidity. For instance, the repellents could not be differen-

Table 2.--Mean bites per test site in one hour exposure in field trial[a].

	0700		0930	
	$\bar{X}$	S_d	$\bar{X}$	S_d
DEET	2.63	2.13	1.00	1.60
SRI-6	3.13	3.68	0.38	0.52
Sulfonamide	0.13[b]	0.35	0.00	0.00
Carbamide	0.00[b]	0.00	0.00	0.00

[a]Test 6 and 7 August 73 on 12 volunteers at a dose rate of 0.48 mg/cm^2.

[b]Significantly different from DEET at 95% level.

Table 3.--Ranking of repellents: Lab and Field.

	LAIR	Camp Lejeune	
	$\bar{X}$ hrs protection to date	Total Bites/hr 6, 7 Aug (0700)	8 Aug
Carbamide	17.4 ± 5.1[a]	0[a]	3[a]
Sulfonamide	14.3 ± 5.6[a]	2[a]	8[a]
SRI-6	8.5 ± 4.9	15	50
DEET	6.6 ± 1.7	21	66
Application rates	0.31 mg/cm^2	0.48 mg/cm^2	0.31 mg/cm^2
Number of volunteers	8-28	12	12

[a]Significantly different from DEET at 95% level.

tiated if all volunteers had had their repellents applied at 0930.

Results from 8 August 1973 (Table 3) show that the carbamide and sulfonamide offer significantly better protection than DEET in decreasing the number of bites. By comparing repellents to DEET, we can obtain information on how the repellents' protective properties under given tests relate to DEET. In laboratory testing (Table 3) we found carbamide and sulfonamide proved to give significantly better protection than DEET, likewise in the field results. Thus, laboratory methods are indicative of the field results under conditions tested and also, carbamide and sulfonamide are superior to DEET under the test procedures used.

Under the test conditions described, SRI-6 was not found to be significantly better than DEET. SRI-6 has poor wash-off resistance (Spencer, 1974) and sweating, a form of wash-off, would detract from this compound's repellent properties in field trials. As a result of poor wash resistance, the SRI-6 compound was not expected to be better than DEET in the field.

SUMMARY.--To determine if our laboratory results would predict a repellent's relative protection time under action field conditions, a field study was undertaken to compare three repellents to the standard military repellent, DEET. Preliminary testing was done to determine the feasibility of our field methods which was found to be satisfactory. Actual field testing produced the same relative comparison to DEET as was determined in our laboratory test (Table 3).

Carbamide and sulfonamide give significantly longer protection time than DEET in the laboratory and significantly less bites than DEET under field conditions, while SRI-6 offered protection which was not significantly different from DEET. For the mosquito species and field conditions encountered, the laboratory screening procedure appears to have predicted the field results.

References Cited

Brodel, C., T. Spencer and W. Akers. 1974. Evaluation of three mosquito repellent screening methods. Rept. No. 18, Dept. of Dermatology Research, Letterman Army Institute of Research, Presidio of San Francisco, Calif. 94129.

Dremova, V. and S. Smirnova. 1970. Effects of repellents on hard (Ixodidae) and soft (Argasidae) ticks. Int. Pest. Control 12:10-14.

Khan, A., H. Maibach and W. Strauss. 1969. Response of the yellow-fever mosquito to varying area of human skin. J. Econ. Entomol. 61:555-557.

Maslii, L. and R. Razbegaeva. 1966. N,N-diakyl alkane sulfonamides. Zhurnal Oreg. Khim. 2(11):2014-2017.

Ostle, B. 1963. Statistics in Research, The Iowa State University Press, Ames, Iowa, U.S.A., 410.

Pervomaiskii, G., V. Osipyan, V. Kazhdan, L. Maslii, A. Shustrov, B. Grabovskii, D. Zhogolev and I. Dunavea. 1967. Voenna-Med. Akad. in Kirova, U.S.S.R. Medl. Parazitol. Parazit. Bolez. 36(6): 730-733.

Selby, S., Ed. 1968. Standard Mathematical Tables, 16th ed. Chemical Rubber Co., Cleveland, Ohio.

Skinner, W., H. Johnson, J. Degraw, D. Skidmore and H. Maibach. 1974. Topical mosquito repellents VIII: alkyltriethylene glycol monoethers. J. Pharm. Sci. (accepted for publication).

Spencer, T., R. Shimmin, S. Bayles and W. Akers. 1974. Insect Repellent Research 1973. Report No. 20, Dermatology Research Division, Letterman Army Institute of Research, Presidio of San Francisco, Calif. 94129

Stepanor, M., V. Osipyan, V. Kazhdan, L. Maslii, B. Grabovskii and I. Dunavea. 1967. Lenningrad Scientific-Research Institute of Chemistry. U. S. Patnt Patent No. 3,624,204.

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