

## COMPARATIVE EFFICACY OF DIETHYLTOLUAMIDE SKIN-APPLICATION REPELLENT (DEET) AND M-1960 CLOTHING IMPREGNANT AGAINST MOSQUITOES IN THE NIPAH PALM-MANGROVE SWAMPS IN MALAYA

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In a set of tests previously discussed in the present series of papers, it was shown that the skin-application repellent diethyltoluamide (deet) was extremely effective against the swarms of mosquitoes in the nipah palm-mangrove swamps of Malaya, but that the protection was limited solely to those parts of the body actually treated with repellent (1, 2). That is, the volunteers sustained mosquito-bites through their untreated clothing or on portions of the exposed skin which had not been covered with repellent. It was, therefore, decided to perform another series of tests to compare the degree of protection offered by a repellent impregnated into the clothing, with that obtained by using deet. The standard U. S. Army clothing impregnant, M-1960<sup>2</sup>, was chosen for this experiment, and the results, presented in this article, indicate that where mosquitoes are as abundant as they are in the nipah palm-mangrove swamps, it is insufficient to rely solely upon the use of either deet repellent or the wearing of M-1960-treated clothing. Simultaneous use of both methods, however, offered virtually solid protection, even when the untreated controls were being attacked by mosquitoes at the rate of 173-244 per man per hour.

### MATERIALS AND METHODS

The present tests were conducted in the nipah palm-mangrove swamps at the Connaught Bridge, Klang, in October and November and the techniques employed were, in the main, the same as those reported in the previous article (1). There were, however, several significant differences, as mentioned below.

All but three of the volunteers in these tests wore new U. S. Army tropical field uniforms, consisting of jungle-green, light weight long-sleeved shirt and trousers, a quarter-sleeve undershirt, and light cotton-rayon socks. (Local low-quarter shoes were worn.) The sleeves of the shirt were worn full-length, as were the trouser legs, which were allowed to

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1. Now with the Army Medical Research and Development Command and the Walter Reed Army Institute of Research, Washington, D. C. respectively.
  2. M-1960 consists of N butylacetanilide (30%), 2-butyl-2-ethyl 1-1, 3-propanediol (30%), benzyl benzoate (30%) and an emulsifier, "Tween 80" (10%).

lie loose and were not tucked into the socks. Certain men wore treated clothing alone, some used deet repellent alone, and others used the combined treatment, i. e., impregnated clothing and application of deet on exposed surfaces.

The treated clothing was impregnated by mixing M-1960 with water at the rate of one part of repellent to 15 parts of water and fully immersing and thoroughly wetting the clothing in the mixture at the rate of 1 gallon of the mixture for 28 uniforms. The clothes were then wrung out and dried, and subsequently washed with soap and cold water, dried and then washed and dried a second time. (This washing procedure was followed in order to minimize the somewhat undesirable characteristics of M-1960, such as the slightly unpleasant odor, and the plasticizing effects upon combs and pens.) The untreated uniforms worn by volunteers serving as controls were also similarly washed with soap and water before issue. Three unprotected men wore only local dress consisting of shorts and a short-sleeved shirt, as in the tests already reported.

Preliminary trials had indicated that volunteers in untreated uniforms were apt to be

Table 1. Protection against mosquitoes in Malaya. Comparison of DEET skin-type repellent, with M-1960 clothing impregnant. (Four replications.)

Category of test group and numbers of men in each	Site of collection	Total numbers of mosquitoes	Mean Collecting Rate <sup>3</sup> ± standard deviation
Controls			
I. Uniformed (6)	Head and limbs only	4,144	173 ± 19
II. Uniformed (6)	Entire body	4,490	206 ± 16
III. Indigenous dress (3)	Entire body	2,909	244 ± 20
DEET skin-repellent alone (uniformed)			
IV. 2 hrs. after application (3)	Entire body	258	22 ± 5
V. 2 hrs. after application (3)	Head and limbs only	3	0.25 ± 0.4
VI. 3 hrs. after application (3)	Entire body	391	33 ± 6
VII. 3 hrs. after application (3)	Head and limbs only	6	0.5 ± 0.6
M-1960 impregnant alone			
VIII. Treated Uniform (3)	Entire body	337	28 ± 6
IX. Treated Uniform (3)	Head and limbs only	330	28 ± 5
X. Treated Uniform (3)	Clothing only	3	0.25 ± 0.4
Both DEET skin-repellent and M-1960-treated uniform			
XI. 2 hrs. after application of deet-repellent (3)	Entire body	5	0.5 ± 0.6
XII. 3 hrs. after application of deet-repellent (3)	Entire body	14	0.5 ± 0.7

3. Mean Collecting Rate — the average number of mosquitoes collected from one volunteer in one hour.

bitten on the head and neck and about the wrists and ankles (despite the socks). Therefore, these areas of the body were treated with skin-application repellent by those volunteers using deet. The compound was applied two and three hours respectively before all the men were exposed simultaneously at 1845 hours for a period of one hour.

Since it was impossible for a volunteer to collect mosquitoes biting inaccessible portions of his own body, in the current experiments the volunteers in each group sat in a circle, and each man assisted his comrades in collecting mosquitoes that alighted on his neighbor's back or on other sites not readily reached by the victim himself. Therefore, the results were tallied and are presented as a "Mean Collecting Rate", rather than as an "Attack Rate". Certain of the men collected mosquitoes from the limbs and head only, while others collected from the entire body, including clothing, and one set caught only those mosquitoes which alighted on the uniforms. The numbers of volunteers and the various categories regarding type of repellent used and method of collection are shown in Table 1.

## RESULTS

From Table 1, it can be seen that unprotected volunteers in uniform captured substantially more mosquitoes when collecting from the entire body (Category II) than did similarly attired men collecting only from the head and limbs (Category 1), viz, a Mean Collecting Rate of 206 versus 173, with an average difference of 33 mosquitoes per man per hour. The controls wearing the indigenous highly-abbreviated dress attracted, on the average, 38 mosquitoes more per man per hour than did their uniformed colleagues, indicating that merely wearing clothing covering the limbs offered some degree of protection. From these data, it is also apparent that using deet or M-1960 alone does *not* provide adequate overall protection. Thus, an average of 22-33 mosquitoes were collected in one hour from the clothing of men who had used only deet repellent, and a mean of 28 mosquitoes per man per hour were collected from uncovered portions of the body in the case of men wearing uniforms treated with M-1960. Nevertheless, both of these compounds were extremely effective *where applied*, as is shown by the fact that the men in groups V, VII and X were virtually free from mosquito-attack. The combination of deet skin-repellent and use of M-1960-impregnated clothing proved to be extremely effective, and the Mean Collecting Rate in these groups of volunteers was 0.5 mosquitoes per man per hour when collecting from the entire body.

The numbers and relative percentages of the various kinds of mosquitoes encountered by the controls in uniform and by the volunteers using deet repellent or M-1960-treated clothing are shown in Table 2.

Of the eighteen types of mosquitoes listed, about four-fifths of the specimens collected by each of these categories of volunteers were *Aedes (Skusea) amesi*, while *Aedes (Aedes) butleri* and *Culex (Neoculex) brevipalpis* were the next most abundant species.

## DISCUSSION

It is worth emphasizing that when control-volunteers in uniform collected mosquitoes from all parts of the body, they averaged about 33 more specimens per hour than did their similarly attired colleagues who collected only from the head and distal portions of the limbs. A similar Mean Collecting Rate of 33 was achieved by those volunteers who

Table 2. Numbers and relative percentages of various kinds of mosquitoes collected by specified volunteers during tests on comparative efficacy of diethyltoluamide (DEET) skin-application repellent and M-1960 clothing impregnant against mosquitoes in the Nipah Palm-Mangrove Swamps, Connaught Bridge, Klang, Selangor. (4 Replications.)

Name of mosquito	Total numbers of mosquitoes and percentages					
	Uniformed controls		DEET repellent <sup>4</sup>		M-1960 clothing <sup>4</sup>	
	No.	%	No.	%	No.	%
<i>Aedes (Aedes) butleri</i>	346	4	32	5	36	5
<i>Aedes (Aedes) sp.</i>	23	@	1	@	0	—
<i>Aedes (Cancraedes) sp.</i>	19	@	1	@	1	@
<i>Aedes (Mucidus) aurantius</i>	53	@	4	@	6	@
<i>Aedes (Skusea) amesi</i>	6,897	80	545	84	551	82
<i>Aedes (Skusea) curtipes</i>	4	@	0	—	0	—
<i>Aedes (Stegomyia) albopictus</i>	83	1	7	1	4	@
<i>Anopheles aurostris</i>	173	2	14	2	13	2
<i>Anopheles hyrcanus</i>	118	1	7	1	13	2
<i>Anopheles kochi</i>	63	@	1	@	0	—
<i>Anopheles sondaicus</i>	43	@	3	@	2	@
<i>Culex (Culex) gelidus</i>	55	@	2	@	1	@
<i>Culex (C.) tritaeniorhynchus</i>	6	@	0	—	0	—
<i>Culex (C.) "vishnui"</i>	21	@	1	@	2	@
<i>Culex (Lophoceratomyia) spp.</i>	164	2	9	1	12	2
<i>Culex (Neoculex) brevipalpis</i>	368	4	26	3	21	3
<i>Mansonia "dives"</i>	172	2	5	@	7	1
<i>Mansonia uniformis</i>	26	@	4	@	1	@
Total numbers:	8,634		658		670	

used deet and collected from the entire body (Category VI), while those deet-treated men who collected only from the portions of the body to which repellent had been applied (Category V) had a Rate of merely 0.25. It therefore may be presumed that these additional mosquitoes were attacking through the untreated clothing. A similar picture of effective protection limited to treated areas was exhibited by the volunteers wearing uniforms treated with M-1960 but who did not use deet (Category IX). They were readily attacked by mosquitoes on the head and limbs, as indicated by the Mean Collecting Rate of 28. From these data it is obvious that, in mosquito-infested terrain, the combined use of skin-application repellent (deet) and clothing-impregnant M-1960 is necessary for adequate protection.

#### SUMMARY

In areas in Malaya heavily infested with mosquitoes, using diethyltoluamide (deet)

4. Virtually all of these mosquitoes came from unprotected parts of the body.

@ = Less than 1%.

skin-application repellent *alone* does not provide adequate protection against attack by these insects. The same is true when using uniforms treated with the standard U. S. Army clothing-impregnant M-1960, in the absence of deet skin-repellent. The combined use of deet and M-1960 provided virtually complete freedom from annoyance by these pests for a minimum period of three hours.

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