

EFFECTIVENESS OF REPELLENT FORMULATIONS CONTAINING DEET AGAINST MOSQUITOES IN NORTHEASTERN THAILAND¹

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ABSTRACT. Formulations of the repellent diethyl methyl benzamide (deet) were evaluated against mosquitoes in field tests conducted in Sisaket Province, northeastern Thailand. Thirty-three percent deet in an applicator stick provided $\geq 87.1\%$ protection against primarily *Culex vishnui*, *Cx. gelidus*, and *Cx. tritaeniorhynchus* for up to 5 h, and 50% deet in ethanol provided $\geq 93.3\%$ protection against *Anopheles* sp. and culicines for up to 8 h. The U.S. Army Extended Duration Repellent Formulation (EDRF), containing 33% deet, 50% deet in ethanol, and 75% deet in ethanol provided complete protection for up to 6 h against primarily *Culex vishnui* and *Anopheles dirus*.

The mosquito repellent diethyl methyl benzamide (deet) was first discovered in 1954 and has since been used throughout the world in numerous formulations (Gupta and Rutledge 1994). Although deet has a broad spectrum of effectiveness against many arthropods, including mosquitoes, the response of different species varies (Rutledge et al. 1978). Field tests conducted against mosquitoes in southeastern Thailand in 1965 reported the effectiveness of 6 repellents (5% dilutions) and showed that only deet protected completely for 45 min or more (Gilbert et al. 1970). In this note we report field studies on the effectiveness of topical repellent formulations containing deet against mosquitoes in northeastern Thailand.

During 1992, tests were conducted in a Royal Thai Army (RTA) camp situated about 1 km from O'Trao refugee encampment, Phusing District, Sisaket Province, northeastern Thailand. The camp was situated in an area where native forest had been cleared extensively for timber production. The relatively few trees that remained were less than 20 years old and interspersed with secondary scrub vegetation.

The repellent formulations tested were 33% deet formulated into an applicator stick (Intergrade Trading Co., Bangkok), and a liquid containing 50% deet in ethanol (Intergrade Trading Co.). A trial of the 33% deet stick formulation was conducted on each of 3 nights in July 1992 and a second trial with 50% deet was conducted on 2 nights in September 1992. Sixteen male Thai soldiers acted as volunteers during the trials. All volunteers wore short trousers, and long-sleeve shirts during the trials. Repellent was applied evenly to the exposed skin of the legs, face, and hands of 8 volunteers; the remaining 8 volunteers were untreated. Both repellents were applied *ad libitum* by the volunteers to ensure an even coverage of the exposed skin, but in this operational trial, the amount applied was not known. The volunteers were assigned to 8 groups of 2 individuals (4 control groups, 4 repellent groups), and each member of a pair collected mosquitoes from his partner. In July 1992, repellent was applied to the test individuals at 1830 h, and in September 1992 tests, repellent was applied at 1800 h on the first night and 1600 h on the second night of testing. For each test all volunteers assembled in their groups and walked about 50-100 m into the surrounding bushland. They sat in a predetermined area and collected all biting mosquitoes using small glass tubes for 20 min each hour from 1900 to 2400 h. The collected mosquitoes for each pair and collection time were placed into a cup, counted, and returned to Bangkok for identification.

In July 1992, a total of 390 mosquitoes, representing 21 species from 4 genera, were collected on the control volunteers. The predominant species collected were *Culex vishnui* Theobald (42.7% of total), *Culex gelidus* Theobald (20%), *Culex tritaeniorhynchus* Giles (12.7%), and *Culex whitmorei* (Giles)

¹ The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense. Mention of a commercial product does not constitute an endorsement of the product by the Department of Defense. The volunteers gave informed consent before participating in the study.

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Table 1. All mosquitoes (mean/pair/night \pm SE) collected hourly on volunteers with skin either treated or untreated with 2 formulations of deet at O'Trao, Phusing District, Sisaket Province, Thailand, July and September 1992.

| Hours after repellent application | July 1992 | | | September 1992 | | |
|-----------------------------------|--------------------------------|---------------------|----------------|-------------------|---------------------|----------------|
| | 33% deet (n = 22) ¹ | Untreated (n = 390) | Protection (%) | 50% deet (n = 12) | Untreated (n = 722) | Protection (%) |
| 0 | 0.4 \pm 0.2 | 3.1 \pm 0.8 | 87.1 | — | — | — |
| 1 | 0.1 \pm 0.1 | 1.8 \pm 0.6 | 94.4 | 0 | 9.3 \pm 2.2 | 100 |
| 2 | 0.3 \pm 0.2 | 4.4 \pm 1.0 | 93.2 | 0 | 12.0 \pm 2.0 | 100 |
| 3 | 0.5 \pm 0.4 | 5.1 \pm 1.1 | 90.2 | 0 | 16.7 \pm 4.6 | 100 |
| 4 | 0.2 \pm 0.2 | 8.9 \pm 3.7 | 98.2 | 0 | 6.6 \pm 2.3 | 100 |
| 5 | 0.4 \pm 0.3 | 9.2 \pm 4.2 | 95.4 | 1.5 \pm 1.5 | 22.4 \pm 5.8 | 93.3 |
| 6 | — | — | — | 0 | 17.4 \pm 4.8 | 100 |
| 7 | — | — | — | 0 | 14.8 \pm 7.3 | 100 |
| 8 | — | — | — | 0 | 18.5 \pm 5.0 | 100 |

¹ Total number of mosquitoes collected by each group.

(7.3%). All are considered potential vectors of Japanese encephalitis (JE) in Thailand (Rattanakul and Panthusiri 1994). In September 1992, 121 *Anopheles* sp. were identified and *Anopheles karwari* (James) (47.9% of *Anopheles* sp.), *Anopheles splendidus* Koidzumi (31.4%), and *Anopheles nivipes* (Theobald) (9.1%) were the predominant species, although none of these are considered to be vectors of malaria in Thailand (Rosenberg et al. 1990). The remaining 601 culicines collected were not identified.

The protection provided by 2 formulations of deet at O'Trao is shown in Table 1. The 33% deet stick formulation provided \geq 87.1% protection against primarily *Culex* spp. for up to 5 h, without a decrease of protection over time. The

small number of mosquitoes collected on the treated volunteers may be attributed to a difficulty in applying the formulation uniformly on the skin. The 50% deet formulation was very effective in protecting treated volunteers against *Anopheles* sp. and culicines for up to 8 h without any loss of activity.

In November 1993, tests were conducted over 2 nights in an RTA camp located in Khukan District, Sisaket Province, Thailand. The camp was located in an area of mature native forest, near a stream. Three formulations containing deet were evaluated; 50% deet in ethanol (Intergrade Co.), 75% deet in ethanol (Royal Thai Army), and the U.S. Army Extended Duration Repellent Formulation (EDRF), containing 33% deet, acrylate polymers, and other inert ingredients (3M

Table 2. Mosquitoes (mean/pair/night \pm SE) collected hourly by volunteers treated with 3 formulations of deet and untreated volunteers at an RTA camp in Khukan District, Sisaket Province, Thailand, November 1993.

| Hours after repellent application | <i>Anopheles dirus</i> | | | All mosquitoes | |
|-----------------------------------|---------------------------|------------------|------------------|--------------------|---------------|
| | EDRF (n = 3) ¹ | 50% deet (n = 7) | 75% deet (n = 5) | Untreated (n = 57) | EDRF (n = 4) |
| 4 | 0 | 0 | 0 | 0.5 \pm 0.5 | 0 |
| 5 | 0 | 0 | 0 | 1.0 \pm 0 | 0 |
| 6 | 0 | 0.5 \pm 0.5 | 0 | 4.0 \pm 1.0 | 0 |
| 7 | 0 | 0 | 0 | 6.5 \pm 2.5 | 0 |
| 8 | 1.5 \pm 0.5 | 2.0 \pm 2.0 | 1.5 \pm 0.5 | 6.5 \pm 0.5 | 1.5 \pm 0.5 |
| 9 | 0 | 1.0 \pm 1.0 | 1.0 \pm 1.0 | 10.0 \pm 1.0 | 0.5 \pm 0.5 |

¹ Total number of mosquitoes collected by each group.

Corp., St Paul, MN). In these tests 4 pairs of volunteers, who wore military shirts buttoned at the wrist and long trousers rolled up to the knee, were used. Each pair of volunteers either tested one of the 3 formulations or was untreated. The repellent was applied under supervision at 1400 h. For liquid formulations, 1 ml of repellent was applied evenly to each leg, including the foot, up to the knee, and 1 ml to the face, neck, and hands. The EDRF was applied according to label instructions by applying a 2.5-ml strip of lotion to each leg and 2.5-ml strip to the face, neck, and hands. Collections began at 1800 h when the volunteers entered the nearby forest and collected all biting mosquitoes using small glass tubes for 45 min, followed by a 15-min rest. Collections were then made each hour for a total of 6 h.

The predominant mosquitoes collected during these tests were *Cx. vishnui* (58.4%) and *Anopheles dirus* Peyton and Harrison (34.7%). The overall density of mosquitoes was low (total collected = 202) during the tests; 50% deet provided protection against all mosquitoes for up to 6 h, and EDRF and 75% deet provided protection for up to 8 h (Table 2).

Recent laboratory and field studies have shown that *An. dirus*, the major vector of malaria in Thailand, is tolerant of formulations containing lower concentrations of deet. In the laboratory, low concentrations of deet ($\leq 35\%$) provided protection against *An. dirus* for ≤ 90 min (Frances et al. 1993). In a subsequent field test at Chanthaburi Province, southeastern Thailand, 25% deet in ethanol provided $>95\%$ protection against *An. dirus* for only 1 h after repellent application (Frances et al. 1996). Previous studies have shown EDRF, which contains 33% deet, provides similar protection against mosquitoes as formulations containing higher concentrations of deet in ethanol (Gupta et al. 1987, Harbach et al. 1990). The results

of the current test also show that EDRF provides similar protection to repellents containing 50% and 75% deet in ethanol, and suggest that these formulations are more effective against *An. dirus* than repellents containing lower concentrations of deet.

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Table 2. Extended.

| All mosquitoes | | |
|----------------------|---------------------|------------------------|
| 50% deet (n = 10) | 75% deet (n = 8) | Untreated (n = 180) |
| 0 | 0 | 29.0 ± 13.0 |
| 0 | 0 | 9.0 ± 6.0 |
| 1.5 ± 1.5 | 0 | 8.5 ± 1.5 |
| 0 | 0 | 13.5 ± 1.5 |
| 2.5 ± 2.5 | 2.0 ± 0 | 13.0 ± 2.0 |
| 1.0 ± 1.0 | 2.0 ± 2.0 | 17.0 ± 3.0 |