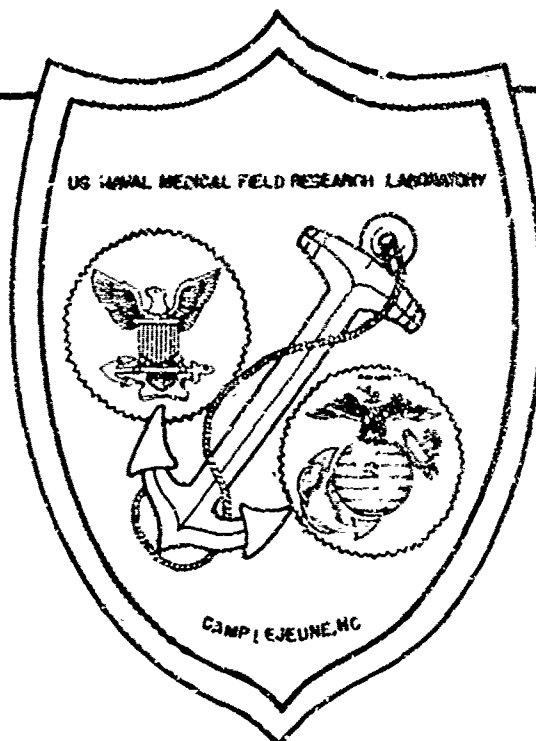


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**FIELD TESTS WITH REPELLENT-TREATED WIDE-MESH
NETTING AGAINST MIXED MOSQUITO POPULATIONS**

by

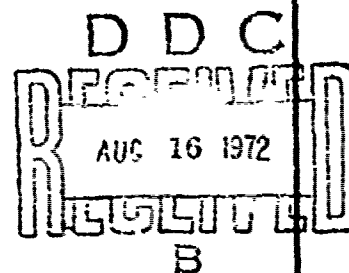
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FIELD TESTS WITH REPELLENT-TREATED WIDE-MESH NETTING AGAINST MIXED MOSQUITO POPULATIONS¹

By Roger H. Grothaus², John M. Hirst³, H. K. Gouck⁴ and D. E. Weidhaas⁴

Abstract: Space repellents were tested against mixed field mosquitoes by impregnating wide-mesh bed nets (8-11 threads/cm). The bed nets were taken to the field where human volunteers entered them and collected any mosquitoes that attempted to feed. Eight repellents were tested during a 3-year period. Ent 15510, 2-[2-(2-Butoxyethoxy)ethoxy]ethyl 3-methylcrotonate; Ent 20830, Diisopentyl malate; Ent 22542, *N,N*-Diethyl-*meta*-toluamide (deet standard); and M-1960 (standard military clothing repellent) all provided less than 90% protection after 1 month of aging. Ent 20297, *o*-Ethoxy-*N,N*-diethylbenzamide, and Ent 20573, 2-[(*p*-Methoxybenzyl)oxy] *N,N*-dipropylacetamide, were placed in the test in 1970 and were still effective after 50 days of aging. Ent 20364, 1-(*o*-Ethoxybenzyl)piperidine, remained effective 359 days after impregnation. Ent 19083, *o*-Ethoxy-*N,N*-dipropylbenzamide was effective after 787 days of aging. All of the repellents were tested at 0.5 gm/gm net weight and 3 were also tested at 0.25 gm/gm net weight.

The use of wide-mesh netting treated with repellents for protection against disease vectors has been shown to be both promising and feasible (Gouck et al. 1967a, Gouck & Moussa. 1969). The advantages of wide-mesh netting over standard, untreated fine-mesh netting (8-11 threads/cm) include good ventilation and improved visibility and hearing (Grothaus & Adams 1971). The wide-mesh netting currently under study is 0.635 cm mesh or 4 threads per inch. Current space repellents that have been tested have not proven adequate in preventing entry of mosquitoes through netting with openings larger than 0.635 cm.

For several years the Agriculture Research Service, U. S. Department of Agriculture, Gainesville, Florida, has been screening compounds to identify more effective space repellents (Gouck et al. 1967b, Gouck et al. 1971). Their work consisted of locating promising compounds using an olfactometer. Only chemicals which prevented *Aedes aegypti* (L.) from flying through netting to reach a human host were selected. The most efficacious compounds

were then taken to the field and tested against wild *Aedes taeniorhynchus* (Wiedemann). These field tests were conducted using a booth-like structure which holds one man. Test netting was placed over panels in the booth. Chemicals which proved effective in field tests were reviewed and some of the more promising compounds were forwarded to this laboratory for further investigations.

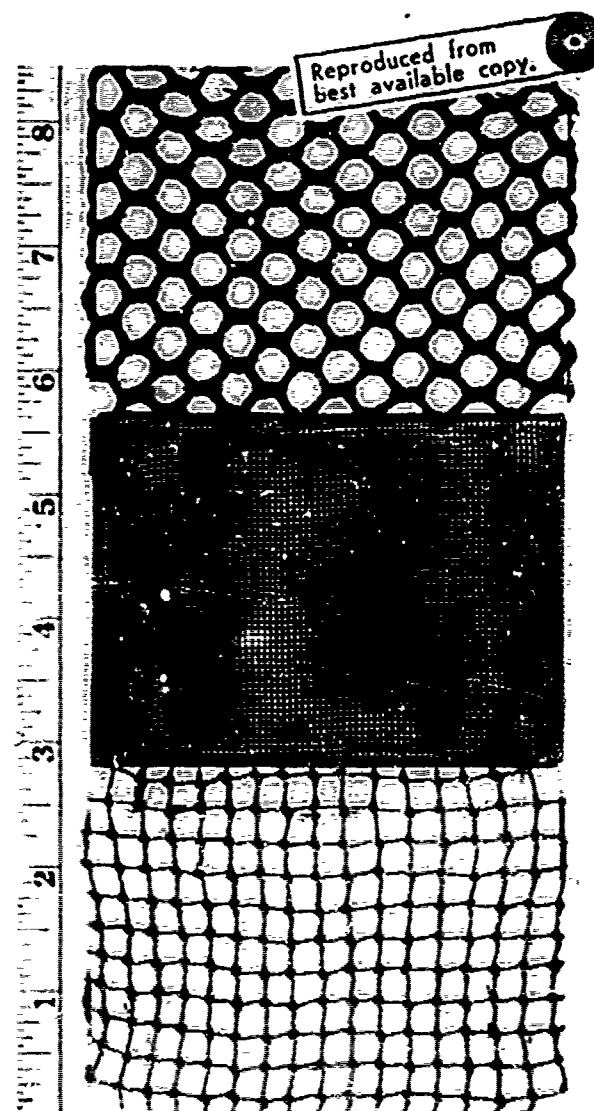


FIG. 1. Top, cotton/polyester netting. Middle, standard fine-mesh netting. Bottom, wide-mesh knotted cotton netting. Scale at left is in inches.

¹The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large. This study was conducted under Bureau of Medicine and Surgery, Navy Department work unit MF12-524.009-8008BX61.

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MATERIALS AND METHODS

The basic testing procedure involved the construction of wide-mesh (0.635 cm) fabrics into standard sized bed nets (1.98 × 0.762 × 1.22 m). The nets were impregnated with test chemicals on the basis of the fabric weight, using acetone as a carrier. Nets were then air dried and placed in individual plastic bags for subsequent testing. Two different fabrics were used in the study. One type of netting consisted of 100% knotted cotton (fig. 1). The second type was composed of 50% cotton and 50% polyester fiber (fig. 1). The latter material proved to have tensile strength and storage life comparable to nylon, but still absorbed the required amount of repellent. The activity of various repellents was evaluated by military volunteers who took the nets to the field and functioned as test subjects. This testing procedure provided information on the durability of the fabrics under field conditions.

A series of nets containing volunteers was arranged in a single row upwind from known mosquito-resting areas. In each experiment, the series contained an untreated net and a net treated with deet, in addition to the test nets. One subject remained outside of the nets and served as the control. Tests were conducted 1 night a week for approximately 6 weeks, mosquito populations permitting, and began approximately 1/2 hr before sunset and ended 1 hr after sunset. Each subject used a small penlight and an aspirator with killing tube (Jackson & Grothaus 1971) to collect all of the mosquitoes attempting to bite him. Collections

were made in increments of 15 min. Specimens were subsequently returned to the laboratory for identification (TABLE 1).

All of the nets were returned to the laboratory after each test and hung in open storage at room temperature. Nets remaining in the study after the summer test period were packed separately in plastic bags and stored at room temperature (19.5–25.0°C) until the following spring. Eight compounds were studied during a 3-year period. All compounds were applied to the nets at a rate of 0.5 gm/gm net weight and 3 of these compounds (Ent 22542, Ent 20573, Ent 20297) were tested at 0.25 gm/gm net weight. The compounds studied included:

- Ent 22542, *N,N*-Diethyl-*meta*-toluamide (deet standard)
- Ent 15510, 2-[2-(2-Butoxyethoxy)ethoxy]ethyl 3-methylcrotonate
- Ent 19083, *o*-Ethoxy-*N,N*-dipropylbenzamide
- Ent 20364, 1-(*o*-Ethoxybenzoyl)piperidine
- Ent 20830, Diisopentyl malate
- Ent 20573, 2-[(*p*-Methoxybenzyl)oxy]-*N,N*-dipropylacetamide (contains 25% *ortho* and 75% *para* isomers)
- Ent 20297, *o*-Ethoxy-*N,N*-diethylbenzamide
- M-1960, mixture composed of 30% benzyl benzoate, 30% *N*-butylacetanilide, 30% 2-butyl-2-ethyl-1,3-propanediol and 10% emulsifier.

RESULTS AND DISCUSSION

Gouck et al. (1967a) established the criterion for effective mosquito protection as a 90% reduction of mosquito penetration through treated netting as compared with untreated netting, based on 2 successive tests. This proved to be a valid method of measuring the actual effectiveness of the chemical compounds in our study. However, in field studies using untreated wide-mesh bed nets, about 87% protection was achieved when compared with an outside control (TABLE 2). Thus, in our field studies, mosquito repellency was occurring from 2 sources. The total protection (average percent effectiveness) of the repellent/net combination was established by comparing mosquitoes collected from an outside control with each repellent net combination. The data on the deet standard (Ent 22542) demonstrate the difference in the comparisons (TABLE 2). The average total effectiveness of the repellent net combination was 98%, but the efficacy of the chemical treatment fell below 90% after 30 days. A similar test was conducted on deet at 0.25 gm repellent/gm net weight, and the average total effectiveness of the

TABLE 1. Mosquito species encountered during repellent-treated wide-mesh netting tests in North Carolina

<i>Aedes</i>	<i>atlanticus</i> Dyar and Knab <i>canadensis</i> (Theobald) <i>cinereus</i> Meigen <i>fulvus fallens</i> Ross <i>irrfirmatus</i> Dyar and Knab <i>solicitans</i> (Walker)* <i>tacniorhynchus</i> (Wiedemann)* <i>texans</i> (Meigen)
<i>Psorophora</i>	<i>ciliata</i> (Fabricius) <i>confinis</i> (Lynch-Arribalzaga) <i>ferox</i> (Humboldt)
<i>Anopheles</i>	<i>barberi</i> Coquillett <i>crucians</i> Wiedemann <i>quadrimaculatus</i> Say
<i>Culex</i>	<i>erraticus</i> (Dyar and Knab) <i>nigripalpus</i> Theobald <i>pipiens quinquefasciatus</i> Say <i>salinarius</i> Coquillett

**A. tacniorhynchus* is the predominant species at Camp Lejeune, constituting 60–70% of the total population. *A. solicitans* constitutes 20–30% of the total population.

TABLE 2. Results of 8 repellents tested on 4-mesh bed nets when exposed to field mosquito populations.

REPELLENT	DAYS OF EFFECTIVE CHEMICAL PROTECTION ¹	AVER. NO. MOSQUITOES COLL./NET/ NIGHT ²	AVER. NO. MOSQUITOES COLL./NIGHT ON OUTSIDE CONTROL	AVER. % EFFECTIVENESS OF REPELLENT/NET COMBINATION	DAYS FROM TREATMENT TO LAST TEST	NET TYPE ³
M-1960 ⁴	30	3.9	165.2	97.6 (2) ⁵	359 ⁶	KC
Ent 15510	30	2.6	165.2	99.4 (9)	359 ⁶	KC
Ent 19083	787	0.1	126.4	99.9 (27)	787	KC
Ent 20297	50	0.1	98	99.9 (9)	50	PC
Ent 20364	359	1.4	165.2	99.2 (9)	359 ⁶	KC
Ent 20573	50	0	98	100.0 (9)	50	PC
Ent 20830	23	3.2	165.2	98.0 (9)	359 ⁶	KC
Ent 22542	30	3	165.2	98.0 (9)	359 ⁶	KC
Check	0	16.9	126.4	86.6 (27)	0	KC
Check	0	13.9	107.9	87.1 (18)	0	PC

¹Day when effectiveness of the chemical decreased to 90% or less as compared to untreated net.

²69% of the mosquitoes collected inside of the nets were *A. taeniorhynchus*, 30% were *A. sollicitans*, and 1% were other species.

³KC = knotted cotton; PC = 50% polyester, 50% cotton.

⁴Standard military clothing repellent.

⁵Total number of weekly tests shown in parentheses.

⁶Test net dropped from study.

repellent/net combination was 97% for 6 weeks, but the chemical treatment failed on the thirty-eighth day. Ent 15510, 20330, and M-1960 impregnated nets were in the same test and they also exhibited high levels of total protection but provided less than 90% effective chemical protection after 30 days. Ent 20830 was ineffective by the twenty-third day. Ent 20364 did not fail during a 359-day test period.

Ent 20573 and 20297 were placed in the test series in 1970 and did not fail during the test period. Ent 19083 was placed in the test program in 1968 and the repellent/net combination has had only 3 mosquito penetrations in 27 weeks of testing. The 3 penetrations occurred during the first 2 tests in 1968. Ent 20573 and 20297 were also tested at 0.25 gm/gm net weight. Both compounds provided 100% treatment effectiveness for 50 days and were retained in the study.

Most of the field data correlated well with the screening data obtained by Gouck et al. (1967a, b). However, M-1960 failed after 30 days in this study as compared to 213 days reported by Gouck et al. (1971). Ent 20830 failed in 23 days as compared with 36-118 days (Gouck et al. 1967a, Gouck et al. 1971). Since the present tests were conducted at different times of the year against mixed species of mosquitoes, variation was anticipated. Different fabrics were also used and may have accounted for some of the variation. The study demonstrated that most of the compounds previously screened for their effectiveness in Florida performed similarly under field conditions in North Carolina.

Possibly the most important data obtained relate to the high percentage of protection obtained be-

cause of the natural repellency of the netting. This was evidenced by deet which failed to provide 90% effectiveness after 30 days, while the combination provided 98% protection in excess of 300 days. Considering present production and development costs, toxicity requirements, and general research costs, deet may have definite advantages over newer compounds regardless of its effective life. The authors had anticipated that under actual field conditions, the use time of a net would be limited to the effective life (90% level) of the chemical. Because of the natural repellency of the netting, it will be necessary to determine the life of production items by studying the total effectiveness of various chemical/net combinations. This should greatly enhance the potential of wide-mesh netting for vector protection.

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