REPELLENCY OF TWO CONTROLLED-RELEASE FORMULATIONS OF DEET AGAINST *ANOPHELES QUADRIMACULATUS* AND *AEDES TAENIORHYNCHUS* MOSQUITOES[1]

C. E. SCHRECK AND D. L. KLINE

U.S. Department of Agriculture, Agricultural Research Service, Insects Affecting Man and Animals Research Laboratory, P.O. Box 14565, Gainesville, FL 32604

ABSTRACT. Two experimental controlled-release repellent formulations containing 35% (3M) and 44% (Biotek) deet, respectively, were compared with a 75% standard formulation of deet used by the military. The military repellent was equal to or significantly better than the formulations in duration of protection against 2 mosquito species in laboratory and field tests, but the formulations contained only 47–59% the amount of deet in the military repellent. In all cases high levels of protection (>95%) were measured, but because of high densities of biting mosquitoes in field tests, this level did not necessarily indicate few bites. In terms of mean bites/test/day by *Anopheles quadrimaculatus* in caged tests, the military repellent had fewest (2) and Biotek had most (41).

INTRODUCTION

In an attempt to extend the duration of protection of the repellent deet (*N,N*-diethyl-3-methylbenzamide, formerly *N,N*-diethyl-meta-toluamide and other isomers), 2 new repellent formulations containing this compound as the active ingredient were developed under contract with the U.S. Army Medical Research and Development Command by independent sources using controlled-release technology. The formulations were intended not only to provide longer protection from bites (≥12 hr), but to be more acceptable cosmetically and easier to apply than the military repellent currently in use.

This paper reports the results of 1) a field study comparing the formulations against natural populations of *Aedes taeniorhynchus* Wiedemann and 2) a laboratory-based study to evaluate and compare the controlled-release repellent formulations with the existing military repellent (75% deet in ethanol) against *Anopheles quadrimaculatus* Say.

MATERIALS AND METHODS

Bioassays of experimental controlled-release repellent formulations against natural populations of *Aedes taeniorhynchus* and caged populations of laboratory-reared *Anopheles quadrimaculatus* were performed using ASTM standard methodology (Anonymous 1983).

Field study. Field tests were conducted against *Ae. taeniorhynchus* at Everglades National Park, Flamingo, Florida. In these tests, 5 participants wore protective clothing including head nets, long sleeved shirts, long pants and field-type footwear. One gram or milliliter of a candidate formulation was applied evenly to the forearm (wrist to elbow) and 1.5 gm or ml to the leg (ankle to knee). Preliminary laboratory tests showed that this amount of repellent formulation completely covered the forearm or leg with a generous but not excessive film of each of the formulations regardless of the arm size of the test participants. Two candidate repellent formulations, one containing 35% deet produced by Personal Care Products, 3M Center, St. Paul, MN, and the second containing 44% deet produced by Biotek, Inc., Woburn, MA, were compared to each other and with equal amounts of the currently used U.S. military all-purpose repellent formulation of 75% deet and 25% ethanol (U.S. National Stock No. 6840-00-753-4963). Volunteers were assigned a randomized sequence of treatments on each limb. Three limbs received a repellent treatment and the fourth limb served as an untreated check. Thus, to eliminate subject and application site differences, volunteers had different combinations applied to the limbs each test day. Participants were cautioned not to rub their treated arms or legs on their clothing or on any other absorbent or abrasive surface. The sleeve or trouser leg of the untreated limb of each volunteer was rolled down except during periodic exposures when timed biting collections were made to determine mosquito attack rates.

Repellents were applied at 0600 hr, and the bioassays were conducted from 0700 to 0900 hr until 1830–1930 hr, weather permitting. Throughout the day participants exposed their

[1] This paper reports the results of research only. Mention of a pesticide does not constitute a recommendation for use by the U.S. Department of Agriculture, nor does it imply registration under FIFRA as amended. Human volunteers who participated in this study gave their free and informed voluntary consent.

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treated arms and legs to natural populations of mosquitoes (>95%) *Ae. taeniorhynchus*. During each exposure period (>12.5 hr), participants circulated in the test area and each recorded the number of bites, if any, on the treated skin of their arms and legs. At hourly intervals throughout the day, the untreated arm or leg of a participant was exposed for 1 min or less, and the number of mosquito bites was recorded. Thus, 10–12 biting counts were recorded by each participant through each day to determine whether there were changes in biting activity.

Outdoor cage study. Failure to find a sufficient number of *An. quadrimaculatus* for field evaluations of the candidate formulations necessitated the use of an outdoor screened cage at Gainesville, Florida, 18.3 × 9.1 × 6.1 m (60 × 30 × 20 ft) high, into which ca. 15,000 laboratory-reared *An. quadrimaculatus* were released. Six volunteers participated in this study and wore protective clothing and head nets as indicated above. Repellents were assigned and applied in the manner described for the field tests, except that the repellents were applied at 0730 hr or 0830 hr and were tested during three 90-min periods (morning, afternoon and evening) until 1945 or 2045 hr. Folding chairs were located 9 m apart on the inside perimeter of the cage—one in each corner and one on each long side equidistant from 2 corners. Volunteers rotated their positions clockwise every 15 min until they had occupied each of the 6 positions for a total of 90 min. At the end of a 90-min test session, the participants left the cage for 2.5 hr (morning) or 3.0 hr (afternoon), after which time they reentered the cage to resume testing.

On the first day, approximately 6,000 avid female *An. quadrimaculatus* were released into the enclosure 1 hr prior to testing. Subsequent releases of approximately 4,000, 3,000 and 2,000 mosquitoes were made on each succeeding day.

Volunteers recorded bite data individually as described earlier. Biting counts on untreated skin were made during each of the three 90-min exposure periods throughout the day and evening.

Data gathered from both field and outdoor cage tests were analyzed to determine 1) duration of complete protection from bites, 2) overall percentage of protection for the entire test period and 3) the total number of bites recorded on each repellent for all tests.

Duration of protection is defined in ASTM document E939-83 as "complete protection time (CPT)—the time from application of the repellent to the time of the first confirmed bite (a 2nd bite by the same species within 30 min of the first)." The CPT data were compared using an ANOVA with a Waller-Duncan multiple range test for differences between means at the 0.05% level of significance.

The percentage of protection from biting for 12 hr or more was determined by the following formula:

$$C = \frac{A}{B}$$

$$\% \text{ protection} = \frac{D-C}{D} \times 100$$

where:

A = total bites on treatment
B = min of exposure to bites
C = bites/min on treatment
D = bites/min on untreated check

RESULTS

The duration of protection data are summarized in Table 1. The 75% deet had a higher CPT (8.2–12.3 hr) than either of the candidate repellent formulations (7.2–11.5 hr) against both mosquito species. Against *Ae. taeniorhynchus* the data showed the 75% deet to be of significantly (0.05% level) longer duration (8.2 hr) in protection from bites than the Biotek product (7.2 hr) and equal to the 3M product (7.6 hr). Against *An. quadrimaculatus*, the data indicated a significantly longer duration of protection (12.3 hr) for the 75% deet over Biotek (10.9 hr) but was not significantly different from 3M (11.5 hr). Candidate repellent formulation and 75% deet comparisons of the percentage of protection from biting in terms of time after treatment are given in Table 2. Against *Ae. taeniorhynchus*, percent protection data were not significantly different and indicated it was not possible to differentiate between treatments even after 13 hr of aging because protection was >98% for all 3 repellents. Although the CPT ranged from 7.2 to 8.2 hr for the 3 formulations, the biting rate on untreated skin (check) was sufficiently high (115–248 bites/min, Table 3) to make the num-

Table 1. Duration of complete protection time (CPT) provided by 2 candidate extended duration formulations containing deet and the military repellent (75% deet) when tested against *Aedes taeniorhynchus* and *Anopheles quadrimaculatus*.

Formulation	% deet	Mean CPT in hours: *Ae. taen.* (field) 20 reps	Mean CPT in hours: *An. quad.* (cage) 24 reps
75% deet	75	8.2 A	12.3 A*
3M	35	7.6 AB	11.5 AB
Biotek	44	7.2 B	10.9 B

*Means with the same letter are not significantly different (0.05% level of confidence; ANOVA with a Waller-Duncan multiple range test).

ber of bites in the treatments appear inconsequential.

Cage test data from assays with the repellents against *An. quadrimaculatus* (Table 2) were similar in percentage of protection against bites to those from the *Ae. taeniorhynchus* field data, and analysis showed there were no statistical differences between treatments until 12 hr after treatment when the Biotek formulation was significantly less effective (0.05% level of confidence; ANOVA) than both the 3M and the 75% deet repellents. However, as before, the biting rate on untreated skin was sufficiently high (means of 6–30 bites/min, Table 3) relative to the numbers biting the treated skin, making it appear there was a high degree of protection from bites. Table 4 compares the overall percentage protection from bites with the total bites recorded in 24 tests. It is evident that a high percentage of protection does not necessarily guarantee few bites. Significantly fewer total bites were observed with the 75% deet standard than with the other formulations against *An. quadrimaculatus*, but not so against *Ae. taeniorhynchus*.

DISCUSSION

The purpose of these assays was to identify the best overall repellent formulation. Three measures of efficacy were made: 1) duration of complete protection from bites, 2) overall protection during a >12-hr day and 3) total number of bites on repellent-treated skin. Of the 3, duration of complete protection was the most useful for determining whether any of the formu-

Table 2. Protection from bites as it relates to time after treatment with each candidate extended duration of 2 repellent formulations and the military repellent (75% deet) when tested against *Aedes taeniorhynchus* and *Anopheles quadrimaculatus*.

Hr after treatment	% protection from bites at indicated hour after treatment					
	Ae. taeniorhynchus (field)[a]			*An. quadrimaculatus* (cage)[b]		
	3M	Biotek	75% deet	3M	Biotek	75% deet
1	–	–	–	100.0	100.0	100.0
2	–	–	–	100.0	99.9	100.0
3	–	–	–	100.0	99.9	100.0
4	99.9	99.9	99.9	–	–	–
5	99.9	99.9	100.0	99.9	99.9	100.0
6	99.9	100.0	100.0	100.0	99.3	100.0
7	99.9	99.9	99.9	99.9	99.1	99.9
8	99.6	99.3	99.9	–	–	–
9	99.6	99.5	99.6	–	–	–
10	99.2	99.0	99.5	100.0	99.9	100.0
11	99.3	99.1	99.1	98.5	96.5	99.9
12	99.5	99.2	99.2	98.5	96.1	99.9
13	99.0	98.8	99.8	99.2	95.3	99.9

[a] Means of 20 tests; not significantly different (0.05% level of confidence; ANOVA).
[b] Means of 24 tests; not significantly different (0.05% level of confidence; ANOVA) until 12 hr after treatment when the Biotek formulation was significantly less effective than both the 3M and the 75% deet repellents.

Table 3. Mean number of bites/min by indicated mosquito species on untreated skin during field and outdoor cage evaluations of 3 repellent formulations.

Hr after tests began	*Ae. taen.* (field)			*An. quad.* (cage)		
	Mean	Range	No. reps	Mean	Range	No. reps
1	–	–	–	–	–	–
2	–	–	–	29.7	4–108	18
3	–	–	–	19.1	1–125	24
4	138.1	11–500	20	19.3	2–57	6
5	152.0	24–400	20	–	–	–
6	115.2	20–600	20	8.5	0–58	18
7	140.8	12–600	20	6.5	0–34	24
8	130.9	4–348	20	5.5	1–13	6
9	215.4	40–800	20	–	–	–
10	160.4	16–620	20	–	–	–
11	221.3	5–660	20	19.4	0–100	24
12	247.9	2–600	20	8.8	0–27	24
13	145.9	52–344	15	8.8	0–31	12

Table 4. Total bites, percentage of protection from bites and mean number of bites/test/day recorded for 2 candidate repellent formulations and 75% deet against *Anopheles quadrimaculatus* in an outdoor cage (data based on 24 assays on skin of arms and legs of 6 test volunteers) and against *Aedes taeniorhynchus* in the field (20 assays on skin of arms and legs of 5 test volunteers).

	Aedes taeniorhynchus (field)				*Anopheles quadrimaculatus* (cage)			
	Repellent formulations			Untreated skin	Repellent formulations			Untreated skin
Measurement	3M	Biotek	75% deet		3M	Biotek	75% deet	
% protection from bites	99.9	99.8	99.9	0.0	98.5	95.1	99.4	0.0
Total bites all tests[a]	2,772 A	3,046 A	2,679 A	398,400[b]	450 A	980 A	36 B	30,000[b]
Mean number of bites/test/day	139 A	152 A	134 A	124,125[c]	19 A	41 A	2 B	3,753[d]

[a] Horizontal figures with the same letter are not significantly different (0.05% level of confidence, ANOVA).
[b] Theoretical estimate of the number of bites that could have been recorded on all volunteers for all tests had they been without protection.
[c] Mean number of bites on untreated skin of 5 volunteers based on 200 1-min biting counts/person taken over 4 days.
[d] Mean number of bites on untreated skin of 6 volunteers based on 168 1-min biting counts/person taken over 4 days.

lations provided >12 hr of protection against bites. The 75% deet was the only formulation to give >12 hr of CPT in tests against *An. quadrimaculatus*. The second most useful measure was total number of bites. When differences in CPT were not significant, total bites provided an indication of a rapid or a slow decline in effectiveness after the CPT was established. Against both species the deet standard had fewer bites than the candidate formulations. The percentage of protection did not make it possible to differentiate between 3 M, Biotek or the deet standard. Obviously when biting rates are at low levels, the calculated percentage of protection will be high, and is not a dependable indicator of efficacy. If this were the sole measure of protection from bites of a disease vector, it could give a false sense of security with regard to infection.

Of the 2 candidates, the 3M product had fewer overall bites (9%), averaged slightly longer in duration of protection and provided a significantly higher percent protection after 12 hr against *An. quadrimaculatus*.

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