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A comparison of the mosquito-repelling efficacy of methyl neodecanamide (MNDA) to that of Deet

THOMAS G. POLEFKA, LI-JUNG LIANG, and
TARACAD NARAYANAN ANANTHAKRISHNAN,
Colgate-Palmolive Company, 909 River Road, Piscataway, NJ 08854 (T.G.P., L.L.), and Entomology Research Institute, Loyola College, Madras-600 034, India (T.N.A.).

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Synopsis

The objective of this small pilot study was to assess the mosquito-repelling efficacy of methyl neodecanamide (MNDA) relative to N,N-diethyl-3-methylbenzamide (Deet) against *Anopheles stephensi, Culex quinquefasciatus,* and *Aedes aegypti* under controlled laboratory conditions. In this study, subjects inserted both forearms (sham- and repellent-treated) into a test chamber containing 50 female mosquitoes. The number of mosquitoes landing on or probing each forearm during the 5-minute "forced-choice" test session was recorded each hour for a total of eight hours. Effectiveness was calculated according to Abbott's formula. Experimental results indicate that topical application of 1% MNDA provided significantly better protection and a broader spectrum of repellency than application of 1% Deet against the three species of mosquitoes. These promising results support further study of MNDA as a topical mosquito repellent.

INTRODUCTION

In some parts of the world, biting insects are nothing more than an annoyance to individuals involved in outdoor activities (1). However, in other parts of the world, mosquitoes and flies represent a major health concern since they are key contributors to the spread of serious diseases such as malaria, filariasis, dengue fever, leishmaniasis, and trypansomiasis (2).

Currently, N,N-diethyl-3-methylbenzamide (Deet) is the most effective and widely used topical insect repellent (3). However, excessive application of this popular insect repellent by consumers may result in serious adverse health effects, such as disorientation, toxic encephalopathy, insomnia, and irritability (4). Indeed, several cases of death have been reported (5). In spite of these adverse reactions, it is unlikely that Deet will be withdrawn from the marketplace anytime soon.

Recently, methyl neodecanamide (MNDA) was shown to be a highly effective cockroach repellent (6,7). Because MNDA and Deet are chemically similar (i.e., both are secondary amides), we decided to assess the mosquito-repelling efficacy of MNDA (relative to

Address all correspondence to Thomas G. Polefka.

Deet) under controlled laboratory conditions against *Anopheles stephensi, Culex quinquefasciatus,* and *Aedes aegypti.*

MATERIAL AND METHODS

Reagent-grade Deet and isopropyl alcohol were purchased from Aldrich Chemical Company (St. Louis, MO). MNDA was prepared by Parrmal Industries (Cuernavaca, Mexico) according to the method described by Steltenkamp *et al.* (6).

EXPERIMENTAL

Uninfected *A. stephensi, C. quinquefasciatus,* and *A. aegypti* were employed in this study. The eggs were hatched, and larvae were reared in muslin-covered plastic trays (50 cm × 19 cm × 15 cm) containing fresh tap water supplemented with a mixture of yeast and dog biscuit. This mixture was changed daily. Pupae were transferred with the aid of a dropper to plastic boxes covered with mosquito netting. Upon emerging, the male mosquitoes were removed and the female mosquitoes were maintained on a diet of 10% sucrose for 4–10 days. Feeding was suspended 24 hours prior to the start of the test.

After giving informed consent, eight healthy adult males (ages 26–56 yrs) were enrolled in the study. However, because only four mosquito test chambers were available for each test session, only four subjects participated on a given test day. For convenience, one species of mosquito was bred at a time and used to complete the evaluation of both repellents. Three separate test sessions, separated by a two-day washout period, were used for each repellent. Finally, to prevent the repellents from interfering with each other, only one repellent was tested during a test session.

To establish a uniform level of cleanliness, the volunteers washed their hands and forearms for one minute with unfragranced soap. The experiment was initiated when a gloved assistant applied 2 ml of the insect repellent (1% Deet or MNDA in isopropyl alcohol) to a randomly assigned hand/forearm and distributed it over the entire skin surface. The contralateral hand/forearm received the vehicle. After ten minutes (to permit evaporation of the isopropyl alcohol), each volunteer inserted his hands into a test chamber (31 cm × 31 cm × 31 cm) containing 50 nulliparous, 4–10-day-old, female mosquitoes (8). A different assistant, unaware of treatment assignments, recorded the number of insects landing/probing per five-minute test session. However, to prevent the mosquitoes from completing their feeding, they were shaken off (8). Once every hour, for a total of eight hours, the hands and arms were re-inserted into the insect chamber and exposed to the insects for five minutes. Between the challenges, the volunteers were permitted to perform light clerical duties but were restricted from washing their hands. Following the completion of each eight-hour test, the volunteers washed their hands/forearms with soap to remove residual insect repellent.

Since the three mosquito species exhibit vastly different feeding schedules (9), the experiments were conducted during species-specific peak feeding periods, namely, 18:00 for *A. stephensi,* 20:00 for *C. quinquefasciatus,* and 07:00 for *A. aegypti.* Although *A. stephensi* is recognized as a nocturnal feeder (9), the species used in this study was crepuscular, showing marked activity at dusk. Similar findings were reported by Reisen and Aslamkham (10) for *A. stephensi* in Pakistan.

The effectiveness of each repellent versus the vehicle control was calculated according to Abbott's formula (11):

$$\text{Percent effectiveness} = \frac{C - T}{C} \times 100$$

where C represents the number of mosquitoes biting/probing the vehicle-treated hand/forearm and T the number of insects biting/probing the repellent-treated hand/forearm during the five-minute challenge session. Percent effectiveness was compared between MNDA and Deet at each time point using Student's paired t-test ($\alpha = 0.05$). A Shapiro-Wilks test indicated that the data, in almost all cases, were normally distributed.

RESULTS AND DISCUSSION

Table I summarizes the results of the "forced-choice" repellency experiment. Each data point represents the mean of three separate trials involving four subjects. Almost 60% of the available *A. stephensi* and *C. quinquefasciatus* landed on or probed the subjects' skin within the five-minute challenge period. For *A. aegypti,* only 40% of the mosquitoes participated in the blood meal search. Although the number of mosquitoes landing or probing remained relatively constant during the first three to four hours, thereafter the numbers of mosquitoes involved decreased substantially, regardless of species. Repletion and adaptation to the stimulus are likely explanations for the diminished activity. Nevertheless, we believe that the "forced-choice" test with a fixed population of mosquitoes, particularly during the early stages of the experiment (i.e., $\leq$4 hours) where 20 or more mosquitoes are involved, provides a reasonable test of a mosquito repellent's efficacy.

The mosquito-repelling efficacy of MNDA and Deet relative to the alcohol vehicle is plotted in Figure 1 as a function of time. Both repellents provided 100% protection

Table I
Number of Mosquitoes Landing on or Probing Repellent/Vehicle-Treated Forearms During Five-Minute Exposure Period*

	Number of mosquitoes landing or probing (Repellent-treated skin/vehicle-treated skin)					
	A. stephensi		*C. quinquefasciatus*		*A. Aegypti*	
Time (hr)	MNDA	Deet	MNDA	Deet	MNDA	Deet
1.0	0/29	0/31	0/28	0/29	0/19	1/23
2.0	0/24	0/29	0/27	0/26	0/18	5/22
3.0	0/26	2/26	0/27	1/25	1/17	6/21
4.0	0/23	4/27	1/23	3/23	1/18	7/16
5.0	1/20	4/24	1/21	3/20	2/15	10/14
6.0	1/19	6/23	2/16	4/19	7/15	13/13
7.0	1/20	7/21	2/13	3/15	8/13	12/14
8.0	2/17	7/19	2/11	3/13	9/12	12/13

* Results represent the means of three separate trials (four subjects per trial). Test chambers were charged with 50 mosquitoes.

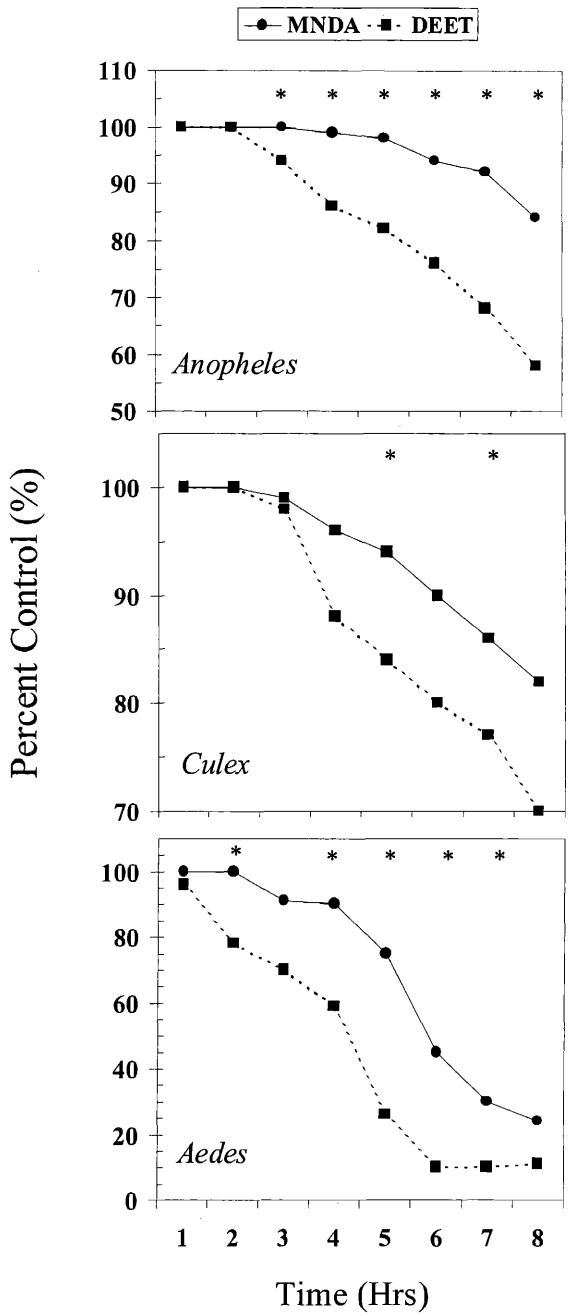

Figure 1. Efficacy of MNDA (——) and Deet (- - - - -) as a function of time versus *Anopheles stephensi, Culex quinquefasciatus,* and *Aedes aegypti.* Each data point represents the global mean derived from three trials with four subjects per trial. Asterisk denotes statistical significance ($p \leq 0.05$) as determined by Student's paired *t*-test. Percent effectiveness = $(C - T)/C \times 100$.

against *A. stephensi* and *C. quinquefasciatus* for the first two hours. However, only MNDA provided 100% protection against *A. aegypti* during the same period. In terms of persistency, both repellents begin to lose efficacy approximately two to four hours after

Table II
Comparative Level of Protection Afforded by 1% Repellent Versus Test Organisms

	Time of protection after application (hr)					
	C. quinquefasciatus		*A. stephensi*		*A. aegypti*	
Level of protection (%)	Deet	MNDA	Deet	MNDA	Deet	MNDA
100	3	3	2	5	0	2
≤90	3	4	2	5	1	2
≤50	8	8	7	8	3	5

application. Although several explanations for diminished repellency are possible (i.e., penetration into the skin, repletion and/or adaptation by the mosquitoes), we believe low application rate (≈25 μg/cm^2) is the dominant reason. Indeed, Maibach *et al.* (12) have reported that the minimum effective dose (MED) for Deet is approximately 16 μg/cm^2. The decision to test 1% repellent in these studies was dictated by the limited toxicity data available to support exposing humans to higher concentrations of MNDA. Nevertheless, the data summarized in Table II supports the efficacy of MNDA and provides impetus to complete the costly toxicity studies necessary to allow MNDA to be tested at levels comparative to that found in products containing Deet.

CONCLUSION

This pilot clinical study showed that 1% MNDA is significantly better than 1% Deet in terms of all measured parameters: fewer landings/probes, greater persistence, and a greater spectrum of repellency.

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REFERENCES

(1) N. R. Read, J. R. Rooker, and J. P. Gathman, Public perception of mosquito annoyance measured by a survey and simultaneous mosquito sampling, *J. Am. Mosq. Control Assoc.*, 10, 79–87 (1994).
(2) World Health Organization, *http://www.ciesin.org/docs/001-613/001-613.html* (2001).
(3) M. Brown and H. A. Adelaide, Insect repellents: An overview, *J. Am. Acad. Dermatol.*, 36, 243–249 (1997).
(4) H. Qui, H. Won Jun, and J. W. McCall, Pharmacokinetics, formulation and safety of insect repellent N,N-diethyl-3-methylbenzamide (DEET): A review, *J. Am. Mosq. Control Assoc.*, 14, 12–27 (1998).
(5) T. G. Osimitz and R. H. Grothaus, The present safety assessment of DEET, *J. Am. Mosq. Control Assoc.*, 11, 274–278 (1995).
(6) R. J. Steltenkamp, R. L. Hamilton, R. A. Cooper, and C. Schal, Alkyl and aryl neoalkanamides: Highly effective insect repellents, *J. Med. Entomol.*, 29, 141–149 (1992).
(7) K. M. Kinscherf, R. J. Steltenkamp, T. F. Connors, and C. Schal, Benefits of cleaning products

containing the repellent methyl neodecanamide against *Blattella germanica* (L.), *Intl. Pest Control,* **38,** 88–91 (1996).
(8) C. F. Curtis, J. D. Lines, J. Ijumba, A. Callaghan, N. Hill, and M. A. Karimzad, The relative efficacy of repellents against mosquito vectors of disease, *Med. Vet. Entomol.,* **1,** 109–119 (1987).
(9) M. Bates, *The Natural History of Mosquitoes* (Macmillan, New York, 1949), pp. 17–22.
(10) W. K. Reisen and M. Aslamkhan, Biting rhythms of some Pakistan mosquitoes (Diptera:Culicidae), *Bull. Entomol. Res.,* **68,** 313–330 (1978).
(11) W. S. Abbott, A method of computing the effectiveness of an insecticide, *J. Am. Mosq. Control Assoc.,* **3,** 302–303 (1987).
(12) H. I. Maibach, A. A. Kan, and W. Akers, Use of insect repellents for maximum efficacy, *Arch. Dermatol.,* **109,** 32–35 (1974).