Journal of the American Mosquito Control Association, 12(2):243–246, 1996

EVALUATION OF A EUCALYPTUS-BASED REPELLENT AGAINST *ANOPHELES* SPP. IN TANZANIA

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ABSTRACT. A eucalyptus-based insect repellent (PMD) with the principal active ingredient *p*-menthane-3,8-diol was evaluated in the field in comparison with deet. In human landing catches in Tanzania, 3 formulations of PMD were tested against *Anopheles gambiae* and *An. funestus.* Repellents, applied to the legs and feet at doses chosen as used in practice, gave complete protection from biting for between 6 and 7.75 h, depending upon the formulation type, with no significant difference between PMD and deet in terms of efficacy and duration of protection.

INTRODUCTION

Repellents have long been used in protection against biting insects, with the main motivation being avoidance of nuisance. However, through their reduction in man–vector contact, repellents can also be regarded as important tools in the prevention of vector-borne disease.

For travellers to tropical countries, malaria continues to pose a serious health risk; in the United Kingdom in 1994 there were 1,887 imported cases of malaria, 11 of which resulted in death (Anonymous 1994[1]). The rapid spread of resistance to antimalarial drugs has increased the importance of supplementary preventative measures. The World Health Organization (1995) currently advises "all travellers should be told that protection from biting mosquitoes is their first line of defence against malaria". The armory of personal protection includes the use of bed nets, suitable clothing, and repellents. Since 1957, the most commonly used insect repellent formulations have contained diethyl methyl benzamide (deet), which is effective against a broad spectrum of insects. There are disadvantages associated with the use of deet, which stem principally from its activity as a solvent of paints, varnishes, and some plastics and synthetic fabrics. There have also been concerns over the toxicity of deet (Miller 1982, Roland et al. 1985), although serious adverse effects are rare (Veltri et al. 1994, Osimitz and Grothaus 1995).

In the search for effective alternatives to deet there has been much interest in natural plant extracts (Opoku et al. 1986, Curtis et al. 1990). The eucalyptus-derived repellent quwenling was reported to have largely displaced the commercial market for di-methyl phthalate in China (Curtis et al. 1990), although it was reported to be somewhat less effective than deet (Schreck and Leonhardt 1991, Collins et al. 1993). Quwenling is made from the waste distillate after extraction of oil from the lemon eucalyptus plant (*Eucalyptus maculata citriodon*) and the principal active component is *p*-menthane-3,8-diol (Schreck and Leonhardt 1991).

A preliminary laboratory evaluation of a new repellent, PMD, similar to quwenling (Trigg and Hill 1996), reported this compound to be almost as effective as deet against *Anopheles gambiae* Giles and also to be effective against midges, ticks, and the stable fly. The repellent (trade name Mosi-guard Natural, MASTA, London, United Kingdom) is produced utilizing lemon eucalyptus oil itself, via an extraction process developed at University College, London. The active component (50%) is principally *p*-menthane-3,8-diol with additional isopulegol and citronellol and the repellent is formulated as a patented mixture of isomers of each.

In this paper a field comparison of PMD with deet against 2 African malaria vectors, *Anopheles funestus* Giles and *An. gambiae* is reported.

MATERIALS AND METHODS

Study area: A field trial was undertaken in June 1995 in the village of Mkuzi, Tanga Region, Tanzania. The village has mostly wattle and daub houses and suitable larval habitats for *An. gambiae* and *An. funestus.*

Repellents and application: Three formulations of PMD (50% AI), a pump spray, a stick, and a gel, were evaluated for efficacy and longevity in comparison with a spray formulation of deet (50% AI, MASTA) against natural populations of mosquitoes. For each trial, 6 experienced insect collectors participated in human night-biting catches having been offered malaria prophylaxis and treatment with sulfadoxine pyrimethamine in the event of contracting malaria infection. On any one day, 2 subjects applied

[1] Anonymous. 1994. Annual malaria statistics report, 1994. Malaria Reference Laboratory, London (unpublished report).

Table 1. Total number of *Anopheles gambiae* and *An. funestus* collected by individual subjects with repellent-treated or untreated skin and protection time until first bite in minutes (PT) over a 3-day period for each PMD formulation tested in comparison with deet spray.

	PMD spray (0.8 g/leg) vs. deet spray (0.8 g/leg)					PMD stick (1.3 g/leg) vs. deet spray (0.8 g/leg)		
	Control	PMD spray		Deet spray		Control	PMD stick	
Person	No.	No.	PT	No.	PT	No.	No.	PT
A	35 (3)[1]	14 (2)	347	19 (1)	374	32 (3)	11 (2)	335
B	68 (1)	17 (2)	359	13 (3)	362	49 (1)	15 (2)	323
C	25 (3)	5 (1)	360	2 (2)	372	33 (3)	4 (1)	470
D	26 (2)	0 (1)	>540	11 (3)	355	36 (2)	9 (1)	357
E	43 (2)	1 (3)	447	0 (1)	>540	17 (2)	3 (3)	375
F	25 (1)	5 (3)	360	3 (2)	490	10 (1)	6 (3)	327
Mean	37.0	7.0	>402.2 (6 h, 42 min)	8.0	>415.5 (6 h, 55 min)	29.5	8.0	364.5 (6 h, 5 min)

[1] The day on which each test was done is shown in parentheses.

deet, 2 applied PMD, and 2 remained as untreated controls. Repellents were applied as evenly as possible to the legs and feet from the knee downwards. It was decided to assess the repellents as used in practice and the choice of dosage was therefore calculated on the basis of what the team members considered adequate to give even and comfortable coverage of their legs. The doses were determined by weighing the containers before and after repellent application and taking the average of all applications.

Repellent was applied 5 h prior to commencing the night catch after preliminary trials indicated that both deet and PMD gave at least 5 h repellency. In the interim period, subjects were careful to keep their clothing away from the treated skin.

Test procedure: In a method adopted from Curtis et al. (1987), subjects sat separately on benches or chairs spaced approximately 10 m apart for a period of 4 h each night from 2200 h until 0200 h. Using flashlights and test tubes they caught those mosquitoes that had landed on the skin and were clearly probing to feed before transferring them into labelled paper cups to be counted and identified later.

Subjects remained seated in the same position on 3 consecutive nights for each phase of the trial; treatments were rotated nightly. This meant that each subject experienced each treatment: a formulation of PMD, deet, and control; the rotation compensated for any positional differences in the number of mosquitoes, and personal differences in persistence of repellent, catching ability, and/or attractiveness to mosquitoes.

RESULTS AND DISCUSSION

The procedure outlined in the Materials and Methods section resulted in the repellents being applied at rates of 0.8 g/leg for PMD spray and deet spray, 2.0 g/leg for PMD gel, and 1.3 g/leg for PMD stick. The results of the night-biting catches are shown in Table 1. *Anopheles funestus* was the most common species biting during the study (69.3% of all mosquitoes collected), followed by *An. gambiae* (29.7%), and 1% other species. When analyzing these data, two likely sources of background variation were considered: 1) day to day variation in the number of mosquitoes caught by the controls, and 2) variation between persons in attractiveness to mosquitoes (Curtis et al. 1987)—a fact apparently illustrated in this data set by person B who was consistently a high scorer regardless of treatment. This was confirmed by a 2-way analysis of variance, allowing for treatments, which showed a highly significant between-person variation in the number of mosquitoes caught ($F = 10.32$, $P < 0.001$).

To compensate for these factors, the number of bites each day on individuals treated with repellent were first subtracted from the mean control catch on that day. Data were then analyzed using the nonparametric Wilcoxon signed rank test, pairing the corrected PMD and deet data for each person. This test was also applied to the time until first bite data (i.e., the time interval between repellent application and the first bite recorded), again pairing the deet and PMD data for each subject.

The result was a nonsignificant difference between PMD and deet both in terms of efficacy ($P > 0.05$) and longevity ($P > 0.05$) of repellency against total anopheline biting for each of the formulations of PMD tested.

At the chosen dose rates, all repellents tested provided greater than 6 h protection from biting.

Table 1. Extended.

PMD stick (1.3 g/leg) vs. deet spray (0.8 g/leg)		PMD gel (2.0 g/leg) vs. deet spray (0.8 g/leg)				
Deet spray		Control	PMD gel		Deet spray	
No.	PT	No.	No.	PT	No.	PT
17 (1)	388	27 (3)	2 (2)	415	9 (1)	420
31 (3)	342	37 (1)	10 (2)	354	37 (3)	320
5 (2)	444	30 (3)	0 (1)	>540	4 (2)	345
4 (3)	361	23 (2)	2 (1)	421	1 (3)	460
3 (1)	385	19 (2)	0 (3)	>540	0 (1)	>540
1 (2)	445	7 (1)	0 (3)	>540	11 (2)	400
10.2	394.2 (6 h, 34 min)	23.8	2.3	>468.3 (7 h, 48 min)	10.3	>414.2 (6 h, 54 min)

Although the gel formulation of PMD gave the longest mean protection time, it is likely that this was due to the application dose (chosen by the team) being the highest of all formulations at 2.0 g/leg. In contrast, where PMD spray and deet spray were compared at the same dose of 0.8 g/leg, the average number of bites and average protection time were very similar.

Between-species differences in sensitivity to the repellents may have been expected as this has been widely documented (Rutledge et al. 1978, Robert et al. 1991). However, a species by repellents chi-square test using the PMD and deet spray formulation data only (as these were applied at the same dose of 0.8 g/leg) showed that there was no difference in sensitivity to PMD and/or deet spray between *An. gambiae* and *An. funestus* ($\chi^2 = 0.005$, $P = 0.94$).

The present study and laboratory investigations (Trigg and Hill 1996) have demonstrated that PMD is an effective repellent against anopheline mosquitoes. As an effective repellent of the malaria vectors *An. gambiae* and *An. funestus*, use of PMD can be regarded as supplementary to bed nets and other measures such as screened windows in the armory of personal protection against the disease. The level and duration of protection by PMD is comparable to that afforded by deet. The repellent PMD has a lemon/menthol smell and, unlike deet, does not possess undesirable solvent properties. Acute toxicological studies have demonstrated minimal toxicity (oral LD_{50} 2,408 mg/kg and dermal LD_{50} >2,000 mg/kg in rats).

It is concluded that PMD is an effective alternative to deet with potential as a means of personal protection against mosquito vectors of disease.

ACKNOWLEDGMENTS

I thank Ali Mtango, Lucy George, William Chambika, Isaya Kibwana, and Stephen Mkongewa of Ubwari Field Station, Muheza, Tanzania, for their participation in the night catches. I am grateful to Jo Morris, Chris Curtis, and Nigel Hill for statistical advice and suggestions for the manuscript.

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