DO WRIST BANDS IMPREGNATED WITH BOTANICAL EXTRACTS ASSIST IN REPELLING MOSQUITOES?

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Summary

A wide range of insect repellent formulations, as well as active ingredients, are currently registered for use in Australia. While topical repellents are most common, there are also commercial products in the form of wristbands impregnated with botanical extracts that purport to repel mosquitoes. In laboratory tests, wristbands impregnated with peppermint oil were tested against the mosquito *Aedes aegypti* to determine their efficacy in repelling mosquitoes from the forearms of human volunteers compared with a commercial DEET-based topical repellent. The wristbands failed to stop landing by the mosquitoes, although the mean landing rate of mosquitoes was significantly lower on forearms in the presence of the wristband compared with untreated controls. The mean landing rate of mosquitoes on forearms treated with DEET was significantly lower than those of forearms in the presences of the wristband. The results indicated that while wristbands impregnated with botanical products may assist in repelling mosquitoes, their inability to completely protect individuals from mosquito bites suggests that they should not be recommended for use in areas of endemic or epidemic mosquito-borne disease.

Keywords. Mosquito repellent, *Aedes aegypti*, wrist band, personal protection measures

INTRODUCTION

Mosquito-borne diseases caused by Ross River virus (RRV), Barmah Forest virus (BFV), Murray Valley encephalitis virus (MVE) and dengue viruses are a major public health concern in Australia (Russell and Kay 2004). The first line of defence against these mosquito-borne diseases is the use of personal protection strategies that may include physical barriers such as bed nets and chemical barriers such as topical insect repellents (Fradin 1998, Frances and Cooper 2007). However, the efficacy of insect repellents and other personal protection strategies can be highly variable (Fradin and Day 2002, Goodyer *et al*. 2010), and there is a need for health authorities to provide the community with accurate information on the most reliable options.

There is a variety of commercial products currently available that are registered as insect repellents with the Australian Pesticides and Veterinary Medicines Authority (APVMA) for use against biting insects. Topical repellents are widely available, with the most effective active ingredients repeatedly shown to be diethyltoluamide (DEET), and picaridin (Fradin and Day 2002, Barnard and Xue 2004, Goodyer *et al.* 2010). Despite the widespread use of DEET-based repellents and the perceived potential to pose health risks (Osimitz and Grothaus 1995), very few adverse health impacts have been reported that have not resulted directly from ingestion, inhalation, exposure to the eyes or excessive application (Qui *et al*. 1998, Sudakin and Trevathan 2003, Goodyer *et al*. 2010). However, botanical based active ingredients are popular with sections of the community looking for alternatives to synthetic products, such as DEET, (Osimitz and Grothaus 1995) and are increasingly common in commercial topical insect repellents. The repellent properties of many plant essential oils, including citronella, eucalyptus, lavender and catmint, have been investigated in laboratory and field evaluations, and they generally offer substantially shorter periods of protection (Barnard 1999, Webb and Russell 2007, Thomas *et al*. 2009, Maguranyi *et al*. 2010).

Extracts from the plant peppermint (*Mentha piperita* L.) have been shown to have both repellent and larvicidal properties (Ansari *et al*. 2000) and, when tested as a topical lotion in combination with other botanical products, peppermint provided up to 55 minutes protection from biting mosquitoes (Fradin and Day 2002). When used as a topical repellent, peppermint oil has been shown to provide complete protection for times between 30 minutes (30% formulation) and 45 minutes (100% formulation) against *Aedes aegypti* L. (Barnard 1999) and when used in combination with other botanical active ingredients, provided between 0-30 minutes protection against *Aedes albopictus* (Skuse) (Barnard and Xue 2004).

Aside from topical applications of repellents, cloth wrist bands impregnated with repellent chemicals are promoted for mosquito protection, but there are few published studies reporting laboratory or field tests of wrist bands. DEET impregnated wrist bands have been shown to provide significant repellency against a range of *Aedes* spp. in North America but, while fewer landing mosquitoes were recorded when volunteers were wearing the wrist bands compared with those without wrist bands, the level of repellency provided by the bands was significantly lower by 20% than DEET topical repellent (Jensen *et al*. 2000). Testing of wrist bands impregnated with the essential oil citronella (25%) in laboratory trials indicated negligible protection times against *Ae. aegypti*, although a control comparison was not presented (Fradin and Day 2002).

The aim of this study was to assess the repellency and protection time provided by a wrist band impregnated with peppermint oil against *Ae. aegypti*, compared with a low dose DEET-based topical repellent in laboratory testing.

MATERIALS AND METHODS

Mosquito species

The mosquito species used in this study was *Ae. aegypti*, a known nuisance-biting pest and vector of dengue viruses commonly used for laboratory testing of mosquito repellents (Barnard *et al*. 2007). The mosquitoes were obtained from colonies raised in the Department of Medical Entomology, Westmead Hospital, and were maintained in controlled environmental conditions at 26.0 ± 2.0°C, relative humidity 65±10% under a photoperiod of 12 light:12 dark cycle with access to a cotton pad soaked in 10% sugar solution.

Mosquito repellents

Repellents used in this investigation were a 10% peppermint oil impregnated wrist band (Gone Insect Repellent, Goldgrade Corporation Pty Ltd, Howrah, Tasmania, Australia) and a commercial topical repellent formulation containing 6.98% DEET (Aerogard® Low Irritant, Reckitt Benckiser, West Ryde, Australia).

Test procedure

The trials were generally based on the methods used for testing topical repellents described in Frances *et al*. (2005a). Adult mosquitoes were held in screened cages at a stocking rate of 20 five- to seven-day old female mosquitoes per cage (20x30x40cm) with continuous access to 10% sucrose solution until 24 h prior to repellent testing. A total of three human volunteers were used in testing with volunteer used for each repellent (wrist band and topical) and control (untreated) test. Prior to repellent testing, forearms of volunteers were placed into a cage of mosquitoes and the number of mosquitoes landing within 1 min was recorded the ensure mosquitoes are sufficiently avid and that the mosquitoes are equally attracted to each volunteer. A minimum of 10 mosquito landings per minute was required for the individual to proceed with testing.

For repellent testing, the forearms (between wrist and elbow) of each volunteer were first divided into two sections of approximately 10cm by making small marks on the forearm with black ink. These sections of the forearm are referred to as either the lower forearm (between wrist and ink mark) or upper forearm (between ink mark and elbow). Each volunteer used one forearm per treatment (i.e. wrist band or topical repellent) or control (no wrist band or topical repellent used). Testing was conducted over three consecutive days with each volunteer tested using each of the three treatments.

While wearing gloves, a freshly opened wrist band was applied to the lower forearm (approximately 5cm from edge of glove). A new wrist band was used by each volunteer. For DEET application, while wearing gloves, 1.0g of repellent was applied evenly to the forearm (between wrist and elbow). Following the attachment of a wrist band or application of topical repellent, the glove was discarded.

Forearms, with either a wrist band, treated with topical repellent or control (i.e. untreated) were exposed to a cage of mosquitoes for 1 minute and the total number of landings (when a mosquito remained on the skin for more than 3 seconds) on the upper and lower section of the arm was recorded. Mosquitoes were brushed from the arm before they had an opportunity to bite or take a bloodmeal. On each day, each treatment, for each volunteer, was tested against mosquitoes on five occasions with a total of 10 min between each exposure of forearms to cages of mosquitoes.

Analysis

The mean landing rate of mosquitoes on the lower and upper forearms in the presence of either the wrist band, topical repellent or control were analysed using two way analysis of variance (ANOVA), and Fisher's least significant difference (LSD) test ($P<0.05$) was used to separate means.

RESULTS

In all replicate tests, mosquito landings were recorded on both upper and lower forearms upon exposure to *Ae. aegypti* in the presence of the wrist bands. Mosquitoes immediately commenced landing on forearms in the presence of wrist bands as soon as they were exposed to mosquitoes. However, there was a significant difference ($F=20.179$, $P<0.001$) in the mean landing rates of mosquitoes on lower forearms (2.67±0.55 mosquitoes per minute) compared with upper forearms (6.13±1.22 mosquitoes per minute) in the presence of the wrist bands (Figure 1).

The highest mean landing rates were recorded for the untreated control forearms, with no significant difference ($F=0.002$, $P=0.959$) between the landing rate on lower forearms (9.13±1.53 mosquitoes per minute) compared with upper forearms (9.26±1.92 mosquitoes per minute). The lowest mean landing rates were recorded for the forearms treated with a topical DEET repellent, with no significant difference ($F=0.152$, $P=0.699$) between the landing rate on lower forearms (0.13±0.23 mosquitoes per minute) compared with upper forearms (0.20±0.19 mosquitoes per minute).

When data for upper and lower forearms were combined, mosquitoes were only recorded landing on forearms treated with DEET on three of the 15 replicate tests with a mean landing rate of 0.3±0.08 mosquitoes per forearm while forearms with wrist band (mean landing rate of 8.8±3.3 mosquitoes per forearm) and untreated controls (mean landing rate of 19.4±5.3 mosquitoes per forearm) recorded landing mosquitoes on all 15 replicate exposures

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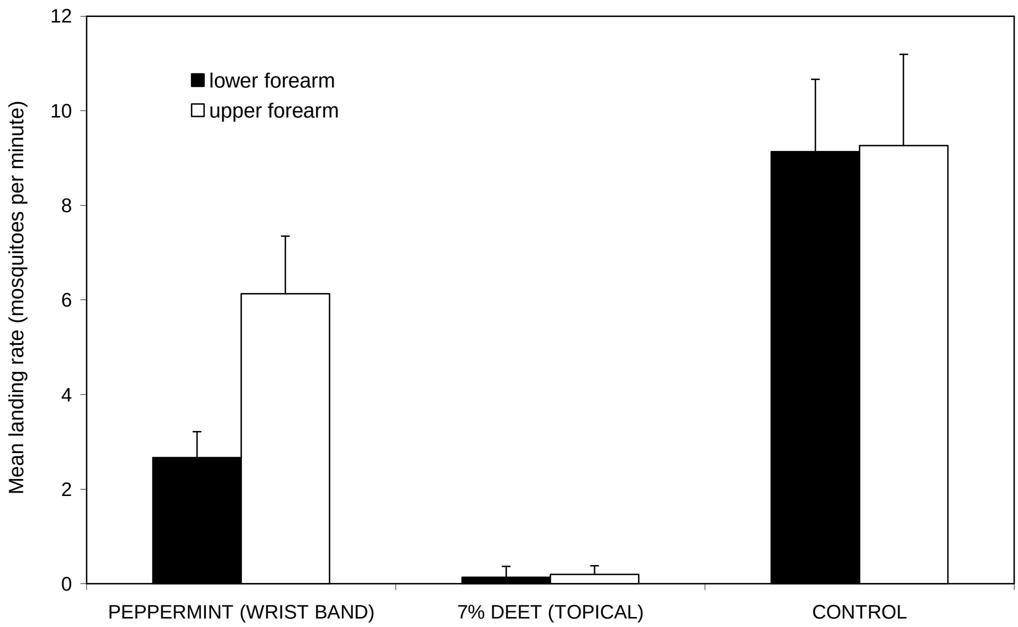

Figure. 1. Mean (±SE) landing rate of the mosquito *Aedes aegypti* on the upper and lower forearms treated with a 7% DEET topical repellent or in the presence of a peppermint oil infused wrist band compared to untreated controls.

DISCUSSION

The key finding from this investigation was that the use of wrist bands impregnated with peppermint oil does not prevent mosquito landings and provides significantly less protection than a topical repellent containing DEET. Although the results indicate that the use of wristbands may assist in repelling mosquitoes, with fewer mosquitoes landing on forearms wearing a wrist band compared with untreated controls, the repellency wasn't sufficient to prevent landings and there is no evidence that the assistance to repellency extends beyond a small area immediately surrounding the wrist band.

An important consideration when assessing the benefits of topical repellents containing DEET is that, even when used at low doses, they can provide protection from biting mosquitoes for over 2 hr (Fradin and Day 2002, Webb and Russell 2009). Mean protection times are typically calculated by taking the time between application of a repellent and the time at which a volunteer receives three or more mosquito bites on exposure to mosquitoes (Frances *et al*. 2005a, Barnard *et al.* 2007). While mean protection times were not calculated in this investigation, for all exposures to mosquitoes by all volunteers, on both upper and lower forearm, at least three landing mosquitoes were recorded, indicating that there is effectively no period of protection provided by the wrist bands.

There are currently two wrist band formulations of mosquito repellent registered with the APVMA and they are classified as a "slow release generator" (flea collars are also included in this formulation classification). The two registered products are Gone Insect Repellent (Goldgrade Coorporation Pty Ltd, Howrah, Tasmania, Australia, APVMA Approval Number 60121/5/0207) that lists an active ingredient of peppermint oil (and is the product used in this trial) and Mosquito-band anti-insect band (Intelligent Health Systems, South Oakleigh, Victoria, Australia, APVMA Approval Number 60836/2/0407) that lists an active ingredient of citronella oil.

There are very few studies that have investigated wrist bands impregnated with synthetic repellents such as DEET or picaridin. However, one study that used DEET impregnated wrist bands found that there was a dose dependent response between repellency and protection times for individuals wearing the products, but that up to 100% repellency and 5 h complete protection from bites could be achieved (Karunamoorthi and Sabesan 2009). When used in a formulation such as impregnated wrist bands, both botanical and synthetic repellents are unlikely to provide complete protection against mosquito bites for extended periods. Any protection provided is also likely to be localized (i.e. wearing wrist bands is not likely to protect the face or legs).

These laboratory tests were only undertaken using one species of mosquito and it may be difficult to determine the repellency of the wristbands in the field against locally important pest mosquitoes. Some studies have indicated that there may be a difference in the response to repellents by different species (Tawatsin *et al*. 2001). However, a more important factor to consider is that the relatively high density of mosquitoes within cage testing is considered substantially higher than would be experienced under field conditions. Field evaluations of mosquito repellents have recorded landing rates of mosquitoes on untreated individuals of 1.2 – 2.3 mosquitoes per minute (Frances *et al.* 2002), 1.1 – 2.1 mosquitoes per minute (Frances *et al*. 2005a), 0.7 mosquitoes per minute (Frances *et al.* 2005b) and 4.3 mosquitoes per minute (Greive *et al.* 2010). These results highlight the variability in landing rates that may be experienced due to differences in the spatial and temporal variability in both mosquito abundance and diversity, but also the relatively greater biting pressure that may be experienced in cages where landing rates are over 10.0 mosquitoes per minute.

Knowledge of personal protection strategies to minimise exposure to biting mosquitoes can vary greatly in the community and it is important that public health messages adapt to the changing demands of the general public, as well as addressing changes in the range of commercial products available, to ensure that there is sufficient awareness of appropriate personal protection strategies (Webb and Russell 2009). When used in conjunction with other protective measures (long sleeved shirts and pants, and topical repellents for the face and neck), wristbands may assist in repelling mosquitoes from the hands and lower forearms and this may be particularly useful when the use of topical repellents on the hands is not desirable (e.g. when fishing). However, as these products are unlikely to prevent mosquito bites, they should not be recommended for use by authorities in areas of endemic or epidemic mosquito-borne disease, and the use of DEET- or picaridin-based topical mosquito repellents should be encouraged to prevent exposure to mosquitoes.

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