Personal protective measures against mosquitoes: insecticide-treated uniforms, bednets and tents

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THE FIRST LINE OF DEFENCE against mosquito-borne diseases, especially malaria, dengue and other arboviruses, is to prevent being bitten. This can be achieved by several integrated methods involving insecticides and repellents. One option is to reduce the biting density by killing adult and larval mosquitoes with insecticides and by using barrier insecticide sprays on tentage and surrounding foliage. These methods are suitable in static base locations, but not for protecting patrolling soldiers. In this situation, individual personal protective measures against mosquitoes are paramount; these include protective dress (long sleeves and trousers) and use of repellents and insecticide-impregnated uniforms and bednets.[1] The combination of repellent use and insecticide-treated uniforms provides effective protection against biting mosquitoes.[2-5]

In an earlier report, we reviewed the use of mosquito repellents by the Australian Defence Force.[6] In this article, we report on the use, effectiveness and safety of insecticide-impregnated military fabrics in the ADF.

Abstract

- Personal protective measures are the first line of defence against mosquito-borne diseases, such as malaria and dengue.
- The use of pyrethroid insecticides to treat military uniforms, mosquito bednets and tentage is part of an integrated approach to minimising the bites of vector and pest mosquitoes suffered by Australian Defence Force personnel.
- The chemical permethrin is currently used by the ADF to treat disruptive pattern camouflage uniforms, mosquito bednets and tent material.
- Permethrin is safe, and effective at reducing biting by mosquitoes. However, methods to increase the persistence of permethrin in treated fabric are needed. Furthermore, it is important to monitor resistance among mosquito populations, to ensure ADF personnel continue to be provided with effective protection.

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Insecticide treatment of uniforms

The disruptive pattern camouflage (DPC) uniforms currently used by the ADF provide some protection against the bites of most mosquito species (Box 1).[7] Adding an insecticide to the uniform provides another layer of protection, further reducing the possibility of mosquitoes (and other arthropods) biting through the fabric. In field tests in Pakistan, a mean of 0.9–1.1 bites per 3-hour test period (primarily from *Aedes albopictus*) was recorded on the untreated United States battle dress uniforms, while no bites were recorded on insecticide-treated uniforms.[8]

The insecticide most commonly used for fabric impregnation is permethrin.[9] This is one of several synthetic pyrethroids developed from pyrethrum, a natural insecticide product of the plant *Chrysanthemum*. Much of the developmental work on the use of permethrin for clothing impregnation was conducted by the US Army and US Air Force. The US Department of Defence sought Environmental Protection Agency (EPA) approval to use permethrin in military fabrics in 1987, and this was obtained in 1990, when permethrin came into field use.[10] The ADF has been using permethrin in DPC uniforms since the early 1990s, and this is ADF policy for deployments to areas or countries with vector-borne diseases. Permethrin is applied to DPC uniforms and mosquito bednets by dipping them in a water emulsion (Box 2) containing 0.6% permethrin for 2 minutes; this should achieve a permethrin concentration of 0.12 mg/cm^2.

Permethrin is lethal to mosquitoes, but also has an irritant or excito-repellent effect, reducing mosquito contact with the fabric and the likelihood of biting.[11] Permethrin-impregnated uniforms also provide good protection against chiggers (the vectors of scrub typhus),[12] the human body

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1 Percentage of mosquitoes feeding (number of mosquitoes tested) through untreated fabrics and on bare skin in the laboratory

Treatment	*Anopheles farauti*	*Anopheles punctulatus*	*Aedes aegypti*
DPCU trouser	0 (100)	0 (60)	65% (58)
DPCU shirt	12% (138)	4% (100)	69% (55)
Bare skin	77% (57)	61% (140)	81% (58)

DPCU = disruptive pattern camouflage uniform.

louse *Pediculis humanus* (the vector of epidemic typhus),[13] sand flies (the vectors of leishmaniasis),[14] and ticks (the vectors of Lyme disease).[15]

2 A retreatment station for permethrin treatment of disruptive pattern camouflage uniforms in Balibo, East Timor, July 2001

Persistence in fabric

An important issue in the use of permethrin-treated fabric is the persistence of the insecticide in the fabric following wearing and washing. In field trials where uniforms were not retreated for 3–6 months, the transmission of malaria and leishmaniasis was not adequately suppressed;[14,16,17] however, these diseases were suppressed when the effectiveness of the treatment was assessed after 4 weeks.[18]

The effectiveness and persistence of an insecticide in a fabric can be determined experimentally in a number of ways: assessing the ability of mosquitoes to feed through the fabric and the time spent in contact with the fabric (probing time) measures the repellent effect, and determining the knockdown effect (immobilisation with or without death) and mortality following exposure measures the toxic effect. Probably most meaningful with regard to personal protection is assessing the ability of the mosquito to feed through the fabric. Laboratory trials most commonly use *Aedes aegypti* for these assessments, as colonies of this mosquito are easy to maintain in large numbers. The use of this species is fortuitous, as it has the greatest ability to bite through untreated fabric (Box 1), so tests against this species would be the most stringent.

Following treatment of the fabric, it is reasonable to expect the protection will diminish over time as the insecticide is lost through normal wear; this process may be accelerated by washing the fabric. One study investigated the wash and wear persistence of permethrin, using the lone star tick to evaluate the protection.[15] After 132 hours of continuous wear, treated clothing provided > 95% protection, but after four washes, only 49% protection was provided. Another study used a weatherometer to simulate conditions in a forested tropical climate to test the effects of weathering on the persistence of permethrin in fabric.[19] After 2 weeks of weathering, the toxic effect of the permethrin treatment was < 2.2% for knockdown and < 11.1% for mortality after 1.5 minutes exposure in *Ae. aegypti*.

Laboratory tests were conducted by the Army Malaria Institute (AMI) with permethrin-treated fabric washed in a commercial washing machine for 30 minutes, using warm water (50° C) containing 4 g/L of laundry detergent. After initial treatment, 100% of mosquitoes (*Ae. aegypti*) were dead 1 hour after a 1-minute exposure. After two warm water washes, the mortality in mosquitoes exposed for 1 minute was less than 5% (unpublished data).

To assess the effects of multiple washes, permethrin-treated DPC uniforms were subjected to five washes in a commercial washing machine. With *Ae. aegypti*, protection against biting provided by untreated shirt fabric was low (average 25%), whereas the range of protection was 84%–99% for treated shirts and 86%–100% for treated trouser fabric over the five washes. Again, the knockdown effect was low: 10% after a 1-minute exposure (unpublished data). Additional trials with three washes supported these data, with no biting of *Ae. aegypti* through the permethrin-treated fabric, but the knockdown effect was reduced from 93% to < 20% after just one wash. Chemical assays showed that the first wash removed 64% of the permethrin, and 80% of the initial permethrin had been removed after three washes.[7]

In field trials on the operational use of permethrin-treated US Army battle dress uniforms conducted during Exercise Tandem Thrust at Shoalwater Bay Training Area in 2001, successful mosquito feeding through the uniforms was observed.[11] Washing permethrin-treated battle dress uniforms once reduced the permethrin concentration in the fabric by 60%, but the permethrin concentration was not reduced following a further 20 washes. In that study, probing time (time spent on the uniform) of *Aedes vigilax* was reduced most on unwashed treated uniforms (7.5-fold reduction), and probing was reduced by 2.5 times on

3 Common method for use of a bednet by soldiers in the field

washed treated uniforms compared with untreated uniforms. The authors concluded that reduced probing time should reduce feeding success and therefore protect against vector-borne disease.[11]

Another study treated plain, non-DPC Nomex flying suits with permethrin (unpublished data). The treated fabrics were analysed to determine the concentration of permethrin; the lighter non-DPC Nomex flying suit absorbed less permethrin emulsion during treatment, and a mean concentration of 0.068 mg/cm^2 was observed, 44% less than the concentration recommended for ordinary DPC uniform fabric (0.12 mg/cm^2).[20] Preliminary studies at AMI have indicated that the concentration of permethrin following treatment is the same for the DPC uniform as the DPC Nomex flying suit and that both treated fabrics are effective in preventing bites of *Ae. aegypti*. Further studies need to be carried out with the non-DPC Nomex suits to determine the effectiveness of treatment against biting mosquitoes.

Various issues are raised in this summary of research. The data show that the persistence of permethrin in fabrics is variable, but the insecticide is lost from the fabric through washing. This loss appears to reduce the toxic effect (knockdown and mortality), but has minimal effect (at least after five washes) on repellency (the ability of the mosquito to bite through the fabric). The issue is when is the concentration, and hence effectiveness, reduced to a point where retreatment is necessary? Despite the results of research conducted to date, US Army doctrine suggests that persistence and effectiveness last much longer than three to five washes. The USAEHA Technical Guide 174, published in June 1991, states that, following treatment with permethrin:

> Do not re-treat the uniforms unless authorized by medical authorities: one treatment is effective in preventing mosquito bites through the fabric for the life of the uniform.[21]

In August 2000, this was changed to read:

> IDA (Individual Dynamic Absorption), 2-gallon sprayer, and factory permethrin treatments provide protection for "over 50 launderings", rather than "life of the uniform". This change was made to increase precision of the guidance because the life of the uniform can vary from a few months on deployment to about 2 years under garrison conditions and the frequency of washing varies from soldier to soldier and with the conditions under which the uniform is used.[22]

The Technical Guide 174[21] cites unpublished data indicating that, even after 50 cold water rinses, permethrin-impregnated fabric caused 100% mortality in mosquitoes and ticks. This appears to be contrary to most published research on the subject.

The individual dynamic absorption method (essentially dipping) involves placing the uniform in a plastic bag containing a permethrin–water emulsion for 2.5 hours, then removing it to be air dried. The aerosol treatment involves spraying the uniforms with 0.5% permethrin. In the factory impregnation method, the fabric is polymer-coated with permethrin after dying and before tailoring; after treatment, the fabric is dried at 130°C. The final concentration is 0.13 mg/cm^2.[23] Evaluation of the effectiveness of these methods with regard to persistence indicates that individual dynamic absorption is superior to the aerosol application method, and the factory treatment is superior to both.[23,24] There was 0.03 mg/cm^2 of permethrin in the factory polymer-coated fabric after 100 washes, whereas there was no measurable permethrin in fabric treated by individual dynamic absorption after 70 washes. The researchers claimed that there was still permethrin activity after 100 washes, based on 100% knockdown in *Ae. aegypti* after 30 minutes of continual exposure following 50 washes and 40 minutes' continual exposure following 100 washes.[23,25] Although the analysis clearly showed greater permethrin retention with the polymer-coated formulation, insecticide efficacy or protection from bites has not been clearly demonstrated. It is unrealistic to believe that a mosquito would be exposed continuously for > 30 minutes in the field — foraging mosquitoes spend less than 3 minutes on permethrin-treated fabric.[26] Most other researchers have used exposure times of 1–3 minutes when assessing knockdown.[7,24,27] More importantly, how effective this treatment method is after 100 or even 50 washes with regard to personal protection (ie, inability of mosquitoes to bite through the fabric) has yet to be investigated.

Overall, available evidence indicates DPC uniforms should be retreated after five washes.

Insecticide treatment of bednets

Bednets have been used for centuries as a barrier against biting mosquitoes. The use of bednets impregnated with insecticides was first trialled in Africa against malaria vectors in the mid 1970s. In the 1980s, a large number of trials were conducted globally using nets impregnated with synthetic pyrethroids — primarily permethrin and deltamethrin.[9] The results were encouraging and, in the mid 1990s, the use of insecticide-treated nets became the cornerstone of the World Health Organization's Roll Back Malaria initiative, aimed at halving the world's malaria by 2010.[28] It should be noted that insecticide-treated nets are only effective while people are under them, thus they are more effective in reducing transmission of malaria than other diseases, as the malaria vector (the *Anopheles* mosquito) usually feeds late at night. Because nets are rarely used during the day or evening, they are not effective against arbovirus vectors, which feed primarily during the day and evening; for example, the dengue vectors, *Ae. aegypti* and *Ae. albopictus*, feed throughout the day, given sufficient shade and humidity.

In laboratory and field trials, insecticide-treated nets reduce the number of mosquitoes that successfully feed through the net and significantly increase the mortality in mosquitoes coming in contact with the net. Additionally, impregnation with insecticide significantly increases the protectiveness of damaged nets.[9]

The current ADF bednets have been in use since the 1960s. They are made from a very durable nylon mesh (mesh size, 43 holes/cm^2), are rectangular, and when erected are 0.55 m high, 0.75 m wide and 2.1 m long. There is no floor, so access is achieved by raising a side of the bednet. The net has four tie-off points that are designed to hold it above a cot fitted with four wooden poles. The use of poles is inconvenient, as they add about 1.5 kg to the weight that a soldier has to carry and are cumbersome in the field. The bednets are difficult to use without a cot and require 5–10 minutes to set up properly. Difficulty in finding four suitable tie-off points often makes it hard for patrolling soldiers to erect bednets correctly, so they are often draped off the backpack and over the sleeping soldier (Box 3). Used in this way, the net may still be effective, as the permethrin treatment will reduce the ability of mosquitoes to feed through the net.

Trials conducted on Buka Island during Operation Bel Isi showed that the ADF-issued bednets treated with permethrin and erected correctly provided > 97.8% protection to sleeping people against the bites of the malaria vector *Anopheles farauti*. Furthermore, fewer mosquitoes gained entry into permethrin-treated ADF bednets than untreated ADF bednets.[29]

The use of bednets by ADF soldiers deployed during INTERFET (1999–2000) was determined by questionnaire. Of 975 soldiers who completed the questionnaire, 757 said they slept under a bednet every night they were in country. Of the 218 soldiers who did not sleep under a bednet every night, many stated that operational or tactical reasons prevented them from doing so. It was shown that the risk of becoming infected with malaria increased significantly when soldiers did not sleep under a bednet every night.[30]

4 Field evaluation of mosquito bednets at Buka, Papua New Guinea, March 1999 (A), and US Army prototype bednet (B)

Although mosquito mortality is important in malaria control programs, the insecticide's ability to reduce biting through the net is more relevant to ADF soldiers and their personal protection. This appears to depend on concentration. In one study, although biting was reduced, 32% of *An. farauti* mosquitoes and 30% of *Ae. aegypti* mosquitoes were able to obtain a blood meal through permethrin-treated net fabric if the sleeper came in contact with the net.[27] However, this was at concentrations of < 0.068 mg/cm^2; at higher concentrations (0.25 mg/cm^2), the malaria vector *Anopheles gambiae* was unable to feed through the net.[26] Biting of any species through nets with permethrin concentrations > 0.2 mg/cm^2 is unlikely.[9]

With the recognised difficulty of erecting the current ADF-issued net, AMI trialled two self-erecting nets for patrolling soldiers. Both types of net were fully enclosed with a floor and were accessed by a zippered opening, the nets were held erect by flexible fibreglass rods (Box 4). These nets were trialled at Buka, Papua New Guinea, during Operation Bel Isi and in East Timor during INTERFET. All nets were treated with permethrin; the two trial nets offered similar protection to the current net when erected correctly. They would offer greater protection than the current net just draped over the sleeping soldier, as commonly used on patrol. However, other considerations limited use of the new nets. They are heavier (1.5 kg v 0.4 kg), bulkier and do not pack well and, although self-erecting, they are difficult to dismantle. This made them unsuitable for patrolling soldiers.

As the nets are treated with permethrin by dipping, the issue of retreatment also applies. Although nets are rarely washed, their use rate can reduce the effectiveness of the insecticide. For nets not in use, insecticide activity remains for > 12 months, but for those used heavily (with daily handling for unwrapping, erecting and wrapping), the effectiveness of the insecticide begins to decline after 2 months.[31] For the ADF, retreatment of nets is advisable every 2 months.

5 Treatment of military tents with bifenthrin at Mt Bundey Training Area, 2003

Insecticide treatment of tents

The use of insecticides, especially permethrin, enhances the barrier effect of tents in preventing the entry of mosquitoes, and biting by mosquitoes within and around treated tents.

This method was first evaluated with the application of repellents such as deet on tent fabrics. In one study, biting by mosquitoes was reduced in and near tents treated with deet.[32] Treatment of the inner walls of tents with permethrin reduces the nuisance of mosquitoes and probably invasive pests,[33] and provides good protection against malaria vectors.[34]

Bifenthrin, a non-alpha-cyano-pyrethroid, has recently been evaluated as a barrier treatment applied to tentage to reduce entry and biting by mosquitoes (Box 5). At the Mt Bundey Training Area, bifenthrin treatment of ADF military tents provided an 81% reduction in entry and 91% reduction in biting inside the tent over a 10-day period.[35] A longer comparative study of the effects of treating military tents with permethrin and bifenthrin was conducted at Wide Bay Training Area. The study showed that barrier tent treatments provide a reasonable increase in preventing the entry of mosquitoes for at least 4 weeks, although there was no significant difference in the protection provided by either bifenthrin or permethrin.[36]

Another method of personal protection against mosquitoes is use of topical repellents. Although strongly recommended, they are not always used.[37] Tent barrier treatment is a method that can be cheaply applied and provides enhanced protection for a number of people. The term "passive prophylaxis" was recently coined to describe the protection provided by permethrin-treated tents in a summer camp site in the US, as the treatment provided prolonged protection and was more effective than repellent alone, because repellent was used inconsistently.[38]

Pyrethroid treatment of tentage is an environmentally safe way of enhancing protection against biting mosquitoes.

Toxicity of pyrethroid insecticides

Permethrin

The acute oral toxicity (LD_{50}) of permethrin for the rat is 3100 mg/kg.[39] Percutaneous absorption of permethrin has been investigated in the rat, rabbit, dog and human, and the degree of absorption is highly species dependent. When applied in an alcoholic vehicle, 60% is absorbed in the rat, 30% in the rabbit, 12% in the beagle and less than 2% in humans. In mammals, permethrin is rapidly detoxified by ester hydrolysis in blood and most body tissues, including the skin. Its major metabolites are almost entirely excreted in urine within 72 hours. Permethrin is metabolised and excreted as inactive metabolites faster than it can be absorbed through the skin, so tissue retention and storage is not a factor. Permethrin does not enter the bloodstream when treated uniforms are worn for a single day.[40]

An important concern in the wearing of permethrin-impregnated clothing for extended periods is the amount of active ingredient in contact with the skin. In a study using mannequins, the amount of fabric in a battle dress uniform was reported as 5.7 m^2 of fabric.[41] It was assumed that soldiers would wear underwear consisting of boxer shorts and singlet, and could potentially have 2.2 m^2 of fabric in contact with the wearer. The US Army recommends permethrin impregnation at 0.125 mg/cm^2 of fabric. Assuming this concentration and a skin surface exposure of 2.2 m^2, about 110 mg of permethrin may reach the skin surface of a man in 1 week.[42] This equals 15.7 mg per day or 0.26 mg/kg per day in a 60 kg individual. Using the reported value for permethrin skin absorption of 2%,[39] the wearer of a treated battle dress uniform for 1 week may be exposed to a systemic dose of 0.005 mg/kg per day. This is one-tenth of the value the EPA has determined as the acceptable daily intake (ADI) of permethrin (0.05 mg/kg per day). Absorption of permethrin into the skin of scabies patients treated with 5% permethrin cream was assessed. Following a mean application of 25 g of cream, the mean estimated absorption in the first 48 hours was 6 mg, which is about 0.5% of the total dose.[43]

Bifenthrin

Bifenthrin has moderate acute toxicity. The acute oral LD_{50} for rats is 632 mg/kg, and the acute dermal LD_{50} in rabbits is > 2000 mg/kg. The product is harmful by inhalation and has an acute inhalation LC_{50} of > 11.58 mg/L per hour. The ADI for humans is 0–0.02 mg/kg body weight. It is non-irritant to skin, virtually non-irritant to the eyes of rabbits, and presents no skin sensitisation on guinea pigs.[44] In India, short-term (7 days) occupational exposure to bifenthrin and deltamethrin was investigated, and it was found that these chemicals should pose no major occupational health hazard to spray workers provided the spraying is done by trained people wearing appropriate protective measures.[45] The current use, including the application of bifenthrin to bednets and tentage, means that humans are unlikely to be exposed in day-to-day life.

Mosquito resistance to pyrethroids

Use of an insecticide on a mosquito population will inevitably produce resistance. This was seen with the organochlorides (DDT) and organophosphates (Malathion) during the 1960s, and was one of the reasons for the failure of the malaria eradication programs that relied on indoor residual spraying to stop malaria transmission.[46] In 1951, insecticide resistance was found in one *Anopheles* species — by 1965, 33 species were resistant.[46] With permethrin, the problem is confounded by cross-resistance developing in DDT-resistant species (as the mode of action of both insecticides is the same), cross-resistance with other pyrethroid compounds, and the large-scale use of permethrin and other pyrethroids in agriculture. Resistance in the major malaria vector *An. gambiae* to pyrethroids (permethrin, deltamethrin and bifenthrin) has been found in a number of West African countries.[47,48] Genes capable of conferring pyrethroid resistance have been found in two other important malaria vectors: *Anopheles funestus* and *Anopheles arabiensis*.[49,50] To delay the spread of resistance, binary mixtures of insecticides are being proposed; the combination of bifenthrin and the organophosphate chlorpyrifos-methyl has shown promising results against pyrethroid-resistant strains of *An. gambiae*.[48] Global monitoring and reporting of insecticide resistance under the auspices of the WHO should provide early warning on changing trends in resistance and enable timely selection of new effective insecticides.

Conclusion

Applying pyrethroids to DPC uniforms, bednets and tentage is a safe and effective way to increase protection against vectors of diseases, such as malaria, dengue and other arboviruses. Technologies that enhance the persistence of permethrin on treated fabric need to be assessed in the Australian context to determine the duration of protection against biting and recommended times to retreatment. Pyrethroid resistance among vectors needs to be monitored continually to ensure that the insecticides used will provide ADF personnel with effective protection.

Competing interests

None identified.

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