

Insecticide-treated clothes for the control of vector-borne diseases: a review on effectiveness and safety

Sarah DeRaedt Banks¹, Natasha Murray², Annelies Wilder-Smith^{2,3,4}, James G Logan¹

1 London School of Hygiene and Tropical Medicine, UK

2 Institute of Public Health, University of Heidelberg, Germany

3 Duke-NUS, Singapore

4 University of Umea, Umea, Sweden

Address for correspondence:

James Logan PhD

Faculty of Infectious Diseases

Department of Disease Control

Keppel Street

London WC1E 7HT, UK

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ABSTRACT

Insecticide-treated clothing has been used by the military and recreational market as personal protection for many years against bites from a variety of arthropods including ticks, chigger mites, sandflies and mosquitoes. Permethrin is the most commonly used active ingredient but others including bifenthrin, deltamethrin, cyfluthrin, DEET and KBR3023 also have been trialled. Treatment is usually done by home or factory dipping. However, new microencapsulation technologies are now available which may prolong the activity of insecticides on clothing, and could help overcome the inevitable reduction in efficacy over time as a result of washing, ultra-violet exposure, and normal wear and tear of the fabric.

The aim of this article is to review the evidence-base for the use of insecticide-treated clothing for protection against bites from arthropods and its effect on arthropod-borne pathogen transmission. Although some studies do demonstrate protection against pathogen transmission, there are surprisingly few, and the level of protection provided is variable depending on the disease and the type of study conducted. For example, insecticide-treated clothing can give between 0 % and 75% protection against malaria and 0% to 79% protection against leishmaniasis. Studies vary in the type of treatment used, the age group of participants, geographical location of the study, and pathogen transmission potential. This makes intervention trials difficult to compare and assess.

Overall, there is substantial evidence that insecticide-treated clothing can provide protection against arthropod bites. Bite protection evidence suggests that insecticide-treated clothing could be useful in prevention of pathogen transmission, but further investigations are required to accurately demonstrate transmission reduction.

Introduction

There are many products and technologies for the prevention of bites from arthropods and to reduce the risk of transmission of vector-borne pathogens. Insecticide-treated bednets (ITNs) are probably one of the most well studied examples with good evidence of efficacy against bites and a significant effect on pathogen transmission (WHO, 2011). As well as the toxic mode of action of the insecticides used in ITNs, the nets themselves act as a physical barrier to prevent biting and some of the active ingredients also can have a repellent effect (Carter, 1989). However, bednets only protect against night-biting arthropods, because of the timing that individuals use bednets, and therefore afford no protection against arthropods that bite during the day. This is of particular concern for diseases such as dengue and yellow fever, the pathogens of which are vectored by day-biting mosquitoes (Hawley, 1988) of the genus, *Stegomyia* (formerly *Aedes*). For protection against those vectors, other technologies may be more appropriate, for example, the use of topically-applied repellents such as *N,N*, diethyl-*m*-toluamide (DEET), p-menthane diol (PMD) and other non-topical repellent active ingredients including those that provide “spatial repellency” (Fradin and Day, 2002). However, repellents are limited by variable user compliance. Short term users, such as vacationers to endemic areas, have greater compliance than long term or routine users like outdoor workers (Achee et al., 2012) and this could greatly affect the effectiveness of repellents as a disease intervention.

Agricultural and wildlife groups, as well as commercial companies, use insecticide-treated clothing to protect workers and the public whilst in fields, forested areas (Breedon et al., 1982, Schreck et al., 1982, Vaughn and Meshnick, 2011). The military has used insecticide-treated uniforms for protection of troops in vector-borne disease endemic areas for many years (Kitchen et al., 2009) and a recent mathematical modelling study demonstrated that insecticide-impregnated school uniforms have the potential to reduce dengue in children by 6% to 55% (Massad et al., 2013).

Despite the common use of insecticide-treated clothing, the evidence for protective efficacy against arthropod bites and disease transmission is not clear or easily accessible. In this review we examine the evidence base for the use of insecticide-treated clothing against insect bites and disease incidence. For the purpose of this review, the term “insecticide-treated clothing” will refer to treated-clothing effective against other arthropods including mites and ticks. We focus on the most commonly used insecticide, permethrin to examine its safety profile and methods of impregnation. Although other insecticides such as deltamethrin, bifenthrin, and cyfluthrin, and active ingredients conventionally used as repellents, including DEET and KBR3023 (Table 1), have been used to treat materials, they are not discussed here.

Methods

We used the following search engines: Pubmed, BIOISIS Citation Index, Medline, Google Scholar, and Armed Forces Pest Management Board Literature Retrieval System. We used the following terms for our search: insecticide-treated clothes, insecticide treated materials, repellent treated materials, permethrin, DEET, polymer coating and textiles, microencapsulation for treated clothing, disease intervention using insecticide treated clothing, pyrethroids, permethrin safety, absorption rates of

permethrin, DEET safety, biting times, guidelines for testing insecticides, and spatial repellents. All articles were located between October 2011 and May 2013.

Active ingredient

Permethrin

The most commonly used active ingredient (AI) is the pyrethroid, permethrin (Table 1). It is thought to act as both a repellent and insecticide and produces Type I responses, which illicit repeated firing of neurons resulting in blocked neural message (Sonderland et al., 2002). The “repellent effect” is defined by increased movement displayed by arthropods, and specifically detachment in ticks, once in contact with the material (Faulde and Uedelhoven, 2006). Permethrin has been shown to be more effective at deterring ticks than DEET, one of the most effective arthropod repellents available (Brown and Herbert, 1997). Not only does permethrin cause “hot-feet”, where insects appear to be standing on a hot surface, repeatedly lifting legs to avoid contact with material, permethrin also knocks down and kills biting arthropods through insecticidal properties (Rossbach et al., 2010). Permethrin has displayed high bite protection against a wide range of biting arthropods including ticks, 97% (Evans et al., 1991), and mites, 74% (Breedon, 1982). Testing with mosquitoes has shown a range of effectiveness from high bite protection against *Stegomyia albopictus*, 100% (Schreck, 1989), to low protection against *Culex sitiens*, 37% (Harbach et al., 1990) (Table 1). While permethrin is a potent insecticide, it has low toxicity in mammals and is used widely in nuisance and disease vector pest control treatment for humans and cattle (Appel et al., 2008, WHO et al., 2005).

Permethrin safety

Despite permethrin’s effectiveness and safety, a small amount of the insecticide is still absorbed through dermal contact and the pyrethroid is listed as a class C carcinogen (Appel et al., 2008, Snodgrass, 1992). This potential risk requires that all contact with permethrin be monitored and the pyrethroid’s metabolism through the body measured. The United States Environmental Protection Agency (EPA) has set up regulations to evaluate all forms of absorption, ingestion, and uptake of permethrin into the body, and only allows those that fall below the maximum daily exposure (USEPA, 2011). Measuring the daily uptake of those using permethrin-impregnated clothing long term, such as military and domestic uniformed personnel, is an important safety consideration (Rossbach et al., 2010). The EPA has calculated non-cancer and cancer risks for military and non-military workers who wear permethrin impregnated clothing. The EPA estimated the clothing (0.125 mg ai/cm²) would be worn 250 days/year for 10 to 35 years. Non-cancer risk (illnesses other than cancer) is determined by calculating Margin of Error (MOE) and only MOEs of greater than 100 are considered below any level of risk. Cancer risk is based on the likelihood of 1 to 3 people in 1 million (1 to 3 X 10⁻⁶) developing cancer to be a negligible risk. These levels are based on dermal absorption rates, body weight, days/year worn, surface area of skin in contact with clothing, and concentration of the active ingredient. EPA studies estimate MOEs of 6,700 and 26,000 for military and non-military respectively. Cancer risk was 1.2 X 10⁻⁶ for military and 3.6 X 10⁻⁶ for non-military workers, both of which fall under their level of concern (USEPA and Prevention, 2009). While some studies have raised concern over potential neurological and cellular negative effects seen when permethrin is used in combination with DEET on rats, the doses used were 0.13 mg/kg/day and the AI was

applied directly to rats (Abdel-Rahman et al., 2004, Abu-Qare and Abou-Donia, 2001). This dose is well above the allowed 0.00068 mg/kg/day for humans (Snodgrass, 1992). The EPA has completed testing for pre- and postnatal studies along with developmental toxicity and has found no evidence that permethrin poses extra risk for children and infants (USEPA and Prevention, 2009). Multiple studies have shown that wearers of insecticide-treated clothing have absorption rates that fall fivefold below the regulations established by the World Health Organization (WHO) and EPA (Snodgrass, 1992, Appel et al., 2008, Rossbach et al., 2010). However, the methods of impregnation do show differing levels of exposure risk. Dipped uniforms have a greater exposure risk than commercial aerosol spray applications (Snodgrass, 1992). Polymer coating techniques appear to be associated with the lowest absorption rate while still being able to impregnate with greater doses of permethrin (Faulde and Uedelhoven, 2006). However, careful monitoring of the products available and the concentrations of permethrin in them is needed to maintain these acceptable daily rates. There is some indication of greater absorption rates of permethrin into the skin occurring in warm climates (Snodgrass, 1992, Appel et al., 2008, Rossbach et al., 2010), however, robust scientific evidence is lacking. The high efficacy and wide range of arthropods that permethrin protects against, along with a low toxicity, makes it an ideal candidate for impregnating clothing.

The Treatment Process

Impregnation of clothing with insecticides and repellents is achieved by binding the AI to the fabrics, utilizing four different techniques: absorption, incorporation, polymer coating, and microencapsulation. All techniques require a fixative, a polymer material also known as a binder, to hold the AI to the fabric throughout wear and washing (Marinkovic et al., 2006). The first method, absorption, allows the AI to bind to material by either spraying or dipping fabric in the insecticide along with a chemical to facilitate the binding process (Schreck et al., 1982). The incorporation method, which is mainly used to treat carpets (Williams et al., 2003), will not be discussed further in this review. The polymer coating method employs a layer of polymers, with the AI bound within, that is coated over the fibre surfaces (Faulde and Uedelhoven, 2006). The final technique is microencapsulation, which is similar to polymer-coating, but encases the AI in a capsule that is mixed into a binding solution. The material is then run through a bath of the AI solution, allowing for a thin layer of polymer which gives the advantage of binding into the fibres (Appel et al., 2008, Marinkovic et al., 2006).

The absorption method can be used for both individual use and large scale factory production. Home spraying or dipping kits are affordable, available worldwide, and an easy to use option for treating clothing with permethrin. However, due to variation in personal application, the coverage may be inconsistent across the article of clothing, leading to areas vulnerable to biting arthropods (Schreck, 1982). Home-dipping kits do not provide long protection times, and reapplication after five washes is often recommended (Kimani, 2006). Alternatively, factory spraying or dipping, which occurs on a large scale in a standardised process, gives a more consistent application to the whole article of clothing. Companies that produce clothing using this method claim that efficacy against biting arthropods lasts up to 70 washes (Vaughn, 2011). Although this type of impregnation is also readily available for the average consumer, it is more expensive than home kits.

The use of polymers to bind permethrin to fabric has been found to provide longer lasting protection than other methods (Faulde, 2006; 2008). The polymer-coating method is done on a large factory scale where the AI is added to the polymer coating before treating the pre-tailored fabric. This method is more expensive than dipping methods, but has the benefit of lasting up to 100 washes (Faulde and Uedelhoven, 2006) and allows for more insecticide to be added with lower absorption rates into the skin (Faulde et al., 2012). Another benefit is the reduction of environmental impact of washing insecticide-treated clothing treated in this manner as there is less run off in the washing liquid (Snodgrass, 1992, Rossbach et al., 2010).

Microencapsulation of permethrin involves enclosing permethrin in a shell of a low molecular pre-polymer or monomer, allowing a specified rate of release, lower absorption by humans, and longer stability of the insecticide (Marinkovic et al., 2006). After the fabric is soaked in microencapsulated permethrin, the material is rolled through a pressing and heating section (Marinkovic et al., 2006). In microencapsulation, the fabric is treated with an ultra-thin layer of polymer that does not just rest on the outside of the fabric, but is thin enough to work its way around individual fibres (Marinkovic et al., 2006). This treatment could allow for longer lasting release and stability of the insecticide without changing the feel, thickness, and appearance of the clothing. Changes to feel, thickness, and appearance may have an impact on compliance, especially in hotter climates where heavier, thicker clothing is not worn. However, no published efficacy studies have been completed on this type of treatment.

Evidence for Efficacy of Impregnated Clothing

The efficacy of insecticide-treated fabrics is usually measured by recording how efficiently the product provides knockdown and killing of biting arthropods. However, many products claim protection against biting and many studies include biting protection as a measure of efficacy. Bite protection is important to quantify as this has the potential to reduce or prevent the transmission of vector-borne pathogens. However, bite protection is a reflection of the AI's repellency and does not consider any of the long term effects of mortality and reducing vector populations. These measures of efficacy can be investigated by performing cone tests recommended by World Health Organisation Pesticides Evaluation Scheme (WHOPES) (WHOPES, 2005) and arm-in-cage tests on a material after weathering and washing is simulated (Gupta et al., 1990). There are currently no WHOPES guidelines for testing of insecticide-treated clothing. Several investigations have shown varying degrees of efficacy against a number of arthropods and these studies are summarised in Table 1.

Bite Protection

Although insecticide-treated clothing can provide some protection against biting, few studies have shown 100% protection against bites using insecticide-treated clothing alone (Table 1). In 1989, Schreck et al. found that bites were mainly distributed on exposed skin when subjects were wearing treated uniforms. The opposite was found when subjects only wore repellent and 100% of the bites were located through the untreated clothing (Schreck, 1989). However, protection was increased to nearly 100% when subjects wore insecticide-treated clothing and a topically applied repellent (Schreck et al., 1982, Schreck, 1989, Pennetier et al., 2010, Sholdt et al., 1989, Schreck et al., 1984).

This combination of repellent and insecticide-treated clothing is recommended by the United States Department of Defense for protection of military personnel against biting arthropods (Board, 2009) and has been shown to give 99.9% protection over a 9 hour day from biting arthropods (Schreck et al., 1984). Despite the success of this combination approach, it has been shown that when both treatments are used, there is an increase in biting on the head (Sholdt et al., 1989).

One factor to consider when interpreting results from bite protection studies is that different fabric treatment processes can result in different levels of bite protection due to variability in absorption and coverage caused by applying the insecticide or repellent to the surface, or soaking it into the material. Duration and type of assay used to assess the treated material also will provide variable results, making direct comparisons difficult (Table 1). Future comparative studies, using standard guidelines, between types of treatment and assays could help to resolve these issues.

Reduction in Insect Populations: Effect of knockdown & mortality

Knockdown and mortality, caused by insecticide-treated materials, will inevitably protect the individual from bites. The ability to knockdown and kill also has the potential to protect at the community level by reducing the population of arthropods in a given area (Seng et al., 2008). This long lasting community effect has been demonstrated in other studies using insecticide treated nets and curtains (Seng et al., 2008, Kroeger et al., 2006), reducing insect populations up to 100 meters away from the intervention (Lenhart et al., 2008). For example, Kimani et al. (2006) collected mosquitoes from the homes of individuals wearing insecticide-treated clothing and compared them with collections from the homes of individuals wearing untreated clothing. Collections were carried out in a baseline survey, then once per month over three months. A significant reduction in total mosquito density was found between treated and untreated homes with treated homes having a reduction in total density, engorged mosquito density, female mosquito density, and *Anopheles* density (Kimani et al., 2006). Rowland et al. (1999) conducted an efficacy test on mortality using individuals wearing treated Chaddars, a large piece of cloth used as a body cover both day and night by women and children, under untreated nets. Human and cow baited traps were used in order to attract host seeking mosquitoes. The trapped mosquitoes were then collected and released into an untreated net with an individual protected with a treated chaddar sitting under the net. A significant reduction in the mosquito numbers inside the untreated net with the individual wearing a treated chaddar was reported for *Culex* spp., *Anopheles stephensi*, and *Anopheles nigerrimus* (Rowland et al., 1999). These two studies demonstrate the potential for insecticide-treated clothing to reduce populations of mosquitoes when they come into contact with the material. The results are promising and the community effects should be investigated further with a longer follow up period and a wider collection radius.

In a similar way to the bite protection studies, a lack of standardized methods is apparent in the studies that have examined effects of impregnated materials on insect populations (Table 1). Duration of exposure, the period of time used to measure knockdown and mortality, definition of bite protection, and concentration of the active ingredient all should be considered but many of these factors are omitted from the published studies. The use of standard methods for all future studies would help to better assess efficacy for insecticide-treated clothing.

Intervention Trials: Effects of insecticide-treated clothing on disease

Several intervention trials have been done using insecticide-treated materials, however, results range from no demonstrated reduction in pathogen incidence to up to 79% reduction (for malaria; Eamsila et al., 1994, Asilian et al., 2003) (Summarized in Table 2).

One study investigated the use of permethrin-treated clothing and bedding and demonstrated a 69% reduction in malaria transmission (Kimani et al., 2006). Similar results (64% reduction in malaria) were found in children aged 0-10 years old in a study completed by Rowland et al (1999) who implemented the use of treated chaddars, patoo (blankets made of thin wool), and top-sheets in an Afghanistan refugee camp in North-Western Pakistan . (Rowland et al., 1999). Additionally, Kimani *et al.*, found reductions in the odds ratios in malaria in subjects between the ages of 5 to 24 and those over the age of 50 (Kimani et al., 2006). In both trials, the clothing and materials treated were the participants' personal items, as opposed to using new clothing. Utilizing existing clothing and habits promotes stronger compliance and greater acceptability. The greater disease risk of the two age groups that had reduced malaria following the intervention in this study, suggests that treated clothing could be a promising disease intervention method.

A large integrated vector management trial in northern Afghanistan used permethrin-treated clothing as one of the interventions against leishmaniasis (Faulde et al., 2009). The trial included treated clothing, skin repellents, bednets and curtains, vector monitoring, habitat cleansing, and health education programs. While the specific effect that treated clothing had on transmission rates is not clear, a pattern of decline was seen over the three year trial. Leishmaniasis fell from 17.5% infection in 2005 to 0% in 2007 (Faulde et al., 2009). This integrated method gave the greatest rate of reduction in disease transmission when compared with the other intervention studies reviewed. However, the study was completed only looking from the trial start date to end date for reduction and had no control group, making it difficult to definitively state that the interventions caused the reduction in disease transmission.

Intervention trials have mainly focused on two diseases, malaria and leishmaniasis. This may be due to the number of studies completed by the military and the relevance of malaria and leishmaniasis to soldier well-being. One study did investigate the effect on scrub typhus transmission in 1947 (Welt, 1947), where four and a half battalions were divided into three test groups. One group received no miticide, the second group had their uniforms sprayed with miticide, and the third group had their uniforms dipped in the miticide. Whilst the study resulted in a high reduction of scrub typhus in groups two and three in comparison to group one, group three slept on cots, while groups one and two slept on the ground. Not sleeping on the ground is a method of reducing scrub typhus, so it is difficult to determine whether the effect was due to clothing or to not sleeping on the ground. Group three, also did not visit the same locations as groups one and two (Welt, 1947). Due to the variations between groups a firm conclusion as to whether the clothing was responsible for the reduction in scrub typhus cannot be made.

Further difficulty arises when comparing trials due to variation between the methods used for each trial. Multiple treatments (e.g. sprayed, hand dipped and factory dipped clothing), duration and follow-up of the trial, education of participants about the intervention, and sample size, are all factors that vary between the trials that have been completed to date (Table 2).

Factors affecting efficacy

Acceptability of, and compliance with treated clothing is a major difficulty for the success of intervention trials. Although not all trials examined here elaborated on factors of acceptability, Rowland et al. and Kimani et al. reported participants had a high and “enthusiastic” (Rowland et al., 1999) acceptance of the treated clothing describing a beneficial reduction in the number of mosquitoes and protection from mosquito bites (Kimani et al., 2006). Eamsila (1994) discussed a general feeling of compliance, but there was no monitoring of use and the authors listed low compliance and acceptability as a potential limitation to the study.

Although no direct link between education and compliance is made in the intervention trials, Faulde et al. study in 2009, discussed above, describes an emphasis on education of use and risks associated with the intervention. In Colombia, while no monitoring was done to ensure compliance the placement of bites and lesions for all but one soldier were on areas not covered by the clothing, indicating compliance (Soto et al., 1995). A third study using treated uniforms as an intervention against cutaneous leishmaniasis for soldiers in Iran made no mention of compliance or location of lesions (Asilian et al., 2003), and therefore it is not known whether there is a correlation between participant acceptance and the success of the trial.

The efficacy of the active ingredient is affected also by exposure to sunlight (ultra-violet), wear of fabric, washing of material (Atieli et al., 2010) and type of fabric (Amalraj et al., 1996). These factors play a role in how efficiently the insecticide remains active in the material. Additionally, the type of fabric affects the original absorption and binding rate of the permethrin to the material (Amalraj et al., 1996). General wear of the insecticide-treated clothing causes deterioration of the fabric and permethrin, sunlight breaks down permethrin, and washing rinses out permethrin (Faulde et al., 2012, Deparis et al., 2004). For most treated fabrics such as bednets and curtains, these factors can be easily monitored and standardised. Bednet exposure to sunlight can be limited, wear is localized to specific regions on the net and washing of bednets occurs less frequently than everyday clothing (Rowland et al., 1999, WHO, 2007, Binka and Adongo, 1997). Clothing, on the other hand, is utilised in a more uncontrolled manner: sunlight is unavoidable in practical terms, wear can be assumed to occur but may vary between persons; and washing of clothing is likely to be significantly more frequent than bednets. The break down, deterioration, and loss of permethrin will affect the pesticides ability to knockdown and kill biting arthropods.

The effect of weathering on treated clothing has been examined also through simulation of environmental factors on material. For example, Gupta et al. (1989) showed that, most permethrin was lost in the first week of simulated weathering. The weathering involves a day and night light cycle along with changes of temperature and humidity to simulate the cycles of outdoor wear, such as tropical to arid environments using a Weather-ometer machine (Gupta et al., 1989). The WHO has a standardised test for washing bednets that also is used for washing clothing (WHOPES, 2005). The method involves cutting four pieces of material and placing in a shaking water bath with soap, followed by two rinses. The material is allowed to dry, then either washed again or tested for knockdown and mortality by exposing mosquitoes to the washed material. It is important to test all clothing and methods of impregnation with these protocols, to gain accurate protective efficacy and active ingredient duration before implementing any intervention studies.

Conclusions

There is clear evidence that insecticide-treated clothing provides protection against bites from a number of arthropods. Also, there is evidence that insecticide-treated clothing can reduce disease incidence, but reduction is likely to be dependent on the targeted vector and the pathogen transmission potential. Other factors that need to be considered when assessing efficacy against pathogen transmission, as evidenced by the studies reviewed here are: time of biting, mechanics behind protection, and AI utilized. The hours of biting of vectors are important to consider due to the fact that during the day most people cannot be under the protection of a bed net and may be working in areas such as forests and fields where the vectors are more numerous. Clothing may offer some protection against night-biting mosquitoes but many factors should be further investigated such as timing of wearing the clothing, duration of wear, and residual activity on the skin after the clothing has been removed.

An understanding of the mechanism behind the protection provided by insecticide-treated clothing would help to determine the most appropriate and efficacious situations to use treated clothing for an intervention. For instance, in areas of insecticide resistance the difference between behavioural or physiological protection becomes paramount to a successful intervention. The true mechanism by which protection is accomplished has yet to be defined. Schreck et al. (1989) investigated the combination of clothing with skin repellents. The study demonstrated that mosquitoes will bite areas of the body that are not covered by treated clothing when a skin repellent is not used. When a repellent is used in combination with treated clothing, almost complete protection is achieved. On the other hand, when untreated clothing is worn, mosquitoes will bite through the clothing regardless of whether a skin repellent is worn along with the treated clothing. This suggests that treated clothing is not necessarily “repellent”, but it does prevent biting on areas covered with the treated material. Further investigations are needed to confirm whether permethrin treated clothing can repel mosquitoes and the mechanisms underlying this behaviour. The distinction between repellent and insecticidal properties is an important factor to consider, especially in areas of permethrin resistance. If treated clothing does actively repel arthropods, it may still provide protection even in places where physiological resistance to pyrethroids is found. Although not discussed in detail in this review, other repellents, such as those normally applied to the skin (e.g. DEET, *P*-Menthane-3,8-*diol* (PMD) and KBR3023), could be used in the situation where permethrin resistance is present (Table 1).

Under the correct circumstances, insecticide-treated clothing is a promising intervention against disease but further studies are needed to define the most appropriate guidelines for testing insecticide-treated clothing. Although guidelines will allow for standardised efficacy testing, the use of insecticide-treated clothing would need careful consideration in each individual field setting. At the London School of Hygiene and Tropical Medicine, we are currently testing permethrin impregnated uniforms under laboratory conditions to measure the efficacy of different types of treatments, including waning efficacy after washing and behavioural response of permethrin resistant mosquitoes to treated fabric (Wilder-Smith *et al.*, 2012b). Furthermore, at the University of Bangkok in Thailand, a randomized controlled school based trial is currently underway to establish the efficacy of impregnated uniforms in school aged children in terms of reduction of dengue infections (Wilder-Smith *et al.*, 2012a). Cost-effectiveness studies and studies on long-term safety of impregnated clothes are being done to assess the value of impregnating clothes or uniforms. For

all future studies, monitoring of the AI effectiveness over time in real world settings should provide a valuable insight into the practicality of long-term use in a field setting.

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Table 1. Studies investigating the different types of treatments and level of protection against biting arthropods.

Sprayed Clothing						
Active Ingredient	Vector	Exposure Time	^a Bite Protection%	Knockdown%	Mortality%	Reference
0.6g/m ² Permethrin (Dragnet 100)	<i>Stegomyia aegypti</i>	3 min	6.1% (±6.1)	NR	84.8% (±8)	Frances, 2003
0.6g/m ² Permethrin (Dragnet 500)	<i>Stegomyia aegypti</i>	3 min	3.3% (±3.3)	NR	97.0% (±3.0)	Frances, 2003
0.6g/m ² Permethrin (Pergrin 500)	<i>Stegomyia aegypti</i>	3 min	6.1% (±6.1)	NR	93.0% (±3.5)	Frances, 2003
0.6g/m ² Permethrin (Dragnet 100)	<i>Anopheles farauti</i>	3 min	NR	100.0%	94.2% (±2.5)	Frances, 2003
0.6g/m ² Permethrin (Dragnet 500)	<i>Anopheles farauti</i>	3 min	NR	100.0%	100%	Frances, 2003
0.6g/m ² Permethrin (Pergrin 500)	<i>Anopheles farauti</i>	3 min	NR	100.0%	100%	Frances, 2003
0.5g/m ² Permethrin (Imperator 25%)	<i>Culex</i> spp.	Dusk till Dawn	69%	NR	27%	Rowland, 1999

0.5g/m ² Permethrin (Imperator 25%)	<i>Anopheles nigerrimus</i>	Dusk till Dawn	65%	NR	4%	Rowland, 1999
0.5g/m ² Permethrin (Imperator 25%)	<i>Anopheles stephensi</i>	Dusk till Dawn	31%	NR	0%	Rowland, 1999
0.5g/m ² Permethrin (Imperator 25%)	<i>Anopheles subpictus</i>	Dusk till Dawn	37%	NR	34%	Rowland, 1999
1.0g/m ² Permethrin (Imperator 25%)	<i>Culex</i> spp.	Dusk till Dawn	69%	NR	25%	Rowland, 1999
1.0g/m ² Permethrin (Imperator 25%)	<i>Anopheles nigerrimus</i>	Dusk till Dawn	62%	NR	10%	Rowland, 1999
1.0g/m ² Permethrin (Imperator 25%)	<i>Anopheles stephensi</i>	Dusk till Dawn	33%	NR	37%	Rowland, 1999
1.0g/m ² Permethrin (Imperator 25%)	<i>Anopheles subpictus</i>	Dusk till Dawn	22%	NR	18%	Rowland, 1999
2.0g/m ² Permethrin (Imperator 25%)	<i>Culex</i> spp.	Dusk till Dawn	76%	NR	44%	Rowland, 1999
2.0g/m ² Permethrin (Imperator 25%)	<i>Anopheles nigerrimus</i>	Dusk till Dawn	43%	NR	40%	Rowland, 1999
2.0g/m ² Permethrin (Imperator 25%)	<i>Anopheles stephensi</i>	Dusk till Dawn	58%	NR	39%	Rowland, 1999
2.0g/m ² Permethrin (Imperator 25%)	<i>Anopheles subpictus</i>	Dusk till Dawn	0%	NR	51%	Rowland, 1999
0.125mg/cm ² Permethrin	<i>Amblyomma americanum</i>	1 hour	100%	NR	100%	Schreck, 1982
0.125mg/cm ² Permethrin	<i>Amblyomma americanum</i>	15 min	98.5%	NR	NR	Evans, 1991
0.125mg/cm ² Permethrin	<i>Ixodes dammini</i>	15 min	100%	NR	NR	Evans, 1991
0.125mg/cm ² Permethrin	Total Ticks	15 min	98%	NR	79.0%	Evans, 1991
0.125mg/cm ² Permethrin	<i>Anopheles dirus</i>	5 min	97%	99.4% (94.4-100)	NR	Eamsila, 94
0.0025g/m ² Bifenthrin (Biflex 10)	<i>Stegomyia aegypti</i>	3 min	3.3% (±3.3)	NR	96.7% (±3.3)	Frances, 2003
0.0025g/m ² Bifenthrin (Biflex 10)	<i>Anopheles farauti</i>	3 min	NR	100%	100%	Frances, 2003
0.05g/m ² Bifenthrin (Talstar 80)	<i>Stegomyia aegypti</i>	3 min	3.3% (±3.3)	NR	96.7% (±3.3)	Frances, 2003
0.05g/m ² Bifenthrin (Talstar 80)	<i>Anopheles farauti</i>	3 min	NR	100%	100%	Frances, 2003

Hand Dipped

Active Ingredient	Vector	Exposure Time	^a Bite Protection%	Knockdown%	Mortality%	Reference
0.125mg/cm ² Permethrin	<i>Stegomyia albopictus</i>	15 min	100%	60%	NR	Schreck, 1989
10g/m ² KBR3020	<i>Stegomyia aegypti</i>	2 hours	62.50% (95% CI 0.29-0.45)	NR	2.04% (95% CI 1.02-4.09)	Pennetier, 2010
10g/m ² KBR3020 & 150 mg/m ² Pirimiphos-methyl (PM)	<i>Stegomyia aegypti</i>	2 hours	76.56% (95% CI 0.18-0.30)	NR	2.48% (95% CI 1.33-4.68)	Pennetier, 2010
0.125mg/cm ² Permethrin	<i>Glossina morsitans centralis</i> (Tsetse Fly)	75 min	34.20%	NR	NR	Sholdt, 1989
0.125mg/cm ² Permethrin & 35% DEET (EDRF) ^{b&c}	<i>Glossina morsitans centralis</i> (Tsetse Fly)	75 min	90.70%	NR	NR	Sholdt, 1989
0.125mg/cm ² Permethrin & 75% DEET (in EtOH) ^d	<i>Glossina morsitans centralis</i> (Tsetse Fly)	75 min	81.40%	NR	NR	Sholdt, 1989
0.125mg/cm ² Permethrin	<i>Amblyomma americanum</i>	15 min	97.5%	NR	NR	Evans, 1991
0.125mg/cm ² Permethrin	<i>Ixodes dammini</i>	15 min	100%	NR	NR	Evans, 1991
0.125mg/cm ² Permethrin	Total Ticks	15 min	97%	NR	69.0%	Evans, 1991
0.125g/cm ² Permethrin	<i>Trombicula</i> spp. (Chigger Mite)	3 days	74.20%	NR	NR	Breeden, 1982
2g/ft ² Benzylbenzoate	<i>Eutrombicula hirsti</i> (Chigger Mite)	1 hour	100	NR	NR	Snyder, 1946
75% DEET	Phlebotomine Sandflies	20 min, September 1980 & January 1981	89% mean (range 68.8-98.1%)	NR	NR	Schreck, 1982
0.125g/m ² Permethrin	Phlebotomine Sandflies	20 min, September 1980 & January 1981	49% (range 6.8-90.5%)	NR	NR	Schreck, 1982
0.125g/m ² Permethrin	<i>Phlebotomus papatasi</i>	1,3,5,7, & 10 min	NR	8, 26, 56, 56, & 84%	30, 73, 82, 91, & 100%	Fryauff, 1996
0.125g/m ² Permethrin	<i>Culex pipiens</i>	1,3,5,7, & 10 min	NR	49, 70, 75, 85, & 98%	56, 72, 66, 76, & 74%	Fryauff, 1996
2.5mg/m ² Deltamethrin	<i>Triatoma sordida</i>	1, 2, & 24hr (7day mortality)	NR	0, 0, & 20%	21.20%	Diotaiuti, 2000

2.5mg/m ² Deltamethrin	<i>Panstrongylus megistus</i>	1, 2, & 24hr (7day mortality)	NR	60, 100, & 100%	100%	Diotaiuti, 2000
2.5mg/m ² Deltamethrin	<i>Rhodnius neglectus</i>	1, 2, & 24hr (7day mortality)	NR	100, 100, & 100%	17.20%	Diotaiuti, 2000
2.5mg/m ² Deltamethrin	<i>Triatoma infestans</i>	1, 2, & 24hr (7day mortality)	NR	30, 40, & 100%	17.60%	Diotaiuti, 2000
5mg/m ² Deltamethrin	<i>Triatoma sordida</i>	1, 2, & 24hr (7day mortality)	NR	0, 0, & 10%	100%	Diotaiuti, 2000
5mg/m ² Deltamethrin	<i>Panstrongylus megistus</i>	1, 2, & 24hr (7day mortality)	NR	45, 100, & 100%	100%	
5mg/m ² Deltamethrin	<i>Rhodnius neglectus</i>	1, 2, & 24hr (7day mortality)	NR	100, 100, & 100%	17.20%	Diotaiuti, 2000
5mg/m ² Deltamethrin	<i>Triatoma infestans</i>	1, 2, & 24hr (7day mortality)	NR	30, 60, & 100%	47%	Diotaiuti, 2000

Factory Dipped

Active Ingredient	Vector	Exposure Time	^a Bite Protection%	Knockdown%	Mortality%	Reference
0.125g/m ² Permethrin	<i>Stegomyia taeniorhynchus</i>	9.5-10 hours	99.9% (mean/day %)	NR	NR	Schreck, 1989
0.125g/m ² Permethrin	<i>Stegomyia aegypti</i>	2 hours	56.25% (95% CI 0.35-0.55)	NR	11.66% (95% CI 8.43-16.30)	Pennetier, 2010
0.125mg/cm ² Permethrin	<i>Culex sitiens</i>	8 hours	37.1	NR	NR	Harbach, 1990
0.125mg/cm ² Permethrin	<i>Aedes vigilax</i>	8 hours	43.1	NR	NR	Harbach, 1990
0.125mg/cm ² Permethrin & 75% DEET (in EtOH)	<i>Culex sitiens</i>	8 hours	72.9	NR	NR	Harbach, 1990
0.125mg/cm ² Permethrin & 75% DEET (in EtOH)	<i>Aedes vigilax</i>	8 hours	83.40%	NR	NR	Harbach, 1990
0.125 mg/cm ² Permethrin & 35% DEET (EDRF)	<i>Culex sitiens</i>	8 hours	78.8	NR	NR	Harbach, 1990
0.125 mg/cm ² Permethrin & 35% DEET (EDRF)	<i>Aedes vigilax</i>	8 hours	93.5	NR	NR	Harbach, 1990
Permethrin	<i>Anopheles spp.</i>	6 hours	48.4% (95% CI 0.63)	NR	11.3% (95% CI 1.66)	Deparis, 2004
Permethrin & 50% DEET	<i>Anopheles spp.</i>	6 hours	44.6% (95% CI 0.93)	NR	11.3% (95% CI 1.27)	Deparis, 2004

0.125mg/cm ² Permethrin Cotton cloth	<i>Pediculus humanus</i> L. (Body lice) (Field strain)	10, 15, 30, 45, 60, 75min & 12hr	NR	0, 23.3, 48.1, 75.7, 90.7, 96.7, 100, 100, & 100%	NR	Sholdt, 1989
0.125mg/cm ² Permethrin NYCO blend cloth	<i>Pediculus humanus</i> L. (Body lice) (Field strain)	10, 15, 30, 45, 60, 75min & 12hr	NR	18.9, 44.4, 82.0, 87.9, 92.5, 100, & 100%	NR	Sholdt, 1989
0.125mg/cm ² Permethrin NYCO blend cloth	<i>Pediculus humanus</i> L. (Body lice) (Field strain)	15, 30, & 60seconds	NR	(68,30,&32%) ^e (64,40,&50%) ^f (100,57,&93%) ^g (100,100,& 100%) ^h	NR	Sholdt, 1989
0.125mg/cm ² Permethrin Cotton cloth	<i>Pediculus humanus</i> L. (Body lice) (Laboratory strain)	15, 30, 45, 60, 75, 90, 105, 120, 135min, & 24 hr	NR	80, 99, 96, 100, 100, 100, 100, 100, 100, 100%	NR	Sholdt, 1989
0.125mg/cm ² Permethrin NYCO blend cloth	<i>Pediculus humanus</i> L. (Body lice) (Laboratory strain)	15, 30, 45, 60, 75, 90, 105, 120, 135min, & 24 hr	NR	72, 96, 100, 100, 100, 100, 100, 100, 100, 100%	NR	Sholdt, 1989
Permethrin ⁱ	<i>Ixodes ricinus</i>	27 weeks	99% ^j , 0.01 Incidence rate ratio (95% CI 0.001,0.11)	NR	NR	Vaughn, 2011

Polymer-coated

Active Ingredient	Vector	Exposure Time	^a Bite Protection%	Knockdown%	Mortality%	Reference
1200mg/m ² Permethrin	<i>Ixodes ricinus</i>	36 hours	95.50%	NR	NR	Faulde, 2008

Hand Applied

Active Ingredient	Vector	Exposure Time	^a Bite Protection%	Knockdown%	Mortality%	Reference
33.25% DEET (EDRF)	<i>Amblyomma americanum</i>	15 min	60.4	NR	NR	Evans, 1991
33.25% DEET (EDRF)	<i>Dermacentor variabilis</i>	15 min	50	NR	NR	Evans, 1991
33.25% DEET (EDRF)	<i>Ixodes dammini</i>	15 min	10	NR	NR	Evans, 1991
33.25% DEET (EDRF)	Total Ticks	15 min	59.8	NR	NR	Evans, 1991

^a Bite protection % Those in bold italics are % blood fed after exposure, those in standard font are reduction in # of bites applied to the skin while concurrently wearing treated clothing. ^bRepellent

^cEDRF, Extended duration repellent formulation or controlled release formulation

^dEtOH, ethanol

^e 15, 30, & 60 seconds exposure at 0.5hours Knockdown f

15, 30, & 60 seconds exposure at 1.0 hours Knockdown g

15, 30, & 60 seconds exposure at 6.0 hours Knockdown h

15, 30, & 60 seconds exposure at 12.0 hours Knockdown i

No concentration of permethrin given; clothing provided by Insect Shield combining factory-based coating technology with a proprietary formulation of permethrin

^jRate of tick bites acquired during work hours was reduced by 99%

NR, Not reported

Table 2. Study characteristics of trials of pesticide-treated clothing against vector-borne pathogen transmission

Location (Author)	Trial characteristics
Afghanistan (Rowland, 1999)	<p>Trial type: Randomized controlled trial, Malaria intervention</p> <p>Study population: 3950 refugees (510 families) from the Adizai refugee settlement. 825 individuals from 102 households were randomly selected. Mean age among the permthrin treated group was 17; mean age in placebo group 19. Diagnosis was verified by microscopy at local health centre.</p> <p>Blinding: Field staff and study participants were blinded to allocation.</p> <p>Intervention: 0.1mg/cm² Permethrin treated chaddars, patoos and top-sheets. Chaddars are piece of material often used for protection against the sun, and biting insects. At night they are used for warmth. During cold months patoos are used as they are a thicker material.</p> <p>Other behaviours recorded: Age, gender, social acceptance of insecticide treatment and compliance, clothing wash habits, insecticidal efficacy against local mosquito population.</p> <p>Outcome: The effect of treated chaddars, patoos, and top-sheets on the reduction of <i>P. falciparum</i> (P < 0.001) was significant, but only borderline for <i>P. vivax</i> malaria (P=0.069). Adjusted odds ratio (95% Confidence intervals) for <i>P. falciparum</i> was 0.51 (0.30, 0.86) and for <i>P. vivax</i> 0.70 (0.43, 1.13). Highest incidence risk group 0-10 years and lowest >20 years identified.</p> <p>Length of Trial: 16 weeks</p>
Thailand (Eamsila, 1994)	<p>Trial type: Randomized controlled trial, Malaria intervention</p> <p>Study population: 403 Thai soldiers aged 18-40 from malaria free areas with little or no aquired immunity. Mid-trial first group of soldiers were sent home and new group of 260 troops replaced them. Three locations were used along the Thai-Cambodia border. Diagnosis was verified through microscopy.</p> <p>Intervention: 0.125mg/cm² Permethrin treated uniforms, and untreated bednets also were available to volunteers.</p> <p>Outcome: The intervention had no significant effect on either <i>P. falciparum</i> or <i>P. vivax</i> malaria incidence over the 13 months.</p> <p>Length of Trial: 13 months</p>
Kenya (Kimani, 2006)	<p>Trial type: Randomised controlled community Trial with a treatment and comparison arm, Malaria intervention</p>

Study population: 198 Somali refugees in Dadaab refugee camp. Multistage cluster sampling was used to select participants. Diagnosis was verified through microscopy.

Blinding: Double blinding of participants and laboratory staff.

Intervention: Personal clothing of the participants was permethrin dipped at a concentration of 15mls of permethrin/4000 mls of water. Repeated every three weeks.

Other behaviours recorded: Baseline data of blood samples, mosquito density, sleeping habits, malaria control practices, age, gender, clothes washing habits, and possession of treated bednets.

Outcome: Owning a bed net reduced the odds ratio of getting malaria to 0.30. The odds ratio post-intervention was reduced by 69% (OR=0.31, P<0.001). Found intervention protective of all age groups except 0-5 years and 25-49 years.

Length of Trial: 3 months

**Colombia
(Soto,1995)**

Trial type: Randomized controlled trial, Malaria & Leishmaniasis intervention

Study population: 172 Colombian soldiers for malaria trial and 286 Colombian soldiers for the leishmaniasis trial. Soldiers were sent to malaria and leishmaniasis endemic areas, and had no previous or current signs of infection. Diagnosis for malaria was verified through microscopy and diagnosis for leishmaniasis was verified through examination of lesions followed by microscopy and electrophoresis.

Blinding: Field staff and study participants were blinded to allocation.

Intervention: 600-712mg/m² Permethrin treated uniforms. Instructed to wear the uniforms day and night.

Other behaviours recorded: Compliance, lesion placement (used as an indicator for compliance), use of other repellents

Adverse effects: 2 of 229 participants reported irritation and pruritis.

Outcome: A significant reduction in malaria of 79% (P=0.015) was reported. Though a lower 75% reduction in leishmaniasis was seen, it was determined to be more significant (P=0.002).

Length of Trial: Malaria study involved 3-5 weeks in endemic area and a 4 week follow-up, in total 8.2 weeks (mean length). Leishmaniasis study was 6-8 weeks in endemic area followed by 12 week follow-up, resulting in 18.6 weeks mean length.

**Iran (Asilian,
2003)**

Trial type: Randomized controlled trial, Cutaneous Leishmaniasis intervention

Study population: 272 Iranian soldiers between the ages of 19 and 24 with no history of having cutaneous leishmaniasis. 134 soldiers in treatment group and 138 in control. Located in Isfahan, and endemic area. Diagnosis was verified by microscopy.

Blinding: Field staff and study participants were blinded to allocation.

Intervention: 850mg/m² Permethrin treated uniforms; uniforms covered whole body except head, neck, hands, and feet. They were instructed to wear them day and night. Told not to wear any insect repellents.

Other behaviours recorded: Compliance, any adverse reactions to the clothing

Adverse effects: None

Outcome: 4.4% in treatment group were infected, and 6.5% in the control group were infected with leishmaniasis. No significant reduction in leishmaniasis transmission was provided by wearing the permethrin treated uniforms (P<0.05).

Length of Trial: 9 months

**Afghanistan
(Faulde, 2009)**

Trial type: Year long program with synergistic personal protection techniques and disease control measures, Zoonotic Cutaneous Leishmaniasis intervention

Study population: German field camp for up to 1200 soldiers in Mazar-e Sharif airport, northern Afghanistan. Diagnosis was verified by PCR

Intervention: Theoretical (not measured after impregnation) concentration of 1300mg/m² permethrin polymer coated uniforms. Theoretical 25mg/m² deltamethrin treated bednets and curtains were completed annually. 0.005% Bromadiolon poison baited traps were used for rodent control. Residual heat fogging of rodent burrows and sand fly breeding sites, and non-residual insecticide fogging near living and work areas. Camp sanitation procedures were implemented, including a high stone wall around the camp; soil compaction and stone paving; removal of upper layer of earth; compaction of earth in the surrounding 100m area; and manual removal of all camp vegetation. Personnel were educated on the threat and transmission of vector-borne diseases, to proper timing and use of the treated uniforms, peak sand fly activity times, the use of repellents, and the safest areas to spend leisure and sport times.

Other behaviours recorded: Surveillance of sand fly and rodent populations throughout the trial.

Outcome: Infection rates of leishmaniasis were reduced. The previous year (2005) to interventions was used as baseline for infection rates. 2005 had 17.5% (14 cases/80 persons) infection rate; 2006 had 0.087% (1 case/1150 persons); and 2007 had 0% (0 cases). Quantified Infection Rates (QIRs) were 2005=0.058, 2006=0.0000055, and 2007=0.0.

Length of Trial: 1 year

* A 3.5m² piece of material used to clothe and protect the wearer; worn both day and night

