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## PREVENTION OF MOSQUITO BITES:

Testing the Residual Effectiveness  
of Permethrin-Treated Clothing



# Environmental Health

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## ABOUT THE COVER



We know that mosquitoes are potential pathogen-transmitting vectors, causing human illnesses such as West Nile virus, Zika, and chikungunya.

There are many ways to prevent these vector-borne diseases, including the use of repellents and insecticides. Permethrin, a repellent approved for human use, has been used to treat clothing to provide a layer of protection to the wearer without the direct application of repellent to the skin. This month's cover article, "Residual Effectiveness of Permethrin-Treated Clothing for Prevention of Mosquito Bites Under Simulated Conditions," evaluated the effectiveness of this clothing and the extent to which fabric type, temperature, and number of washes affected mosquito knockdown and mortality, and permethrin content.

See page 8.

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# Residual Effectiveness of Permethrin-Treated Clothing for Prevention of Mosquito Bites Under Simulated Conditions

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**Abstract** Biological hazards such as exposure to ticks and mosquitoes can affect health. Permethrin-treated clothing is available to the public. We don't currently understand, however, the effects of environmental factors such as fabric type, washing, sunlight, and temperature on permethrin content in treated clothing with respect to mosquito knockdown and mortality. We evaluated the extent to which fabric type (100% cotton denim jeans, 100% polyester work shirt, 35% cotton/65% polyester work shirt), light exposure (0 or 100%), temperature (18 °C, 32 °C), and number of washes (0, 3, 12, 36) affected mosquito knockdown 2 hours post-exposure, mosquito mortality 24 hours post-exposure, and permethrin content. All fabrics used in this study were treated with permethrin at a concentration of 125 µg/cm<sup>2</sup>. Denim fabric having no washes and no light exposure showed the highest amount of permethrin. Washing and light exposure significantly reduced the ability of permethrin-treated fabrics to induce mosquito knockdown and/or mortality under the simulated conditions used for this test. Temperatures tested did not affect permethrin content or mosquito knockdown and mortality. Long-lasting impregnation of uniforms protects against mosquito bites under simulated laboratory conditions. Employers and employees should consider the use of permethrin-impregnated clothing and uniforms in addition to daily repellent sprays.

## Introduction

Biological hazards such as arthropod bites can affect health and include exposure to potential pathogen-transmitting vectors such as mosquitoes. Mosquito bites can cause local to systemic allergic reactions, depending on immune response (Crisp & Johnson, 2013). Work attire differs among state and consulting foresters, park rangers, etc. Consequently, variation in protection from vectorborne disease may exist and affects risk assessments.

In the U.S., mosquitoes transmit viral pathogens that can cause human illnesses such as West Nile encephalitis (approximately 1,000 cases/year in nonoutbreak years), La Crosse encephalitis (approximately 100 cases/year), and Eastern equine encephalitis (approximately 10 cases/year) (Centers for Disease Control and Prevention [CDC], 2016). Foresters are at a higher risk of contracting vectorborne pathogens than the general public due to the outdoor nature

of their work (Covert & Langley, 2002). A study of U.S. National Park Service employees at the Great Smoky Mountain and Rocky Mountain National Parks showed an increase in zoonotic infections in workers related to job description (e.g., more infections in resource managers compared with administrators) (Adjemian et al., 2012).

Permethrin is a repellent/insecticide approved for human use by the U.S. Environmental Protection Agency (U.S. EPA). This pyrethroid is an effective repellent and can be lethal against arthropods including ticks and mosquitoes (Miller, Wing, Coper, Klavons, & Kline, 2004; Young & Evans 1998). Clothing can be treated with permethrin using a variety of methods such as spraying, dipping, polymer coating, and microencapsulation. The latter technique is reported to be the most resistant to washing; however, published reports evaluating this method are lacking (Banks, Murray, Wilder-Smith, & Logan, 2014).

An 8-night field study in Iranian military personnel showed that permethrin-soaked uniforms (information on fabric type not provided) treated with 125 µg/cm<sup>2</sup> permethrin provided 73%, 87%, 90%, 84%, and 79% protection, respectively, against night-biting mosquito species *Culex bitaeniorhynchus*, *Cx. tritaeniorhynchus*, *Cx. perexiguus*, *Cx. theileri*, and *Anopheles stephensi* (Khoobdel et al., 2006).

A similar study evaluating repellency of permethrin-treated military uniforms (information on fabric type not provided) treated with 125 µg/cm<sup>2</sup> permethrin showed 89% protection against *Cx. pipiens* (Khoobdel et al., 2006). The aforementioned studies showed no significant difference in perme-

thrin content in uniforms over the course of the study; however, no attempt was made to wash and/or expose the clothing to environmental conditions and no information was provided on the fabric type of the uniforms.

Another study by German military personnel evaluated polymer-coated permethrin-impregnated uniforms (65% cotton/35% polyester; reported to remain effective for up to 100 washings) for 6 months under tick-infested field conditions (Faulde et al., 2015). The study showed up to 99.6% tick bite reduction in personnel properly wearing uniforms, although washing methods and/or frequency of washing was not noted. The U.S. military uses permethrin-treated uniforms to limit casualties due to vectorborne disease; they emphasize that proper wearing of the uniform is important for prevention of mosquito and tick bites (Shultz, 2001).

Others have shown that natural fabrics display repellent/insecticidal properties for longer than synthetic materials such as polyester (Wood et al., 1999). Environmental variables, however, may impact these properties and permethrin will be lost over time with normal wear (Frances, Watson, & Constable, 2003). Permethrin-treated fabrics (115–147  $\mu\text{g}/\text{cm}^2$  applied by dynamic absorption method, i.e., sprayed on with total absorption and no dripping) were exposed to various degrees of weathering (e.g., temperature, xenon light, humidity, water spray to simulate rain) for 9 weeks (Gupta, Rutledge, Reifenrath, Gutierrez, & Korte, 1989). The same study showed 50% cotton/50% nylon twill fabric was 93% effective against *Aedes aegypti* bites for 6 weeks, while 100% cotton poplin fabric showed 92% mosquito repellency for only 3 weeks.

The toxic effect (0–2% knockdown) of permethrin in weathered fabrics decreased more rapidly than the repellent effect against *Ae. aegypti*. Chemical tests using gas chromatography (GC) showed a decrease in permethrin after the first week of weathering (Gupta et al., 1989). Another study that washed permethrin-treated (treatment method not reported) fabric with warm water and 4 g/L laundry detergent in a commercial washing machine found 100% and <5% knockdown of *Ae. aegypti* after the first and second wash, respectively (Frances & Cooper 2007). Conversely, another study showed that fabric from Iranian military uniforms retained 75% of the initial permethrin ( $93.5 \pm 2.7 \mu\text{g}/\text{cm}^2$ )

after being soaked for 12.5 hours in water (Khoobdel, 2010).

In 2003, U.S. EPA approved (registration #74843-2) the proprietary use of Insect Shield (www.insectshield.com) factory-sprayed or dipped permethrin-treated clothing in commercially available apparel. Insect Shield reports that treated clothing (fabric tested: 50% cotton/50% nylon) can be washed up to 70 times before losing effectiveness. U.S. EPA requires that the active ingredient (permethrin) be expressed as a percentage of weight of the active compared to the overall product weight. The weight of garments (and the fabrics from which they are made) can vary, e.g., denim fabric in jeans might weigh 200  $\text{g}/\text{m}^2$  compared with a military uniform at 100  $\text{g}/\text{m}^2$ . Therefore, the jean might get roughly twice the amount of active ingredient as the Army uniform, so that each fabric type gets about 125  $\mu\text{g}/\text{cm}^2$  (J. Griffin, of Insect Shield, personal communication, February 17, 2014).

A nonrandomized pilot study ( $N = 16$  participants) of outdoor workers in the North Carolina Division of Water Quality showed a 93% reduction in tick bites for workers who wore Insect Shield-treated clothing for 7 months (Vaughn & Meshnick, 2011). A separate pilot study evaluating permethrin-treated clothing in foresters working in the Central Appalachian region of the U.S. showed that control participants received fewer tick bites compared with foresters wearing treated clothing; however, more control participants were exposed to at least one bite compared with foresters in the treatment group (Richards, Balanay, & Harris, 2015).

A large-scale study evaluating the effectiveness of Insect Shield-treated uniforms in state forestry and recreation/parks employees in North Carolina concluded that the clothing was less effective at repelling ticks in the second year of wear (38% protection) compared with the first (83% protection) (Vaughn et al., 2014). While the aforementioned study showed that permethrin-treated uniforms were effective, it left two gaps in knowledge: How well does permethrin work against mosquitoes and why does its efficacy disappear? Limited studies have evaluated the extent to which environmental conditions impact mosquito knockdown for permethrin-treated fabrics (Schreck, Mount, & Carlson, 1982).

While state forestry personnel wear uniforms, consulting foresters wear a variety of

work clothing, including shirts of lightweight fabric and jeans that may be twice the weight of military or other uniforms. With thousands of state and consulting foresters in the U.S., safety concerns and/or disparities in protection within this group or workers must be addressed.

## Materials and Methods

### Permethrin Treatment of Clothing

Two sets of three types of clothing were used: A) 100% cotton, i.e., denim jeans (Grainger, Lake Forest, IL); B) 100% polyester, i.e., lightweight work shirt (Grainger, Lake Forest, IL); and C) 35% cotton/65% polyester, i.e., U.S. Forest Service uniform field shirt (Human Technologies Corporation, Utica, NY).

One set of each type of clothing was sent to Insect Shield for treatment with permethrin. All fabrics used in this study were treated with permethrin at a concentration of 125  $\mu\text{g}/\text{cm}^2$  (J. Griffin of Insect Shield, personal communication, February 17, 2014). Two replicate swatches (5  $\text{cm}^2$ ) were cut from each treated and untreated fabric and used for experiments for a total of 192 swatches (Table 1). Temperature treatments (18 °C and 32 °C) were based on temperatures during spring (Weather Underground, 2017a) and summer (Weather Underground, 2017b) months in North Carolina.

### Simulated Environmental Exposure

The washing portion of the study lasted for 36 days (one day per wash). Clothing swatches were soaked for 10 min in containers with 250 mL cold tap water and 1 mL of All Free and Clear detergent, and then rinsed in cold tap water for 15 s. This process was carried out twice and swatches were air dried in incubators overnight (18 °C or 32 °C) between daily successive washings. Washing treatments include no washes (i.e., new garment), three washes (i.e., number of washes garment would receive in 1 week), 12 washes (i.e., number of washes garment would receive in 1 month), and 36 washes (i.e., number of washes garment would receive in 1 season).

Clothing in simulated sunlight groups were hung in an incubator (18 °C or 32 °C) and exposed to light from a xenon lamp (i.e., 3 wash group: 72 h light exposure; 12 wash group: 288 h; 36 wash group: 864 h) (estimated sunlight exposure of outdoor worker, assuming 8 hours of daily exposure). Fabrics

in the zero wash group served as a control for the xenon light treatment and were exposed to light for 864 h. Fabrics in the group experiencing no light were kept in an incubator with no light.

### Mosquito Experiments

Mosquito knockdown/mortality experiments were conducted for each fabric swatch. *Ae. albopictus* (F<sub>13-15</sub>) originating from New Orleans, Louisiana, were used for knockdown/mortality experiments. The colony had no history of insecticide exposure. Mosquitoes were reared under standard conditions (Richards, Anderson, & Alto, 2012) and eggs were hatched in plastic rearing pans (12 cm x 8 cm x 5 cm) with 1.0 L of tap water and 200 mg larval food (1:2 mixture of brewer's yeast and liver powder).

Larvae were fed every other day for approximately 4 days. Pupae were transferred to 25 mL plastic cups containing 20 mL water, and adults were allowed to emerge in square cages (33 cm<sup>3</sup>) and provided 20% sucrose *ad libitum*. For each group and replicate (N = 192), approximately 12 female mosquitoes were transferred via mechanical aspirator to clear plastic cones (65 mm length x 15 mm stem diameter) (Fisher Scientific) placed over each fabric swatch and held for 3 min, to approximate the World Health Organization Pesticides Evaluation Scheme (WHOPES) (World Health Organization, 2013).

After the fabric exposure period, mosquitoes from each replicate were aspirated from funnels and transferred to separate 0.5 L cardboard cages (Instawares) with mesh screening on top; they were provided with 20% sucrose *ad libitum*. Mosquitoes were held in an incubator at 28 °C for the duration of the experiment. The extent to which mosquitoes were knocked down (i.e., lying on back or side and unable to fly) was assessed and recorded at 2 hours post-exposure (hpe) and mortality was assessed at 24 hpe (as in WHOPES).

### Permethrin Content in Fabrics

We adapted published methods (Gupta et al., 1989) to analyze permethrin content. After mosquito experiments were complete, each fabric swatch (N = 192 swatches) was transferred to individual 60 mL amber glass vials containing 40 mL acetone and soaked for 6 hours to elute permethrin. A portion of the extract (1 µL) was analyzed directly by capil-

| Fabrics | # of Washes | Light (%) | Temperature (°C) | Permethrin |
|---------|-------------|-----------|------------------|------------|
| A, B, C | 0           | 0         | 18               | Yes        |
| A, B, C | 0           | 100       | 18               | Yes        |
| A, B, C | 3           | 0         | 18               | Yes        |
| A, B, C | 3           | 100       | 18               | Yes        |
| A, B, C | 12          | 0         | 18               | Yes        |
| A, B, C | 12          | 100       | 18               | Yes        |
| A, B, C | 36          | 0         | 18               | Yes        |
| A, B, C | 36          | 100       | 18               | Yes        |
| A, B, C | 0           | 0         | 32               | Yes        |
| A, B, C | 0           | 100       | 32               | Yes        |
| A, B, C | 3           | 0         | 32               | Yes        |
| A, B, C | 3           | 100       | 32               | Yes        |
| A, B, C | 12          | 0         | 32               | Yes        |
| A, B, C | 12          | 100       | 32               | Yes        |
| A, B, C | 36          | 0         | 32               | Yes        |
| A, B, C | 36          | 100       | 32               | Yes        |
| A, B, C | 0           | 0         | 18               | No         |
| A, B, C | 0           | 100       | 18               | No         |
| A, B, C | 3           | 0         | 18               | No         |
| A, B, C | 3           | 100       | 18               | No         |
| A, B, C | 12          | 0         | 18               | No         |
| A, B, C | 12          | 100       | 18               | No         |
| A, B, C | 36          | 0         | 18               | No         |
| A, B, C | 36          | 100       | 18               | No         |
| A, B, C | 0           | 0         | 32               | No         |
| A, B, C | 0           | 100       | 32               | No         |
| A, B, C | 3           | 0         | 32               | No         |
| A, B, C | 3           | 100       | 32               | No         |
| A, B, C | 12          | 0         | 32               | No         |
| A, B, C | 12          | 100       | 32               | No         |
| A, B, C | 36          | 0         | 32               | No         |
| A, B, C | 36          | 100       | 32               | No         |

*Note.* Each group was replicated once for a total of 192 fabric swatches. Fabrics tested were A) 100% cotton (denim jeans), B) 100% polyester (lightweight work shirt), and C) 35% cotton/65% polyester (U.S. Forest Service uniform field shirt).

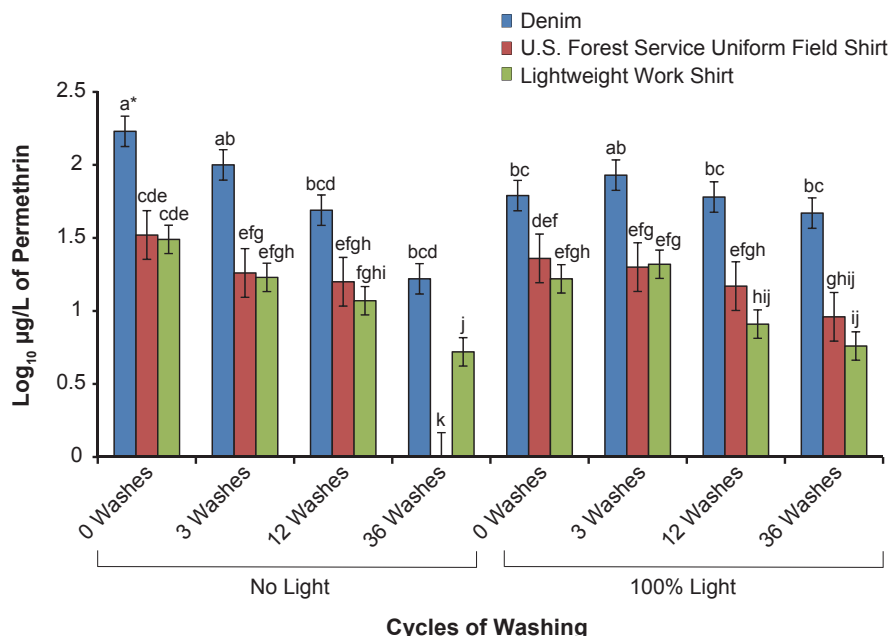
lary GC with flame ionization detector (GC-FID) using an Agilent GC 6850.

The capillary column used was DB-5MS (5% phenyl-methylpolysiloxane), 15 m x 0.25 mm (intra diameter), 0.25 µm (film thickness) (Agilent Technologies). The injector and detector temperatures were set at 250

°C and 260 °C, respectively. The oven temperature was programmed from 200 °C–250 °C (Hengel, Mourer, & Shibamoto, 1997) at 10 °C/min and held for 7 min, with a total run time of 12 min. Nitrogen was used as both carrier (32.6 mL/min) and make-up (10 mL/min) gas, and hydrogen was used as

FIGURE 1

### Permethrin Content (µg/L) in Fabrics Experiencing Different Number of Washes and Light Exposure



Fabrics are denim (100% cotton), U.S. Forest Service uniform field shirt (35% cotton/65% polyester), and lightweight work shirt (100% polyester).

\*The letters above each bar represent differences or similarities between treatment groups. Treatment groups with the same letter are not significantly different by means comparison. Treatment groups with a different letter are significantly different by means comparison.

TABLE 2

### Analysis of Variance Showing Differences in Permethrin Quantities Between Fabrics, Light Exposures, Temperatures, and Number of Washes

| Variable               | df (numerator, denominator) | F     | p-Value           |
|------------------------|-----------------------------|-------|-------------------|
| Fabric                 | 2, 83                       | 92.49 | <b>&lt; .0001</b> |
| Light                  | 1, 83                       | 0     | .965              |
| Temperature            | 1, 83                       | 0.41  | .526              |
| Washes                 | 3, 83                       | 27.84 | <b>&lt; .0001</b> |
| Light and washes       | 3, 83                       | 5.51  | <b>.002</b>       |
| Light and fabric       | 2, 83                       | 3.75  | <b>.028</b>       |
| Temperature and washes | 3, 83                       | 0.02  | .996              |
| Temperature and fabric | 2, 83                       | 0.34  | .711              |

Note. Significant values are shown in bold.

the detector gas (30 mL/min). A permethrin stock solution was prepared by dissolving 0.01 g permethrin (99.0% Crescent Chemical) in acetone (40 mL) and was used to prepare the calibration standards.

Five-point calibration curves were used at the beginning and end of each set of samples and the average of the standards was used to generate the calibration curve for quantification. The linearity of the detector response was checked before conducting analysis by using these calibration curves.

### Statistical Analyses

Normality was verified with Kolmogorov-Smirnov tests. The proportions of mosquitoes knocked down at 2 hpe and dead at 24 hpe were placed into five categories: 1)  $x < 0.20$ , 2)  $0.40 > x > 0.19$ , 3)  $0.6 > x > 0.39$ , 4)  $0.80 > x > 0.59$ , and 5)  $x > 0.79$ . Multinomial logistic regression ( $p < .05$ ) was used to predict the likelihood of mosquito knockdown and mortality on the basis of several independent variables (i.e., fabric type, light exposure, pesticide treatment, temperature, and wash frequency) (SAS Institute). Analysis of variance (ANOVA) was used to evaluate differences in permethrin content within treatment groups. Permethrin quantities were log-transformed prior to using ANOVA to improve normality. If significant differences were observed, then a Duncan test was used to determine differences in the means. Spearman rank correlation coefficient tests were used to compare mosquito knockdown at 2 hpe and/or mortality at 24 hpe to permethrin concentration.

### Results

#### Permethrin Content

The number of washes ( $p < .0001$ ) and fabric type ( $p < .0001$ ) significantly affected permethrin content (Figure 1, Table 2). Light alone did not affect permethrin content; however, fabrics subjected to washing treatments under different lighting and temperature conditions (light and washes:  $p = .002$ ; light and fabric:  $p = .033$ ) affected the permethrin content. Fabrics washed 0 or 3 times showed significantly higher permethrin content than fabrics washed 12 or 36 times. Denim fabric having no washes and no light exposure showed significantly higher permethrin ( $180.5 \pm 36.2$  µg/L) than all other fabrics (Figure 1). After 36 washes, no permethrin was detected in

the U.S. Forest Service uniform field shirt not exposed to light; however, a low level of permethrin was detected in the same type of shirts that were exposed to light (Figure 1). Temperatures tested here showed no effects on permethrin content.

### Mosquito Knockdown and Mortality

Permethrin content was correlated with the proportion of mosquitoes knocked down at 2 hpe ( $r_s = 0.412$ ,  $p < .0001$ ) and dead at 24 hpe ( $r_s = 0.265$ ,  $p = .0090$ ). Fabric washing and light exposure significantly reduced the ability of permethrin-treated clothing to induce mosquito knockdown (washing:  $p < .0001$ , 37–60% reduction; light:  $p = .009$ , 7% reduction) and/or mortality (washing:  $p < .0001$ , 24–35% reduction; light:  $p < .0001$ , 12% reduction) (Table 3, Figures 2 and 3).

Fabrics receiving no washing showed the highest mosquito knockdown and mortality and fabric type did not impact mosquito effects. The highest mosquito knockdown at 2 hpe and mortality at 24 hpe was observed for the unwashed lightweight work shirt (100% polyester) not exposed to light (88% knocked down, 70% mortality). The lowest numbers of mosquitoes knocked down after 2 hpe ( $N = 0$ ) were observed for the U.S. Forest Service uniform field shirt (35% cotton/65% polyester) washed 36 times and either exposed to light or no light and the lightweight work shirt washed 36 times and exposed to light. No mosquito mortality 24 hpe was observed in the lightweight work shirt washed 36 times and exposed to light. Temperatures tested showed no effects on mosquito knockdown and mortality.

### Discussion

Long-lasting permethrin-treated clothing offers an alternative to repeated application of insect repellents to skin and clothing. Field evidence, however, suggests a reduction in effectiveness of permethrin-treated clothing against tick bites after 1 year (Vaughn et al., 2014). In this simulated laboratory study, we found that both permethrin content and mosquito knockdown activity decreased with washing and exposure to light. Although no permethrin was detected in the U.S. Forest Service uniform field shirt (no light exposure) after 36 washes, we did detect permethrin in the same work shirt that had been exposed to light. We hypothesize there might have been variation in permethrin

TABLE 3

**Logistic Regression Testing the Relationships of Categorized Proportions of *Aedes albopictus* at 2 hpe (Knockdown) and 24 hpe (Mortality) After Exposure to Different Fabric Types, Light Treatments, Pesticide Treatments, Temperatures, and Number of Washes**

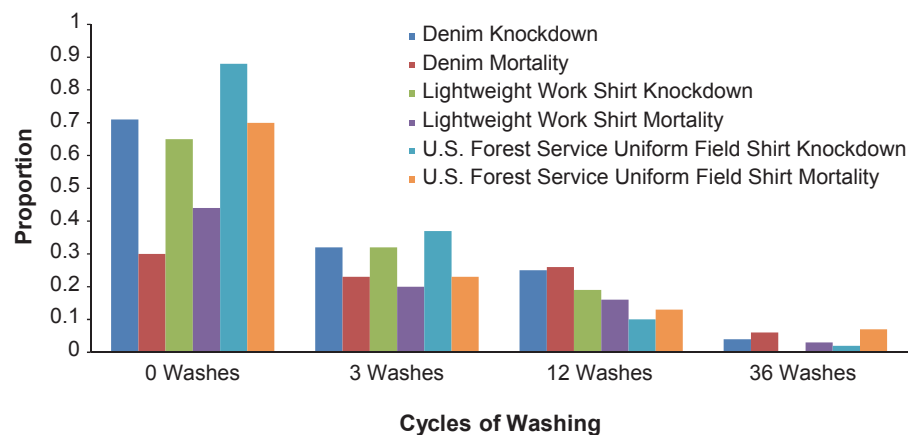
| Variable         | 2 hpe |          |                   | 24 hpe |          |                   |
|------------------|-------|----------|-------------------|--------|----------|-------------------|
|                  | df    | $\chi^2$ | p-Value           | df     | $\chi^2$ | p-Value           |
| Fabric           | 2     | 5.61     | .061              | 2      | 3.08     | .214              |
| Light            | 1     | 6.86     | <b>.009</b>       | 1      | 26.50    | <b>&lt; .0001</b> |
| Pesticide        | 1     | 80.85    | <b>&lt; .0001</b> | 1      | 57.39    | <b>&lt; .0001</b> |
| Temperature      | 1     | 1.93     | .165              | 1      | 3.62     | .057              |
| Washes           | 3     | 66.89    | <b>&lt; .0001</b> | 3      | 28.28    | <b>&lt; .0001</b> |
| Likelihood ratio | 8     | 217.29   | <b>&lt; .0001</b> | 8      | 108.76   | <b>&lt; .0001</b> |

hpe = hours post-exposure.

Note. Significant values are shown in bold.

FIGURE 2

**Proportion *Aedes albopictus* Experiencing Knockdown (2 hpe) or Mortality (24 hpe) After Being Exposed to Different Fabrics (Not Exposed to Light) for 3 Minutes**



hpe = hours post-exposure.

Fabrics are denim (100% cotton), U.S. Forest Service uniform field shirt (35% cotton/65% polyester), and lightweight work shirt (100% polyester).

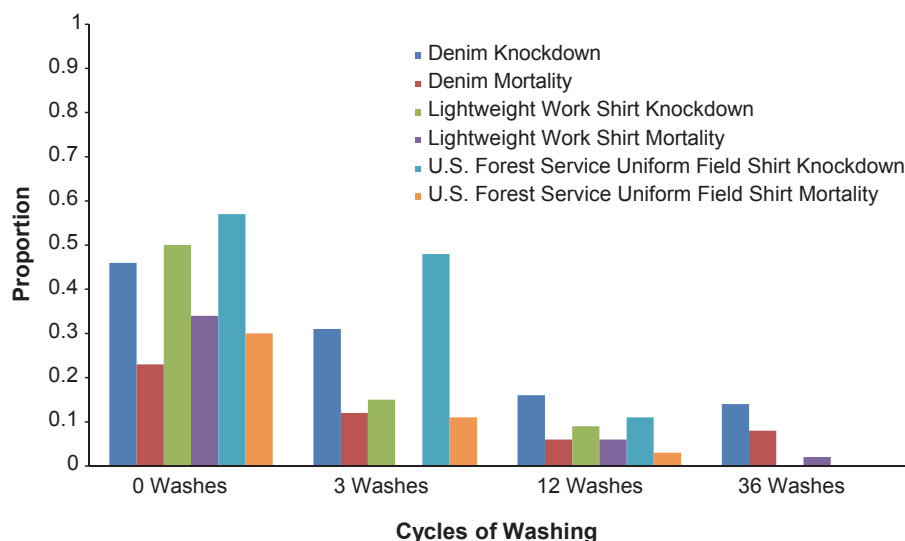
content across the shirt, possibly due to the fabric type. Further evaluation is warranted. While permethrin content remained high for 36 washes in some fabrics, mosquito knockdown activity was reduced substantially between 12 and 36 washes. This weakening of response happened more quickly than had previously

been observed (U.S. Environmental Protection Agency, 2016a, 2016b).

Permethrin content, but not mosquito knockdown activity, varied by fabric type. Denim (100% cotton) exhibited higher permethrin content than both the typical work shirt fabric (100% polyester) and the

FIGURE 3

**Proportion *Aedes albopictus* Experiencing Knockdown (2 hpe) or Mortality (24 hpe) After Being Exposed to Different Fabrics (Exposed to Light) for 3 Minutes**



hpe = hours post-exposure.

Fabrics are denim (100% cotton), lightweight work shirt (100% polyester), and U.S. Forest Service uniform field shirt (35% cotton/65% polyester).

U.S. Forest Service uniform field shirt fabric (35% cotton/65% polyester). Although denim showed the highest permethrin content due to higher fabric weight/area, the treated surface area (5 cm<sup>2</sup>) to which mosquitoes were exposed was the same for all fabrics. Consequently, mosquitoes showed no significant differences in knockdown or mortality among the tested fabrics. Additional mosquito species and populations should be tested to determine repeatability of these effects.

Another study evaluating 0–55 washings for military uniforms (65% cotton/35% polyester) soaked with a synthetic pyrethroid against *Ae. aegypti* showed that mosquito repellency (but not mortality after 24 h) decreased after 25 washings (Sukumaran, Sharma, Wasu, Pandey, & Tyagi, 2014). The same study showed 100% mortality (after 24 h) for mosquitoes exposed to fabrics that had been washed up to 55 times.

Gas chromatograph analysis of permethrin-treated military clothing (40% Permanone or 27.5% Ptamex) worn in the field, washed four times, and then exposed (in the labora-

tory) to *Ae. aegypti* and *An. quadrimaculatus* Meigen showed 5% loss of permethrin from wear and 49% loss after washes (Schreck et al., 1982). The permethrin clothing treatment in the aforementioned study, however, differed from our study. An adapted WHOPES method was used on different types of bed nets (polyethylene, polyester) using a variety of washing (hand, machine) and drying (sun, shade, hanging, laid on ground) methods and showed that hand washed nets hung to dry in the shade maintained the highest insecticidal properties (Atieli, Munga, Ofulla, & Vulule, 2010). It is not necessary, however, to wash bed nets as frequently as clothing worn in the field, because nets are not as likely to get dirty as frequently as clothing.

Our results demonstrate that, regardless of fabric type, washing 12–36 times progressively decreases the effectiveness (measured here using knockdown and mortality) against mosquitoes. Assuming weekly washing, repellent activity would last 4–9 months; however, this duration would depend on washing method and other environmental factors. Thus, foresters and other

outdoor workers should have multiple sets of treated work clothing and/or carry permethrin spray to ensure protection from mosquito exposure for each season. A cost-effectiveness analysis could be conducted to compare the cost and ease of use for permethrin treatment of garments to daily personal application of insect repellants, e.g., Insect Shield treatment costs \$7.95–\$9.95/garment compared to popular products such as Off! Deep Woods (N, N-diethyl-meta-toluamide, or DEET) (SC Johnson) or Repel Permethrin (permethrin) (Spectrum Brands) that cost approximately \$0.34–\$0.68/mL. Compliance with these preventive measures, however, is usually low.

Workers also could dip their own clothing in commercially available products containing permethrin; however, this do-it-yourself method may increase health risks due to higher than normal pesticide exposure levels (Pages et al., 2014). The absorption of permethrin from two types of commercially available impregnated clothing was tracked using urinary metabolites (Rossbach, Niemietz, Kegel, & Letzel, 2014). They concluded that permethrin content in the body for those wearing permethrin-treated clothing would be higher than for those not wearing this clothing, and that this burden would increase with daily use. The authors suggest unwashed clothing would result in greater permethrin uptake compared to uptake after several launderings. At the U.S. Army-recommended permethrin-treatment level of 125 µg/cm<sup>2</sup> and estimated 2% skin absorption (Taplin & Meinking 1990), someone wearing this clothing for a week would be exposed to 35 µg/kg/week. This level is lower than the U.S. EPA recommendation of 350 µg/kg/week (Frances & Cooper 2007).

## Conclusion

Limitations and considerations for future studies are as follows. Differences observed in mosquito knockdown in the current study compared with previous assays could have been due to our use of cones instead of petri dishes to measure knockdown activity (J. Griffin, Insect Shield, personal communication, September 4, 2014). Additionally, the difference could be due to the use of different fabric, conditions of washing, and mosquito species/populations. The impacts of permethrin-treated clothing on knockdown for dif-

ferent mosquito species and other types of biting flies could be evaluated in a separate study.

Others have shown that the WHOPES washing method (for bed nets) is gentler than field methods used such as rubbing with hands and/or rocks (Ateili, Munga, Ofulla, & Vulule, 2010). It is currently unknown whether most workers such as foresters wash their uniforms in washing machines, by hand, or dry clean their uniforms; this remaining question should be investigated further in a separate study to fully characterize the reduction in mosquito-repellent efficacy due to washing.

There is currently no WHOPES washing method for insecticide-treated clothing (Faulde et al., 2015). We tested only two temperatures in this study due to funding constraints; however, future studies could assess a range of temperatures and humidity levels that would represent a variety of different environments.

Variation in ultraviolet light from our xenon light source might not precisely approximate sunlight conditions, but was used as a proxy for natural conditions in this laboratory simulation. A cost-benefit analysis would be useful for employers and individuals working in outdoor environments where arthropod exposure is common.

Results from this laboratory study show that long-lasting impregnation of uniforms negatively impacts mosquitoes; this finding may translate to protection from bites in the field on body parts that are covered with clothing, albeit for less than 1 year. Field studies are needed to evaluate the bite protection of different types of fabric under a variety of environmental conditions. The risk of mosquito- and tick-borne disease is high among outdoor workers, such as foresters, throughout the world. Employers and employees should recognize occupational health risks and consider the use of perme-

thrin-impregnated clothing and uniforms in addition to standard prevention practices such as periodic tick checks. 🐛

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