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Assessing Durability and Safety of Permethrin Impregnated Uniforms Used by Outdoor Workers to Prevent Tick Bites after One Year of Use

Stephanie L. Richards,[1,5] Jeffrey Driver,[2] Megan C. Dyer,[3] Thomas N. Mather,[3] Sheana Funkhouser,[4] Cedar Mitchell,[4] Jo Anne Balanay,[1] Avian White,[1] and Steven Meshnick[4]

[1]Environmental Health Sciences Program, Department of Health Education and Promotion, College of Health and Human Performance, East Carolina University, 300 Curry Court, Greenville, NC 27858, USA, [2]risksciences LLC, 5285 Gulf of Mexico Dr., Longboat Key, FL 34228, and University of South Florida, College of Public Health, 13201 Bruce B Downs Blvd, Tampa, FL 33612, USA, [3]Center for Vector-Borne Disease, University of Rhode Island, 45 Upper College Rd, Kingston, RI 02881, USA, [4]Department of Epidemiology, University of North Carolina Gillings School of Global Public Health, 135 Dauer Dr, Chapel Hill, NC 27599, USA, and [5]Corresponding author, e-mail: richardss@ecu.edu

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Abstract

Long lasting permethrin-impregnated (LLPI) clothing can retain permethrin and repel ticks for up to three months and without exceeding EPA-approved safe levels; however, little is known about longer term effects of wearing LLPI clothing. Here, permethrin content was measured in new forester pants soon after initial impregnation (Insect Shield) and again one year later after being repeatedly worn by foresters in the field. Urine samples were collected from foresters for biomonitoring of permethrin metabolites at multiple time intervals (pre-use, one-month, three-to-four-months, and one-year post-use). Lethality against nymphal *Ixodes scapularis* Say was measured in clothing after one year of wear by foresters. Furthermore, to test potential variability in permethrin impregnation of different batches of clothing, separate sets of clothing were anonymously sent to Insect Shield for permethrin treatment over a period of three months and permethrin was quantified. Results demonstrated 33% of participants' pants had no measurable permethrin after one year of wear and permethrin content and tick mortality varied significantly between clothing. Only two of the participants' clothing resulted in ≥ 30% tick mortality after one year of wear. Significant differences were observed in 3-PBA and trans-DCCA, but not cis-DCCA metabolites in participants over the four measured time points and were higher than general United States population levels. This study provides practical information on the safety (measured by urinary metabolites) over time of LLPI clothing. It also provides snapshots (pre-washing and after one year of wear) of effectiveness of LLPI clothing as personal protective equipment against ticks for outdoor workers.

Key words: permethrin, LLPI, forester, tick protection

Due to the nature of their work, people who work outdoors are at greater risk for exposure to ticks (Niscigorska et al. 2003, Kaya et al. 2008, Lee et al. 2014); thus, these employees (e.g., foresters) are also at an increased risk of tick-borne diseases (Covert et al. 2002, Cinco et al. 2006, Wallace et al. 2016). Permethrin, a synthetic pyrethroid, is registered by the United States Environmental Protection Agency (US EPA) as an active ingredient (AI) used in insecticides for mosquito/tick control and has been the primary AI used to impregnate clothing for arthropod bite prevention (EPA 2009, 2017). One of the recommendations provided by the National Institute for Occupational Safety and Health (NIOSH) for protecting workers from tick-borne diseases is the treatment of clothing and gear with permethrin (NIOSH 2010, 2011). Wearing long-lasting permethrin-impregnated (LLPI) clothing is one protective measure to reduce exposure to ticks and other vectors and is frequently cited as a best practice for tick bite protection (Miller et al. 2011, EPA 2017, Prose et al. 2018). The LLPI clothing is different than self-applied permethrin as it is a proprietary, factory treatment that binds the permethrin to fabrics. Studies have demonstrated the effectiveness of LLPI clothing in protecting outdoor workers (including military personnel) against various species of ticks (Vaughn et al. 2011, 2014, Faulde et al. 2015, Richards et al. 2015, Mitchell et al. 2020) and mosquitoes (Londono-Renteria et al. 2015). Routine wear, sunlight exposure, washing, and drying of clothing reduces permethrin content, which reduces protection against arthropods. For example, in a recently conducted double-blind randomized placebo trial, LLPI clothing was shown to provide a two-year protective effect of 58% against tick bites for outdoor workers in the Northeastern U.S., with reduced protection in the second year compared to the first year (Mitchell et al. 2020).

The EPA states that permethrin is poorly absorbed through the skin, and that factory-treated LLPI clothing is unlikely to cause significant immediate or long-term adverse effects to wearers (EPA 2017). The World Health Organization (WHO) reports the acceptable daily intake (ADI) of permethrin is 50 mcg/kg of body weight (WHO 1999). Biomonitoring studies have assessed the absorption of permethrin into the body of outdoor workers who used LLPI clothing (Appel et al. 2008, Rossbach et al. 2010, Kegel et al. 2014, Proctor et al. 2014). An exposure level of 5–6 µg permethrin/kg of body weight (this was < ADI) was estimated for soldiers wearing permethrin-impregnated uniforms (Appel et al. 2008). Rossbach et al. (2016) found permethrin uptake by foresters who wore LLPI clothing for three months was below the ADI value. Others reported that longer duration of wear (Proctor et al. 2014) and smoking while wearing the clothing (Kegel et al. 2014) increased permethrin exposure/absorption. It is important to investigate permethrin uptake in long-term wearers of LLPI clothing to better assess any associated potential risks.

The aims of this study are to: 1) compare permethrin content in uniform pants at the initial impregnation point with content after one year of field wear and washing/drying by outdoor workers; 2) determine the amount of permethrin absorbed by outdoor workers wearing LLPI clothing over a one year period; 3) determine the lethality for nymphal *Ixodes scapularis* Say of unworn new LLPI clothing and worn clothing; and 4) evaluate the extent to which there may be variability in permethrin content of different batches of LLPI clothing.

Materials and Methods

Participants

Twelve participants were recruited between May and July 2016 from outdoor workers at the North Carolina (NC) Divisions of Parks & Recreation and Wake County Parks in central NC. Eligible and enrolled participants were over 18 yrs of age, gave informed consent, and wore a uniform at least four days each week during the spring/summer seasons. Workers were excluded if they were pregnant or planned to become pregnant over the one-year study duration, had a known allergy or sensitivity to insecticides or were non-English speakers. Non-English speakers were excluded from this pilot study because study staff only spoke English and would not have been able to obtain consent. Study personnel contacted workers and invited them to participate in the study. The study design and procedures were approved by an institutional review board at the University of NC Chapel Hill (#15-1770). All participants provided written, informed consent.

Study Procedures

At the beginning of the study, participants were asked to send spring or summer uniform items (except underwear/briefs) to Insect Shield (Greensboro, NC) for standard LLPI treatment (treated in summer 2016). Participants sent half of their uniforms to be treated and, upon receiving those back, sent the other half. Participants were instructed to notify study personnel if they ordered new clothing during the study. Spot urine samples were collected from each participant at the following time points during the study: 1) before wearing LLPI clothing, 2) after one month of first LLPI clothing use, 3) after three to four months of first LLPI clothing use, and 4) after one year of LLPI clothing use. Urine samples were collected following standard procedures (Sullivan et al. 2019) and were aliquoted and stored at −80°C and then shipped on dry ice to the Centers for Disease Control and Prevention (CDC) environmental health laboratory for testing (part of the CDC's National Biomonitoring Program through Division of Laboratory Sciences) (CDC 2021a, b).

At the end of the study (summer 2017) after one year of wearing LLPI clothing, participants completed a questionnaire indicating frequency of uniform wear, personal use of insecticides or repellents, and tobacco use and other demographic information. At this time, participants also were asked to submit one set of uniform items to study personnel. Clothing items were sent first to the University of Rhode Island for tick-killing efficacy studies and then were sent to East Carolina University where permethrin retention studies were conducted on clothing.

Laboratory Analysis of Worn LLPI Clothing

Participant-worn LLPI clothing was evaluated for permethrin retention and tick mortality, along with unworn LLPI and unworn untreated clothing as controls. Three replicate swatches from each pair of pants were collected. For LLPI and untreated controls, three replicate swatches were also taken from each pair of pants (total of six replicate swatches) per control type (two treated pants and two untreated pants).

Permethrin Retention

Swatches cut from forester worn LLPI pants, as well as unworn untreated and unworn LLPI pants, were used to assess the amount of permethrin retained. A separate test was conducted to investigate potential differences in permethrin impregnation between different batches treated by Insect Shield (treated in summer 2018). Unworn pants were anonymously sent to Insect Shield five different times over a period of three months (samples NC A–E) and investigators also tested swatches from these pants for permethrin retention and tick-killing efficacy. Permethrin content was determined as described previously (Richards et al. 2018). The Collaborative International Pesticides Analytical Council (CIPAC) method 331/LN/M/3 is the base method for permethrin extraction from fabrics (e.g., mosquito nets) and shows extraction efficiency using heptane (CIPAC 2009). Here, we have adapted that method using acetone as the solvent as other studies have also done with a variety of tested fabrics (Pennetier et al. 2013, Dieval et al. 2017, Ghamari et al. 2019).

Briefly, each pants swatch was transferred to separate amber glass vials containing 40 mL acetone and soaked for 1 h to extract permethrin in a water-filled Sonicator (Ultrasonic Bath, Fisher Scientific, Kennesaw, GA). Extracts (1.5 mL) from these vials were transferred to 1.5 mL amber gas chromatograph (GC) vials and 1 µL of this extract was analyzed directly by capillary GC with flame ionization detector (GC-FID) using an Agilent GC 6850 (Agilent Technologies, Alpharette, GA).

Tick Bioassays

Nymphal blacklegged ticks (*Ixodes scapularis*) were used in these experiments to follow up on recent observations of reduced protection from LLPI clothing after one year of wear (Mitchell et al. 2020). Nymphal ticks were derived in the laboratory from wild-caught host-seeking females fed on rabbits in the laboratory to produce eggs, and then fed as larvae on hamsters (Mather and Mather 1990) (University of Rhode Island Institutional Animal Care and Use Committee approved protocol AN08-04-017, originally dated June 2008). Engorged larvae were held under 23.5°C/>95% RH and 14h light/10h dark until molting. Molted nymphs were held in the incubator for an additional two to three months before being used in forced exposure bioassay experiments.

To determine tick mortality, 10 lab-reared nymphal blacklegged ticks were placed on horizontally oriented pants swatches (three replicate swatches from each pair of pants) for three minutes before being collected using a tweezer and placed into a plastic snap cap vial with a modified mesh cap and plaster of Paris substrate, and were then held at room temperature in humidified chambers (90–95% RH). After 24 h, ticks were examined to determine their vigor (Eisen et al. 2017) by removing them from vials and testing their responses to gentle physical prodding with tweezers and human breath. Briefly, ticks exhibiting normal movement and responses to stimuli were classified as "alive". Ticks not moving or moving in an uncoordinated way (i.e., some leg movement, but not able to walk or flip over) after response to stimuli were classified as "dead".

Laboratory Analysis of Participant Urine Samples

There are only a few major metabolites of permethrin. We chose three metabolites to estimate permethrin bioavailability. The selected metabolites (i.e., cis-3-[2,2-dichlorovinyl]-2,2-dimethylcyclopropane carboxylic acid, or "cis-DCCA", trans-3- [2,2-dichlorovinyl]-2,2-dimethylcyclopropane carboxylic acid, or "trans-DCCA", and 3-phenoxybenzoic acid, or 3-PBA) are used to estimate urinary concentrations of permethrin and other pyrethroids (cyfluthrin and cypermethrin) as part of the CDC's National Biomonitoring Program (CDC 2021a, b). While each moiety (alcohol and ester) produces metabolites common among pyrethroids, when data are collected for both background and prospective exposure (i.e., before and after wearing permethrin-impregnated clothing), and the estimated dose from the metabolite of each moiety of permethrin are comparable, it is possible to have reasonable confidence in estimating permethrin-specific bioavailability and not other possible pyrethroids with a metabolite common to one moiety or the other. The third and only other significant metabolite of permethrin in mammals (4-hydroxy-3-PBA), is no more definitive for permethrin than 3-phenoxybenzoic acid (3-PBA) itself.

Urine samples were analyzed for the three permethrin metabolites (cis-DCCA; trans-DCCA; and 3-PBA). These target analytes were extracted and concentrated from urine by offline solid phase extraction, separated by high-performance liquid chromatography using a gradient elution program, and analyzed by isotope dilution tandem mass spectrometry as described previously (Davis et al. 2013). Accuracy and precision for each analytical run were monitored using calibration standards, reagent blanks, and quality control materials. Limits of detection for cis-DCCA, trans-DCCA, and 3-PBA were 0.5, 0.6, and 0.1 μg/L, respectively. Urinary creatinine (mg/dL) was also measured at CDC using an enzymatic reaction. Participant urinary creatine measurements are provided as Supplementary Material to facilitate normalization of the metabolite concentrations and comparison of the results of this study to datasets of other studies. Metabolite concentrations less than the level of detection (LOD) were replaced with a value equal to one half of the LOD for the statistical analysis. Metabolite concentrations were used to estimate the dose of permethrin absorbed by participants.

Calculation of Absorbed Dose

The stoichiometry of metabolites (fraction of dose excreted in urine as metabolite) from permethrin was obtained from Ratelle et al. (2015) based on a human metabolism study. The absorbed dosage was estimated from metabolite concentrations based on the general methodology described by Fortin et al. (2008) as:

$$\begin{aligned}\text{Dosage} &= [\text{Metabolite x Urine Excreted x} \\ &\quad (\text{MWpermethrin} = \text{MWmetabolite})] \\ &\quad / [\text{Fraction Metabolite in Urine x Body Weight (kg)}]\end{aligned}$$

Where Dosage = μg permethrin absorbed per kg body weight; Metabolite = μg metabolite per L urine (Table 1); Urine excreted = L urine excreted per day (assumed to be 1.5 L/day (Aylward et al. 2018); MWpermethrin = Molecular weight of permethrin (391 daltons); MWmetabolite = Molecular weight of metabolite (Table 1); Fraction in urine = Fraction of dose excreted in urine as metabolite (Table 1); and Bodyweight = Bodyweight (kg) for each participant.

Statistical Analyses

SAS 9.4 (SAS Institute, Cary, NC) was used for statistical analyses. Analysis of variance (ANOVA) was used to evaluate differences ($P < 0.05$) in permethrin content between LLPI pants submitted by different participants, untreated and LLPI controls. Permethrin quantities were log-transformed (x + 1) to improve normality. When significant differences were observed, a Duncan test was used to further evaluate the differences. The pharmacokinetics of excretion of permethrin metabolites was analyzed as described by Ratelle et al. (2015) to calculate the absorbed permethrin dosage. Repeated measures ANOVA was used to evaluate differences ($P < 0.05$) in metabolite quantities between participants across baseline, one month, three-four month, and one year testing periods. When significant differences were observed, a Duncan test was used to further evaluate the differences. Spearman's correlation analysis was used to measure correlation between multi-month averages of permethrin metabolites and permethrin quantity in participants' clothing ($P < 0.05$). Tick mortality was analyzed using Pearson's chi-square tests to evaluate independence in contingency table analyses and used Tukey's HSD ($P < 0.05$) for significance testing.

Results

Table 1 shows a summary of permethrin content in clothing, *Ixodes scapularis* bioassay results, and urine metabolites (3-PBA, cis-DCCA, trans-DCCA) in 12 participants. Table 2 shows self-reported demographic features, use of treated clothing, and application of insect repellents for all study participants.

Permethrin Content in Clothing

Each of the 12 participants submitted one pair of LLPI uniform pants that they had worn throughout the one-year period of study. Untreated unworn pants and unworn LLPI pants (stored with investigators until the testing period) were used as negative and positive controls, respectively. Unworn, LLPI control pants demonstrated 14.37 μg/cm^2 permethrin in six pants swatches (Table 1). Unworn, untreated control swatches from pants had no permethrin. After one

Table 1. Summary of urine metabolite, permethrin retention in clothing, and tick bioassay results

Control/participant ID	Permethrin equivalent daily dose (μg/kg/day). Multi-month average[a]			Mean ± SD permethrin content (μg/cm²)[b]	Nymphal Tick Bioassays[c]	
	3-PBA	cis-DCCA	trans-DCCA		Mean tick mortality (%)	*P*-Value
Untreated controls	–	–	–	0.0 ± 0.0	0/60 (0)	-
Treated controls	–	–	–	14.37 ± 0.93	57/60 (95)	<0.001*
NC201	1.15	0.46	0.94	11.60 ± 1.41	4/30 (13)	1.000
NC202	1.00	0.42	0.81	0.0 ± 0.0	1/30 (3)	1.000
NC203	7.63	0.42	2.75	9.48 ± 1.10	3/30 (10)	1.000
NC205	0.95	0.08	0.31	0 ± 0.0	0/30 (0)	1.000
NC206	4.64	0.40	2.83	0 ± 0.0	2/30 (7)	1.000
NC212	6.95	0.57	3.92	12.42 ± 1.56	9/30 (30)	0.766
NC213	10.89	3.20	5.16	23.97 ± 3.65	18/30 (60)	0.007*
NC216	3.71	1.01	2.36	3.85 ± 0.25	6/30 (20)	0.993
NC217	0.55	0.14	0.30	0.0 ± 0.0	3/30 (10)	1.000
NC218	7.45	0.94	3.50	8.80 ± 3.25	7/30 (23)	0.965
NC219	2.98	0.40	1.26	7.07 ± 0.60	4/30 (13)	1.000
NC220	0.70	0.17	0.36	41.20 ± 9.42	29/30 (97)	<0.001*

[a]Permethrin equivalent dose estimates based on the unweighted average value derived across each of the urinary metabolites measured at one month, three to four months, and 1 year post use of LLPI; metabolites measured were 3-PBA: 3-Phenoxybenzoic acid; cis-DCCA: Cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid; and trans-DCCA: Trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid.

[b]Mean permethrin content was calculated as the mean of three pants swatches (65% polyester, 35% cotton) tested from the same pair of pants (after one year of wear by participants).

[c]Mean tick mortality was calculated as the mean of 10 lab-reared nymphal blacklegged ticks being applied to each of the three pants swatches tested from the same pair of pants after one year of wear by participants (30 nymphs total) and assessed as dead from visual inspection at 24 h. Significant differences ($P < 0.05$) between participant pants and treated controls when compared to untreated controls using Tukey HSD test are marked with (*).

year of wear, 67% ($N = 8$) of participants' pants had average permethrin levels higher than that measured in the untreated controls (range 3.85–41.2 μg/cm²). The other 33% ($N = 4$) of participants' pants had no measurable permethrin after one year of wear. The mean permethrin content in pants varied between participants after one year of wear (range 0–41.2 μg/cm²). The pants from two participants (NC213 and NC220) contained permethrin levels of 23.97 ± 3.65 and 41.20 ± 9.42 μg/cm², higher than the positive control (14.37 ± 0.93 μg/cm²), even after one year (Table 1) and were the only samples submitted from participants which had a statistically significant tick mortality ($P < 0.05$). Significant differences were observed in fabric permethrin content between participants after one year of wear (df = 11,26; $F = 293.02$; $P < 0.0001$) with participant NC220 having permethrin content significantly ($P < 0.05$) higher than all other participants.

Ixodes scapularis Bioassays

Of the three-minute exposure nymphal blacklegged tick bioassays conducted on pant swatches collected from subjects after one year of wear, only two of the 12 pairs of pants tested were still effective at killing ≥ 30% of the ticks and these results were significantly ($P < 0.05$) different from the untreated unworn pant controls (Table 1). In contrast, all unworn treated clothing samples submitted anonymously (NC-A, NC-B, NC-C, NC-D, and NC-E) delivered a lethal dose of permethrin to ≥ 97% of the exposed nymphal blacklegged ticks (Table 3) which was significantly different ($P < 0.001$) from the untreated unworn control pants.

Urine Metabolites

The 12 participants had urine samples taken prior to wearing LLPI clothing (baseline), and at time points of one month, three to four months, and one year after starting the trial. The median level of urinary permethrin metabolites from all study participants increased in concentration over baseline levels at both one month and three to four months after participants began wearing LLPI clothing (Fig. 1); however, after one year of wearing LLPI clothing, levels of permethrin metabolites in urine samples decreased. Estimated permethrin equivalent doses for each of the three metabolites measured is listed for each participant (Table 1). Significant differences were observed in 3-PBA and trans-DCCA, but not cis-DCCA metabolites in participants over the four measured time points (3-PBA:

Table 2. Self-reported demographic features, use of treated clothing, and application of insect repellents for all study participants ($N = 12$)

Age (years) (mean ± SD)	44.5 ± 9.6
Male (%)	9 (75)
Height (in) (median [IQR])	71.5 [68.2, 73.2]
Weight (lbs)[a] (median [IQR])	212.0 [191.2, 265.0]
Tobacco use (%)	3 (25)
Position title (%)	
Maintenance	4 (33)
Park ranger	5 (42)
Superintendent/ emergency management	3 (25)
Years in current position (median [IQR])	6.8 [3.8, 10.4]
Use of InsectShield®-treated work clothing (%)	
All of the time	4 (33)
Most of the time	6 (50)
Half of the time	2 (17)
Less than half or none of the time	0 (0)
Use of any repellent on skin (%)	7 (58)
DEET (%)	6 (50)
Picaridin (%)	1 (8)
Lemon eucalyptus (%)	1 (8)
Use of any repellent on clothing (%)	5 (42)
Permethrin[b] (%)	1 (8)
DEET (%)	4 (33)
Picaridin (%)	0 (0)
Lemon eucalyptus (%)	0 (0)

[a] Weight values were missing for two participants

[b] Permethrin applied once per month

Table 3. Permethrin content in batches of unworn pants sent anonymously to Insect Shield and treated on different days

Batch	Date Treated	Mean ± SD permethrina content (μg/cm2[a])	Mean tick mortality (%)[b]
Untreated control[c]	N/A	0.0 ± 0.0	0/60 (0.00)
NC-A	7/8/18	97.65 ± 12.56	29/30 (97)*
NC-B	7/30/18	73.57 ± 3.19	29/30 (97)*
NC-C	date unknown	80.52 ± 2.66	30/30 (100)*
NC-D	8/16/18	117.25 ± 9.90	30/30 (100)*
NC-E	9/6/18	96.88 ± 15.91	30/30 (100)*

[a]Mean permethrin content was calculated as the mean of three pants swatches (65% polyester, 35% cotton) tested from the same pair of pants.

[b]Tick mortality was calculated as the mean number of dead ticks observed 24 h after 10 lab-reared nymphal blacklegged ticks were applied for three minutes to each of three pants swatches tested from the same pair of pants (30 nymphs total). Significant differences ($P<0.05$) observed in mortality in ticks exposed to unworn treated control pants compared to unworn untreated control pants using Tukey HSD test are marked with (*).

[c]Untreated clothing is made from the same material as treated clothing but is unworn and not treated with permethrin.

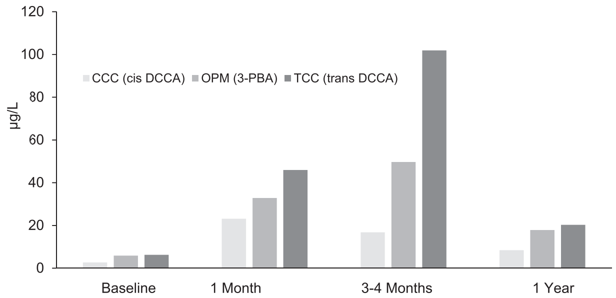

Fig. 1. Temporal permethrin metabolite concentrations (median μg/L) in urine of outdoor workers wearing impregnated clothing. Temporal permethrin metabolite concentrations (median μg/L) in urine of outdoor workers wearing impregnated clothing increased in concentration over baseline levels at both 1 mo and 3–4 mos, but decreased to near baseline levels after one year.

df = 3, 44; F = 3.50; P = 0.0.023; trans-DCCA: df = 3,43; F = 4.76; P = 0.006; cis-DCCA: df = 3, 44; F = 1.85; P = 0.152).

Parametric correlation analyses of the metabolite data demonstrated associations between the three markers of exposure in about half the participants (42–58%) at each time of sampling. A significant ($P < 0.05$) correlation was observed between multi-month averages of 3-PBA and cis-DCCA (42% of participants), 3-PBA and trans-DCCA (58% of participants), and cis-DCC and trans-DCCA (58% of participants) (Table 4). Three participants (NC212, NC216, NC218) demonstrated positive correlation and one participant (NC213) demonstrated negative correlation between permethrin detected in the clothing and urinary metabolites (Table 4).

Unworn pants treated in different batches over a three-month period in 2018 demonstrated a range of permethrin content from 73.57–117.25 μg/cm^2 (Table 3). Significant differences were observed in permethrin content between batches (df = 4, 10, F = 8.26, P = 0.003). The batch of pants (NC-D) treated on 8/16/18 demonstrated significantly ($P < 0.05$) higher permethrin content than the other batches. Batches NC-A and NC-E had significantly higher permethrin content than batches NC-B and NC-C.

Table 2 shows information collected from the 12 participants after one year of wearing LLPI clothing. Most participants (83%) reported wearing the LLPI clothing either all the time or most of the time at work, while 17% wore it only half of the time. More than half (58%) of participants wore repellent (i.e., DEET, picaridin, lemon eucalyptus) on their skin and 42% applied repellent (including permethrin) directly to their clothing. One participant (NC217) reported applying permethrin to their LLPI clothing once per month; however, no permethrin was detected on the clothing after 12 months.

Discussion

Results of this study indicate that treated and unworn commercial LLPI clothing can provide >95% mortality (Tables 1 and 4) to nymphal blacklegged ticks exposed for three minutes, despite occasional batch-to-batch variability in the amount of permethrin retained on the clothing. Different permethrin concentrations were observed in untreated controls used at the beginning of the study in Table 1 (14.37 μg/cm^2) compared to different batch treatments throughout the year shown in Table 3 (range 73.57–117.25 μg/cm^2). Insect Shield applies permethrin by weight so that may explain these differences (i.e., lighter weight fabrics are treated at a lower level than heavier fabrics). The reason for the difference in levels by fabric type is so that wearers receive equal protection, regardless of clothing type (lighter fabrics need less permethrin than heavier fabrics to give the same amount of protection). After one year of wearing and laundering (albeit this was not numerically tracked), the amount of permethrin retained in the clothing was reduced by nearly one half and in such cases, the tick killing ability also was reduced. Ticks experienced > 30% mortality on only two pairs of pants that had been worn over the one-year period, while the other pairs of pants resulted in 0–30% tick mortality. This observation that permethrin content in LLPI clothing can fade over extended time of being worn and laundered suggests the need for annual or semi-annual re-treatment or replacement to maintain protective efficacy. It was expected to have zero or low permethrin content in clothing after one year of wearing and washing and this was the case in five of 12 participants. Variability in permethrin content detected in the pants of outdoor workers after one year of wear was likely the result of variables such as washing/drying frequency, washing technique, type of detergent, sunlight or heat exposure, variation in permethrin added during the impregnation process, personal application of permethrin to clothing, or other unknown factors. None of these variables were assessed in this study. It was assumed that participants wore their treated clothing as directed; variation in LLPI treated clothing use throughout the study period is possible but was not assessed or corrected for. Repeated washing has been shown to reduce the knockdown and/or killing ability of LLPI fabric for vectors (Most et al. 2017, Richards et al. 2017, Connally et al. 2019). Connally et al. (2019) tested ticks on clothing worn by participants for 16 d (over an 8-wk period) and after 16 wash/dry cycles and found similar results, albeit the participants wore the clothing for a shorter duration than in the current study. The same study also reported differences in permethrin lost in shirts (90%) compared to pants and socks (50–60%), which was expected likely due to different fabric weights and materials. Washing technique (machine washing vs. simulated hand washing) and heat exposure (i.e., ironing and/or clothes dryer) also can significantly

Table 4. Significant Spearman correlation between urine metabolites (3-PBA, cis-DCCA, trans-DCCA) in 12 participants and permethrin detected in clothing

Participant ID	3-PBA and cis-DCCA *r* (*P*)	3-PBA and trans-DCCA *r* (*P*)	cis-DCCA and trans-DCCA *r* (*P*)	Permethrin in clothing *r* (*P*)
NC201	1.0 (< 0.0001)	1.0 (< 0.0001)	1.0 (< 0.0001)	-
NC202	-	-	1.0 (< 0.0001)	-
NC203	-	-	1.0 (< 0.0001)	-
NC205	-	1.0 (< 0.0001)	1.0 (< 0.0001)	-
NC206	-	1.0 (< 0.0001)	-	-
NC212	-	-	1.0 (< 0.0001)	1.0 (< 0.0001) for 3-PBA, cis-DCCA, and trans-DCCA
NC213	-	-	-	-1.0 (< 0.0001) for 3-PBA and trans-DCCA
NC216	1.0 (< 0.0001)	-	-	1.0 (< 0.0001) for 3-PBA, cis-DCCA, and trans-DCCA
NC217	-	1.0 (< 0.0001)	-	-
NC218	1.0 (< 0.0001)	1.0 (< 0.0001)	1.0 (< 0.0001)	1.0 (< 0.0001) for 3-PBA, cis-DCCA, and trans-DCCA
NC219	1.0 (< 0.0001)	1.0 (< 0.0001)	1.0 (< 0.0001)	-
NC220	1.0 (< 0.0001)	1.0 (< 0.0001)	1.0 (< 0.0001)	-

Where "-" is indicated, *P*-value was not significant.

affect the extent of permethrin reduction on treated clothing (Banks et al. 2015). In our study, two participants had higher permethrin content in their pants after 1 yr of wear than the unworn LLPI controls, suggesting that these participants likely self-applied permethrin-based repellents to their clothing.

This study is unique in that it evaluates long-term (1 yr) exposure to LLPI and its impacts on urine metabolites, tick mortality, and permethrin retention. Baseline, but not multi-month average levels of permethrin measured here in urinary concentrations (µg/L) of cis-DCCA (Fig. 1; baseline: 2.72 µg/L; multi-month: 16.13 µg/L) and trans-DCCA (Fig. 1; baseline: 6.26 µg/L; multi-month: 56.05 µg/L) among most participants in this study were comparable to, for example, general US population levels of trans-DCCA reported in 2001–2002 (2.56 µg/L; ages 20–59 yrs), 2011–2012 (4.33 µg/L; ages > 20 yrs), or 2013–2014 (6.30 µg/L; ages 20–59 yrs) at the 95th percentile (CDC 2021a; see table on page 182; CDC 2021b; see table on page 180). Baseline, but not multi-month levels of permethrin measured here in urinary concentrations of 3 PBA (Fig. 1; baseline: 5.89 µg/L; multi-month: 33.47 µg/L) among most participants in this study were comparable to, for example, general US population levels of 3 PBA reported in 1999–2000 (3.21 µg/L; ages 20–59 yrs), 2001–2002 (3.25 µg/L; ages 20–59 yrs), 2007–2008 (6.65 µg/L; ages 20–59 yrs), 2009–2010 (6.95 µg/L; ages 20–59 yrs), 2011–2012 (7.32 µg/L; ages > 20 yrs), or 2013–2014 (7.36 µg/L; ages 20–59 yrs) at the 95th percentile (CDC 2021a; see table on page 190; CDC 2021b; see table on page 184). Comparisons between baseline levels of trans-DCCA or 3 PBA in some participants were comparable, but slightly higher or lower than levels in the general US population and the degree of difference depended on the year of baseline population used for comparison. Cis-DCCA levels among the US population were not reported in CDC so are not compared here (CDC 2021a, b).

Appel et al. (2008) discussed that only *ca*. 50% of permethrin is absorbed from clothing (indicating 25 mcg/kg/day would be the internal dose if 50 mcg/kg/day is the ADI). Some participants in the current study had dose levels close to 25 mcg/kg/day, for comparison. Another study evaluating 3-PBA and cis- and trans-DCCA in Army personnel found permethrin in participants higher than the general public, but still within EPA's ADI (Maule et al. 2019). The same study mentioned that military personnel may wear their uniforms longer than the typical eight-hour workday, increasing permethrin exposure. Permethrin absorption/uptake in military personnel was also 60–90% higher under hot and humid conditions, compared to more normal conditions (Maule et al. 2020, Proctor et al. 2020). This effect is generally increased by energy expenditure (e.g., physical activity) and percent body fat (Proctor et al. 2018); however, the number of times a uniform is laundered (hence reducing permethrin) content impacts permethrin absorption (Scarpaci et al. 2020). It should be noted that rate of urinary flow or creatinine excretion may vary between individuals and can potentially impact comparisons of urinary metabolites (Fortin et al. 2008) and this should be considered when comparing metabolites between different studies.

The practical information gained here will inform outdoor workers and employers on efficacy and safety of this clothing over time. The public also has access to commercially available permethrin-treated clothing from a variety of vendors and should be aware of safety precautions and recommendations for washing/wearing and retreatment/replacement, depending on fabric weight/type and other factors. The small sample size of 12 participants should be considered when interpreting results. We expected loss of permethrin retention and effectiveness over time (Faulde et al. 2016, Sullivan et al. 2019, Mitchell et al. 2020). Our findings that permethrin content varies between treatment batches of permethrin impregnation are interesting and may help explain some of the variation in efficacy of LLPI clothing for different individuals.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

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