HHS Public Access

Author manuscript

Toxicol Lett. Author manuscript; available in PMC 2021 March 19.

Published in final edited form as:

Toxicol Lett. 2018 December 15; 299: 210–217. doi:10.1016/j.toxlet.2018.10.001.

Role of Body Composition and Physical Activity on Permethrin Urinary Biomarker Concentrations While Wearing Treated Military Uniforms

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Abstract

Wearing of permethrin treated clothing is becoming more prevalent in military and outdoor occupational and recreational settings, as a personal protection measure against vector borne diseases transmitted through arthropods (e.g., malaria, Lyme disease). The goal of the study was to prospectively examine permethrin exposure among new U.S. Army recruits who had just been issued permethrin-treated uniforms over a 10-week military training period and whether individual body composition (percent body fat, %BF) and physical workload (total energy expenditure, TEE) influenced the exposure. Exposure was assessed by quantification in urine of three permethrin metabolites, 3-phenoxybenzoic acid (3-PBA), and *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid. Although there was individual variability, urinary concentrations and estimated dose levels decreased over the 10-week period. Mixed models demonstrated that 10% higher %BF was significantly associated with 4.42% higher 3-PBA concentrations and a 10% higher daily TEE was significantly associated with a 10.57% higher 3-

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PBA concentrations. Additional factors influencing exposure included sex, number of uniform launderings, and wear-time (hours per previous day).

Keywords

permethrin; military; uniforms; body composition; percent body fat; energy expenditure

1. Introduction

Permethrin ((+/−)-3-phenoxybenzyl-3-(2,2-dicholorovinyl)-2,2-dimethylcyclopropanecarboxylate) is a synthetic pyrethroid pesticide, which is derived from naturally occurring chemicals found in chrysanthemum flowers. It is one of the most widely used pesticides due to its low acute toxicity in mammals and its high effectiveness as both an insecticide and insect repellant (WHO, 2005). Permethrin acts as a neurotoxicant on insects by inhibiting closure of the sodium ion channels in nerve cells (Vijverberg and van den Bercken, 1990), triggering prolonged excitation of affected cells in the body. Insect mortality usually occurs soon after uptake of the pesticide. In humans, localized acute permethrin exposure can result in paresthesia, characterized by sensations of prickliness, itching, and tingling on the skin. Higher doses have been associated with more systemic toxicity with reports of headaches, dizziness and, in severe cases, coarse muscular fasciculation, seizures, and disturbances of consciousness (i.e., excessive drowsiness and coma) (He et al., 1989). Recent animal and select human studies have suggested that permethrin exposure affects the dopaminergic system (Carloni et al., 2013), renal functioning (Lebov et al., 2016), and neonatal neurodevelopment (Nasuti et al., 2017). The EPA has classified oral exposure to permethrin as “Likely to be Carcinogenic to Humans” by the oral route (USEPA, 2007).

Exposure to permethrin can be assessed by the measurement of three urinary metabolites of permethrin: 3-phenoxybenzoic acid (3-PBA), and *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid (*cis*-DCCA and *trans*-DCCA) (Choi et al., 2002, Starr et al., 2008). As permethrin is lipophilic, unmetabolized concentrations may accumulate in various fatty tissues. Animal studies have found that permethrin persists the longest in adipose and brain tissue (Casida et al., 1983) with a half-life of 4 to 5 days (Hallenbeck 1985). Recent studies evaluating permethrin absorption in human subjects demonstrate a mean half-life of 38.5 hours when absorbed dermally via treated clothing (Rossbach et al., 2014).

The primary source of exposure to permethrin among the U.S. general population is through routine low dose ingestion of permethrin from permethrin-treated foods and contaminated drinking water (Riederer et al., 2008). The use of permethrin embedded clothing and gear for outdoor recreational and military use has become more common, resulting in an additional potential source of permethrin exposure via dermal absorption. Studies have demonstrated that permethrin-treated clothing is effective as an insect repellant, with its high contact toxicity and knockdown effect well-documented (Faulde et al., 2003; Gopalakrishnan et al., 2014) and has shown high efficacy in preventing sand fly, tick and mosquito bites (Gopalakrishnan et al., 2014). Previous research has also shown that wearing

permethrin-treated clothing increases concentrations of urinary biomarkers of permethrin (Proctor et al., 2014; Rossbach et al., 2010) through dermal absorption (Appel et al., 2008; Tomalik-Scharte et al., 2005). Various factors have been demonstrated to modify exposure in humans, including clothing wear-times and launderings (Proctor et al., 2014; Rossbach et al., 2016), physical workload (Rossbach et al 2014), and environmental temperature (Rossbach et al 2014; Proctor et al., under review).

Use of permethrin-treated combat uniforms to minimize risks of vector-borne diseases in the U.S. Armed Forces has existed under several different protocols over time. In the early 2000s, treated uniforms were available for issue to individuals for use during deployments to regions of the world where vector-borne diseases were endemic. In 2013, the U.S. Army implemented a new policy requiring the use of permethrin-treated Army combat uniforms regardless of the Soldier's role or location (Bernier and Perry, 2012). Based on a review of the scientific literature, since the introduction of permethrin-treated clothing into commercial markets there have been no significant reported increases in acute, short-term health impacts associated with short-term exposure to permethrin. However, minimal research has examined the potential health impacts of chronic exposure to permethrin-treated garments over prolonged periods of time, such as daily wear across multiple weeks, months or years.

The current study prospectively evaluates whether percent body fat (%BF), a measure of body composition, and total energy expenditure (TEE), an indicator of physical activity, during a 10 week initial military training period with U.S. Army recruits affect permethrin exposure as measured by urinary permethrin biomarkers (3-PBA, *cis*-DCCA, and *trans*-DCCA). Based on permethrin's lipophilic properties in animal research, we hypothesized that having higher percent body fat (%BF) would result in higher urinary concentrations of permethrin biomarkers while wearing permethrin-treated military uniforms. Also, we hypothesized that factors that influence metabolism, such as increased physical activity or workload, would affect the dermal absorption of permethrin and result in higher urinary biomarker concentrations. We also explored whether permethrin exposure was associated with significant reporting of health symptoms or functional health over the course of the 10-week recruit training period.

2. Materials and Methods

The protocol was reviewed and approved by the U.S. Army Research Institute of Environmental Medicine Institutional Review Board (IRB) and the U.S. Army Medical Research and Materiel Command IRB. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory did not constitute engagement in human subjects research. The investigators adhered to the policies for protection of human subjects as prescribed in Army Regulation 70–25, and the research was conducted in adherence with the provisions of 32 CFR Part 219. All participants gave their informed consent prior to the research study. Approval to conduct this research with U.S. Army recruits was also obtained from the U.S. Army Training and Doctrine Command, Center for Initial Military Training.

2.1 Study Participants and Design

The study was a prospective cohort study with data collection conducted at a U.S. Army Basic Combat Training (BCT) site during the fall of 2015. All recruits were eligible for inclusion in the study. It was determined *a priori* that a sample size of 60 individuals, with a 25% drop-out rate (or n=45), would be sufficient to test the study hypotheses (with simulations indicating at least 80% power of showing a within-subject association at the 2-tailed $p<0.05$ level)Approximately 250 recruits were briefed about the study and 91 provided consent to be considered for the final study group. Sixty recruits were selected at random from the pool to serve as the final study population.

Data were collected during three separate Sessions over the 10-week BCT period, for a total of 15 study days (Table 1). Session 1 took place during the first week of the BCT training cycle and included 2.5 days of data collection; Session 2 was approximately 4 weeks later at the midpoint of BCT and included 5.5 days of data collection; Session 3 was approximately 4 weeks later, during the last week of training prior to graduation and involved 5.5 days of data collection. On each study day, participants reported to the study team in the morning prior to starting their daily activities for 10–30 minutes between the hours of 0400–0500 and again in the evening after completion of the majority of their daily duties for 10–30 minutes between 2000–2100.

Each participants had received a newly issued Army permethrin-treated uniforms approximately 4 days prior to the start of data collection procedures. All Army issued uniforms are required to meet EPA, Federal Insecticide, Fungicide, and Rodenticide Act, and Army permethrin-treated fabric levels and efficacy (% bite protection) standards. Regulated fabric permethrin concentration requirement ranges from a minimum of 0.095 $mg \cdot cm^{-2}$ to a maximum of 0.135 $mg \cdot cm^{-2}$.

2.2. Procedures

2.2.1. Questionnaire—A baseline questionnaire, given to participants at the start of the study, asked participants to provide basic demographic information (e.g., sex, age, education), and to report on lifestyle behaviors (e.g., smoking and alcohol habits prior to BCT) and prior pesticide exposures. Functional health was assessed with the Veterans RAND 12-item Health Survey (VR-12; Kazis et al., 1999; Selim et al., 2009) and the Medical Outcomes Study (MOS) cognitive functioning scale (MOS CF, Stewart 1992). The VR-12 queries for self-appraisal of somatic (“physical”) health and emotional (“mental”) impacts on day-to-day functioning (e.g., accomplishing less than usual). Responses are computed to provide physical and mental component summary (PCS, MCS respectively) scores and are standardized to U.S. population norms (mean=50, SD=10). The MOS CF assesses the functional impacts of thinking and attention on day-to-day functioning and is scored on a 0–100 scale. Higher VR-12 and MOS cognitive functioning scores indicate better functioning. A 25-item health symptom checklist utilized in prior studies involving military occupational exposures to neurotoxicants (e.g., Proctor et al., 2011; Proctor et al. 1998) was utilized to assess current health symptoms. A composite summary score (maximum range: 0–72) of responses to 18 health symptoms of interest, pertaining primarily to musculoskeletal, neurological and dermal symptoms, was computed. Participants were

asked how often they experienced each symptom in the past week (Never, Rarely, Sometimes, Often, or Very Often), and the response was scored from 0–4 with 0 being Never and 4 being Very Often. The responses were summed for each participant to provide an overall indicator of the severity of health symptoms they were experiencing.

The VR-12, MOS cognitive functioning scale and health symptom checklist were given again on Session 2 – Day 5 and Session 3 – Day 5 to evaluate changes in health symptoms or general functional health over the 10-week study period.

2.2.2. Body Composition—Each participant's height (cm), weight (kg), and measurements for computation of %BF were gathered (Table 1) starting on Session 1 – Day 1. Height, measured by use of a SECA 2017 stadiometer (with shoes removed), was only measured on Session 1 – Day 1. Weight (wearing shirts, shorts, and socks only) was measured for a total of 8 times over the study using a Doran DS6150 Remote Indicator Scale. Percent BF was calculated (Siri, 1993) using the three site skinfold technique (Jackson and Pollack, 1978) using a Harpenden (Baty International, 2010) skinfold caliper and skinfold measurements were performed a total of 6 times over the study. Also at the first data collection visit, during Session 1 – Day 1, body fat measurements were collected using the Army's standard method of measuring body circumferences (Department of the Army, 2013) for comparison. Body mass index (BMI) was calculated from collected height and weight data (CDC, 2009); body surface area was computed using the DuBois formula (Wang et al., 1992).

2.2.3. Activity Tracking—At each evening study visit, participants completed a one page form asking about their activities and uniform wear during the previous 24 hour period. Specifically, participants were asked to report what time of day they went to bed and awoke each day, number of hours of sleep, and number of hours wearing their uniform. Also, as the washing of uniforms was performed by the individual recruits on his/her time schedule and number of times laundered directly impacts uniform permethrin concentration, participants were asked about the number of times the uniform worn had been washed. All laundering was done at the same location by all participants. Additionally, physical activity levels were tracked for the first 48 hours of each of the three Sessions (from Day 1 AM to Day 3 AM) by having recruits wear an activity monitor (Philips brand Actical (Heil, 2006) on their right leg (at their ankle or above their boots). The monitors recorded data on the participants' movements and computed the estimated energy expenditure (kcal, in 1 min intervals). Raw data were consolidated into individual-level average daily activity levels to provide assessment of average daily energy expenditure. Additionally, the doubly-labelled water (DLW) method, which is considered the gold standard for determining TEE, was used in this study (DeLany et al., 1989). Participants ingested 90 g of a mixture of 99.9% atom percent excess $^{2}H_{2}O$ and 4.5 g of 10% $H_{2}O^{18}$ at their morning visit on Day 1 of Session 2 and Session 3. Eight spot urine samples (1 pre-dose and 7 post-dose) were collected over the next 5 days and isotopic enrichment was measured using Isotope Ratio Mass Spectrometry (IRMS) to compute average kcal·day^{-1} expended over five study days in both Sessions 2 and 3 (Day 1 AM to Day 6 AM).

2.2.4. Biological Samples—Every day throughout each of the 3 study Sessions, spot urine samples were collected from each participant (Table 1). In total, 5 samples were collected from each individual during each of the three Sessions for the quantification of permethrin biomarkers and creatinine; 5-mL aliquots were frozen and shipped overnight in batches to the CDC, where they were analyzed by semi-automated solid phase extraction followed by high performance liquid chromatography-isotope dilution tandem mass spectrometry (Davis et al., 2013). Limits of detection (LOD) were 0.1 $\mu g \cdot L^{-1}$ (3-PBA), 0.5 $\mu g \cdot L^{-1}$ (*cis*-DCCA), and 0.6 $\mu g \cdot L^{-1}$ (*trans*-DCCA).

A total of eight 10-mL aliquots from the collected urine samples in Sessions 2 and 3 were frozen and sent overnight to the Pennington Biomedical Research Center for the $^{2}H_2O$ and H_2O^{18} isotope analyses, following the DLW protocol. Results provided the average daily energy expenditure (kcal) over the respective two 5.5 day Session periods examined.

2.3. Statistical Analyses

Descriptive statistics of the demographics of the study group at baseline, along with body composition metrics and average permethrin metabolite concentrations over the study period, were examined. Comparisons were performed between baseline characteristics to establish whether there appeared to be any selective loss to follow up among participants. Chi-square tests were performed for categorical variables (sex) and t-tests (VR-12 scores, height, weight) or Wilcoxon rank-sum tests (biomarkers, age) were run for continuous variables depending on the normality of the data. Reports of changes in health symptoms and functional health over the course of the study were examined via repeated measure ANOVAs. Changes in %BF between Session 1 – Day 1 and Session 3 – Day 6 were evaluated by Paired-tests. Correlations examined the relationship between Day 1 measured %BF with the skinfold caliper and taping methods. Two-sample (pairwise) Wilcoxon tests were performed to compare biomarker concentrations within and across Sessions.

Cis-DCCA and *trans*-DCCA concentrations were summed to yield a total DCCA concentration ($\sum$DCCA). No adjustments based on LOD were necessary, as permethrin biomarkers were detected in all samples analyzed. Correlations between the permethrin biomarkers, %BF, TEE, uniform laundering, and wear times were determined.

Using a method described and applied in Appel et al. (2008) and followed in Proctor and colleagues (2014), the average daily permethrin doses for each Session ($\mu g \cdot kg^{-1} \cdot day^{-1}$) were estimated. First, estimated daily creatinine excretion was calculated for each permethrin collection based on the participant's baseline age and race and the most recently measured weight. This was done using an equation outlined by Ix and colleagues (2011): estimated creatinine excretion rate ($g \cdot day^{-1}$) = 879.89 + 12.51*weight (kg) – 6.19*age (years) + (34.51 if black) – (379.42 if female). Next, creatinine adjusted $\sum$DCCA ($\mu g \cdot g^{-1}$ creatinine) was multiplied by estimated daily creatinine ($g \cdot day^{-1}$) to yield a daily biomarker dose. The dose ($\mu g \cdot day^{-1}$) was converted using the molar mass ratio of permethrin to $\sum$DCCA (391/209=1.87) to estimate permethrin dose per day ($\mu g \cdot day^{-1}$). Finally, daily permethrin dose was divided by each participant's body weight (kg) to compute daily dose estimates ($\mu g \cdot kg^{-1} \cdot day^{-1}$), which were then averaged also over each Session.

Urine samples with creatinine levels outside the 20–350 mg·dL^{-1} were excluded from the primary analyses, as samples outside that range may be too dilute or concentrated. In addition, excluding creatinine concentrations outside this range eliminates subjects with kidney dysfunctions or hydration issues that may affect results. As a result, 25 samples from the Session 1 data were excluded (251 of 276 samples included), 4 samples from Session 2 (224 of 228 samples included), and 6 samples from Session 3 (209 of 216 samples included).

Linear mixed model (LMM) analyses, using a spatial power correlation structure, were performed to examine the association between %BF and physical energy expenditure and urinary permethrin biomarker concentrations. Separate analyses were run for 3-PBA and ∑DCCA. The permethrin biomarkers, creatinine, %BF, and TEE values were log-transformed to preserve the assumption of normality for the residuals.

Model 1 examined the independent relationship between %BF and permethrin biomarker concentrations and utilized data from the 6 visits when %BF was determined. Model 2 evaluated the relationship between TEE and permethrin biomarker concentrations. In Model 3, both %BF and TEE were analyzed in the same model to evaluate their mutually adjusted effect on permethrin biomarker concentrations. Models 2 and 3 were restricted to analysis of data collected only at 2 time points (i.e., Session 2 – Day 6 and Session 3 – Day 6) when TEE was determined.

All models were adjusted for sex, age, creatinine, days in BCT, number of times the participant's uniform had been washed, and the number of hours that the uniform had been worn during the day prior to collection. Parameter estimates (exponentiated betas) were obtained using maximum likelihood estimation in order to interpret effect of %BF and TEE on permethrin exposure.

Post hoc sensitivity analyses were performed to examine the effects of creatinine exclusion criteria, as decisions about exclusions reduced the sample size available. The final adjusted model (Model 3) was performed i) with no participant exclusions due to creatinine concentrations, and ii) excluding participants with creatinine levels with a somewhat more restrictive range, outside the range of 30–300 mg·dL^{-1}.

All study analyses were performed using SAS, version 9.4 (SAS Institute, Inc., Cary, NC).

3. Results

The age range of the male and female participants at the start of BCT was between 18 and 29 years, and the range in %BF and BMI was between 3.67-26.51 and between 20.11-32.34, respectively. (Table 2). The majority (70%) of those who started the study self-reported as white, Caucasian, with 15% identifying as Hispanic, Latino; 7% as black, African American, 5% as multiracial, and 3% as Asian/Pacific Islander. A total of 28 (47%) reported using any type of tobacco product (cigarettes, chewing tobacco, e-cigarettes) prior to BCT; tobacco use was not permitted during BCT.

Over the course of the study, eight participants dropped out of the study at/during Session 2, and an additional eight at/during Session 3. Of the total 16 (26.75%) who discontinued participation, eight had left recruit training before its conclusion (or were injured and not able to complete with their group) and the other eight opted to discontinue participation in the study at various time points over the study. For the latter, no specific reasons were provided. There were no significant differences in baseline characteristics (means and ranges) between those who started the study (n=60) and those who completed all Sessions (n=44).

Between the start and end of BCT, health symptom reporting did not vary significantly (study cohort average score was 5.67 (5.59) on Session 1 – Day 1 compared to score of 5.07 (7.99) on Session 3 – Day 5). However, the average was higher at Session 2 – Day 5 (10.10 (8.80). The most frequent health symptom at all 3 Sessions was stomach cramps. There were no significant changes in the reported physical, mental, or cognitive functional health measures over the study.

The average number of cumulative uniform launderings for the issued uniform worn the day before each of the six collection points were: Session 1 – Day 1: 1.28 (0.97, range: 0–4 washings); Session 1 – Day 3: 1.36 (0.96, range: 0–4); Session 2 – Day 1: 5.87 (2.29, range: 2–15); Session 2 – Day 6: 6.15 (2.59, range: 2–11 washings); Session 3 – Day 1: 9.02 (5.53, range: 1–25 washings); and Session 3 – Day 6: 9.79 (6.51, range: 0–28 washings). Over all study days, the range in number of hours uniforms were worn varied between 0 and 18.75 hours·day^{-1}. The average number of hours that the uniform had been worn during the day before the six body fat collection points were: Session 1 – Day 1: 11.72 (2.97), Session 1 – Day 3 3.08 (4.38), Session 2 – Day 1 – 8.64 (3.22), Session 2 – Day 6 – 9.11 (2.64), Session 3 – Day 1 – 13.35 (3.68), and Session 3 – Day 6 – 10.60 (5.37).

Figure 1 presents the average creatinine-adjusted concentrations of 3-PBA, *cis*-DCCA, and *trans*-DCCA. Biomarker concentrations were at their highest at the onset of the study and declined gradually to their lowest concentrations by the end of the study. Within Study Sessions, there were no significant differences between concentrations over the Session time. However, across Study Sessions, there were significant differences ($p<0.05$) between each Session 3 values and those measured at all timepoints in Sessions 1 and 2, and there were some significant differences between Session 1 and Session 2 measured levels, particularly when comparing the first three timepoints in Session 2 to those measured in Session 1. The estimated mean daily dose of permethrin (μg·kg^{-1}·day^{-1}) on Session 1 – Day 1 was 8.55 μg·kg^{-1}·day^{-1} (ranging between 2.0– 22.1) and the average computed for each of the 3 Sessions was 5.88 (SE = 0.16), 4.59 (SE = 0.15), and 1.71 μg·kg^{-1}·day^{-1} (SE = 0.05), respectively.

Significant Spearman correlations between %BF and all three creatinine-adjusted biomarker concentrations were found (Table 3). Spearman correlations were used as biomarker data was not found to be normally distributed via Shapiro-Wilk normality testing. Body surface area was positively correlated with %BF ($\rho = -0.190$, $p=0.0010$) but not with permethrin biomarker concentrations. Spearman correlations were also calculated between the Session 1

– Day 1 skinfold and Army circumference methods of determining %BF. Results were ρ = 0.8156 (p<0.0001) among males and ρ = 0.8286 (p<0.0001) among females.

Table 4 presents a summary of the body composition measures, at baseline and over the study period by Session. While body weight and BMI remained steady throughout the study period among both males and females, a significant decline in %BF was noted between the first and last study day in males (Paired t=9.81, p<0.0001) and females (Paired t=7.09, p<0.0001).

The effects of %BF and TEE on 3-PBA and ∑DCCA are shown in Table 5. %BF was a significant predictor of ∑DCCA concentrations in the mixed models (Model 1) and TEE was significantly associated with 3-PBA concentrations in Model 2. Both higher %BF and TEE were significant positive predictors of 3-PBA when together in the same model, and %BF also was a significant predictor of ∑DCCA (Model 3). In Model 3, a 10% higher %BF is associated with 4.42% higher 3-PBA concentrations; 10% higher %BF is associated with 4.32% higher ∑DCCA concentrations; and a 10% higher TEE is associated with a 10.57% higher 3-PBA concentrations. Higher TEE was not significantly associated with ∑DCCA (Models 2 and 3).

The sensitivity analyses, examining creatinine exclusion criteria, found no difference in the Model 3 results for 3-PBA with no exclusions (%BF: F=5.90, p=0.0224; TEE: F=5.19, p=0.0312) nor under stricter criteria (%BF: F=5.41, p=0.0292; TEE: F=5.53, p=0.0276). Similarly, no differences were noted when examining ∑DCCA concentrations with respect to %BF (no exclusion: F=4.92, p=0.0354, stricter criteria: F = 5.56, p=0.0265). The results for TEE looking at ∑DCCA concentrations remained not significant (no exclusion: F=1.34, p=0.2575, stricter criteria: F = 1.06, p=0.3133).

4. Discussion

The results of this study demonstrate two important insights pertaining to factors impacting permethrin exposure from the wearing of treated military uniforms. First, a significant positive association between %BF and both 3-PBA and ∑DCCA was observed (Model 3), with adjustment for TEE. Second, a significant and independent relationship between higher daily TEE and higher 3-PBA (Models 2 and 3) was observed; the relationship was not observed with ∑DCCA. The exposure can be attributed directly to the wearing of permethrin treated military uniforms as the results document that the number uniform launderings and hours worn have on exposure biomarkers concentrations. Specifically, the standardized beta coefficients across the models demonstrate that these uniform characteristics contribute to the biomarker concentration to a somewhat comparable degree as do %BF or TEE. The study found no evidence suggesting elevated health symptom reports or effect on functioning while wearing permethrin-treated uniforms.

Through the prospective study design, we observed the natural time course of permethrin biomarker concentrations among Army recruits wearing newly issued permethrin-treated uniforms over a 10-week time period. During the first week of BCT, the estimated permethrin dose was at its highest on Session 1 – Day 1, with an individual maximum

estimated dose of 22 μg·kg^{-1}·day^{-1} close to the ADI assuming 50% absorption of oral dose (i.e., 25 μg·kg^{-1}·day^{-1}). On average, the estimated dose on Session 1 – Day 1 was about 3 times lower and by Session 3 – Day 6 had dropped to about 12 times lower than the ADI. Other studies have reported maximum estimated dose levels in the 5–6 μg·kg^{-1}·day^{-1} range (Appel 2008, Rossbach et al., 2014, Proctor et al. 2014), which is 4–5 times lower than the 25 μg·kg^{-1}·day^{-1} among persons wearing permethrin-treated clothing with additional laundering(s). The gradually increasing peak shown in Figure 1 over the Session 2 period is likely due to the increased uniform wear-time, as participants reported wearing their uniforms 11–12 hours·day^{-1} on Session 2 – Days 2–4 and greater than 9 hours on Day 5.

Only a limited number of studies (Appel 2008; Kegel et al 2014) examining permethrin exposure as a result of wearing permethrin-treated clothing have included females, but the number have not been sufficient to determine if sex differences were present in permethrin exposure. In our study females represented 30% of the study cohort, sufficient to both provide descriptive results of differences in body composition and physical activity between men and women and examine the main effect of sex on permethrin exposure. However, he study design did not permit sufficient sample size to adequately power analyses involving second- and third-order interaction effects in the LMMs, such as, sex-%BF or sex-TEE, or sex-%BF-TEE interactions effect on biomarker concentrations, which would be quite informative. Further studies with larger sample sizes are warranted. In addition, it would be interesting to explore whether the use of topically applied lotions or personal care products may have an effect on dermal absorption of permethrin, especially since males and females may use these products differentially.

Key strengths of the study include both the population studied and the environment in which the study was conducted. The involvement of new Army recruits in this study, with the inclusion of females, permits generalizations to anyone wearing permethrin-treated clothing (e.g., new Army personnel, as well as outdoor workers or recreational users) for the first time and for extended periods of time. In addition, the BCT environment provided considerably more control over a number of situational and environmental conditions than is typical for field-based research, to include more consistent climate/temperature conditions, regulated diet and exercise (alcohol, tobacco, and dietary supplements are prohibited during BCT; medications are restricted to those prescribed at sick call), and highly structured daily routines. In effect this minimized residual confounding by essentially matching the population on external factors that could potentially effect the association between %BF and TEE on permethrin exposure. Also, permethrin concentration in new Army uniforms is regulated, within a range, so all participants were exposed to similar levels at the outset of the study.

As noted, the urinary concentrations of permethrin biomarkers declined over the 10-week assessment period. It would be valuable to examine if and how this decline continues by following participants for additional weeks and months to determine if a steady–state level is reached during longer-term routine wear of permethrin-treated clothing.

4.1. Conclusions

In conclusion, overall, %BF and TEE were associated with permethrin exposure as determined by urinary permethrin biomarkers (3-PBA and/or *cis*-DCCA, *trans*-DCCA, and ∑DCCA) from wearing permethrin-treated uniforms during a 10 week BCT period. While there was individual variation in the range of estimated permethrin doses observed over the 10-week period, none of the dose levels were above WHO exposure guidance and no significantly elevated health symptom reports while wearing permethrin-treated uniform were noted.

Acknowledgements

We thank the U.S. Army Basic Combat Training (BCT) recruits who were participants in the study, as well as the locale BCT site and Training and Doctrine Command, Center for Initial Military Training (CIMT) personnel for their support and generous participation in the project. We are grateful to the additional USARIEM study team personnel for their assistance in the data collection process. We acknowledge and appreciate staff at the Pennington Biomedical Research Center, Baton Rouge, LA and the Centers for Disease Control and Prevention (CDC), Atlanta, GA for their support and timely analyses of samples.

Funding

This work was supported by the US Army Medical Research and Materiel Command through a grant award (W81XWH-14-1-0130; PI: S. P. Proctor) to the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.

Appendix A

Table A.1 -

Linear Mixed Regression Model Results for trans-DCCA

Model Type	Variable	Beta Estimate	F statistic (p)	AIC
Model 1 Adjusted*, %BF	%BF	0.2577	**4.34 (0.0386)**	366.4
Model 2 Adjusted*, TEE (kcal·day^{-1})	TEE	0.4788	0.98 (0.3321)	111.2
Model 3 Adjusted*, Both %BF and TEE (kcal·day^{-1})	%BF	0.4090	**4.47 (0.0447)**	
	TEE	0.4867	(0.3113)	108.6

Table A.2 -

Linear Mixed Regression Model Results for cis-DCCA

Model Type	Variable	Beta Estimate	F statistic (p)	AIC
Model 1 Adjusted*, %BF	%BF	0.2902	**5.39 (0.0214)**	336.7
Model 2 Adjusted*, TEE (kcal·day^{-1})	TEE	0.4734	0.88 (0.3581)	118.0
Model 3 Adjusted*, Both %BF and TEE (kcal·day^{-1})	%BF	0.5178	**7.48 (0.0113)**	
	TEE	0.5204	1.18 (0.2870)	112.8

*Model adjusted for sex, age, days in BCT, creatinine concentration, number of hours uniform worn the day prior, and number of times uniform worn day before had been laundered

AIC=Akaike Information Criteria

cis-DCCA= *cis*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid

trans-DCCA= *trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid

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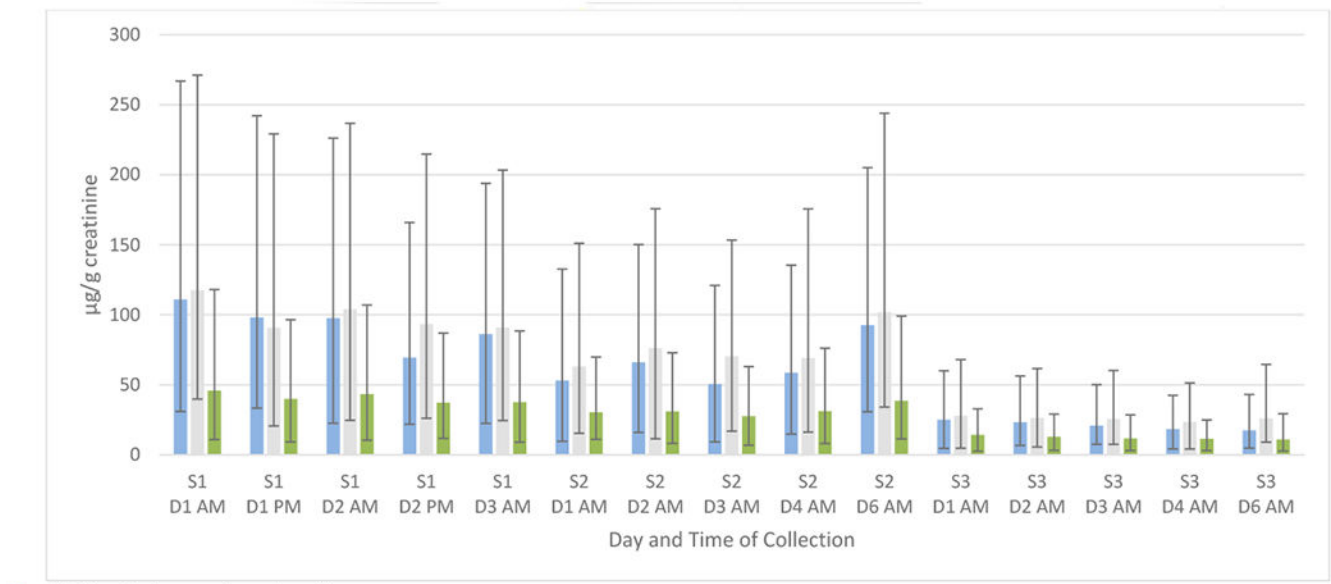

Figure 1.

Median Creatinine-Adjusted Concentration ($\mu g \cdot g^{-1}$ creatinine) of Permethrin Biomarkers with Interquartile Ranges over Study Period

Table 1:

Data Collection Timeline of Study

	Start of BCT Session 1 1st week					Middle of BCT Session 2 5th week								End of BCT Session 3 Last week						
Day	1	2	3			1	2	3	4	5	6			1	2	3	4	5	6	
Urine																				
AM	X	X	X			X	X	X	X	[X]	X			X	X	X	X	[X]	X	
PM	X	X				[X]	[X]							[X]	[X]					
%BF	X		X			X					X			X					X	
Height	X																			
Weight	X		X			X		X			X			X		X			X	

X sample or measurement collected

[X] urine sample collected but not analyzed for permethrin metabolites

BCT: Basic Combat Training

%BF: Percent Body Fat

Table 2.

Descriptive characteristics of study cohort at study start and those who completed prospective study

Descriptive Mean (SD) or N (%)	**Baseline Cohort, Session 1 - Day 1 N=60 who started study**	**Baseline Cohort, Session 1 - Day 1 N = 44 who completed study**
Age (years), mean (SD)	20.77 (2.72)	21.01 (2.77)
Male, N (%)	40 (70.0%)	30 (68.2%)
Height (cm), mean (SD)	172.29 (9.83)	172.07 (9.93)
Weight (kg), mean (SD)	72.95 (13.13)	72.82 (12.74)
Body Mass Index, mean (SD)	24.43 (3.31)	24.52 (3.38)
%BF, mean (SD)	14.75 (5.82)	14.72 (5.72)
Body Surface Area (m^2), mean (SD)	1.85 (0.19)	1.85 (0.20)
3-PBA ($\mu g \cdot L^{-1}$), mean (SD)	235.46 (182.33)	257.79 (183.60)
trans-DCCA ($\mu g \cdot L^{-1}$), mean (SD)	250.59 (214.84)	275.58 (224.78)
cis-DCCA ($\mu g \cdot L^{-1}$), mean (SD)	105.64 (85.48)	115.50 (85.50)
VR-12, Physical Component Score, mean (SD) *	54.67 (4.23)	54.35 (4.44)
VR-12, Mental Component Score, mean (SD) *	53.81 (8.19)	54.67 (4.23)
MOS Cognitive Functioning Scale, mean (SD) *	84.92 (19.52)	84.32 (20.05)
Health Symptom Score, mean (SD) *	5.54 (6.00)	5.67 (5.59)

* n = 59

3-PBA= 3-phenoxybenzoic acid

cis-DCCA = *cis*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid

trans-DCCA = *trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid

%BF = percent body fat

Table 3:

Correlations between creatinine-adjusted concentrations of permethrin biomarkers and Covariates of Interest

Variable	Spearman ρ (p-value): 3-PBA	Spearman ρ (p-value): *trans*-DCCA	Spearman ρ (p-value): *cis*-DCCA
%BF	0.318 (<0.0001)	0.304 (<0.0001)	0.308 (<0.0001)
TEE (DLW), kcal·day^{-1}	0.127 (0.2515)	0.062 (0.5761)	0.099 (0.3721)
Body Surface Area (m^2)	−0.008 (0.8860)	−0.035 (0.5060)	−0.053 (0.3104)
# Uniform launderings	−0.580 (<0.0001)	−0.530 (<0.0001)	−0.494 (<0.0001)

3-PBA= 3-phenoxybenzoic acid

cis-DCCA= *cis*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid

trans-DCCA= *trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid

%BF= percent body fat

TEE= total daily energy expenditure, average energy expenditure per day over Session measured

Table 4.

Changes in Body Composition and Physical Activity Characteristics over Study Time Period (n=44*)

	Session 1 - Day 1	Session 1 - Day 3**	Session 2 - Day 1	Session 2 - Day 6**	Session 3 - Day 1	Session 3 - Day 6	Repeated ANOVA F (p-value)
	Mean (STDDEV)						
Weight (kg), all	72.81 (12.74)	71.90 (12.65)	72.51 (11.15)	71.98 (10.86)	72.10 (9.79	72.07 (9.92)	0.04 (0.9989)
Males	77.20 (11.01)	75.94 (11.26)	76.40 (9.21)	75.71 (8.91)	75.34 (7.97)	75.29 (8.20)	0.17 (0.9721)
Females	63.41 (11.25)	63.54 (11.49)	64.44 (10.70)	64.26 (10.70)	65.14 (9.95)	65.16 (9.98)	0.07 (0.9965)
Percent Body Fat, all	14.72 (5.72)	14.43 (5.97)	12.21 (5.13)	11.88 (4.98)	11.97 (5.27)	11.89 (5.03)	**2.74 (0.0196)**
Males	12.36 (4.85)	11.92 (5.00)	9.75 (3.88)	9.30 (3.49)	9.09 (3.29)	9.19 (3.20)	**3.99 (0.0019)**
Females	19.79 (3.88)	19.62 (4.28)	17.48 (3.11)	17.22 (2.88)	18.15 (2.75)	17.65 (2.91)	1.55 (0.1834)
BMI, all	24.52 (3.38)	24.21 (3.35)	24.48 (2.83)	24.34 (2.73)	24.32 (2.39)	24.31 (2.45)	0.07 (0.9965)
Males	25.02 (3.47)	24.56 (3.46)	24.79 (2.84)	24.62 (2.74)	24.41 (2.38)	24.40 (2.49)	0.20 (0.9626)
Females	23.45 (3.03)	23.49 (3.11)	23.84 (2.79)	23.76 (2.73)	24.11 (2.48)	24.11 (2.44)	0.15 (0.9801)
Actical (kcal) – 2 day avg, all		3653.93 (735.32)		3602.78 (675.76)		3382.04 (558.20)	1.70 (0.1355)
Males		3876.43 (676.73)		3806.17 (641.37)		3538.68 (499.64)	2.00 (0.0811)
Females		3157.60 (626.33)		3199.51 (550.56)		3046.37 (543.85)	0.21 (0.9561)
DLW (kcal) – 5 day avg, all	-	-		4004.15 (580.88)		3850.98 (611.69)	1.23 (0.2703)
Males	-	-		4279.29 (445.00)		4096.37 (500.18)	2.05 (0.1583)
Females	-	-		3411.54 (349.45)		3325.14 (492.76)	0.27 (0.6065)

* Of n=44 (30 males; 14 females)

** For Session 1 – Day 3 and Session 2 – Day 6 n=43 (29 males; 14 females); 1 male participant missing that time point assessment

Table 5:

Results from Linear Mixed Models Evaluating Effect of %BF and TEE on Permethrin Biomarkers

	LN 3-PBA				LN ∑DCCA			
Model	**Beta**	**Std. B**	**SE**	**F statistic (p)**	**Beta**	**Std. B**	**SE**	**F statistic (p)**
Model 1 *,								
%BF	0.2170	0.1008	0.132	2.71 (0.1016)	0.2619	0.1367	0.122	**4.63 (0.0328)**
Sex	0.1925	0.0870	0.139	1.92 (0.1715)	0.0916	0.0465	0.128	0.51 (0.4775)
# hrs worn	0.0223	0.1208	0.005	**24.25 (<.0001)**	0.0227	0.1380	0.004	**28.36 (<.0001)**
# times washed	−0.0347	−0.1641	0.011	**9.35 (0.0026)**	−0.0237	−0.1262	0.011	**5.09 (0.0252)**
Days in BCT	−0.0206	−0.0202	0.003	**67.51 (<.0001)**	−0.0168	−0.0184	0.002	**52.26 (<.0001)**
AIC	372.2				339.1			
Model 2 *								
TEE, kcal·day^{-1}	1.0463	0.1609	0.497	**4.44 (0.0449)**	0.4517	0.0781	0.479	0.89 (0.3548)
Sex	0.5843	0.2640	0.186	**9.82 (0.0032)**	0.3602	0.1829	0.186	3.75 (0.0600)
# hrs worn	0.0264	0.1426	0.013	4.12 (0.0528)	0.0157	0.0953	0.012	1.74 (0.1986)
# times washed	−0.0237	−0.1122	0.011	**4.38 (0.0462)**	−0.0083	−0.0444	0.011	0.61 (0.4414)
Days in BCT**	−1.5874	−1.5572	0.102	**241.2 (<.0001)**	−1.4155	−1.5606	0.092	**239.5 (<.0001)**
AIC/N	116.5				109.9			
Model 3 *								
%BF	0.4533	0.2106	0.186	**5.93 (0.0223)**	0.4447	0.2322	0.189	**5.56 (0.0265)**
TEE, kcal·day^{-1}	1.0539	0.1621	0.475	**4.93 (0.0357)**	0.4753	0.0822	0.462	1.06 (0.3133)
Sex	0.2895	0.1308	0.217	1.77 (0.1905)	0.0760	0.0386	0.218	0.12 (0.7293)
# hrs worn	0.0252	0.1359	0.013	3.86 (0.0605)	0.0138	0.0839	0.012	1.36 (0.2550)
# times washed	−0.0256	−0.1212	0.011	**5.48 (0.0275)**	−0.0100	−0.0538	0.010	0.94 (0.3416)
Days in BCT**	−1.5839	−1.5538	0.102	**240.3 (<.0001)**	−1.413	−1.5579	0.092	**233.8 (<.0001)**
AIC	112.5				106.3			

*Model adjusted for sex, age, days in BCT, creatinine concentration, number of hours uniform worn the day prior, and number of times uniform worn day before had been laundered

AIC=Akaike Information Criteria

** Session 2 – Day 6 collection used as reference point

3-PBA = 3-phenoxybenzoic acid

∑DCCA= *cis*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid) + *trans*-DCCA (*trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid)

%BF= percent body fat; TEE= total daily energy expenditure, average energy expenditure per day over Session measured (kcal·day^{-1})