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ORIGINAL PAPER

Long-lasting permethrin-impregnated clothing: protective efficacy against malaria in hyperendemic foci, and laundering, wearing, and weathering effects on residual bioactivity after worst-case use in the rain forests of French Guiana.

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Abstract Personal protective measures against hematophagous vectors constitute the first line of defense against arthropod-borne diseases. However, guidelines for the standardized testing and licensing of insecticide-treated clothing are still lacking. The aim of this study was to analyze the preventive effect of long-lasting polymer-coated permethrin-impregnated clothing (PTBDU) against malaria after exposure to high-level disease transmission sites as well as the corresponding loss of permethrin and bioactivity during worst-case field use. Between August 2011 and June 2012, 25 personnel wearing PTBDUs and exposed for 9.5 person-months in hyperendemic malaria foci in the rain forest of French Guiana contracted no cases of malaria, whereas 125 persons wearing untreated uniforms only, exposed for 30.5 person-months, contracted 11 cases of malaria, indicating that PTBDU use significantly ($p = 0.0139$) protected against malaria infection. In the field, PTBDUs were laundered between 1 and 218 times (mean 25.2 ± 44.8). After field use, the mean remaining permethrin concentration in PTBDU fabric was 732.1 ± 321.1 min varying between 130 and 1270 mg/m^2 (mean 743.9 ± 304.2 mg/m^2) in blouses, and between 95 and 1290 mg/m^2 (mean 720.2 ± 336.9 mg/m^2) in trousers. Corresponding bioactivity, measured according to internal licensing conditions as KD_{99} times against *Aedes aegypti* mosquitoes, varied between 27.5 and 142.5 min (mean 47.7 ± 22.1 min) for blouses, and between 25.0 and 360 min (mean 60.2 ± 66.1 min) for trousers. We strongly recommend the use of long-lasting permethrin-impregnated clothing for the prevention of mosquito-borne diseases, including chikungunya, dengue, and zika fevers, which are currently resurging globally.

Keywords Permethrin-impregnated clothing · Malaria prevention · Long-lasting activity · Personal protection · Laundering resistance · Weathering effects

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Introduction

Among the increasing number of vector-borne diseases that are currently emerging or resurging worldwide, few are vaccine-preventable. Prophylactic drugs are available only for malaria, one of the most frequent and important vector-borne diseases, but drug resistance is on the increase and spreading. For this reason, personal protective measures against hematophagous vectors constitute the first line of defense against arthropod-borne diseases in endemic areas (Faulde et al. 2006). To date, the synergistic use of skin repellent formulations combined with long-lasting insecticide-treated fabrics, including clothing, tents, and netting, is highly recommended for the protection of at-risk personnel (WHO 2001a, b; Faulde et al. 2008; Pennetier et al. 2010; Banks et al. 2014). Because of its highly advantageous properties which include excito-repellency, hot-feet, knockdown, kill, and residual activity, the synthetic residual pyrethroid permethrin has been widely used since decades as an effective arthropod contact repellent for fabric impregnation (US Armed Forces Pest Management Board 2009; Vaughn et al. 2014; Faulde et al. 2016).

Because soldiers deployed to active vector-borne disease foci are at an increased risk of contracting vector-borne diseases, permethrin-impregnated battle dresses have chiefly been developed for and used by armed forces since the 1940s (Pagès et al. 2010). Initially, battle dresses were impregnated with insecticides by dipping or spraying, but the residual activity was short. In recent years, many armies have developed their own long-lasting impregnated battle dress uniforms using different types of impregnation on various fabrics. At this time, no World Health Organization Pesticides Evaluation Scheme (WHOPES) or other national or public health guidelines exist for the standardized testing and licensing of insecticide-treated clothing, which is also commercially available in the civilian market (Faulde et al. 2016). In order to guarantee that wearing the impregnated fabric will be protective and safe, both the initial concentration and release rate of permethrin should be monitored through an appropriate quality assurance procedure during the production process (Appel et al. 2008). This is why different internal testing and licensing specifications have been developed among national forces and agencies.

In order to ensure that manufacturers fully comply with minimum quality requirements, especially those concerning protective efficacy and user safety, the German Armed Forces (Bundeswehr) implemented the standardized testing and licensing algorithm TL 8305-0331 (WIWeB 2016), launched in 2002 and revised to accord with increased technical and scientific knowledge or specific force health protection needs during military deployments. The yellow fever mosquito, *Aedes aegypti*, has been identified as the most suitable biosensor for testing the toxicological effects of pyrethroids on fabrics against vector mosquitoes, especially when monitoring for low doses or cutoff values of less residual impregnation methods (Faulde et al. 2016, Osborne et al. 2016). In a comparison of the residual bioactivities and laundering resistances of five commercially available, factory-treated permethrin-impregnated fabrics designed for the prevention of mosquito-borne diseases, extremely high variability in initial permethrin concentration, residual bioactivity, and permethrin loss during laundering was observed (Faulde et al. 2016). The resulting data indicated that only one of the examined products completely met all the necessary efficacy and safety requirements required by TL 8305-0331 (Faulde et al. 2016).

To date, most studies on impregnated fabrics efficacy have been conducted under laboratory conditions prior to and after laundering, or under experimental conditions. The protective effect of impregnated clothes against mosquito bites has, however, been demonstrated in field or near-field conditions in two recent studies (Londono-Renteria et al. 2015, Osborne et al. 2016). To our knowledge, only one study has attempted to investigate the protective effect of permethrin-impregnated clothing against vector-borne diseases under field conditions in disease-endemic areas (Soto et al. 1995). In a double-blind, randomized study on Colombian soldiers wearing permethrin-impregnated battle dress uniforms under non-combat conditions during a 4- to 6-week period, an incidence reduction of 80% against malaria and 78% against cutaneous leishmaniasis was shown (Soto et al. 1995). Unfortunately, permethrin concentrations, residual activity, bioactivity, laundering, and environmental effects were not monitored during this relatively short field study.

Further, it is well known that soldiers and relief agency personnel operating in vector-borne disease-endemic regions where they are exposed to active disease foci are at an increased risk of contracting vector-borne diseases (Pagès et al. 2010). Malaria in particular has been of special military and civil health importance during recent missions worldwide (Faulde 2006; Pagès et al. 2010). In December 2010, a notable *Plasmodium vivax* malaria outbreak occurred among French forces involved in the “Harpie” mission (Pommier de Santi et al. 2016a). This mission was launched in 2008, with the goal of stemming the increase in illegal gold mining in the rain forests of French Guiana (Pommier de Santi et al. 2016b). Overall, 72 *P. vivax* malaria cases, including 3 mixed *P. vivax/P. falciparum* infections, were reported between December 2010 and April 2011, resulting in an overall attack rate of 26.5% (72/272) among French military personnel. Two illegal gold mining sites located in remote areas within the rain forest were identified as primary infection sites (Pommier de Santi et al. 2016a). Retrospective analyses showed that the main drivers of this outbreak were the lack of adherence by military personnel to malaria prevention combined with a high level of malaria transmission at illegal gold mining sites within the rain forest (Pommier de Santi et al. 2016a). Also, since the former French cotton PTBDUs were heavy and not able to dry in the humid Guiana forest, French forces had been equipped with fast-drying but non-impregnated synthetic fabrics prior to their deployment.

The purposes of this study were to (a) analyze the preventive effect of long-lasting factory-treated permethrin-impregnated Bundeswehr battle dress uniforms (PTBDUs) during exposure to high-level malaria transmission sites in the French Guiana rain forest following the malaria outbreak described (Pommier de Santi et al. 2016a, b); (b) investigate laundering, wearing, and weathering effects on residual permethrin content and bioactivity after military worst-case use in rain forests; and (c) determine whether the permethrin impregnation method used for fabric treatment remains in accord with previous efficacy results stemming from the standardized licensing requirements of German Armed Forces algorithm TL 8305-0331.

Materials and methods

Procurement of fabrics

PTBDUs, specifically designed for tropical wet climatic conditions, were supplied by UTEXBEL S.A. (Ronse, Belgium). Blouses and trousers were polymer-coated with permethrin (cis:trans = 25:75%) at a batch-specific initial concentration of 1300 mg a.i./m^2, as previously described (Faulde and Uedelhoven 2006). The military uniform fabric used for permethrin treatment consisted of 65% cotton and 35% polyester fibers, with a medium-specific weight of 220 g/m^2.

Personnel, field use, and laundering of fabrics

Thirty newly produced PTBDUs were used between August 2011 and June 2012 by 25 personnel of two combat units primarily involved in the French operation Harpie (Pommier de Santi et al. 2016b). The personnel were selected for their increased exposure times during duty in malarious areas, especially the hyperendemic malaria foci of the gold mining sites Dorlin and Repentir (Pommier de Santi et al. 2016a, b). While serving in malaria foci, only PTBDUs were worn. During jungle duty, laundering of PTBDUs was carried out individually as frequently as required or desired, either by machine laundering or hand washing with detergent, and the number of machine or hand washings was recorded. Blouses and trousers were always washed simultaneously. The soldiers may also have been "drenched" during tropical rain showers or when crossing tropical rivers, swamps, or ponds, which may have affected overall laundering frequency. The number and type of launderings, mechanical characteristics, and user friendliness were recorded for each soldier, using a self-administered form. The soldiers were told that the German PTBDUs were permethrin-impregnated. The concurrent negative control consisted of 125 personnel wearing untreated French battle dress uniforms (NTBDUs), but all such personnel experienced field conditions identical to those wearing PTBDUs. The NTBDU-wearing personnel were selected on the basis of their estimated lower exposure times in hyperendemic malaria foci. Throughout the study, all the military personnel used malaria chemoprophylaxis at 100 mg of doxycycline per person per day during their entire stay in the rain forest, a regimen that was extended for an additional 4 weeks after leaving the malaria transmission area. The number of person-months of exposure in malaria transmission areas was analyzed for both groups. Environmental conditions and activity patterns during duty were described and site maps were provided by Pommier de Santi et al. (2016a).

Malaria case definitions

Laboratory-confirmed *P. falciparum* malaria cases (Pommier de Santi et al. 2016a) were considered new by excluding suspected cases of *P. falciparum* recrudescence (i.e., a new malaria attack after a first attack in the preceding 3 weeks but without a new mission into malarious areas). Cases of recrudescence can occur when treatment with ACT (Riamet®) is not completed by the patient, or the drug is not taken as prescribed.

Laboratory-confirmed *P. vivax* malaria cases (Pommier de Santi et al. 2016a) were considered new, excluding relapses. For both the temperate long latency and the tropical frequent relapse phenotype (White 2011), all malaria attacks occurring up to 90 days after the first attack were systematically excluded.

Permethrin quantification

Quantification of permethrin in washed and unwashed fabrics was carried out using the validated method of the Bundeswehr Research Institute for Materials, Fuels, and Lubricants published elsewhere (Faulde et al. 2003).

Test mosquitoes

Aedes aegypti mosquitoes from a continuously (≥40 years) reared colony were raised from eggs (Berlin strain) obtained from the German Federal Environmental Agency in Berlin. Eggs were deposited, and hatched larvae were fed according to a standardized protocol described elsewhere (Faulde et al. 2016).

Insecticidal testing procedures

Insecticidal activity was determined by the "cone test" as described by Faulde et al. (2016). When testing residual 99% knockdown (KD_{99}) activity of insecticide-impregnated fabric according to the TL 8305-0331 licensing protocol, a mean KD_{99}-time < 71.5 min has to be reached for *Ae. aegypti* after 100 defined machine launderings, according to EN ISO 6330:2012 (International Organization for Standardization 2012), in order to ensure sufficient protective efficacy during field use. All tests were replicated tenfold.

Statistical analyses

Values were reported as mean ± standard deviation (SD). The malaria protection rate of the PTBDU group was tested against the negative control group (NTBDU) using the one-sided Z-score test at the 5% level (statistical significance). Wearing, weathering, and environmental effects as measured by residual permethrin concentration versus number of launderings and residual permethrin amount versus KD_{99} values after field use against laboratory results were analyzed at the 5% level (statistical significance) by two-way analysis of variance (ANOVA) or Levene test ANOVA and error degrees of freedom using the SPSS 8.0 program (SPSS Software GmbH, Munich, Germany).

Results

The PTBDU group, consisting of 25 personnel, was exposed for 9.5 person-months (0.38 months per person) within hyperendemic malaria foci without experiencing any cases of malaria, whereas the 125 persons of the NTBDU control group were exposed for 30.5 person-months (0.24 months per individual) and experienced 11 cases of malaria (Table 1). Of these, eight cases were due to *P. vivax*, three cases were caused by *P. falciparum*, and none were mixed infections. Malaria incidence rates were zero per 100 exposed person-months for the PTBDU group, and 36.1 per 100 exposed person-months for the NTBDU group (Table 1). When considering overall exposure time to hyperendemic malaria foci summarized as person-months within the NTBDU group, an estimated mean of 3.4 malaria cases should have occurred within the PTBDU group, indicating that permethrin-impregnated clothing significantly (Z-score = −2.202; p = 0.0139) prevented malaria infection.

Of the 30 permethrin-impregnated battle dress uniforms worn during field duty, 23 (76.7%) complete uniforms were sent back for further investigation. The number of total (machine plus manual) launderings with detergent varied from 1 to 218 (mean 25.2 ± 44.8). Machine laundering was performed between 1 and 112 times (mean 14.1 ± 23.4), whereas uniforms were hand-washed between 0 and 106 times (mean 10.4 ± 22.1).

Mean KD_{99} times varied between 27.5 and 142.5 min (mean 47.7 ± 22.1 min) for blouses, and between 25.0 and 360 min (mean 60.2 ± 66.1 min) for trousers, yielding an overall mean KD_{99} rate of 54.0 ± 49.7 min. Data indicate that, during worst-case field use, loss of residual bioactivity was higher in PTBDU trousers when compared to that in PTBDU blouses (Fig. 1). Among the 23 PTBDU blouses tested for KD_{99}, only one (4.3%) exceeded the required cutoff value (71.5 min) at 142.5 min (sample 11), whereas three (13.0%) PTBDU trousers failed to meet the necessary knockdown efficacy requirement, with KD_{99} times of 88.5 min (sample 7), 91.7 min (sample 13), and 360 min (sample 11) (Fig. 1). Exceeding the KD_{99} cutoff value was directly linked to laundering frequencies of 218 times for blouses and >54 times for trousers, indicating that higher laundering frequencies led to lower remaining bioactivity (Fig. 1).

Overall, the mean remaining permethrin concentration in the PTBDU fabric was 732.1 ± 321.1 mg/m^2 and varied between 130 and 1270 mg/m^2 (mean 743.9 ± 304.2 mg/m^2) for blouses, and between 95 and 1290 mg/m^2 (mean 720.2 ± 336.9 mg/m^2) for trousers, showing that the mean loss of permethrin was slightly higher in trousers than in blouses (Fig. 2). KD_{99} times exceeded the required efficacy of the complete PTBDU subsequent to 218 field launderings, leading to a remaining permethrin concentration of 130 mg/m^2 in the blouse, and 95 mg/m^2 in the trouser fabric (Fig. 2). Unlike in blouses, two more PTBDU trousers did not reach the 100 launderings-KD_{99} cutoff value, showing remaining permethrin amounts of 160 mg/m^2 after 75 launderings (sample 7) and 190 mg/m^2 after 54 launderings (sample 13). While all the blouses met the TL 8305-0331 licensing requirements after worst-case field use in the rain forest of French Guiana, two trousers (8.7%) did not. In contrast to the KD_{99} values and corresponding residual permethrin concentrations obtained after 100 defined launderings during the licensing process (Faulde et al. 2016), data gathered during this study indicate that either the laundering procedures carried out individually under field conditions negatively influenced the stability of the permethrin impregnation and/or abrasive or substantial environmental effects took place during field use.

No significant differences in bioavailability (p > 0.05, df = 109) were found when comparing the 99% knockdown rates of residual permethrin concentrations in PTBDUs obtained after field use with those obtained after laboratory testing (Fig. 3a). Accordingly, the PTBDU trousers after field use showed no significant difference (p > 0.05; df = 109) when compared with the corresponding laboratory-tested PTBDU trousers (Fig. 4a), indicating that remaining permethrin contents embedded in the polymer layer onto the fibers were fully bioavailable after both laboratory testing and worst-case field use and were, therefore, effective against test mosquitoes.

When comparing the residual amounts of permethrin after field launderings with permethrin contents after defined, standardized machine laundering according to EN ISO 6330 (p > 0.05; df = 109), residual permethrin amounts after field

Table 1. Malaria incidence rates among wearers of permethrin-impregnated (PTBDU) versus untreated (NTBDU) clothing during duty in French Guiana from August 2011 to June 2012

BDU type	Number	Person-months exposed	Malaria cases contracted	Malaria incidence rate (p 100 person-months)	Z-score	*p*
PTBDU	25	9.5	0	0	2.202	0.0139
NTBDU	125	30.5	11	36.1		

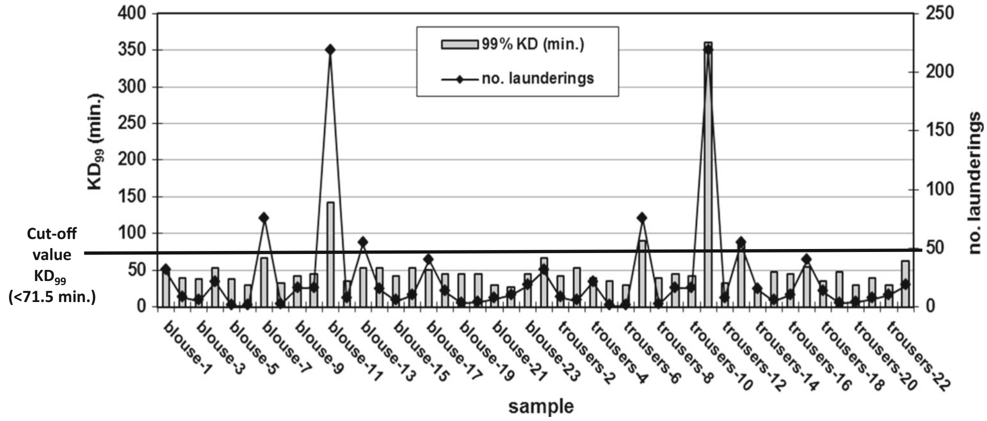

Fig. 1 Mean residual bioactivity as measured by KD_{99}-values and corresponding laundering frequency of permethrin-impregnated clothing after use in the primary rain forest of French Guiana

use were highly variable with respect to number of launderings (Fig. 3b). As an example, a residual permethrin amount of approximately 400 mg/m^2 resulted from between 5 and 21 field washings, representing a difference of 16 washing-equivalents. But, five standardized laboratory launderings resulted in a remaining permethrin concentration of 1250 mg/m^2 in blouses and trousers, while, after five field washings, the PTBDU sample 3 had a residual permethrin concentration of 860 mg/m^2 in the blouse and 410 mg/m^2 in the trouser, and the sample 15 had 930 mg permethrin/m^2 in the blouse and 670 mg/m^2 in the trouser. Thus, a threefold higher permethrin loss was observed in the trouser sample 3. Consequently, the mean additional permethrin loss was 1.4-fold in the blouses and 2.3-fold in the trousers, including a 1.7-fold higher loss in the field-worn trousers when compared to the corresponding blouses. Our data indicate that, besides the well-documented permethrin loss due to detergents used during the washing processes, an additional permethrin loss due to abrasive and/or weathering effects took place. At a given permethrin concentration, the high variability in numbers of field launderings did not differ significantly ($p > 0.05$; df = 109) among the blouses and trousers (Fig. 4b). Generally, TL 8305-0331 requirements were fulfilled after worst-case field use except for two trousers (PTBDU samples 7 and 13) that exceeded the required minimum KD_{99} bioactivity below 100 launderings due to excess permethrin loss.

Discussion

The long-lasting permethrin-impregnated Bundeswehr battle dress uniform is significantly effective against malaria infection during exposure in hyperendemic malaria foci under worst-case environmental and duty conditions. Nevertheless, the percentage protective efficacy remains unclear because—unlike those in the NTBDU group—no one in the PTBDU cohort contracted malaria. On the basis of exposure time and the 11 malaria cases experienced by the NTBDU group, 3.43 malaria cases would theoretically be expected to occur within the PTBDU group, when wearing NTBDUs, i.e., wearing PTBDUs should reduce malaria incidence rates ≥3.4-fold. Within the hyperendemic malaria study sites, which were characterized by high-level co-occurrence of *P. vivax* and *P. falciparum* transmission, *P. vivax* cases were prevalent (Pommier de Santi et al. 2016b). According to recent studies, various anopheline mosquito species may be involved in the

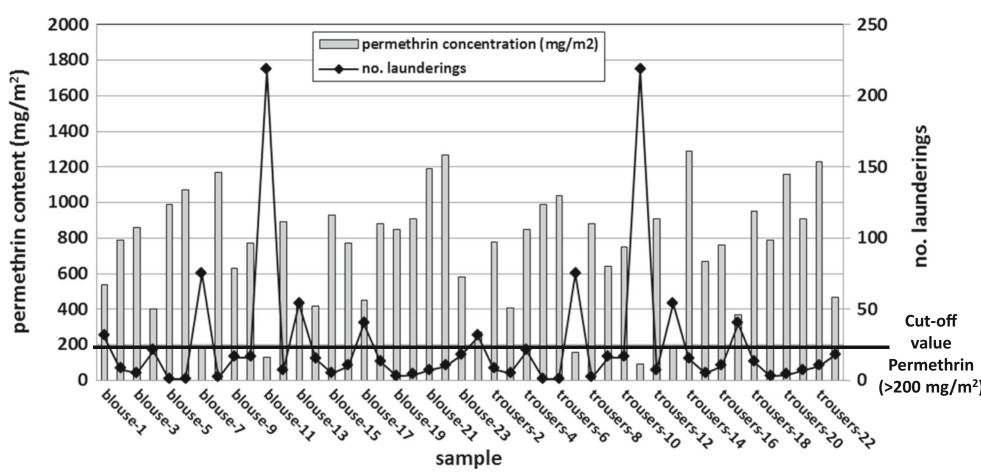

Fig. 2 Residual permethrin concentrations and corresponding laundering frequency of permethrin-impregnated clothing after use in the primary rain forest of French Guiana

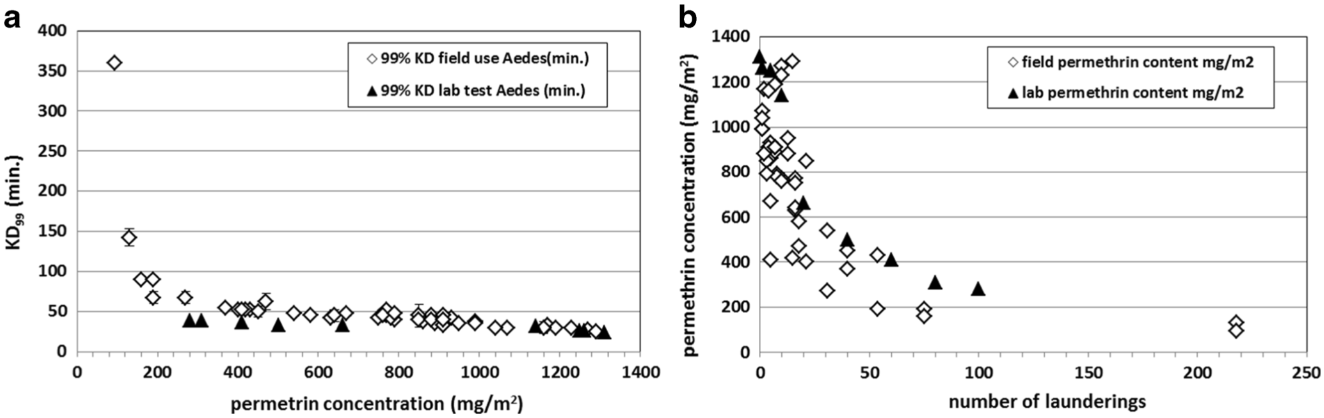

Fig. 3 **a** Residual permethrin concentrations and corresponding mean bioactivity as measured by KD_{99}-values of permethrin-impregnated clothing after use in the primary rain forest of French Guiana vs. laboratory results. **b** Residual amount of permethrin after laundering in the field vs. standardized laboratory machine launderings according to EN ISO 6330

focal transmission of malaria in French Guiana. Although *Anopheles darlingi* has long been considered the principal malaria vector in the French Guiana rain forest, other sylvatic species may transmit *P. falciparum* and *P. vivax* in artificially altered and/or deforested areas. For example, in recent years, *An. marajoara*, *An. nuneztovari* s.l., and *An. inini* have been identified as main vectors chiefly responsible for malaria outbreaks reported in French forces (Dusfour et al. 2012; Pommier de Santi et al. 2016a; Pimenta et al. 2015). And, in the Amazonian forest, additional sylvatic vectors that are also present in French Guiana have been found infected with *Plasmodium* spp.; some of these may be involved in malaria transmission at sites that are ecologically favorable to them (Dusfour et al. 2012; Pommier de Santi et al. 2016a; Pimenta et al. 2015). *Anopheles darlingi* and *An. marajoara* are considered primary neotropical malaria vectors that share similar lowland jungle habitats but differ in certain behavioral traits (Sinka et al. 2010). Unlike *An. darlingi*, *An. marajoana* is reported to favor wetland habitats that have undergone anthropogenic environmental change as at gold mining sites (Sinka et al. 2010). Under such conditions, *An. marajoara*, formerly considered a secondary vector of minor importance, assumes a dominant role relative to *An. darlingi* (Moreno et al. 2007). However, in our study, permethrin-induced bite protection was directed against both primary *Anopheles* vectors.

In South America, cases of malaria associated with gold mining are a public health concern, especially in rain forest sites where mining is illegal (Pommier de Santi et al. 2016a, b). Such sites may serve to further the geographical spread of malaria (Douine et al. 2016). It is therefore hardly surprising that the movements of illegal gold miners have recently been identified as a major reason for the nationwide re-emergence of malaria in Venezuela, where sylvatic and rural transmission cycles may spill over into urban areas, with epidemic effects (ProMED 2016). Besides chemoprophylactic regimens, personal protective measures against mosquito bites, especially the use of long-lasting factory-treated permethrin-impregnated clothing, may prevent both infection and the spread of disease.

The minimum effective permethrin concentration on treated clothing may differ markedly among vector arthropod species and families (Faulde et al. 2016). Although effective

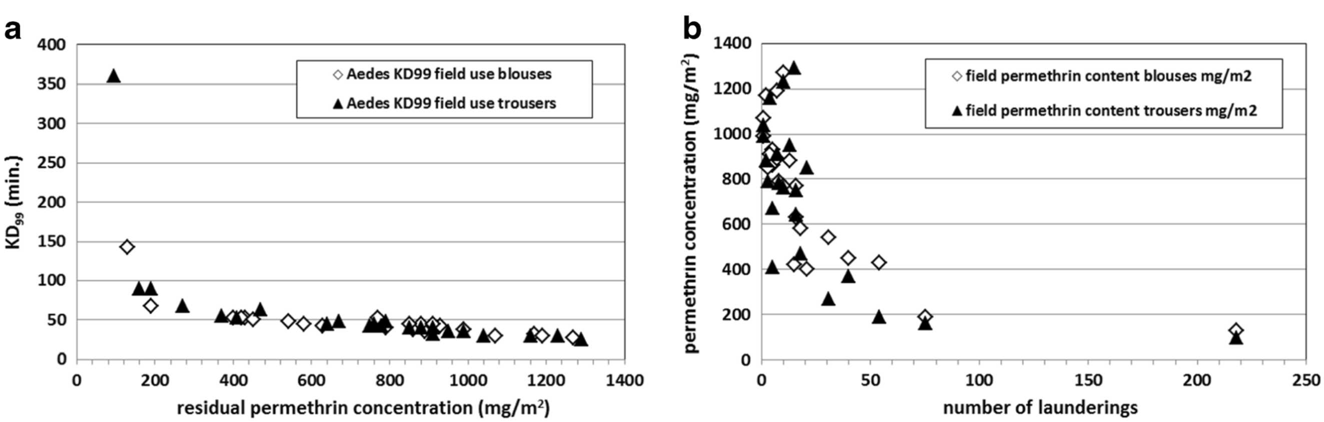

Fig. 4 **a** Residual permethrin concentrations and corresponding mean bioactivity as measured by KD_{99}-values of permethrin-impregnated battle dress uniform trousers vs. blouses after use in the primary rain forest of French Guiana. **b** Residual amount of permethrin in impregnated battle dress uniform trousers vs. blouses after field laundering

doses on impregnated fabrics could be as low as between 25 and 200 mg permethrin/m^2 against sand flies (Burgess et al. 1988), between 80 and 100 mg permethrin/m^2 against anopheline mosquitoes (Darriet et al. 1984), and between 62 and 250 mg permethrin/m^2 against *Amblyomma americanum* ticks (Schreck et al. 1982), low and/or sublethal permethrin concentrations may increase insecticide resistance and result in undesired behavioral changes especially in tick vectors (Faulde et al. 2006; Faulde et al. 2012). For example, low doses of permethrin may stimulate or accelerate attachment to impregnated clothing in the camel tick, *Hyalomma dromedarii* as well as in female *Dermacentor reticulatus* ticks (Fryauff et al. 1996; Buczek et al. 2015). Also, reports on some commercially available permethrin-impregnated fabrics designed for gardeners and foresters have described increased findings of dead but engorged ticks on the human body (Faulde et al. 2016). It is, therefore, highly recommended to employ long-lasting impregnation methods while aiming at remaining minimum residual permethrin concentrations ≥200 mg permethrin/m^2 during field use in order to ensure sufficient protective efficacy while simultaneously avoiding undesired behavioral side effects and/or pyrethroid resistance.

Our results clearly indicate a laundering-independent excess loss of permethrin that was not linked to changes in bioavailability because the permethrin concentration vs. KD_{99} value-ratio was unchanged. Suggested reasons for this excess permethrin loss are weathering effects due to frequent contact with water (rain, swimming though ponds and rivers), heat, sunlight, and other environmental factors influencing the chemical stability of the permethrin molecule, and/or abrasive factors that result in damage to the fabric surface on which permethrin is embedded in a plastic layer (Faulde et al. 2006). Such effects may be due to crawling over soil, sand, or rocks; vigorous hand cleaning with brushes with soap; or contact with caustic chemicals.

In concordance with the EU Biocides Regulation 528/2012, it is strongly recommended that manufacturers of impregnated clothing provide data on concentrations, migration rates, homogeneity on impregnated fabrics, protective efficacy, and laundering resistance of the insecticide used for their products (Appel et al. 2008). This information is critical to designing safe and effective personal protection products. Although the polymer-coating method has been shown to provide excellent laundering resistance, residual stability, and long-term efficacy, our results point to the need for further improvements in residual permethrin contents and bioavailability, especially when garments are intended for long-term worst-case field use.

Despite documented permethrin loss from laundering, weathering effects. and mechanical abrasion during military worst-case use in the tropical rain forest, long-lasting factory-based polymer-coated permethrin-impregnated clothing provided excellent protection against bites of infectious anopheline mosquitoes, thereby reducing malaria incidence rates significantly when military personnel were operating in high-transmission foci area. In the light of this, our method is highly recommended for personal protection against mosquito-borne diseases of public health importance, including chikungunya, dengue, and zika fevers, all of which are currently resurging globally.

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Conflict of interest The authors declare that they have no conflict of interest.

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