

Contact Toxicity and Residual Activity of Different Permethrin-Based Fabric Impregnation Methods for *Aedes aegypti* (Diptera: Culicidae), *Ixodes ricinus* (Acari: Ixodidae), and *Lepisma saccharina* (Thysanura: Lepismatidae)

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ABSTRACT The effectiveness and residual activities of permethrin-impregnated military battle dress uniforms were evaluated by comparing a new company-manufactured ready-to-use polymer-coating method with two "dipping methods" that are currently used to treat uniforms. Residual permethrin amounts and remaining contact toxicities on treated fabrics before and after up to 100 launderings were tested against *Aedes aegypti* (L.), *Ixodes ricinus* (L.), and *Lepisma saccharina* (L.). The residual amount of permethrin was considerably higher with the polymer-coating method: 280 mg a.i./m² after 100 launderings, compared with 16 and 11 mg a.i./m², respectively, obtained when using the two dipping methods. Hard ticks were most susceptible to the new polymer-coating method, resulting in prelaundering 100% knockdown times of 7.0 ± 0.9 min, whereas equivalent times for the dipping methods were 7.9 ± 0.35 min and 8.0 ± 0.54 min, respectively. After 100 launderings, 100% knockdown of *I. ricinus* nymphs was reached at 15.2 ± 1.04 min using the polymer-coating method, compared with 178.8 ± 24.7 min and 231 ± 53.6 min, respectively, using the dipping methods. Similar results were obtained for *Ae. aegypti* and *L. saccharina*, indicating that the polymer-coating method is more effective and efficient when compared with the dipping methods.

KEY WORDS arthropods, repellents, uniforms, permethrin

AMONG THE MANY VECTOR-BORNE diseases that are currently emerging or resurging worldwide, few are vaccine-preventable. Prophylactic drugs are available for malaria, but in many parts of the world drug resistance is on the increase and spreading geographically. For this reason, personal protective measures against vectors constitute the first line of defense against arthropod bites and arthropod-borne diseases. A major advance for protection of personnel at high risk (e.g., soldiers, travelers, and outdoor workers) has been the development of topical repellent formulations and residual insecticides that can be impregnated into clothing, tents, and netting (WHO 2001a, b).

Permethrin, a synthetic pyrethroid insecticide, which combines the essential qualities of repellency, hot-feet, knockdown and kill, has been widely used for decades as an arthropod contact repellent in fabric impregnation. The following characteristics of permethrin favor its use (Burgess et al. 1988, Croft et al. 2001): high level of potency against a wide range of arthropods, multiple repellent and toxicological ef-

fects on arthropods, rapid reactivity, low mammalian toxicity, excellent photostability, and resistance to weathering when applied to durable goods. To date, three methods have been developed for permethrin-based fabric impregnation:

1. Treatment of fabrics by dipping or spraying, leading to absorption of permethrin onto the surface of the fibers (absorption method), the method currently in widest use to prevent arthropod bites. Various formulations are currently commercially available (Evans et al. 1990, Eamsila et al. 1994, Carnevale and Mouchet 1997).
2. "Eulanisierung" of wool or silk fibers using solutions under heat and salt gradients to bind permethrin into the fibers (incorporation method), thus preventing infestation and damage of woolen or silk fabrics by clothes moths and keratin-eating beetles; this method has been developed by the Bayer Company, Leverkusen, Germany (Zimmermann and Höcker 1988).
3. Specific polymerization of permethrin onto the fiber surface (polymer-coating method), thereby enhancing weathering and laundering resistance as well as long-term residual activity against arthropod vectors; fabrics are factory-treated during production and are thus ready-to-use.

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This method has been developed only recently and suggests that the nature of the permethrin polymerization process on the fibers' surface is critical to insecticidal or acaricidal activity as well as long-term residual activity and laundering resistance (Gupta et al. 1990, Fryauff et al. 1996).

The purpose of the current study was to compare a newly developed factory-treatment permethrin-binding method with two different commercially available dipping methods used for the prevention of arthropod bites. This was accomplished by examining the levels of residual permethrin in treated military uniforms after up to 100 launderings. The remaining contact toxicities were measured by knockdown effect of corresponding washed and unwashed uniform samples against adult *Aedes aegypti* (L.), and *Lepisma saccharina* (L.), and nymphal *Ixodes ricinus* (L.).

Materials and Methods

Test Arthropods. *Ae. aegypti* eggs from a continuously (≥ 40 yr) reared colony (Monheim strain) were obtained from the Bayer Company. Eggs were deposited in distilled water and hatched larvae were fed with homogenized Tetramin fish food until adult emergence. A regimen of 27°C, 70% RH, and a photoperiod of 12:12 (L:D) was maintained throughout the study. Adults were fed with 10% sucrose solution, and only nonblood fed female mosquitoes, 5–8 d old, were used in permethrin studies. *Ixodes ricinus* nymphs were collected by flagging in natural habitats on Kuehkopf Mountain, Koblenz, Germany, then transported in glass vials at 18°C, 90% RH, and used directly in laboratory tests. Laboratory colonies of *L. saccharina* were reared in glass basins at 24°C, 80% RH, under a 12 h photoperiod, and fed on a standard diet consisting of (by weight) 60% wheat flour, 18% oat flakes, 11% sugar, 8.4% milk powder, and 2.6% egg-white.

Permethrin Impregnation of Fabric. The manufactured permethrin impregnated military uniforms were supplied by UTEXBEL S.A. (Ronse, Belgium). Fabrics were polymer-coated with permethrin (cis:trans = 25:75) after the dyeing process, and before tailoring. This yielded a theoretical permethrin concentration of 1300 mg a.i./m², and fabrics were dried by in-process-heating at 130°C. Uniform impregnation using the Peripel 10 "dipping method" (AgrEvo, Berkhamsted, Herts, United Kingdom) was carried out by dissolving 18.5 ml of Peripel 10 solution in 500 ml water (dilution rate: 1:27). The garment was rolled, tied, and incubated for 2.5 h in a glass basin (30 cm in diameter). This yielded a theoretical permethrin concentration of 650 mg a.i. permethrin (cis:trans = 25:75)/m², and the garment was air-dried for ≈ 5 h. The Insect/Arthropod Repellent Fabric Treatment (IARFT) method (Coulston Products Inc., Easton, PA) was applied by dissolving a solution of 40% permethrin (EC) in 500 ml of water. The garment was rolled, tied, and incubated for 2.5 h in a plastic bag containing the repellent solution. Subsequently, the uniform was removed and allowed to dry thoroughly (≈ 5 h), thus

yielding a theoretical permethrin concentration on the fabric of 1250 mg a.i. permethrin (cis:trans = 40:60)/m², respectively. The standard military uniform fabric used for each permethrin treatment consisted of 80% cotton and 20% polyester fibers, with a specific weight of 300 g/m².

Scanning Electron Microscopy (SEM) Analysis of Permethrin-Treated Fabrics. SEM analysis of treated fabrics was performed using a JSM-6400 scanning electron microscope from JEOL (Tokyo, Japan). Samples were coated with gold for 5 min according to the method described in the operation manual provided by the manufacturer (Operation Manual, 1991, Fisons Instruments, Uckfield, United Kingdom), using a Polaron SC 500 Sputter Coater (Fisons Instruments, Uckfield, United Kingdom).

Laundering Procedure. Permethrin-treated fabrics were washed according to European Norm (EN) 26330:1993/ISO 6330:1984 in a washing machine model A2 (front-loading, horizontal rotating drum type, model W 406–2 TMT, Miele Company, Gütersloh, Germany) at 60°C. It was adjusted for delicate textiles (procedure No. 3A, using a total washing time of 90 min, including one laundry and four rinse cycles, air-dry material load: 2 kg). A commercially available light-duty detergent was used for laundering (Burti, Burnus GmbH, Darmstadt, Germany). Samples were taken after 1, 5, 10, 20, 40, 60, 80, and 100 launderings and air-dried.

Permethrin Quantification. Measurement of permethrin in washed and unwashed fabrics was accomplished using the validated method of the Federal Armed Forces Research Institute for Materials, Explosives, Fuels, and Lubricants. Samples were taken from different locations on the material and cut into pieces $\approx 0.5 \times 0.5$ cm. All pieces were combined and mixed thoroughly. Fifty milliliters of toluene (Merck, Darmstadt, Germany) were added to an aliquot (5 g) of each sample. For extraction, the samples were treated in an ultrasonic bath. Toluene extracts were dried over sodium sulfate. After the addition of hexachlorobenzene (Fluka Chemie, Deisenhofen, Germany), an internal standard analysis was performed by capillary gas chromatography/mass spectrometry in the selected ion monitoring (SIM) mode using a HP 5989B mass spectrometer combined with a HP 5890 gas chromatograph, both from Agilent Technologies (Waldbronn, Germany). The permethrin quantification performed according to the method defined by the Eurachem/CITAC Guide shows an accuracy of $\pm 5\%$. However, the uncertainty of the results is known to be up to $\pm 30\%$, because of the nonhomogeneous distribution of the permethrin remaining in treated fabric after repeated launderings.

Testing Procedures. Plastic test tubes from the WHO insecticide susceptibility kit (WHO 1970) were used. Test fabrics after 0, 1, 5, 10, 20, 40, 60, 80, and 100 launderings, as well as negative controls, were taped to cover all inner surfaces of the tubes. Adult *Ae. aegypti* were stored for 30 min at +10°C before testing to reduce motility, collected in glass test tubes, and quickly transferred into the WHO plastic test tubes.

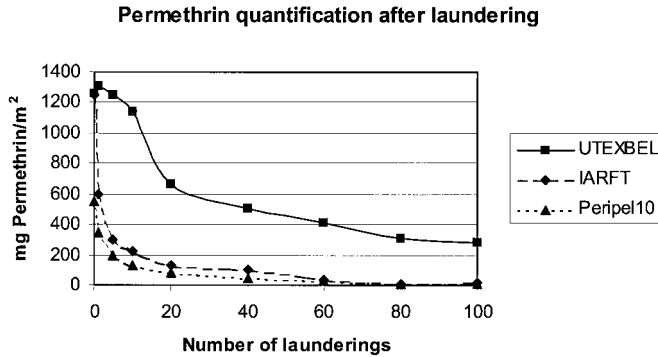


Fig. 1. Residual quantities of permethrin prior to and after 1, 5, 10, 20, 40, 60, 80, and 100 launderings.

I. ricinus nymphs were transferred using tweezers, and *L. saccharina* were placed into the WHO tubes using a tiny brush. Ten test arthropods were exposed simultaneously per run. The time of exposure necessary to obtain knockdown was measured. Knockdown (according to the definitions of the WHO and the Federal Environmental Office, Berlin, Germany) was defined as follows: *Ae. aegypti* adults and *I. ricinus* nymphs: inability to move/migrate; *L. saccharina*: inability to move and lying upside down (Hoffmann 1995).

Data Analysis. Arthropod tests were replicated 10 times per wash group and impregnation method. Values were reported as mean \pm SD. Differences in mean knockdown time of the repellent formulations tested were analyzed by one-way analysis of variance (ANOVA), ANOVA F value with treatment, and error degrees of freedom (df). The knockdown effect measured was tested against the residual error at the 5% level (statistical significance). The differences between the least squares means (LS means) and the P values associated with these differences were computed and compared using a two-sided t -test (comparing treatment groups) or one-sided t -test (comparing control to treated groups) at the fifth percentile of significance with the SPSS 8.0 program (SPSS Software GmbH, Munich, Germany).

Results

The measured initial permethrin quantities obtained after treatment of fabrics as well as the remaining amounts of permethrin after 0, 1, 5, 10, 20, 40, 60, 80, and 100 launderings are depicted in Fig. 1 for all three methods investigated. The quantitative loss of permethrin after laundering is almost identical for the IARFT and the Peripel 10 methods, the higher amount of permethrin remaining in the IARFT formulation being because of the double dose of permethrin used for initial treatment. With the UTEXBEL method, higher residual quantities of permethrin were detected, and 280 mg a.i./m² were still present on the fabric after 100 launderings. This amount is equivalent to the quantity of permethrin remaining after three launderings using Peripel 10 and after six launderings using IARFT.

SEM analyses showed that fibers treated by the dipping methods (Peripel 10 and IARFT) appeared smooth and even (Figs. 2A and B), whereas the polymer-coating method of UTEXBEL yielded smooth-surfaced, polymer-layered, cross-linked fibers before laundering (Fig. 2C). The amount of cross-linkage steadily decreased after repeated washings, indicating a mechanical disruption of the fibers' surface structure. The situation after 10 launderings is shown in Fig. 2D.

The time frame for obtaining 100% knockdown of *I. ricinus* nymphs constantly exposed to permethrin treated fabrics and the effects of laundering is shown in Fig. 3A. With unwashed, treated fabrics, 100% knockdown was obtained after 7.0 ± 0.9 min with the UTEXBEL method, versus 7.9 ± 0.35 min with IARFT, and 8.0 ± 0.54 min with Peripel 10. Knockdown activity of the polymer-coating method was significantly higher when compared with the IARFT ($F = 37.6$; $df = 1.263$; $P < 0.0001$) and Peripel 10 ($F = 37.6$; $df = 1.263$; $P < 0.0001$) dipping methods, whereas IARFT statistically showed no better knockdown activity when compared with Peripel 10 ($F = 37.6$; $df = 1.412$; $P < 0.052$). All impregnation methods showed significant differences when compared with the negative control group ($F = 39.1$; $df = 1.283$; $P < 0.0001$), which showed no knockdown effect in *I. ricinus* nymphs after 8 h of exposure. Both dipping methods yielded exponential graphs when knockdown time was compared with the number of launderings, whereas the UTEXBEL method showed a linear relationship. After 100 launderings, 100% knockdown of *I. ricinus* nymphs was achieved after 15.2 ± 1.04 min using UTEXBEL, 178.8 ± 24.7 min using IARFT, and 231.0 ± 53.6 min using Peripel 10. The knockdown activity remaining in fabrics treated by the UTEXBEL method after 100 launderings was comparable to the results obtained after 20 launderings with Peripel 10, and after 28 launderings with IARFT.

The 100% knockdown time for adult *Ae. aegypti* exposed to washed and unwashed permethrin-treated uniforms is depicted in Fig. 3B. Again, knockdown activity of the polymer-coating method was significantly higher when compared with the IARFT ($F = 39.1$; $df = 1.293$; $P < 0.0001$) and Peripel 10 ($F = 39.1$;

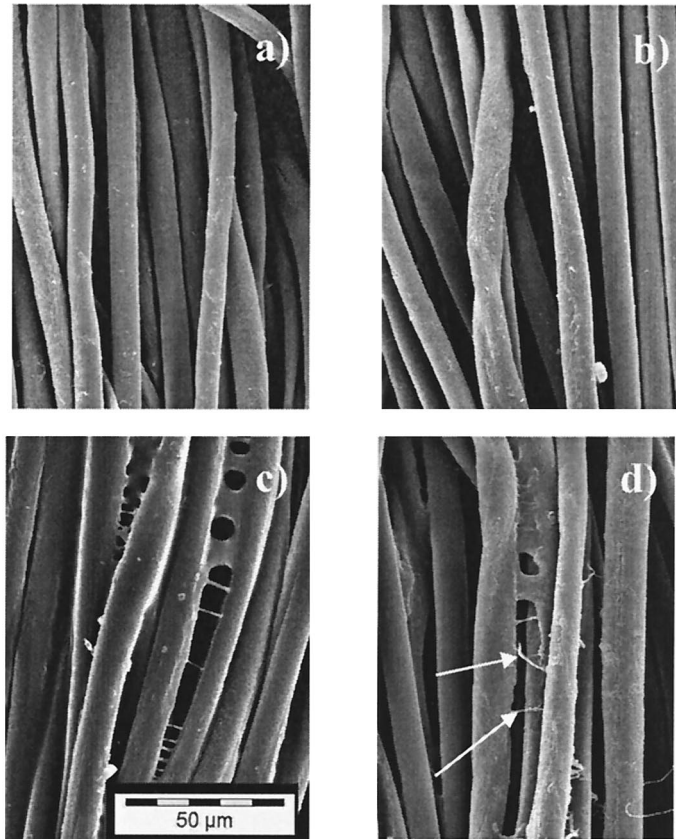


Fig. 2. Scanning electron microscopy results (magnification rate: 500x): a) fabric fibers treated with Peripel 10 prior to laundering; b) fabric fibers treated with IARFT prior to laundering; c) fabric fibers treated with the polymer-coating method prior to laundering; d) fabric fibers treated with the polymer-coating method after 10 laundrings (arrows indicate disrupted polymer cross-linkages).

$df = 1.293$; $P < 0.0001$) dipping methods. IARFT showed significantly better knockdown activity when compared with Peripel 10 ($F = 27.2$; $df = 1.587$; $P < 0.009$). All impregnation methods showed significant differences when compared with the negative control group ($F = 54.5$; $df = 1.209$; $P < 0.0001$), which showed no knockdown effect in adult *Ae. aegypti* after 8 h. When using unwashed treated fabrics, 100% knockdown was achieved after continuous exposure for 10.2 ± 2.7 min with IARFT, 10.8 ± 5.0 min with Peripel 10, and 15.8 ± 5.1 min with UTEXBEL. After 100 laundrings, the resulting knockdown time was 38.3 ± 5.1 min using UTEXBEL, and 240.4 ± 97.0 min using IARFT. No remaining 100% knockdown activity could be detected after 100 laundrings using the Peripel 10 impregnation method. Knockdown time after 100 laundrings with the UTEXBEL method was comparable to 15 laundrings with Peripel 10, and 34 laundrings with IARFT.

For *L. saccharina*, a crawling, permanently surface-exposed species widely used for testing insecticidal activities on surfaces, the required time for 100% knockdown following continuous exposure to permethrin-treated washed and unwashed uniform fabrics is shown in Fig. 3C. In this case, knockdown

activity of the polymer-coating method was significantly higher when compared with the IARFT ($F = 56.5$; $df = 1.407$; $P < 0.0001$), and Peripel 10 ($F = 56.5$; $df = 1.407$; $P < 0.0001$) dipping methods. IARFT impregnation showed significantly higher knockdown activity when compared with Peripel 10 ($F = 28.7$; $df = 1.306$; $P < 0.0001$). All methods investigated showed significant differences when compared with the negative control group ($F = 59.3$; $df = 1.212$; $P < 0.0001$) which showed no knockdown effect after 24 h of exposure. Unwashed, treated fabrics showed 100% knockdown after 5.5 ± 2.6 min with IARFT, 10.0 ± 0.7 min with Peripel 10, and 13.8 ± 2.7 min with UTEXBEL. After 100 laundrings, the measured knockdown time was found to be 31.2 ± 15.4 min with UTEXBEL, and 153.6 ± 31.2 min with IARFT. No 100% knockdown activity could be detected after 100 laundrings using Peripel 10 treatment. Knockdown activity subsequent to 100 laundrings with UTEXBEL-impregnated fabrics was comparable to that obtained after 12 laundrings with Peripel 10, and after 34 laundrings with IARFT.

The kinetics (mean value $n = 10$) of the knockdown effect in *I. ricinus* nymphs continuously exposed to UTEXBEL-treated fabrics after 0, 1, 5, 10, 20, 40, 60, 80,

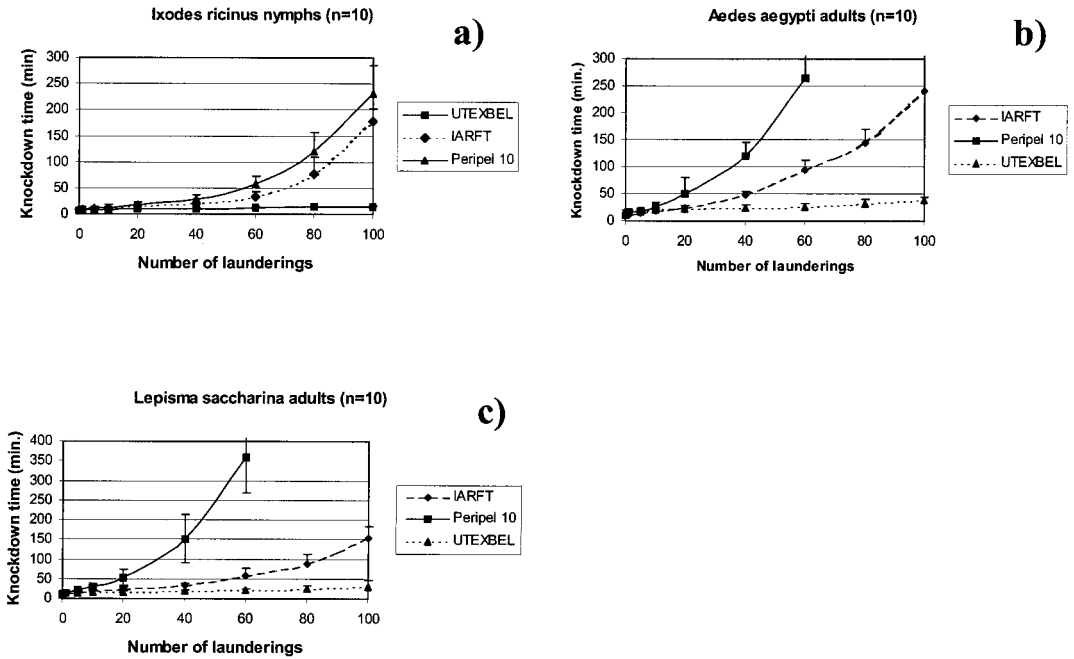


Fig. 3. Time necessary to achieve 100% knockdown in: a) *Ixodes ricinus* nymphs; b) *Aedes aegypti*; and c) *Lepisma saccharina* prior to and after laundering of fabric impregnated with permethrin according to the UTEXBEL, IARFT, and Peripel 10 methods.

and 100 launderings, and a negative control, are shown in Fig. 4. The resulting sigmoid graphs reveal the dose-dependent effect of the remaining bioactive permethrin concentration on the fibers' surface.

Discussion

In contrast to our results for *I. ricinus* nymphs, the UTEXBEL-impregnated fabric showed lower initial knockdown activity against *A. aegypti* and *L. saccharina*. The reason for this result remains unclear and should be further investigated. Nevertheless, the UTEXBEL-impregnated fabric containing 1250 mg a.i.

permethrin/m² before washing generally showed lower initial knockdown activities when compared with the IARFT method containing the same amount of permethrin. We believe that diffusion processes from the permethrin-containing polymer may be chiefly responsible for reduced biocidal activity, in which case only part of the total amount of permethrin is bioavailable directly on the fibers' surface. This assumes an equilibrium between absorbed and bioavailable permethrin, which commonly occurs when treating plastic surfaces with pyrethroids during pest control operations (Hoffmann 1995). Additionally, our results reveal statistically significant differences in

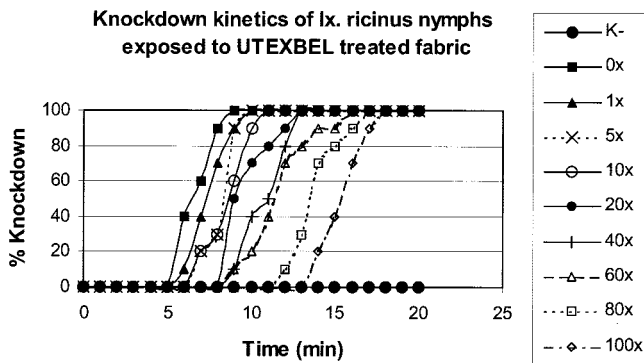


Fig. 4. Kinetics of the knockdown effect in *Ixodes ricinus* nymphs continuously exposed to UTEXBEL-treated fabric after 0, 1, 5, 10, 20, 40, 60, 80, and 100 launderings. K- = negative control.

bioavailable permethrin when comparing different permethrin impregnation formulations and treatment methods.

Recently published studies have led to the assumption that permethrin-treated clothing, bed-nets, curtains, and tents are effective means for reducing the incidence of arthropod-borne diseases (Heal et al. 1995, Hewitt et al. 1995, Clarke et al. 2001, Faulde 2001). However, these methods must be as safe as possible to humans, as well as cost effective and easy to apply. This last factor is especially important because permethrin-treated equipment is likely to be used by thousands of inexperienced personnel in the armed forces, as well as by tourists, refugees, and disease-threatened populations in the Third World. The newly established, highly effective polymer-coating method described herein provided protection from vector arthropods even after 100 launderings without retreatment. Furthermore, military clothing usually does not sustain repeated machine launderings, especially when worn under deployment conditions, so residual activity is likely to exceed the lifetime of treated fabrics. Laundering by hand may increase residual permethrin quantities because mechanical disruption of the permethrin-containing polymer layer would probably be less than that associated with laundry machines.

Interestingly, low permethrin concentrations on treated cloth fabrics may result in stimulation of attachment in some hard tick species as an unintended result of sublethal exposure to permethrin (Fryauff et al. 1994). Such behavior was investigated in the camel tick, *Hyalomma dromedarii* Koch, with results suggesting the premature or excess release of a neurosecretory substance that elicits attachment. With the dipping or spraying method, low residual concentrations of permethrin, such as those resulting from frequent launderings, may result in an accumulation of camel ticks on the human body and subsequent engorgement, especially when the amount of available bioactive permethrin is sublethal. Therefore, in marked contrast to the polymer-coating method, in which high residual concentrations of permethrin remain after laundering, clothing that is dipped or sprayed must be continually retreated to maintain sufficient permethrin to ensure lasting repellent, knockdown, and kill activities (Evans et al. 1990, Dobrovorsky et al. 1999).

An additional problem stemming from individual impregnation of fabrics by dipping or spraying is the increased rate of exposure to highly concentrated permethrin solutions and formulations, potentially resulting in increased permethrin incorporation by inhalation or skin contact, thus simultaneously increasing the health risk to workers. When using the UTEXBEL method, exposure to permethrin is reduced by the higher binding efficacy of the polymer layer on the fiber surfaces, which also minimizes contamination of waste water during laundering and dermal incorporation of permethrin. For the IARFT dipping method, the exposure dose to humans wearing permethrin-treated military clothing is predicted to be 6×10^{-4}

mg/kg/d (Snodgrass 1992). To date, similar studies quantifying exposure doses to humans from the coating method have not been conducted, though these would be of special interest when analyzing the health threat/benefit ratio in occupational medicine.

Efforts to minimize human exposure to permethrin, especially when used in combination with N,N-diethyl-m-toluamide (DEET) and pyridostigmine bromide, are being closely followed by toxicologists, because the toxicological effects of these chemicals when used in concert are widely thought to be one of the causes of Gulf War Syndrome (Pennisi 1996, Plapp 1999, Hoy et al. 2000). Although this hypothesis remains unproved, recent studies suggest that simultaneous incorporation of permethrin, DEET, and pyridostigmine bromide in sufficiently high doses may cause diffuse neuronal cell death and cytoskeletal abnormalities (Abdel-Rahman et al. 2001). It may also generate free radical species, thus increasing the levels of the oxidative stress marker 3-nitrotyrosine in rats (Abu-Qare et al. 2001). Moreover, some recent studies indicate that DEET, but not permethrin, can act systemically to cause signs of toxicity when the two chemicals are simultaneously applied dermally, especially on mouse skin (Baynes et al. 1997, Young 1998). Further, it has been demonstrated that DEET decreases percutaneous permethrin absorption in mice (Baynes et al. 1997).

We strongly recommend use of the permethrin polymer-coating method because of its high and long-lasting efficacy, uniform and standardized method of application, cost effectiveness, minimal exposure threat to humans, and reduced logistical and time constraints stemming from ready-to-wear, factory-based impregnation techniques for the treatment of clothing, bednets, and other fabrics. Although our results confirm that ticks are most susceptible to permethrin (Rey 1998, Ho-Pun-Cheung et al. 1999), this method can also be used universally for protection against other hematophagous arthropod vectors, such as body lice, that are of great health importance, especially among today's increasingly numerous refugee populations (Sholdt et al. 1989).

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