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CONTACT TOXICITY OF PERMETHRIN-IMPREGNATED MILITARY UNIFORMS TO *CULEX PIPIENS* (DIPTERA: CULICIDAE) AND *PHLEBOTOMUS PAPATASI* (DIPTERA: PSYCHODIDAE): EFFECTS OF LAUNDERING AND TIME OF EXPOSURE[1]

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ABSTRACT. The effects of laundering and time of exposure on the insecticidal activity of military uniform fabric impregnated with permethrin at 0.125 mg active ingredient (AI)/cm^2 are reported from susceptibility tests with laboratory-reared mosquitoes, *Culex pipiens*, and sand flies, *Phlebotomus papatasi*. Knockdown/mortality resulting from exposure of groups of female insects for periods of 1, 3, 5, 7, and 10 min was recorded and compared among 5 different treatment/wash groups (untreated/unwashed, treated/unwashed, treated/1-wash, treated/2-wash, treated/3-wash). Laundering was by machine washing with detergent and warm water followed by hot-air machine drying. Post-exposure assessments at intervals from 0 to 60 min and at 24 h showed that knockdown for each exposure time and wash group was initially low but increased steadily during the first hour post-exposure. Sand flies were less sensitive than mosquitoes to knockdown during the first 60 min after contact with treated/unwashed fabric; however, 24-h mortality rates for sand flies were higher as compared with mosquitoes. The permethrin remaining after a 3rd wash had little knockdown effect on mosquitoes but was toxic to sand flies at each of the 5 exposure times. Significant reductions in the knockdown effectiveness of permethrin-treated fabric to mosquitoes and sand flies was associated with single and repeated washings of the fabric.

INTRODUCTION

A major advance in the prevention of arthropod bites and vector-borne diseases in military personnel has been the development of topical repellent formulations and a long-lasting insecticide that can be impregnated into uniforms, tents, and netting. Permethrin, an insecticide with important repellent property against many arthropod species, was recently adopted for wide-scale use as a fabric impregnant by the U.S. Department of Defense. This compound is currently recommended for personal protection against biting flies, mosquitoes, ticks, mites, and fleas and confers protection even after repeated washing of the treated fabric (Schreck et al. 1978, 1982a).

With numerous reports of repellent effectiveness against arthropods of public health importance (Breeden et al. 1982, Schreck et al. 1984, 1986; Wirtz et al. 1986), there is a tendency to assume that permethrin is similarly effective against other man-biting arthropod species. Schreck et al. (1979) cautioned against this assumption and stressed the need for evaluations against each target species. For example, permethrin-treated uniforms provided more than 97% protection against the mosquito *Aedes taeniorhynchus* Wied. (Schreck et al. 1984) but only 49% protection against Panamanian sand flies (Schreck et al. 1982b). Permethrin-treated uniforms provided only 13% more protection than untreated uniforms in preventing human contact and biting by sand flies in the Sinai desert (Dees et al. 1987[8]). Although permethrin is known to cause rapid knockdown and mortality of many biting dipteran species, certain Panamanian sand flies are able to bite and engorge to repletion even after 15 min of contact exposure with permethrin-treated fabrics (Schreck et al. 1982b). Similarly, *Phlebotomus papatasi* Scopoli, a vector of *Leishmania major* and phleboviruses, exhibits probing behavior during direct contact with permethrin-treated uniforms and readily attacks skin surfaces previously covered by the treated fabric (Dees et al. 1987[8]). Despite precautions that included the issue

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[8] Dees, W. H., S. Gaber, F. A. Abdel A'al and H. A. Hanafi. 1987. Evaluation of clothing impregnants and skin repellents against medically important arthropods of Northeast Africa. Annu. Meeting Entomol. Soc. Am., Nov. 29–Dec. 3, 1987, Boston, MA. Unpublished report.

of permethrin-impregnated uniforms, and a main deployment during the cooler winter period, leishmaniasis was the dominant arthropod-borne disease that occurred among U.S. troops during Operations Desert Shield/Storm (Kreutzer et al. 1993, Magill et al. 1993, Persian Gulf Veterans Coordinating Board 1995). These observations suggest that the repellency of impregnated permethrin may not provide an effective first line of defense against all human-biting dipteran species. Where the protective repellent effect of permethrin is questionable, or lacking, contact toxicity becomes an important line of defense against insect bites and vector-borne pathogen transmission.

The present paper reports the results of contact toxicity tests with washed and unwashed permethrin-treated uniform fabric against laboratory-reared *Culex pipiens* Linn. and 2 strains of *P. papatasi.* Knockdown and mortality at different exposure times were compared within and between species and wash groups.

MATERIALS AND METHODS

Insects: Laboratory colonies of *P. papatasi* originating from Israel and India in 1978 were reared according to the methods of Modi and Tesh (1983). A colony of *Cx. pipiens* originating from Gharbaya, Egypt, in 1987 was reared in distilled water and fed Tetramin® fish food until adult emergence. A regimen of 27°C, 70% RH, and a photoperiod of 12:12 h (L:D) was maintained throughout the study. Female sand flies and mosquitoes, aged 5–7 days old and fed on 30% sucrose, were used for testing.

Fabric treatment: Tropical weight, woodland camouflage Battle Dress Uniform (BDU) fabric of 100% cotton was treated with an aqueous suspension of permethrin (Permanone 40% emulsifiable concentrate, Fairfield American Corp., Rutherford, NJ) to achieve a deposition rate of 0.125 mg active ingredient (AI)/cm^2 of fabric. Sufficient liquid was used to saturate each BDU fabric sample without runoff. Saturated fabrics were placed in plastic bags for 24 h to enhance liquid penetration and then air dried. Five treatment/wash groups of fabric were prepared: untreated/unwashed (control), treated/unwashed, treated/1-wash, treated/2-wash, and treated/3-wash. Permethrin-impregnated fabrics were machine laundered with warm water and Tide® detergent and were hot-air machine dried as described by Schreck et al. (1982a). Prepared test fabrics were kept separately in sealed plastic bags and stored for 4–6 months at room temperature in darkness until tested.

Testing procedure: A modification of the World Health Organization (W.H.O.) procedure for determining insecticide susceptibility of adult mosquitoes (W.H.O. 1970) was employed. Test fabrics were taped to cover all inner surfaces of the plastic tubes from the W.H.O. kit. Ten or 15 females were aspirated into the tube for an exposure period of 1, 3, 5, 7, or 10 min. At the end of the exposure period, the insects were transferred to a screen-topped clear plastic container, provided a cotton wick saturated with 30% sucrose, and observed at 0, 5, 10, 15, 30, and 60 min and at 24 h for knockdown and mortality. Knockdown, defined as the inability to fly, and mortality were recorded at each post-exposure interval. Sand flies and mosquitoes exposed to untreated/unwashed fabric for 10 min served as controls. Tests were replicated 5–10 times per wash group and exposure time with separate groups of 10–15 female insects.

Data analysis: Differences in percentage of knockdown and mortality between treatment/ wash groups within an exposure period, between exposure times within a wash group, and between species/strains were compared by one-way analysis of variance and Bartlett's test for homogeneity of variance using EpiStat version 5.01b (CDC, Atlanta, GA, 1991). For data not normally distributed and where Bartlett's test showed the variances in samples to differ significantly, the Kruskal-Wallis nonparametric test was applied (Sokal and Rohlf 1981). Knockdown rates at 15 min (KD_{15}) post-exposure and mortality rates at 24 h (MR_{24}) post-exposure were selected for statistical comparison because these time points were considered to represent short- and long-term toxic effects or recovery.

RESULTS

Mean percentages of knockdown and 24-h mortality of each wash group and exposure time were plotted as a function of post-exposure observation time for *Cx. pipiens* (Fig. 1). Knockdown and mortality effects of impregnated permethrin on *Cx. pipiens* were dependent on exposure time, post-exposure time, and number of washes. Knockdown after all contact times with treated/unwashed fabric rose sharply during the first 15–30 min post-exposure (Fig. 1) and rose more slowly or negligibly after exposure with treated/washed fabric. No knockdown was recorded immediately after 3 min of exposure to treated/1- or 2-wash fabric, but after 15 min, 29 and 19%, respectively, of the mosquitoes were unable to fly. Differences in KD_{15} and MR_{24} attributable to exposure time were statistically significant in only the treated/3-wash group (Table 1). However, significant differences in KD_{15} and MR_{24} were associated with single and multiple machine washings at the 3-, 5-, 7-, and 10-min exposure times (Table 1). Ten minutes of contact

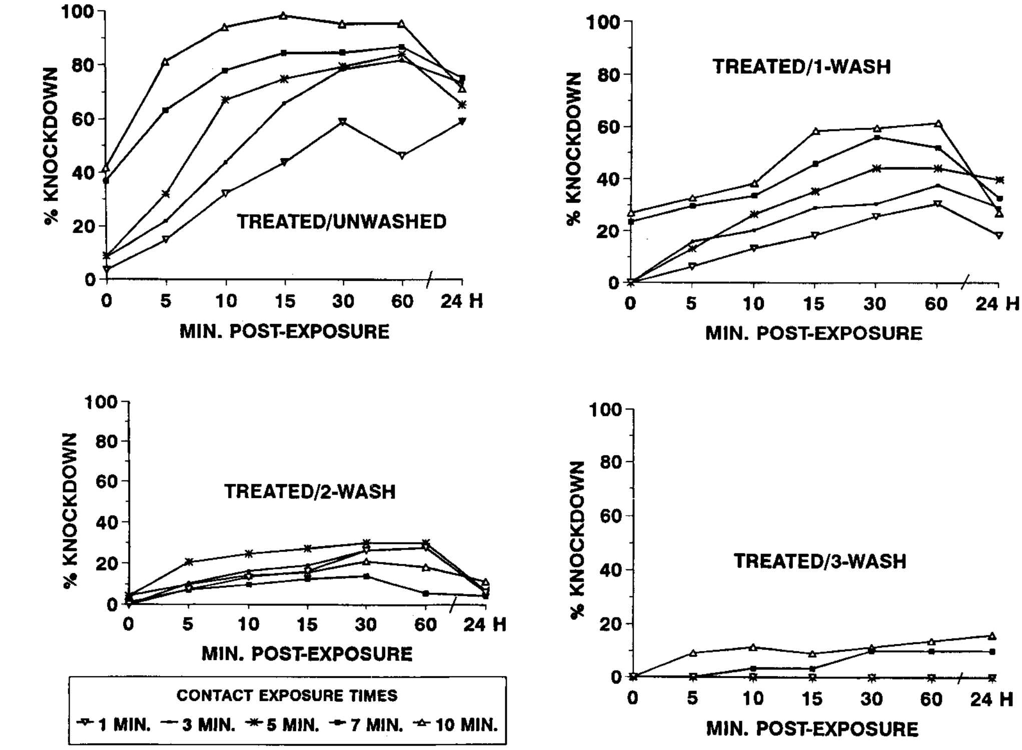

Fig. 1. Contact toxicity of unwashed and washed permethrin-treated fabric to female *Culex pipiens* (Egyptian strain). Knockdown was assessed at 0, 5, 10, 15, 30, and 60 min post-exposure. Mortality was assessed at 24 h post-exposure.

with treated/unwashed fabric produced 98% KD_{15}; whereas contact with treated/3-wash fabric for the same time period resulted in only 10% KD_{15}. Mosquito mortality at 24 h, like knockdown, was primarily dependent on the wash factor and secondarily dependent on exposure time. One minute of exposure to treated/unwashed fabric killed 56% of the mosquitoes, and 10 min of exposure increased the kill only to 74%. The discrepancy between the maximum

Table 1. *Culex pipiens* (Egypt): Summary effect of exposure to unwashed and washed permethrin-treated uniform fabric at 15 min and 24 h post-exposure.

	Percentage of knockdown at 15 min post-exposure (KD_{15})					Percentage of mortality at 24 h post-exposure (MR_{24})				
Exposure (min)	0-wash	1-wash	2-wash	3-wash	*H*-statistic for wash groups	0-wash	1-wash	2-wash	3-wash	*H*-statistic for wash groups
1	49	21	20	0	7.8	56	15	7	0	16.0*
3	70	29	22	0	11.4*	72	29	6	0	19.6*
5	75	32	31	0	11.2*	66	37	7	13	12.3*
7	85	44	12	0	15.7*	76	29	4	3	15.9*
10	98	55	16	9	21.6*	74	28	11	17	15.2*
H-statistic for exposure times	11.4*	8.7	1.8	10.3*		6.8	2.6	2.6	9.5*	

* Denotes that KD_{15} or MR_{24} values were different at $P < 0.05$ level of significance (Kruskal-Wallis H is equivalent to chi-square).

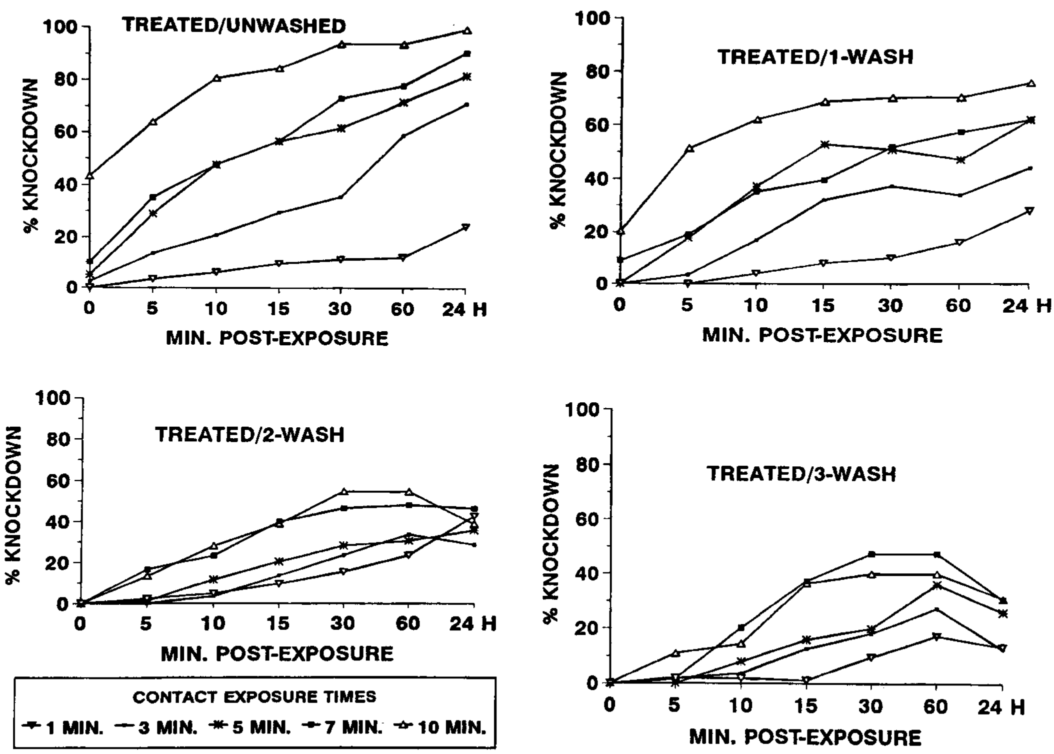

Fig. 2. Contact toxicity of unwashed and washed permethrin-treated fabric to female *Phlebotomus papatasi* (Israeli strain). Knockdown was assessed at 0, 5, 10, 15, 30, and 60 min post-exposure. Mortality was assessed at 24 h post-exposure.

knockdown achieved within 60 min and the endpoint MR_{24} values in treated/unwashed, 1-, and 2-wash groups shows that post-exposure recovery occurred at virtually all exposure times (Fig. 1). Knockdown and mortality among controls were negligible.

Mean percentages of knockdown or mortality for each exposure time, post-exposure time, and treatment/wash group for the Israeli and the Indian strains of *P. papatasi* are shown in Figs. 2 and 3, respectively. Both sand fly strains appeared to be less sensitive than *Cx. pipiens* to knockdown during the first 60 min after contact with treated fabric. This effect was most pronounced in assays performed with treated/unwashed fabric, where knockdown rates of mosquitoes exceeded those of the sand fly strains at virtually all contact times and post-exposure observation points. In contrast, assessment at 24 h revealed that mortality rates for both strains of *P. papatasi* were greater than those of the mosquitoes at each exposure time in the 4 treatment/wash groups compared. Sand fly strains did not show recovery from knockdown as did *Cx. pipiens*. Within the treated/unwashed and treated/1- and 2-wash groups, the Indian strain of *P. papatasi* demonstrated 24-h mortality rates that were generally equal to or greater than the maximum knockdown achieved in 60 min. Mortality at 24 h in the Israeli strain of *P. papatasi* was consistently greater than 60-min knockdown for the treated/unwashed and treated/1-wash groups. Also in contrast to *Cx. pipiens,* sand fly strains demonstrated consistent significant differences in KD_{15} atributable to exposure time in each treatment/wash group and fewer significant reductions in KD_{15} and MR_{24} within exposure times that could be associated with single and multiple washings of treated fabric (Tables 2 and 3). The 3rd washing of treated fabric, which caused little intoxication of mosquitoes, produced knockdown and mortality rates in sand flies that were comparable to those sustained under exposure to treated/2-wash fabric (Figs. 2 and 3). No knockdown or mortality occurred in either strain exposed to untreated/unwashed control fabric.

DISCUSSION

The contact toxicity and resulting knockdown effectiveness of permethrin-treated fabric against

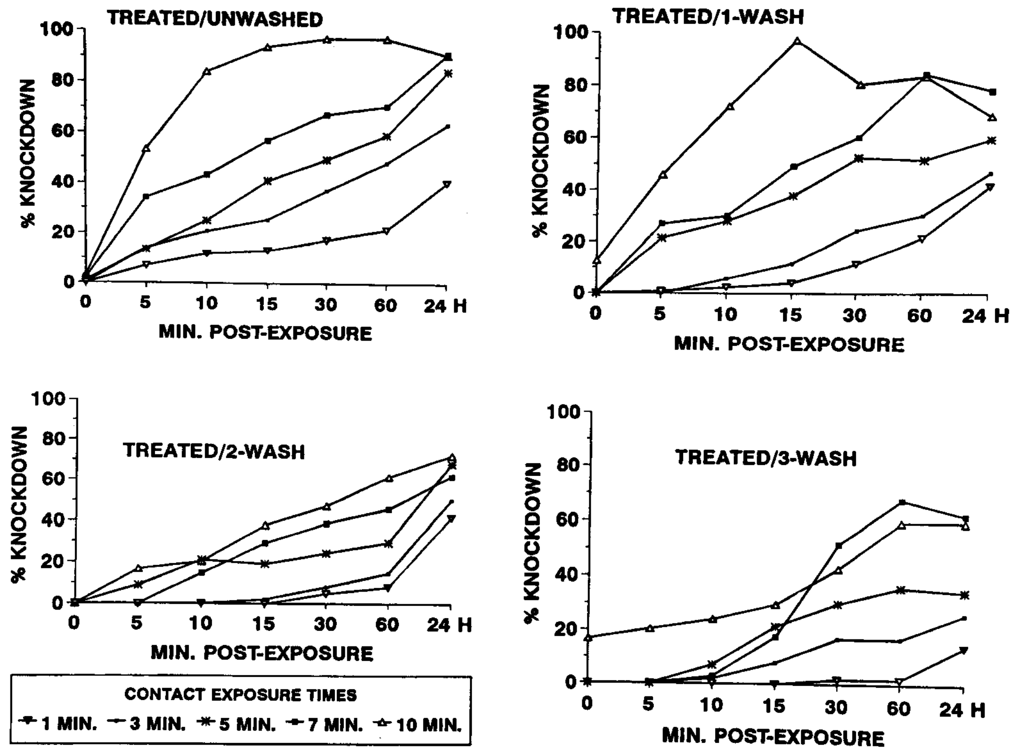

Fig. 3. Contact toxicity of unwashed and washed permethrin-treated fabric to female *Phlebotomus papatasi* (Indian strain). Knockdown was assessed at 0, 5, 10, 15, 30, and 60 min post-exposure. Mortality was assessed at 24 h post-exposure.

Cx. pipiens and 2 geographic strains of *P. papatasi* decreased significantly after single and multiple launderings. Permethrin susceptibility also differed between the mosquito and sand fly species tested. *Culex pipiens* was more susceptible to rapid knockdown than was *P. papatasi*, but the sand fly strains recovered less frequently from knockdown and had higher mortality rates for each of the treatment/wash groups and exposure times evaluated.

Table 2. *Phlebotomus papatasi* (Israel): Summary effect of exposure to unwashed and washed permethrin-treated uniform fabric at 15 min and 24 h post-exposure.

	Percentage of knockdown at 15 min post-exposure (KD_{15})					Percentage of mortality at 24 h post-exposure (MR_{24})				
Exposure (min)	0-wash	1-wash	2-wash	3-wash	*H*-statistic for wash groups	0-wash	1-wash	2-wash	3-wash	*H*-statistic for wash groups
1	8	8	10	20	1.4	30	24	45	14	5.9
3	26	32	16	13	1.1	73	44	33	14	13.0*
5	56	52	23	16	8.9*	82	65	40	26	9.0*
7	56	39	48	42	0.9	91	62	56	36	6.8
10	84	71	42	39	8.5*	100	77	44	34	12.0*
H-statistic for exposure times	21.7*	13.3*	24.9*	14.1*		28.3*	13.4*	0.7	2.0*	

* Denotes that KD_{15} or MR_{24} values were different at $P < 0.05$ level of significance (Kruskal-Wallis *H* is equivalent to chi-square).

Table 3. *Phlebotomus papatasi* (India): Summary effect of exposure to unwashed and washed permethrin-treated uniform fabric at 15 min and 24 h post-exposure.

Exposure (min)	Percentage of knockdown at 15 min post-exposure (KD_{15})					Percentage of mortality at 24 h post-exposure (MR_{24})				
	0-wash	1-wash	2-wash	3-wash	*H*-statistic for wash groups	0-wash	1-wash	2-wash	3-wash	*H*-statistic for wash groups
1	14	5	0	0	8.6*	42	45	43	15	4.8
3	25	13	3	9	3.4	63	49	55	29	2.2
5	41	38	18	25	3.0	84	65	65	40	4.2
7	52	48	30	20	9.6*	92	80	64	69	2.1
10	93	78	40	29	19.4*	91	67	76	63	1.0
H-statistic for exposure times	24.7*	28.8*	24.9*	14.1*		10.4*	5.3	3.6	7.8	

* Denotes that KD_{15} or MR_{24} values were different at $P < 0.05$ level of significance (Kruskal-Wallis *H* is equivalent to chi-square).

The residual insecticidal activity of impregnated permethrin has been studied under a variety of conditions and against a number of arthropod species. The general consensus is that permethrin is stable in sunlight (Schreck et al. 1982a, 1982b), persists through long periods of wear and natural weathering (Schreck et al. 1980, Gupta et al. 1989), and remains impregnated in fabrics at protective levels even after repeated cold-water rinsings (Schreck et al. 1978). However, residual activity of impregnated permethrin declined considerably as a result of agitated cold-water laundering with soap (Snow et al. 1987, Rozendaal 1989), and the most significant decline in residual activity resulted from machine washing with soap and hot water (Schreck et al. 1978). About half of the original concentration of permethrin at the standard application rate (0.125–0.20 mg AI/cm^2) persisted through 4 detergent/warm-water machine washings and 4 hot-air machine dryings. This residual permethrin caused 100% knockdown of a laboratory strain of *Aedes aegypti* (Linn.) but only after an exposure period of 35 min (Schreck et al. 1982a). In comparative bioassays, *Ae. aegypti* was more susceptible to knockdown by permethrin than was *Anopheles gambiae* Giles *sensu strictu,* which in turn was more susceptible than *Culex quinquefasciatus* Say (Rozendaal 1989). Rozendaal (1989) also reported large variations in the time required to achieve 100% knockdown of laboratory-reared *Cx. quinquefasciatus* due to survival of less susceptible individuals. In the present study, 10 min of contact exposure to treated/unwashed fabric failed to produce 100% knockdown within 1 h with *Cx. pipiens* and 2 strains of *P. papatasi.* Penner and Wilamovsky (1987) based the permethrin sensitivity of laboratory-reared *P. papatasi* (Israel) on a contact period of 60 min and a 24-h mortality assessment. Under those conditions, an LC_{90} of about 0.02 mg AI/cm^2 was determined for the toxicant. After 3 launderings, the permethrin remaining from an initial application rate of 0.125 mg AI/cm^2 probably still exceeds this LC_{90} (Schreck et al. 1982a). However, 60 min of sustained contact seems far in excess of the actual contact time that sand flies might have with the treated uniform under natural field conditions. In addition, laboratory-reared insects, raised under optimal conditions, may be larger, healthier, and more tolerant of permethrin than are those typically found in nature (Penner and Wilamovsky 1987).

Our results show a response of *P. papatasi* to contact exposure with impregnated permethrin that supports field observations of persistent sand fly contact and human-biting activity despite the use of treated/unwashed BDUs (Schreck et al. 1982b, Dees et al. 1987[8]). Unlike *Cx. pipiens,* which demonstrated differences in knockdown response that were primarily dependent on the wash factor, *P. papatasi* demonstrated differences in knockdown that appeared to be more dependent on exposure time. Brief contact exposure, more closely approximating the contact that sand flies actually make while attempting to obtain human blood, did not induce rapid and significant knockdown results. Among sand flies that underwent 3 min of contact with treated/unwashed fabric, more than 63% remained unaffected and potentially able to bite for at least 30 min post-exposure. However, despite a comparatively delayed knockdown response to unwashed permethrin-treated fabric, both strains of *P. papatasi* remained sensitive to intoxication through multiple washes of the fabric.

It is important to reiterate that our study did not intend to measure the "first-line" repellent effect of impregnated permethrin. Prompted by reports that suggested a questionable repellent effect against sand flies, we evaluated strictly the "second-line" knockdown effect of contact exposure. The results obtained should not be construed as definitive. Additional study of the contact behavior and feeding response of *P. papatasi* against treated fabrics under field and laboratory conditions will reveal more fully the protective efficacy of permethrin-impregnated uniforms against this vector.

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