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SCIENTIFIC NOTE

COMPARATIVE TOXICITY OF PERMETHRIN- AND BIFENTHRIN-TREATED CLOTH FABRIC FOR *ANOPHELES FARAUTI* AND *AEDES AEGYPTI*[1]

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ABSTRACT. In this laboratory study, we applied 3 formulations of permethrin (Peregin® 500 EC, Dragnet® 500 EC, and Dragnet® 100 ME) and 2 of bifenthrin (Biflex® 10 ME and Talstar® 80 SC) to swatches of Australian military shirt fabric. The knockdown and mortality of *Anopheles farauti* and *Aedes aegypti* after exposure to treated fabrics were compared. The mortality of *An. farauti* exposed to permethrin-treated swatches for 3 min in World Health Organization test kits was 94.2–100% after initial treatment and fell to <28% after 2 cold water washes, and knockdown was <20% after 3 washes. The mortality of *An. farauti* exposed to bifenthrin-treated swatches was initially 100% and remained >55% after 3 washes, whereas knockdown was <25% after 2 washes. Mortality of *Ae. aegypti* exposed by tarsal contact to permethrin- and bifenthrin-treated fabrics was 84.8–100% prior to washing and fell to <21% and <40%, respectively, after 1 cold water wash. The ability of *Ae. aegypti* to obtain a blood meal through treated fabrics was variable, and a small percentage (0–6.1%) of mosquitoes obtained a blood meal through fabrics after initial treatment. The effect of cold water washing on the persistence of both chemicals in fabric by chemical assays showed that between 58% and 66% of both chemicals was lost from the test fabric after a single wash.

KEY WORDS *Anopheles farauti, Aedes aegypti,* bifenthrin, permethrin

The wearing of clothing treated with permethrin to protect people against mosquito vectors of disease has been advocated in recent years. Military forces deployed to areas where malaria, dengue, and arboviruses are endemic have used this method (Horosko and Robert 1996, Young and Evans 1998).

Earlier studies showed that a combination of the wearing of permethrin-impregnated military uniforms and application of repellents containing *N,N*-diethyl-*3*-methylbenzamide (deet) on the exposed skin provided the best protection against mosquitoes (Gupta et al. 1987, Harbach et al. 1990). In some instances, the wearing of permethrin-treated uniforms alone provided better protection against mosquitoes than did untreated uniforms, but this protection was less than that provided by the combination of permethrin-treated uniforms and topically applied deet (Schreck et al. 1984). This finding prompted 2 groups to evaluate the effectiveness of permethrin-treated uniforms in protecting soldiers against malaria. In a trial in northeastern Thailand, the use of permethrin-treated uniforms did not reduce the incidence of malaria in Thai soldiers over a 6-month period (Eamsila et al. 1994). However, in a later study in Colombia, permethrin-treated uniforms provided better protection against malaria than untreated uniforms over a 4-week period (Sota et al. 1995).

Bifenthrin is a non-alpha cyano pyrethroid used against a range of agricultural pests and, recently, as an insecticide treatment for mosquito bednets (Hougard et al. 2002). The chemical has a relatively low irritant and knockdown effect compared with permethrin and deltamethrin. Bifenthrin caused a higher mortality by allowing mosquitoes to rest on treated surfaces for longer periods (WHO 2001).

The aim of this laboratory study was to compare the knockdown and mortality of mosquitoes exposed to shirt fabric impregnated with 3 formulations of permethrin and 2 formulations of bifenthrin. Because pyrethroids are lost from clothing primarily as a result of laundering (Schreck et al. 1982), the persistence of the formulations in treated fabric after washing in cold water was also compared.

The following insecticide formulations were evaluated during this study: 1) Dragnet® 100 ME, containing 100 g/liter permethrin (25:75 cis:trans) as a micro emulsion, produced by FMC, Australia; 2) Dragnet® 500 EC, containing 500 g/liter permethrin (25:75 cis:trans) as an emulsifiable concentrate, produced by FMC, Australia; 3) Peregin® 500 EC, containing 500 g/liter permethrin (25:75

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Table 1. Knockdown (±SE) after 60 min and mortality (±SE) after 24 h of *Anopheles farauti* after a 3-min exposure to cloth treated with insecticides.[1]

	Number of cold water washes							
	0		1		2		3	
Treatment	KD (%)	Mort (%)	KD (%)	Mort (%)	KD (%)	Mort (%)	KD (%)	Mort (%)
No treatment	0	2.0 ± 2.0	0	2.0 ± 2.0	0	4.0 ± 2.5	4.4 ± 2.7	6.6 ± 4.4
Dragnet 100 ME	100	100	81.2 ± 7.8	46.6 ± 14.6	8.0 ± 2.0	8.0 ± 3.8	18.0 ± 5.0	38.0 ± 8.0
Dragnet 500 EC	100	100	88.4 ± 5.0	70.4 ± 4.9	28.0 ± 3.2	20.2 ± 7.0	12.0 ± 5.4	22.0 ± 7.2
Peregin 500 EC	100	94.2 ± 2.5	91.8 ± 3.8	63.8 ± 6.4	31.0 ± 5.1	27.2 ± 5.5	18.8 ± 6.1	23.2 ± 6.3
Talstar 80 SC	100	100	44.0 ± 8.1	97.8 ± 2.2	5.8 ± 2.4	88.6 ± 5.4	1.8 ± 1.8	70.6 ± 6.1
Biflex 10 ME	100	100	39.6 ± 4.3	95.8 ± 2.6	0	55.8 ± 9.9	18.2 ± 3.2	59.8 ± 7.0

[1] KD, knockdown; Mort, mortality.

cis : trans) as an emulsifiable concentrate, produced by Aventis, Australia; 4)Talstar® 80 SC, containing 80 g/liter bifenthrin as a suspension concentrate, produced by FMC, Australia; and 5)Biflex® 10 ME, containing 10 g/liter bifenthrin as a micro emulsion, produced by FMC, Australia.

Australian Defence Force Disruptive Pattern Camouflage Uniform shirt fabric, made of 50% cotton/50% polyester, was used. A total of 60 swatches from this material measuring 15 cm × 12 cm were prepared. The test insecticides were mixed with water at the following rates: 60 ml formulation/liter water for Dragnet 100 ME, 12 ml/liter for Dragnet 500 EC and Peregin, 2.5 ml/liter for Biflex 10 ME, and 6.25 ml/liter for Talstar 80 SC. The emulsion was mixed in a small hand-held sprayer and applied at a rate of 100ml/m^2. The swatches were weighed before and after treatment to estimate the application rate of insecticide. Swatches were allowed to air dry in the laboratory before being wrapped in aluminum foil. The 5 test chemicals were applied to 10 swatches each, with an additional 10 swatches left untreated. Five swatches of each treatment and controls were used for exposure to mosquitoes, and five of each treatment were used for chemical analysis.

Mosquitoes used in the study were from colonies maintained in our laboratory in Brisbane. They were *Anopheles farauti* Laveran, 6–7-day-old nulliparous females from a colony established from Rabaul, Papua New Guinea, in 1972, and 2–6-day-old nulliparous female *Aedes aegypti* (L.) from a colony originally established from the Queensland Institute of Medical Research, in 1981.

A contact bioassay and a biting bioassay were conducted on the test fabrics. The contact bioassay used World Health Organization (WHO) susceptibility test kits, which allowed mosquitoes to be exposed to fabrics for short times. Five replicate groups of *An. farauti* females, each of 10 mosquitoes, were exposed for 3 min after initial treatment and then after each cold water wash. Test swatches were rolled and fixed into the test kit treatment cylinder with 2 metal clips. After exposure to the test fabrics, the mosquitoes were transferred to the holding cylinder of the WHO kit. The cylinders were placed into a polystyrene container and covered with moist cotton wool. The knockdown of mosquitoes was scored 60 min after exposure, and mortality was scored at 24 h. A mosquito was scored as knocked down if it was lying on its back or side and was unable to maintain flight after a gentle tap on the cylinder.

The biting bioassay involved placing 7–10 *Ae. aegypti* adults between the lid of a petri dish (8.5 cm diameter, 1.3 cm high) and a card. Three replicate groups of mosquitoes were tested for each treatment after initial treatment and after each cold water wash. For each biting test, the test fabric was held tightly over the stomach of a human volunteer, and then the card was removed to expose the mos-

Table 2. Biting and mortality (±SE) of *Aedes aegypti* after exposure to cloth treated with insecticides for 3 min.[1]

Treatment	Number of cold water washes							
	0		1		2		3	
	Fed (%)	Mort (%)	Fed (%)	Mort (%)	Fed (%)	Mort (%)	Fed (%)	Mort (%)
No treatment	32.1 ± 6.6	6.7 ± 6.7	14.6 ± 5.5	5.0 ± 5.0	20.8 ± 15.0	3.3 ± 3.3	3.7 ± 3.7	11.1 ± 0
Dragnet 100 ME	6.1 ± 6.1	84.8 ± 8.0	6.4 ± 3.2	9.7 ± 0.3	3.7 ± 3.7	15.3 ± 9.7	0	3.3 ± 3.3
Dragnet 500 EC	3.3 ± 3.3	97.0 ± 3.0	0	18.5 ± 5.0	0	5.6 ± 5.6	0	10.0 ± 0
Peregin 500 EC	0	93.0 ± 3.5	0	20.9 ± 1.3	0	3.3 ± 3.3	0	6.4 ± 3.2
Talstar 80 SC	3.7 ± 3.7	100	9.1 ± 9.1	39.1 ± 6.6	4.2 ± 4.2	7.4 ± 3.7	12.1 ± 12.1	6.1 ± 6.1
Biflex 10 ME	3.3 ± 3.3	96.7 ± 3.3	7.5 ± 3.9	32.7 ± 24.2	7.4 ± 3.7	20.4 ± 8.6	9.1 ± 5.3	3.0 ± 3.0
Bare skin	—	—	47.0 ± 19.7	9.1 ± 9.1	23.3 ± 14.5	3.7 ± 3.7	25.8 ± 8.7	6.4 ± 3.2

[1] Mort, mortality.

quitoes to the fabric for 3 min. The card was then returned and the mosquitoes were liberated into a 250-ml cup. Sixty minutes later, the number of bloodfed mosquitoes and knockdown were recorded, and mortality was recorded at 24 h. The biting response of a sample of test mosquitoes was also assessed by exposing them to the bare stomach of the volunteer.

At the conclusion of each round of mosquito bioassays, all swatches were washed by hand in cold water containing 4 g/liter of a commercial washing powder (Septone, Blue Lustre). Each swatch was washed gently by kneading in detergent water for a total of 4 min, then rinsed twice with fresh water for 1 min per rinse.

A subsample of 3 replicate swatches (5 × 6 cm) of each treatment was obtained after initial treatment and after each cold water wash for chemical analysis of permethrin and bifenthrin. Because only 4 rounds of chemical tests were completed, some swatch material remained. The active ingredient was extracted from the swatches with 100% acetone in an ultrasonic bath for 10 min. The analysis was conducted by high-performance liquid chromatography fitted with a photodiode array detector with 85% acetonitrile as the mobile phase.

The knockdown and mortality of *An. farauti* after 3 min of exposure to cloth treated with 5 insecticides are shown in Table 1. Because of the variability, valid statistical analysis was not possible. The mortality of mosquitoes exposed to permethrin-treated swatches was 94.2–100% after initial treatment, and this fell to <28% after 2 cold water washes. Knockdown was initially 100% and fell to <25% after 3 washes. By contrast, the mortality of mosquitoes exposed to bifenthrin was >55% even after 3 cold water washes.

The biting of *Ae. aegypti* through cloth fabric is shown in Table 2. The number of mosquitoes feeding through untreated and treated fabric varied, but a small percentage of mosquitoes fed through treated fabrics after initial treatment.

The persistence of active ingredient in treated fabrics is shown in Table 3. In the current experiments, between 62% and 67% of permethrin was lost from swatches after a single cold water wash, and 79–82% was lost after 3 washes (Table 3). The effect of washing on bifenthrin-treated cloth was similar to permethrin and is also shown in Table 3. With Talstar 80 SC–treated cloth, 63.2% of the active ingredient was lost after the first wash and 85% after 3 washes. For Biflex 10 ME, 58.4% of active ingredient was lost after 1 wash and 82% after 3 washes. Despite the relatively low amount of active ingredient remaining in bifenthrin-treated swatches, there was relatively high mortality in *An. farauti* after exposure to the cloth for 3 min (Table 1).

This study has shown that fabric impregnated with both permethrin and bifenthrin was toxic to *An. farauti* and *Ae. aegypti* after initial treatment. However, 61–66% of permethrin and 58–63% of

Table 3. Mean concentration (±SE) of permethrin and bifenthrin in shirt fabric after initial treatment and after cold water washing.

Number of washes	Permethrin content (mg/m²) Dragnet 100 ME	Dragnet 500 EC	Peregin 500 EC	Bifenthrin content (mg/m²) Talstar 80 SC	Biflex 10 ME
0	633.0 ± 32.6	617.0 ± 22.0	644.0 ± 10.1	38.1 ± 0.9	58.2 ± 1.2
1	214.3 ± 3.2 (66.1)[1]	236.3 ± 4.3 (61.7)	228.0 ± 4.0 (64.6)	14.0 ± 0.2 (63.2)	24.2 ± 1.5 (58.4)
2	198.7 ± 11.1 (68.6)	221.7 ± 13.2 (64.1)	192.3 ± 1.7 (70.1)	6.8 ± 0.5 (82.2)	17.4 ± 0.5 (70.1)
3	117.0 ± 11.0 (81.5)	132.7 ± 9.1 (78.5)	124.0 ± 2.1 (80.7)	5.7 ± 0.4 (85.0)	10.5 ± 1.9 (82.0)

[1] Numbers in parentheses show the percentage of active ingredient lost compared with the initial treatment.

bifenthrin were lost after the first cold water wash. After 3 washes, knockdown and mortality of *An. farauti* were low (<38%). Despite the loss of 82–85% of the active ingredient from fabric, the overall mortality of *An. farauti* exposed to bifenthrin-treated fabric was greater than 55%, even after 3 cold water washes. The biting of *Ae. aegypti* through treated fabric varied, but 0–6.1% of mosquitoes obtained blood through fabrics after initial treatment.

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