REPELLENTS AND OTHER PERSONAL PROTECTION STRATEGIES AGAINST *AEDES ALBOPICTUS*[1]

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ABSTRACT. Five chemical repellents, a controlled-release repellent formulation, Avon Skin-So-Soft® bath oil and permethrin-impregnated clothing fabric were assayed for personal protection against bites of *Aedes albopictus*. On skin the chemical repellents provided significant ($P = 0.05$) protection from biting; however, *Ae. albopictus* was more sensitive to the repellents than the standard, *Ae. aegypti*. Two experimental repellents provided 6–7 h protection from bites, 25% deet in ethanol provided > 8 h protection, a controlled-release formulation containing 35% deet provided > 10 h protection, and the Avon product provided 0.64 h protection from bites. Permethrin-treated fabric provided complete protection from mosquito bites through 0–5 washings. Repellent products containing ≥ 12% deet should provide satisfactory protection against *Ae. albopictus*; the use of permethrin-impregnated clothing should provide additional protection.

INTRODUCTION

In 1985, *Aedes albopictus* (Skuse), a vector of dengue and several other pathogenic arboviruses (Shroyer 1986), was found in large numbers in Harris County, Texas (Sprenger and Wuithiranyagool 1986). It occurs in Florida, and according to the Centers for Disease Control (CDC 1987), *Ae. albopictus* has been found in Delaware, Illinois, Indiana, Maryland, Missouri and Ohio, and now it is believed to be present in most states east of the Mississippi (Hawley 1988, Moore et al. 1988). Continued dispersal of this mosquito into heavily populated areas of the northeastern United States is projected (Nawrocki and Hawley 1987).

Preliminary tests by the Centers for Disease Control indicate *Ae. albopictus* is tolerant to several insecticides. Khoo et al. (1988) reported malathion resistance and a tolerance for bendiocarb in populations of *Ae. albopictus* in Harris County, Texas. On this basis, chemical management of this mosquito may prove to be very difficult.

To date, the results of personal protection research with *Ae. albopictus* have been largely inconclusive. Traub and Elisberg (1962a, 1962b) reported deet (formerly *N,N*-diethyl-*meta*-toluamide, the new American Chemical Society nomenclature is *N,N*-diethyl-3-methylbenzamide) to be an effective repellent against a number of mosquito species in Malaya, but *Ae. albopictus* comprised < 1% of the field population during their 1-h test. In Pakistan, Sholdt et al. (1988) reported repellency of *Ae. albopictus* for ≤ 10 h with a 35% deet formulation; however, biting rates on untreated skin in this study were also low. Consequently we do not know whether, under severe challenge, currently used repellents and other personal protection tactics are effective against *Ae. albopictus*.

Reported here are the results of tests evaluating: 1) Five repellents including deet (test standard) against *Ae. albopictus* and *Ae. aegypti* (Linn.), 2) a controlled release formulation containing deet, 3) two dosages of deet in ethanol and 4) Avon Skin-So-Soft®. The latter is a bath oil sometimes used as a repellent (Schreck and Kline 1981) for biting midges and mosquitoes. Also reported here are the biting behavior and knockdown responses of *Ae. albopictus* after contact with unwashed and washed permethrin-treated clothing fabrics.

MATERIALS AND METHODS

The *Ae. albopictus* used were the progeny of wild larvae collected in Jacksonville, FL, in December 1987. After emergence as adult mosquitoes, they were placed in screened cages (97 × 81 × 69 cm) at 27°C and 70% RH. Bloodmeals were taken from the arms of human volunteers; a solution of 10% sugar (by volume) and water was available in cages at all times. Eggs were accumulated in 473-ml (16-oz) plastic cups lined with filter paper and half-filled with water. After 2–3 days the water was decanted, the filter paper (with eggs) air dried and the filter paper stored at 70% RH. Before use, eggs were removed from the filter paper with a stiff brush, poured into small plastic vials to which water was added and the vials capped and shaken vigorously. Once the eggs hatched the contents of each vial were emptied into a rearing tray (45 × 56 × 7.6-cm deep) containing 3 liters of well water (27°C) to which was added a 50-ml slurry containing 3

[1] Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the United States Department of Agriculture.

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parts liver powder and 2 parts brewers yeast. After 2 days, an additional 75 ml of the slurry was added; pupae were normally harvested on the fifth day. Pupae were placed in water-filled cups in separate stock cages (37 × 38 × 46 cm) for emergence; a solution of 10% sugar (by volume) and water was provided for the emerging adults. Female mosquitoes were removed in lots of ca. 500 at 4 days postemergence and placed in clean cages for testing (Posey and Schreck 1981).

Aedes aegypti colonized originally from wild stock collected at Orlando, FL, in 1939 were used as a standard test species for comparison. The same rearing procedure was used as for *Ae. albopictus* except that mosquitoes were offered bovine blood through a membrane.

Repellency tests: The test procedure used to determine duration of repellency was described by Schreck (1985). The efficacy of 25% ethanol solutions of the following 4 repellents and deet (standard) were compared using both *Aedes* sp.: dimethyl phthalate (DMP), 1,3-ethylhexanediol (EHD), 1-(3-cyclohexen-1-ylcarbonyl)-2-methylpiperidine (CYM) and 1-(3-cyclohexen-1-ylcarbonyl)-piperidine (CYP). Avon Skin-So-Soft, 12.5% and 25% deet in ethanol, and a controlled-release repellent formulation containing 35% deet developed for military use by the Minnesota Mining and Manufacturing Company (3-M), St. Paul, MN (and recently adopted as the U. S. Armed Services standard topical repellent), were evaluated separately in unpaired tests for repellency to *Ae. albopictus*.

The repellency tests were conducted by spreading 1 ml of test chemical (25% ethanol solution of technical grade) evenly on one forearm of a volunteer and the 25% deet standard on the other forearm. Each treated arm was exposed to ca. five-hundred 5- to 6-day-old female *Ae. albopictus* or *Ae. aegypti* for 3 min at approximately 30-min intervals until repellency was lost. Effectiveness of repellency was based on complete protection time, i.e., the time between treatment and the first confirmed bite (one bite followed by another bite within 30 min). Student's *t*-test was used to analyze the paired test data. In separate tests, 1 ml of Avon Skin-So-Soft, the 2 deet/ethanol formulations and the controlled release formulation were each applied to a forearm and tested in the manner cited above against *Ae. albopictus*. Tests were repeated (4–16 times depending on formulation) on the arms of 3 volunteers on different days and using different mosquito populations. The duration of protection was calculated as an average of the responses on each test date for each material.

Permethrin-treated fabric tests: One hundred percent cotton fabric was impregnated by the method described by Schreck et al. (1984). Permethrin from Permanone® (40% emulsifiable concentrate (EC), supplied by Fairfield American Corp., Frenchtown, NJ) was applied as an aqueous suspension at the rate of 0.125 mg permethrin (AI)/cm^2 of cloth. Fabric swatches measuring 30.6 (base) × 23.2 (sides) × 23 (end) cm were washed 0, 1 and 5 times in a commercial washing machine using warm water and detergent. Duplicate untreated fabric swatches were subjected to the same washing procedure. For bioassay purposes, a swatch was draped over the top of the forearm, pulled tight from below to form a sleeve and stapled closed for the length of the forearm. Masking tape was used to seal the junction of the sleeve and a glove worn to cover the hand and wrist.

The test comprised 2 parts. In the first part, a volunteer introduced the left arm which was covered with an untreated swatch into a test cage containing one hundred 5- to 6-day-old female *Ae. albopictus* for a 15-min test period. Knockdown counts were recorded at the end of the 15-min test and again after 45 min (none of these mosquitoes was observed to recover during this time). All mosquitoes were then aspirated from the cage, anesthetized with carbon dioxide and crushed on paper to determine the number of individuals that obtained a bloodmeal. In the second part of the test, a treated swatch was placed on the right arm of a volunteer and introduced into a 2nd cage with mosquitoes. Each test was repeated 3 times—each time using new mosquitoes. The number of bites through untreated and treated swatches in each trial was recorded and mean percent protection from bites calculated after correction for the proportion of the mosquito population that did not bite through the untreated fabrics (Abbott 1925).

RESULTS

Results of tests of the 4 technical grade repellents in ethanol compared with a deet standard are summarized in Table 1. Mean protection provided by DMP and EHD was 3.2 h and 4.3 h, respectively, against *Ae. albopictus*; against *Ae. aegypti* these values were 0.7 h and 1.4 h, respectively. The protection periods provided by the deet standard were 7.9 h and 8.7 h with *Ae. albopictus*, and 6.9 h and 5.0 h with *Ae. aegypti*. One - (3 - cyclohexen - 1 - ylcarbonyl) - piperidine and CYM provided protection of 6.4 h and 7.1 h, respectively, against *Ae. albopictus*, and 3.9 h and 5.7 h against *Ae. aegypti*. For CYM, these protection periods were not significantly different from the deet standard for *Ae. albopictus*

Table 1. Duration of protection from bites of *Aedes albopictus* and *Ae. aegypti* on human skin treated with 4 mosquito repellents (25% technical repellent in ethanol) in direct comparison tests with a 25% deet standard.

Chemical	Mean duration of complete protection from bites (h)*			
	Aedes albopictus		*Aedes aegypti*	
	Candidate repellent	Deet std	Candidate repellent	Deet std
Dimethyl phthalate (DMP)	3.2	7.9	0.7	6.9
1,3-Ethyl hexanediol (EHD)	4.3	8.7	1.4	5.0
1-(3-Cyclohexen-1-ylcarbonyl)-2-methylpiperidine (CYM)	7.1a	8.2a	5.7c	7.8c
1-(3-Cyclohexen-1-ylcarbonyl)-piperidine (CYP)	6.4b	8.3b	3.9	7.3

* Mean of 6 tests. Means in the same row for each mosquito species followed by the same letter are not significantly different (Student's *t*-test $P = 0.05$); all others are significantly different.

Table 2. Duration of protection from bites of *Aedes albopictus* on human skin in indirect comparisons of 2 concentrations of deet in ethanol, an experimental repellent formulation, and Avon Skin-So-Soft®.

Formulation or common name	Concentration of deet (%)	No. tests	Mean duration of complete protection from bites (h)
Controlled-release repellent formulation	35.0	4	>10.0**
Deet	25.0	16	8.2
Deet	12.5	6	6.3
Avon Skin-So-Soft*	0	8	0.6

* Product containing mineral oil, isopropyl palmitate, diisopropyl adipate, fragrance, dioctyl sodium sulfosuccinate and benzophenone-11; tested at full strength.
** Tests terminated at 10 h.

and *Ae. aegypti*. However, *Ae. aegypti* was less sensitive to CYP repellent than deet.

Results of tests of 2 concentrations of deet in ethanol, the controlled-release repellent formulation and Avon Skin-So-Soft are summarized in Table 2. Deet at a concentration of 12.5% provided 6.3 h protection against *Ae. albopictus* compared to 8.2 h at 25%. The controlled-release formulation (35% deet) provided > 10 h protection from bites. Avon Skin-So-Soft, with a mean duration of complete protection from bites of 0.64 h, was ca. 10 times less effective than the lowest concentration of deet (12.5%). Complete protection against bites of *Ae. albopictus* was provided by clothing fabric treated with permethrin even after 5 launderings (see Fig. 1). Knockdown of *Ae. albopictus* after 1 h ranged from 60% at 0 washes to 26% after 5 washes.

DISCUSSION

All the repellents tested provided long-lasting protection against bites of *Ae. albopictus*. Furthermore, *Ae. albopictus* was more sensitive to the repellents than was *Ae. aegypti*. Dimethyl phthalate and EHD are often combined with deet in commercially prepared repellent formulations. However, CYM and CYP are experimental chemicals, and although each appears about as effective as deet against *Ae. albopictus*, neither is available for general use.

Concentrations of deet as low as 12.5% provide > 6 h of protection; 35% concentrations of deet in a controlled-release formulation should provide essentially all-day protection from *Ae. albopictus* and *Ae. aegypti*.

Avon Skin-So-Soft bath oil gave very short duration of protection against *Ae. albopictus*, and although different test methods were used, these results are similar to those reported by Rutledge et al. (1982) in tests with *Ae. aegypti*.

Protection against *Ae. albopictus* from permethrin-treated clothing was very good, and on the basis of the fabric swatch tests, results were comparable to that reported by Sholdt et al. (1988) and Schreck et al. (1984) for this and other mosquito species.

In summary, commercially available repellents containing ≥ 12% deet should furnish satisfactory protection against the bites of *Ae. albopictus*. Permethrin could provide an effective additional personal protection strategy if used in combination with a repellent formulation containing deet.

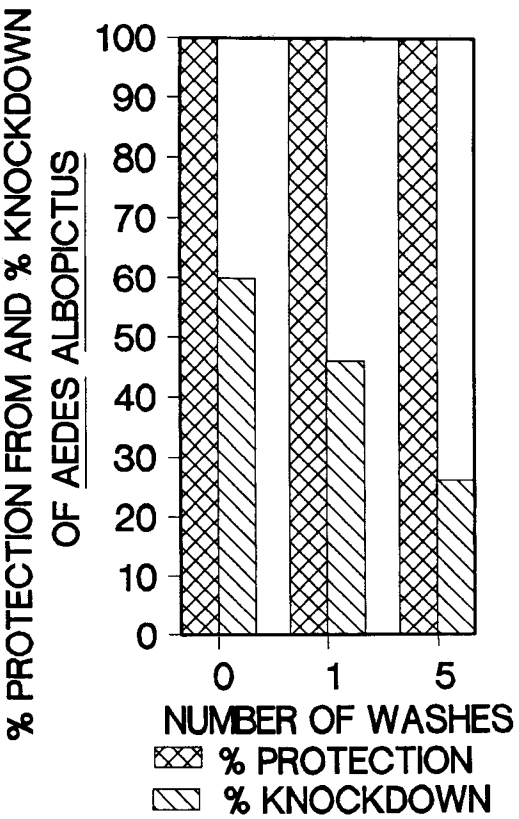

Fig. 1. Comparisons of the effects of washing on permethrin-impregnated clothing fabric (100% cotton) in terms of protection from bites through the cloth and knockdown effect on *Aedes albopictus* after contact with the treatment.

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