EFFICACY ASSESSMENT OF QUWENLING, A MOSQUITO REPELLENT FROM CHINA[1]

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ABSTRACT. Quwenling, an insect repellent product of China derived from extracts of the lemon eucalyptus plant (*Eucalyptus maculata citriodon*), was evaluated. Laboratory tests compared Quwenling with deet against *Anopheles albimanus, An. quadrimaculatus, Aedes aegypti, Ae. albopictus* and field tests with *Ae. taeniorhynchus.* Cloth treated with Quwenling at >2× the dosage of deet was effective against 2 of 4 species tested (*Ae. albopictus* 29 days, *An. quadrimaculatus* 28 days). On the skin of volunteers at 2× the dosage of deet, the duration of protection for Quwenling was significantly less than deet with *Ae. aegypti* and *Ae. taeniorhynchus*, and was not significantly different with *Ae. albopictus.* Both repellents were ineffective against the anopheline species. As a topically applied mosquito repellent, Quwenling has a shorter duration of effectiveness than deet.

INTRODUCTION

Of 65 formulations of non-US produced repellent products identified in a recent worldwide survey by the US Armed Forces, 33 contained deet (*N,N*-diethyl-3-methylbenzamide, formerly *N,N*-diethyl-*meta*-toluamide). The remainder contained natural oils such as citronella, or the contents were not revealed.

One of the non-deet formulations, Quwenling, was said to be an effective mosquito repellent commonly used in China (Curtis et al. 1990). Previously, dimethyl phthalate was thought to be the repellent of choice in China, so it was with considerable interest that we evaluated Quwenling as a repellent.

Samples were provided by B. A. Schiefer, US Army Medical Materiel Development Activity, Ft. Detrick, MD 21701. The formulation was a clear pale blue liquid with a definite aromatic odor packaged in 50-ml plastic squeeze bottles. Raised print (in Chinese) on both sides of the bottle was translated as follows: "prevents biting from mosquitoes and 4 other insects including ticks, external use only," registration no. 10, 1985. Printed matter from the manufacturer, Nanyue Pharmaceutical Factory, 394 Tongfong Road, Hengyang, Hunan Province, stated that laboratory tests with mosquitoes "prove that it is more effective but less toxic than DETA" (deet).

MATERIALS AND METHODS

According to Curtis et al. (1990), Quwenling is reportedly made from the waste distillate after extraction of lemon eucalyptus oil from the plant, *Eucalyptus maculata citriodon.* The major ingredient in the waste distillate was reported to be "*p*-menthene-diol-3,8," but no other details on ingredients were provided (Curtis et al. 1990). Personal communication with C. F. Curtis and Lu Baolin, coauthor-editor and coauthor, respectively, of the publication cited above, revealed that the product contains 30% active ingredients (AI) in a non-specified alcohol solution. The sample in our possession was analyzed chemically at the USDA/ARS Insect Chemical Ecology Laboratory, Beltsville, MD. The analysis indicated *p*-menthane-3,8-diol (correct name for *p*-menthene-diol-3,8) was the major component along with lesser amounts of other terpene-type alcohols. The most abundant minor component was dioctyl phthalate and traces of an ethyl ester of hexanoic or heptanoic acid. P-menthane-3,8-diol and/or similar compounds were tested prior to 1955 by the USDA/ARS Medical and Veterinary Entomology Research Laboratory, Gainesville, FL. At that time they were considered to possess a low order of effectiveness and did not warrant further study. Dioctyl phthalate, for example, provided protection from bites of *Aedes aegypti* (Linn.) for only 23 min (avg. 4 tests).

Initially, our sample of Quwenling was evaluated with laboratory-reared mosquitoes in a routine screening test, comparing it with a standard as described by McGovern et al. (1978). Eight grams containing 30% AI (mostly *p*-methane-3,8-diol) of the formulation was applied to a 300-cm^2 area of a cotton stocking; thus, the dosage

[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. Human subjects participating in this study gave free and informed voluntary consent.

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rate was 8 mg AI/cm^2. After 2 h, the treated stocking was placed over an untreated nylon stocking on the arm of a volunteer and exposed for 1 min sequentially in 4 cages (35 × 35 × 45 cm) containing laboratory reared *Ae. aegypti, Ae. albopictus* (Skuse), *Anopheles quadrimaculatus* Say or *An. albimanus* Wied. Mosquitoes in each cage were of mixed sexes of which ca. 1,000 were 5- to 8-day-old females never having fed on blood. Test exposures were repeated at 24 h and then at weekly intervals until 5 bites were received in the 1-min test period. Numbers of days to the first bite and to 5 bites in 1 min were recorded. The standard repellent, deet, was tested concurrently at the usual dosage of 3.3 mg AI/cm^2. Deet is effective on cloth against *Ae. aegypti* and *An. quadrimaculatus*; however, it was ineffective against *An. albimanus* in this test. When the mosquitoes are challenged with an arm in an untreated stocking, biting rates are usually >60 bites/min. At no time during the tests was lack of biting pressure observed.

A second series of tests was performed on the skin of volunteers as described by Schreck (1985). For these evaluations, Quwenling was again used full strength (30% AI) from the container. The standard was 15% deet in ethanol solution. One milliliter of a repellent was spread evenly over the forearm of a volunteer, wrist to elbow. After 15 min, with the hand protected by a glove, the arm was exposed sequentially in 4 cages, each containing 200 blood-hungry females, 5–7 days old, of the species used in the screening test. Exposure time was 3 min at approximately 30-min intervals thereafter until the repellents (paired on each arm of each of 4 individuals) failed to protect against biting. Effectiveness was based on duration of complete protection, that is, the time between treatment and the first confirmed bite (a bite followed by another within 30 min).

A third series of tests was conducted in the field (Everglades National Park, September 1990) with *Ae. taeniorhynchus*. Repellents were applied to skin and compared as in the second series of tests described above, but the deet standard was 25% in ethanol solution, and treated arms were exposed continuously to natural populations of mosquitoes until the first confirmed bite was received. Three volunteers participated in 5 paired tests over 3 days during which mean temperatures ranged from 22.4 to 30.9°C and mean RH from 62 to 100%. Bite counts (47) on untreated skin of 3 volunteers, taken at various intervals (morning, noon and afternoon) during this time, averaged 57 bites/min (range, 0 to >200 bites/min).

The paired data from the second and third series were analyzed by Student's *t*-test to determine if there were significant differences (5% level) between the deet standard and Quwenling.

RESULTS

Presented in Table 1 are the results of the initial screening tests on cloth. Quwenling at >2× the dose of the deet standard was effective only against *Ae. albopictus* (29 days) and *An. quadrimaculatus* (28 days). Deet was effective against *Ae. albopictus, Ae. aegypti* and *An. quadrimaculatus* for 21 days, but was ineffective as was Quwenling against *An. albimanus*. Repellents that provide 11 days or more of protection in cloth tests against a standard series of species are considered promising. Since the cloth test does not necessarily assure that a material will be effective when applied to human skin, further tests on skin are necessary (Table 2).

Both Quwenling and deet were ineffective in repelling the anopheline species. These results are not new for deet. The insensitivity of *An. albimanus* to deet has been amply documented (Rutledge et al. 1978, 1983; Schreck 1977, 1985).

Table 1. Repellency of Quwenling when applied to cotton cloth at 8 mg AI/cm^2 and compared with a deet standard at 3.3 mg/cm^2 in tests against 4 species of laboratory reared mosquitoes.

Species	No. days to the 5th bite	
	Quwenling	Deet
Aedes aegypti	1	21
Ae. albopictus	29	21
Anopheles quadrimaculatus	28	21
An. albimanus	0	0

Table 2. Mean duration of protection from bites of mosquitoes during paired tests with Quwenling (ca. 0.5 mg AI/cm^2) and a deet standard (ca. 0.25 mg AI/cm^2, except where noted) each applied as 1-ml aliquots to the arms of 3–4 volunteers.

Species	No. tests	Mean no. hours protection from bites	
		Quwenling	Deet
Aedes aegypti	8	1.1 ± 1.4 a	4.8 ± 1.9 b
Ae. albopictus	8	5.6 ± 1.6 a	6.6 ± 1.9 a
Ae. taeniorhynchus	5	0.3 ± 0.1 a	1.3 ± 0.4 b*
Anopheles albimanus	6	<15 min a	<15 min a
An. quadrimaculatus	6	<15 min a	<15 min a

* Deet tested at 25% in ethanol.

Paired test data with each species analyzed using Student's *t*-test; same letters in rows indicate no significant difference at 5% level of confidence.

There is also evidence that when applied to skin, deet does not adequately protect from bites of *An. quadrimaculatus* (Schreck 1977). However, as was seen in the initial test (Table 1), deet-treated cloth proved quite effective against this species.

Mean duration of protection from bites of *Ae. aegypti* for Quwenling was 1.1 h, and for deet it was 4.8 h. Although there was variation in times of protection between individuals and within individuals over days, at the 15% concentration deet was significantly more effective (5% level) than the Quwenling product (30% AI in alcohol) against this species.

With *Ae. albopictus* , we found no significant difference (5% level) between Quwenling and deet. Duration of protection was 5.6 h and 6.6 h for each repellent, respectively. These results agree closely with earlier data we reported (Schreck and McGovern 1989) in that the mean duration of protection of deet at 12.5% in ethanol against *Ae. albopictus* was 6.3 h (6 tests).

With *Ae. taeniorhynchus*, duration of protection by Quwenling (0.3 h) was significantly less (5% level) than that of deet (1.3 h). For both repellents, the periods of protection during the field trials were many-fold less than those of the laboratory study. This may be attributable to warm, moist weather conditions plus the high densities and aggressiveness of mosquitoes encountered during the field tests (mean biting rate on untreated skin of 3 people, 3 days, 47 observations = 57 bites/min).

DISCUSSION

The discussion on the repellency of Quwenling presented by Curtis et al. (1990) cites Li et al. (1974) as the source of data. Both laboratory and field evaluations were made. In one laboratory test, Quwenling and deet were compared at 1.5 mg/cm^2 of a 50% formulation of each repellent on the skin of mice against *Ae. aegypti* and *Ae. albopictus*. This is equivalent to 0.75 mg AI/cm^2. Quwenling was reported to last 8.2 and 12.5 h and deet 5.2 and 8.3 h in duration of protection against the respective *Aedes* species. In a second test [Curtis et al. (1990) page 80, Table 3], at 5 different dosages on humans (indicated "mice" in the text, but corrected by Lu Baolin in personal communication), protection time of Quwenling against *Ae. aegypti* was proportional to the amounts applied. However, at a dose on human volunteers 1.7× *less* (0.44 mg AI/cm^2) than that used on mice (0.75 mg AI/cm^2), Quwenling protected >1.5× *longer* (13 h) against *Ae. aegypti*. Unfortunately, in these tests, no comparisons were made with a deet standard, which perhaps might have provided added data to help clarify these unexpected results.

With the paired tests we conducted on human skin against *Ae. aegypti* and *Ae. albopictus*, somewhat less than the 0.75 mg AI/cm^2 dosages of the repellents Li et al. (1974) used on mice were applied. The amounts we tested were 1/3 (0.25 mg AI/cm^2) that of the deet and 2/3 (0.5 mg AI/cm^2) that of the Quwenling they evaluated on mice. However, this amount of Quwenling was about equal to 0.44 mg AI/cm^2, the amount used in their tests with humans and reported on page 80, Table 3, cited above. With *Ae. aegypti*, we found the average duration of protection on humans for deet (4.8 h) was >4.4× that of Quwenling (1.1 h), the latter only 8.5% of the protection time (13 h) they had reported.

The same table (Table 3 referred to above) did not provide data for human tests with *Ae. albopictus*, whereas Table 4 (same page) gave results but could not be compared because dosage rate/unit area was not given.

These significant contrasts between the published data and our data could indicate: 1) the mice and humans used in these studies are not comparable as test subjects, 2) the strains of *Ae. aegypti* and *Ae. albopictus* used in these independent experiments are biologically not comparable in sensitivity to the repellents, and 3) the repellency assessment procedures are not comparable.

The low order of effectiveness of Quwenling against *Ae. aegypti* and *Ae. taeniorhynchus* indicates that its duration of activity on human skin compares poorly to that of deet against these species. Yet, we found both repellents provided effective long-lasting protection against *Ae. albopictus* when they were tested on cloth (21+ days) and when they were tested on human skin (about 6 h).

Had deet been tested in the present study at a dosage (25%) approximating that of Quwenling (30%), it would be expected to have lasted >8 h (Schreck and McGovern [1989] reported deet at 25% in ethanol with a mean protection time of 8.3 h in 24 tests). Thus, the duration of protection for deet, if compared in equal proportions on skin to Quwenling against *Ae. albopictus*, would be substantially longer. Based on these findings, 1) Quwenling is not a better mosquito repellent than deet, and 2) although an aggressive biter of unprotected skin, *Ae. albopictus* is likely a poor choice, particularly as a single source of test data, for selecting and recommending general purpose repellents.

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