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LABORATORY EVALUATION OF A NEW REPELLENT CAMOUFLAGE FACE PAINT[1,2]

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ABSTRACT. A combined formulation of the U.S. Army's camouflage face paints and the new extended duration topical insect/arthropod repellent was tested on human volunteers to determine repellency and duration of protection (2–12 h) against *Aedes aegypti* and *Anopheles stephensi*. Under laboratory conditions (27°C and 80% RH), the face paint/repellent formulation provided ≥95% repellency or better for up to 6 h for *Ae. aegypti* and 8 h for *An. stephensi*. An analysis of variance showed no significant differences in the repellent efficacy of the 4 camouflage colors: white, green, loam, and sand.

INTRODUCTION

The use of topical repellents is an economical and practical means of preventing the transmission of arthropod-borne diseases to the individual soldier in the field. In continuing efforts to reduce the morbidity and mortality of military personnel caused by arthropod-borne diseases, the Walter Reed Army Institute of Research is studying various means of incorporating repellents into topical lotions and creams to enhance effectiveness, persistence, and user acceptance.

The utilization of sustained-release technology for topical insect repellents has provided extended protection against biting arthropods (Gupta and Rutledge 1989). The new extended duration topical insect/arthropod repellent formulation (EDTIAR) is a multipolymer sustained-release formulation of *N,N*-diethyl-3-methylbenzamide (deet), and has numerous advantages over presently available repellents including: lower deet concentration, an extended protection time, greater user acceptance, and reduced plasticizing effects.

The insect repellent (U.S. National Stock Number 6840-00-753-4963) cannot be used with currently issued camouflage face paints due to the solvent properties of the repellent, which cause the paint to run. However, preliminary tests have indicated that when camouflage face paint and EDTIAR are applied separately they are compatible, but EDTIAR must be applied first. By incorporating polymer sustained-release technology, Natick Research, Development, and Engineering Center, Natick, MA, has developed a compatible combination of camouflage face paints and deet insect repellent. The new combination repellent/face paint can be applied to the skin in a one-step application. This face paint repellent formulation is part of the continuing effort to provide maximum protection to individual soldiers in the field.

This study was conducted to evaluate effectiveness of the camouflage face paint repellent formulation when applied to exposed human skin (areas of the body not protected by clothing) to repel laboratory-reared mosquitoes for extended time (2–12 h).

MATERIALS AND METHODS

The active ingredient in the repellent is 30% *N,N*-diethyl-3-methylbenzamide (deet). The components of the face paints are ceresine wax, mineral oil, talc, and color mixture. This combination formulation is in kits containing 4 face paint colors: loam (light green), green, sand (beige), and white. Each color was tested separately with and without deet in sustained-release polymer formulation.

The mosquito species used in the study were *Aedes aegypti* (Linn.) and *Anopheles stephensi* Liston. Mosquitoes were reared and maintained at 27°C, 80% RH, and a 12:12 h light : dark photoperiod. Larvae were fed a diet of floating catfish food (Continental Grain, Chicago, IL), and adults were maintained on a 10% sucrose solution. Mosquitoes used for experimentation were nulliparous females between 5 and 15 days of age. The sugar solution was removed from the experimental insects 10–12 h prior to host exposure, and a pledget saturated with water was substituted for the sugar solution.

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[1] Opinions and assertions contained herein are private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army. Use of trade names does not imply official endorsement or approach of the products mentioned.

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

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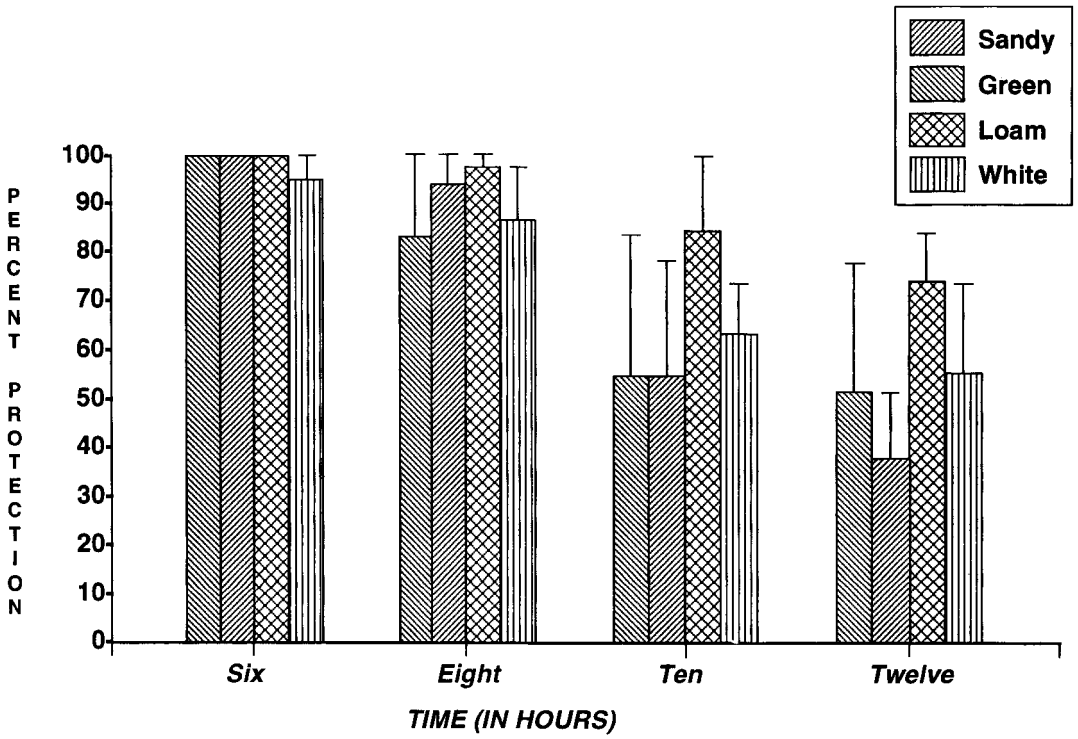

Fig. 1. Mean percent protection of face paint/repellent combination against *Aedes aegypti*. Percent protection was calculated by Abbott's formula.

The test method was a modification of the American Society for Testing and Materials Standard E951-83 (Anonymous 1983). Assignment of the test arms to the 10 treatments (4 colors of formulation with deet and 4 colors without deet, 2 controls on bare skin) for each test trial was determined by a computer-generated randomization list. The repellent (deet) was not diluted for testing and each of the 4 paint colors were applied as a pure color on the forearms by the 5 volunteers. Volunteers were instructed to apply enough of the combination formulation to adequately cover the designated areas of the forearms, but were not restricted to the amount applied. The quantity of repellent face paint formulation used by each test subject was determined by weighing the container before and after the application of each compound. Following the completion of each 12-h test, treated arms were cleansed with cold cream and mild soapy water to remove formulated face paints from the treated surfaces. The control was the 2 untreated forearms.

At the start of each test, a clear transparent plastic cage (4 × 5 × 18 cm) containing 15 mosquitoes was secured to each forearm with Velcro® strips. Then a plastic slide was withdrawn thus exposing the treated/control surfaces of the forearms to biting mosquitoes. The number of mosquitoes biting in the cage was recorded at the end of 90 sec. If all mosquitoes were observed to bite prior to the designated 90-sec time interval, the test for that forearm was stopped. New mosquitoes and cages were used for each test. Used cages were cleaned prior to reuse. The same testing procedure for each mosquito species (*Ae. aegypti* and *An. stephensi*) was repeated at 2-h intervals on the same day, with one species immediately following the other mosquito species. Thus, 7 tests of each species were conducted on each face paint formulation at 0, 2, 4, 6, 8, 10, and 12 h after application on the skin. The above testing procedure was replicated on 3 separate days. Test volunteers were allowed to resume their normal duties following each test. Volunteers were cautioned not to rub, scratch, or wash the treated areas for the duration of the 12-h test period. All tests were conducted in an environmental chamber under identical environmental and diurnal conditions (27°C, 80% RH).

Mosquito biting counts were analyzed as a 4-factor analysis of variance to check for any significant differences ($P \geq 95$) between the face paint colors (with and without deet) and protec-

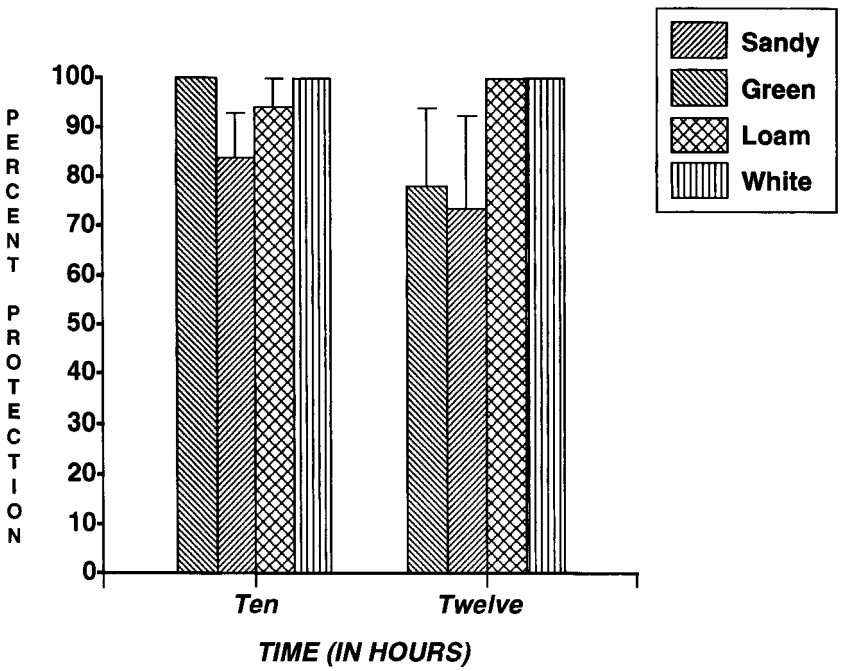

Fig. 2. Mean percent protection of face paint/repellent combination against *Anopheles stehensi*. Percent protection was calculated by Abbott's formula.

tion time at the 5% level of significance. Percent repellency was determined from the total number of bites on the control and repellent-treated test volunteers by Abbott's formula (Abbott 1925).

RESULTS AND DISCUSSION

All 4 face paint formulations (with deet) provided ≥95% or better biting protection from *Ae. aegypti* and *An. stephensi* up to 6 and 8 h, respectively (Figs. 1 and 2). However, the repellency for deet-formulated face paints fell below the acceptable 95% protection level at 8 h for *Ae. aegypti* (Fig. 1) and at 10 h for *An. stephensi* (Fig. 2). This indicates that *An. stephensi* is more sensitive to the face paint/repellent combination than *Ae. aegypti* and thus is repelled for a longer time. However, the results are somewhat in contrast with the findings of Gupta and Rutledge (1993) who reported a 97–100% repellency for *Ae. aegypti* and *An. stephensi* for up to 12 h after EDTIAR and face paints were applied separately. The differences in repellency observed between the 2 studies can be partially attributed to the difference in the biting affinities of the mosquito colonies used and also to the difference in face paint formulations utilized for both studies. The face paint formulation used by Gupta and Rutledge (1993) contained various cosmetic additives and fixatives to facilitate application that were not present in the face paint formulated for this study. When face paint only was applied as a treatment, Gupta and Rutledge (1993) reported up to 70% repellency for the first hour after treatment, with lower levels of repellency existing up to 10 h following face paint application. Some of the cosmetic ingredients used in the earlier face paint and EDTIAR formulation may have contributed to the higher levels of repellency observed.

In the current study, statistical analyses showed that there were no significant differences in the percent repellency of any of the face paints (without deet) and the control. In addition, the color of face paint formulation did not have a measurable effect on mosquito repellency. An analysis of variance (ANOVA) was conducted to determine if the amount of face paint formulations applied by the test subjects was a factor in the percent of mosquito repellency. The ANOVA indicated that the quantity of formulation applied did not play a significant role in repellency.

In the present study conducted under mild climatic conditions (27°C, 80% RH), the face paint/repellent combination provided adequate protection for a minimum of 8 h against the mosquitoes tested. It has been shown that the longevity of effectiveness of controlled-release repellent formulations varies under different climatic conditions (Gupta and Rutledge 1989). It

seems apparent that a final camouflage face paint insect repellent formulation including additives, fixatives, and excipients similar to that of EDTIAR may even extend the duration of protection of the camouflage face paint/repellent combination tested in these studies. Therefore, it is highly recommended to prepare a camouflage face paint insect repellent with all the inactive ingredients and then field test for stability, effectiveness, and longevity for protection against biting insects/arthropods.

In summary, camouflage face paints can be formulated with the new controlled-release repellent formulation without reducing the effectiveness of the repellent against mosquitoes. Advantages of the combined face paint repellent formulation are: 1) camouflage face paints and repellent can be applied in a one-step application, 2) a reduction of storage space and weight compared with 2 separate products, 3) a lower concentration of deet can be used, and 4) it is more economical to produce.

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