

EFFECT OF SKIN ABRASIONS ON THE EFFICACY OF THE REPELLENT DEET AGAINST *Aedes aegypti*^{1,2}

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ABSTRACT. Abrasion of repellent-treated human skin affected the efficacy of a sustained-release insect repellent containing *N,N*-diethyl-3-methylbenzamide (deet) against bites of *Aedes aegypti*. Skin treated with repellent when abraded up to 30 times showed significantly lower protection than unabraded skin against mosquito bites for 10 h. The mean value of the kinetic coefficient of friction during skin abrasion by clothing (battle dress uniform fabric) for repellent-treated skin (0.159 ± 0.003) was significantly higher than untreated skin (0.122 ± 0.005). Repellent-treated skin appeared stickier than the untreated skin. An increase in the number of skin abrasions by clothing resulted in a reduced duration of protection against mosquito bites.

KEY WORDS Diptera, Culicidae, mosquito, vector, human protection, friction machine

INTRODUCTION

Arthropod-borne diseases such as malaria, dengue, encephalitis, and filariasis are still major health threats causing human morbidity and mortality worldwide. The use of topical repellents to prevent arthropod bites is an effective personal protective measure to reduce or prevent transmission of these diseases. Insect repellents may be as economical as vector control operations and an alternative to chemical vector control (Gupta and Rutledge 1994). Furthermore, topical repellents can give immediate protection to individuals exposed in areas where suppression of arthropod vectors is not practical or feasible.

N,N-Diethyl-3-methylbenzamide (deet) is an effective broad-spectrum repellent, and is the main ingredient in many repellents currently available for use against insects and other arthropods affecting humans (Gupta and Rutledge 1989). The duration of protection of most deet repellents may be shortened by various factors including sweating, contact with water, and abrasion (or rubbing away by friction) of repellent-treated skin by vegetation or clothing (Smith et al. 1963, Rutledge et al. 1986, Solberg et al. 1995), an especially important concern for military use. Earlier studies (Rutledge et al. 1986) have established that excessive evaporation (i.e., evaporation more than the minimum effective evaporation rate required to repel the target

species) accounts for a substantial fraction of the repellent lost from the skin. Other physical properties of repellents include lubrication and wear-resistance. Abrasion has been believed to play a significant role in repellent loss from skin but the mechanical parameters of this process are unknown. Force of friction is the degree of force that resists the sliding of one material surface over another substrate or material. The interaction of lubrication and wear-resistance properties with skin does not appear to be a linear function of the applied force. The force of friction decreases with increasing applied force within a certain range of values (Rutledge et al. 1986). Abrasion is defined as the wearing away of repellent due to friction resulting from skin rubbings by clothing. In vivo skin friction measurements have been calculated by El-Shimi (1977), who also determined the effect of 2 different substances (i.e., talcum powder and silicone oil) on the coefficient of friction of skin.

Our general goal is to understand the physico-chemical effects of the skin on the repellent efficacy, which lead to better understanding of how repellents might be optimally used by soldiers under field conditions. The information gained may improve our ability to make precise recommendations regarding repellent formulation for use by the service members. In this study we evaluated the effect of repeated clothing abrasions on repellent efficacy against adult mosquitoes when repellent was topically applied to human skin in the laboratory.

MATERIALS AND METHODS

Mosquito species: The mosquito species used was *Aedes aegypti* (Linn.), from Walter Reed Army Institute of Research (WRAIR) colonies that originated from Bangkok, Thailand. Mosquitoes were reared at 27°C and 80% relative humidity (RH) on a 12:12 (light:dark) h photoperiod. Larvae were fed a diet of catfish chow (Continental Grain Company, Chicago, IL), about 0.5-1.0 g in 2,500 ml of water, and adults were maintained on a 10% sucrose solution. About 10-12 h prior to host expo-

¹ Opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the U.S. Department of the Army. Use of trade names does not constitute an official endorsement or approval of the products mentioned. Human subjects participating in this study gave free and informed voluntary consent.

² The investigators had adhered to the policies for protection of human subjects as prescribed in the U.S. Army Regulation 70-25.

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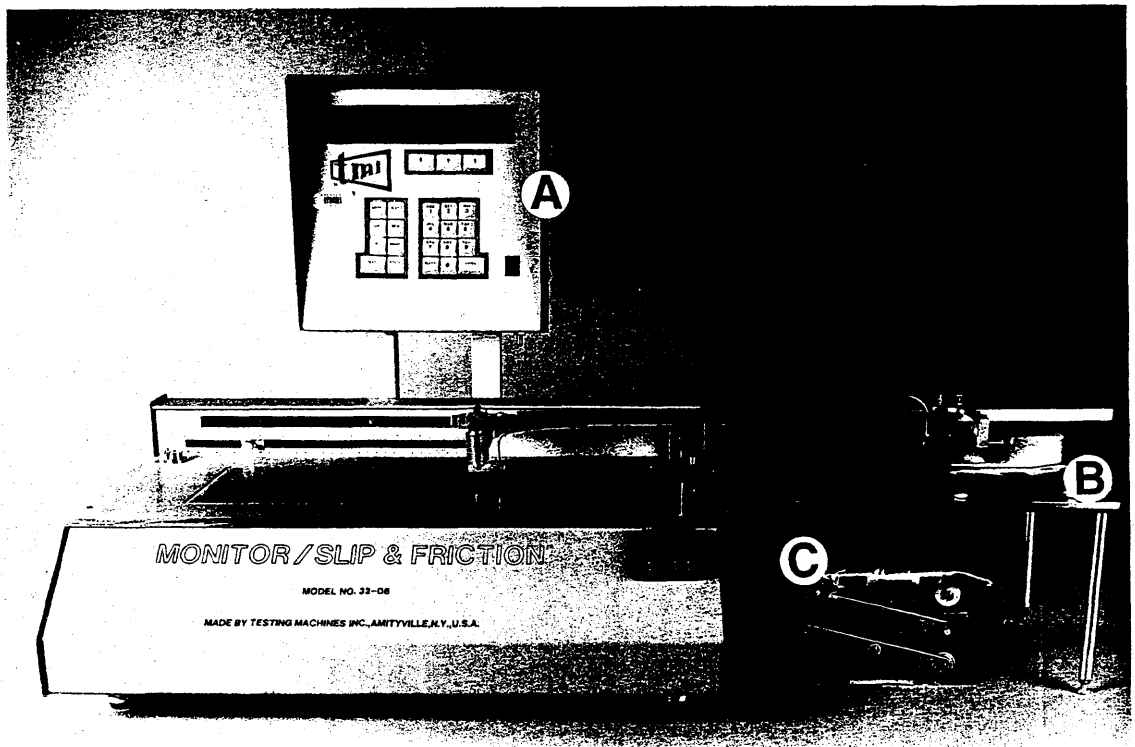


Fig. 1. Frontal view of the slip and friction tester machine showing the liquid crystal display screen and key panel (A), the sled (B), and the adjustable plate (C) to keep the arm secured underneath the sled.

sure, the test insects were provided with pledgets saturated with water as a substitute for sucrose solution. Mosquitoes used in the experiments were nulliparous females 6–13 days old.

Repellent and abrasion machine: An extended duration topical insect/arthropod repellent lotion (3M Consumer Specialties Division, St. Paul, MN; NSN 6840-01-284-3982) was used in this study. It contained an acrylate polymer formulation of deet (active ingredient: *N,N*-diethyl-*m*-toluamide, 31.58%; other isomers, 1.75%) that sustained the release of the chemical from the skin.

The skin was abraded using a TMI® Monitor/Slip and Friction Testing Machine Model 32-06-00 (Testing Machines, Inc., Amityville, NY) to provide measured, uniform, and repeated abrasions to the volunteer's repellent-treated forearms (Fig. 1). The machine was standardized and calibrated using a calibration fixture supplied by the company before the start of the experiment. The machine is designed to test the coefficient of starting friction (static friction) and the sliding friction (kinetic friction) between 2 specimens, or between a specimen (clothing) and a substrate (skin). The coefficient of friction (COF) is a ratio of the frictional force to load or weight that presses 2 surfaces together. The testing machine has a direct-driven movement of the pulling arm, eliminating the use of cables,

which often tangle and can lead to error; a sled is attached to the moving drive arm. The fabric was wrapped around the sled (Fig. 1B) in such a manner that it came in contact with selected skin area during the abrasion process. The forearm was positioned under the sled of the machine (Fig. 2), and abraded along the treated or untreated skin. The forearms of volunteers were abraded 1, 5, 10, 20, and 30 times after application of repellent and subsequently exposed to mosquito bites.

Repellent test: Tropical battle dress uniform fabric (MIL-C-342 [Natick Research Development and Engineering Center, Natick, MA]; 100% cotton, 7-oz. poplin cloth) printed with a 4-color, woodland camouflage pattern was cut into specimens (22 × 22 cm) and mounted on the sled of the machine. The fabric rubbed the skin of the volunteer during the abrasion process. Both forearms of the volunteer were rubbed with the same abrasion treatment; however, only one arm (the treated arm) was treated with repellent prior to abrasion. During the first day of the test, the forearm of each volunteer that was to serve as the treated arm for the entire day was selected. The treated arm (e.g., the forearm selected during the first day for repellent treatment) was alternated for each day of the study.

The repellent was applied evenly to the forearms (flexor region) of 3 volunteers. One forearm of each

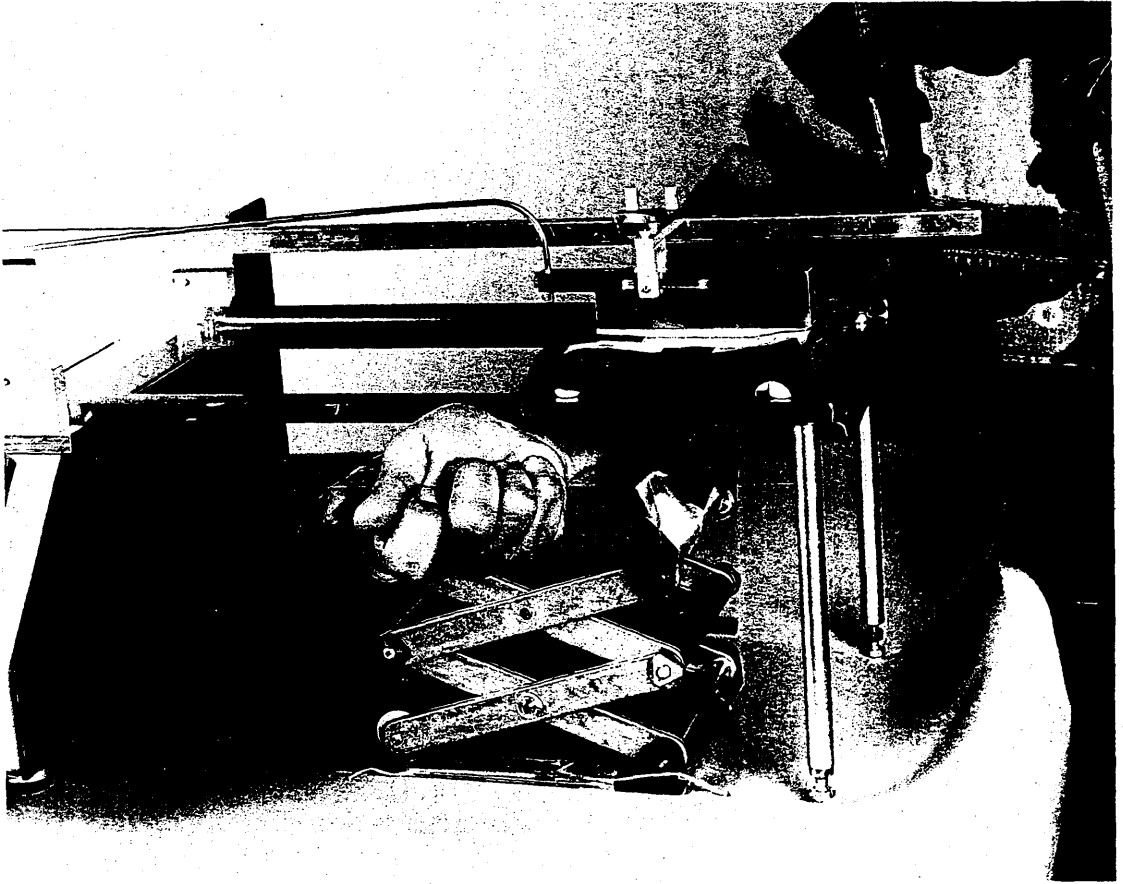


Fig. 2. Volunteer's forearm in position during abrasion process.

volunteer was treated with repellent and the other forearm was left untreated and served as control. An approximately 52.5-cm² area of the skin was treated with a known amount of insect repellent and was allowed to air-dry for 5 min prior to abrasion procedure.

The efficacy test method used in this study was similar to the procedure described by Hoch et al. (1995). At the start of each test, a clear transparent plastic cage (18 × 5 × 4 cm) containing 15 nulliparous adult female mosquitoes was secured to the flexor region of the forearm with Velcro® (USA, Inc., Douglas, AZ) tape, and a slide was withdrawn to expose the treated skin to the mosquitoes. The number of biting mosquitoes was recorded at the end of 90 sec by visual indication of blood in the insect abdomen. If all mosquitoes were observed to bite prior to the 90-sec time limit, the test was stopped. This procedure was repeated every 2 h for 10 h using fresh cages of mosquitoes each time. Thus, the forearms were exposed to mosquitoes at 0, 2, 4, 6, 8, and 10 h after the skin abrasion process. The test volunteers were allowed to resume their normal duties following each test. They were warned not to scratch, rub, or wash the treated areas

of forearms during the 10-h test period. All tests were conducted in the laboratory at 25 ± 1.0°C. The research protocol was reviewed and approved by the WRAIR Scientific Review Committee for scientific merit and adherence to scientific goals. Ethical, informed consent, confidential, risk, benefit, and remuneration aspects of the protocol were reviewed and approved by the WRAIR Human Use Review Committee. Final approval was made by the Human Subjects Research Review Board.

Statistical analysis: For kinetic COF data, statistical analysis was by single classification of variance with unequal sample sizes, using the $\ln(x + 1)$ transformation to normalize distribution and control variance. Means were compared using the Student-Neuman-Keuls test (Sokal and Rohlf 1995). Percentage protection was determined from the total number of bites on the control and repellent-treated test volunteers by Abbott's formula (Abbott 1925). A 2-way analysis of variance was done on the percentage protection of repellent by time as influenced by the number of abrasions, using the general linear model (GLM) procedure (SAS Institute 1990). Means were separated using Tukey's

Table 1. Mean (\pm SE) values of the kinetic coefficient of friction on repellent-treated and untreated forearm.

| Num-ber of abra-sions | Num-ber of repli-cations | Mean values of kinetic coefficient of friction ¹ | |
|-----------------------|--------------------------|---|----------------------|
| | | Untreated arm | Treated arm |
| 1 | 3 | 0.130 \pm 0.042 a | 0.120 \pm 0.024 a |
| 5 | 15 | 0.126 \pm 0.005 a | 0.157 \pm 0.157 ab |
| 10 | 30 | 0.110 \pm 0.003 a | 0.167 \pm 0.167 ab |
| 20 | 60 | 0.104 \pm 0.015 a | 0.134 \pm 0.134 ab |
| 30 | 90 | 0.138 \pm 0.004 a | 0.176 \pm 0.004 b |

¹ Means in the same column followed by the same letter are not significantly different ($P \leq 0.05$; Student-Newman-Keuls test [SAS Institute 1990]).

studentized range test ($P \leq 0.05$) (SAS Institute 1990).

RESULTS AND DISCUSSION

The mean weight of insect repellent formulation applied by individual volunteers to their forearms (flexor region) was 1.1 ± 0.002 mg/cm². There were no significant differences in amounts of the repellent applied among volunteers for different abrasion treatments.

Mean values of the kinetic COF on the forearms of volunteers are shown in Table 1. No significant differences were found in kinetic COF values among unabraded forearms without repellent application as compared to repellent-treated forearms. Insect repellent-treated forearms that were abraded 30 times had significantly higher kinetic COF values than those abraded only once ($P \leq 0.05$, Student-Newman-Keuls test). When analyzed separately, repellent-treated forearms (0.159 ± 0.003) had significantly higher kinetic COF values com-

pared to nontreated forearms (0.122 ± 0.005) ($P \leq 0.0001$).

The percentage of biting protection provided by deet repellent against *Ae. aegypti* during the 10-h period is shown in Fig. 3. No significant reduction was found in protection against mosquito bites up to 4 h after application, except for forearms abraded 30 times ($P \leq 0.05$, Tukey's studentized range test). At 6 h forearms with 20 and 30 abrasions had significantly lower protection ($P \leq 0.05$). At 8 h forearms that were unabraded (82%) and those abraded only once (68%) had significantly higher protection than those abraded 5-30 times ($P \leq 0.05$). All abraded forearms had significantly lower protection than unabraded ones for 10 h ($P \leq 0.05$); however, no significant protection differences were observed among abraded forearms.

Our results showed that abrasions of the skin affected the duration of protection provided by insect repellent containing deet. As early as 4 h after receiving abrasions, repellent-treated forearms that were abraded 30 times had the lowest protection against mosquito bites. Unabraded forearms had the highest mosquito protection among repellent-treated arms for 10 h. Smith et al. (1963) reported that insect repellents, when applied on the skin, are lost over time by evaporation. In our study, mechanical abrasion of the skin seems to accelerate the evaporation of the repellent, resulting in decreased protection from mosquito bites. Abrasion also might have physically affected the adjuvant or polymer-fixative component of the repellent that controls the slow release of the active ingredient (Mehr et al. 1985); this requires further investigation.

Although absorption and evaporation have been intensely studied as modes of loss of deet from the skin (Reifenrath and Spencer 1989), skin abrasion has been largely neglected. Prior to the present

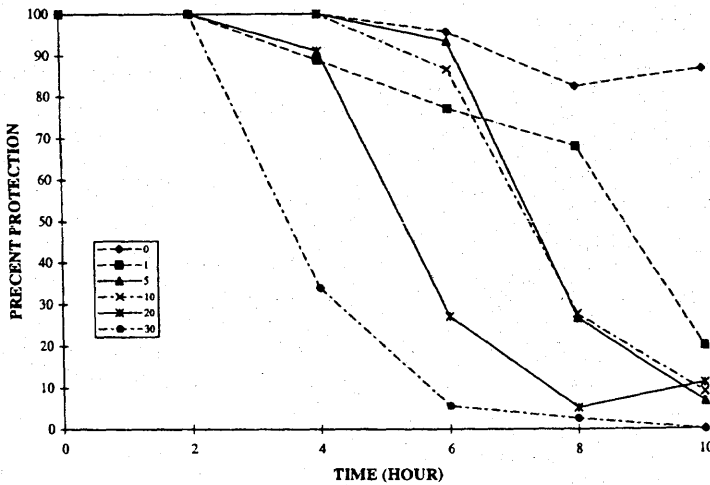


Fig. 3. Percentage protection from *Aedes aegypti* bites provided by insect repellent deet after fabric abrasions ($n = 0, 1, 5, 10, 20, \text{ and } 30$ abrasions).

study, only Gilbert et al. (1957) and Khan et al. (1977) had studied the loss of deet by abrasion. Khan et al. (1977) concluded that deet mixed in polymer formulation remained more effective as compared to dimethyl phthalate after the treated skin was swiped by tissue papers. The present study was the first attempt using a TMI Monitor/Slip and Friction Testing Machine to provide measured, uniform, and repeated abrasions to human skin to determine the effect of deet on the coefficient of friction of fabric and human skin.

Figure 3 shows that abrasion greatly reduces the protection otherwise provided by deet against mosquitoes. In future studies it will be important to quantitate the levels of abrasion actually experienced by individuals using deet in the field and to determine the mechanical, physiologic, and other mechanisms by which abrasion reduces the efficacy of deet. It may then be possible to extend the protection period of deet by reformulation to reduce abrasive loss.

In addition to skin abrasions, the effects of additional factors (i.e., water exposure, perspiration, temperature, safety, cosmetic acceptability) must be included in evaluating long-lasting repellents against arthropod vectors of human diseases.

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