LABORATORY EVALUATION OF CONTROLLED-RELEASE REPELLENT FORMULATIONS ON HUMAN VOLUNTEERS UNDER THREE CLIMATIC REGIMENS[1,2]

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ABSTRACT. Six controlled-release personal-use topical insect/arthropod repellent formulations of diethylmethylbenzamide (deet) were evaluated in an environmental chamber on volunteers for repellency against the mosquitoes *Aedes aegypti* and *Ae. taeniorhynchus* under three climatic regimens: basic variable high humidity (tropical environment), basic constant high humidity (forested and wet environment) and basic hot (hot-dry environment). The best protection under all the climatic regimens was provided by the Biotek formulation. In a tropical environment, some formulations induced more biting from mosquitoes than the concurrent untreated control in the late hours of the testing. Repellency was not directly related to the deet concentration in the various controlled-release repellent formulations.

INTRODUCTION

Inorganic and botanical materials such as alum, citronella and pennyroyal have been used as insect repellents since ancient times. Although these materials are highly effective, they last for only a short time on the skin. The first practical synthetic repellent was developed by the Standard Oil Development Company in 1929. This material, dimethyl phthalate, is effective for about two hours, depending upon the amount applied, number and kinds of insects present, time of day, weather, activity of the user and other factors. The next major advance in repellent technology was the discovery of diethylmethylbenzamide (also known as diethyltoluamide, "deet") by the U.S. Department of Agriculture in 1954. Deet is still regarded as the best broad spectrum repellent known, but its short protection/repellency time has been recognized as a deficiency for many years.

In recent years the insect repellent program at Letterman Army Institute of Research (LAIR) has been directed towards the exploitation of modern sustained-release technology (Reifenrath and Rutledge 1983, Mehr et al. 1985) to develop a controlled-release insect repellent formulation for topical use that will provide extended protection against biting arthropods, be safe and pleasant to use and be compatible with plastics, synthetic fabrics and similar materials. The sustained-release technology is already widely employed in the formulation of drugs, fertilizers, pesticides, perfumes, toiletries and other products.

Basic research at LAIR in the late 1970s and early 1980s established the physical parameters and theoretical framework which demonstrated the feasibility of polymer and microcapsule mechanisms to release deet at a predetermined rate. The formulations tested in those early studies utilized microcapsule and polymer systems designed to provide continuous long-term release of the active ingredient, including microcapsules and microparticles, and film-forming polymers. In microcapsule formulations, the active ingredient is contained in tiny capsules produced by coacervation, interfacial polymerization, extrusion and other processes. The release rate is determined by the size and number of the microcapsules, the composition and thickness of the microcapsule walls, the concentration and properties of the excipient, and other additives used. These formulations may also contain free active repellent in addition to that contained in the microcapsules. In polymer systems, the active ingredient is formulated with a polymer that will form a thin film over the skin. This film acts as a reservoir for the active ingredient and slows its absorption and evaporation. In microparticulate controlled-release systems, the active ingredient is absorbed on the surface of microparticles and released slowly over time.

In the present study (phase I), we evaluated six prototype Extended Duration Topical Insect/Arthropod Repellent (EDTIAR) formulations in an environmental chamber for effectiveness against laboratory reared mosquitoes under three different climatic regimens: basic variable high humidity (tropical environment), basic constant high humidity (forested, rainy and wet environment) and basic hot (hot-dry environment). The results obtained from this study were used in conjunction with other data, including cosmetic evaluation and field tests against natural populations, to select two can-

[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.

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

didate EDTIAR formulations for further development and testing in phase II of the program.

MATERIALS AND METHODS

Mosquito Species: The yellow fever mosquito colony, *Aedes aegypti* (Linn.), used in the experiments was obtained from the University of California at San Francisco in 1974. Rearing procedures were similar to those of Mehr et al. (1985). The colony of a salt marsh mosquito, *Ae. taeniorhynchus* (Wiedemann), was obtained from the Insects Affecting Man and Animals Research Laboratory, U.S. Department of Agriculture, Gainesville, FL, in 1976. Mosquitoes were reared and maintained at 27 ± 3°C and 80 ± 10% relative humidity and under 12:12 hr photoperiod. Larvae were reared on a diet of floating catfish food (Continental Grain, Chicago, IL). The adults were maintained on 10% sucrose solution. The mosquitoes used were nulliparous females of mixed age in the range of 5 to 15 days.

EDTIAR Formulations: The active ingredient of all six formulations was diethylmethylbenzamide. All other components of the formulations, including excipients, additives, and structural elements, were specified to be inert ingredients. The formulations evaluated were two microencapsulated formulations: 1) Controlled Release Insect Repellent (38.5% deet)—Bend Research Inc., Bend, OR (Bend) and 2) Insect Repellent (53% deet) provided by Southern Research Institute, Birmingham, AL (SRI); three polymer formulations: 3) Controlled Release Personal Use Arthropod Repellent Formulation (49% deet)—by Hercon Division of Health-Chem Corporation, South Plainsfield, NJ (Hercon), 4) Javelin Insect Repellent (14.5% deet)—Javelin Corporation, Redwood City, CA (Javelin) and 5) Controlled-Release Personal Use Arthropod Repellent Formulation (28.5% deet)—Personal Care Products Laboratory, 3M, St. Paul, MN (3M); and a microparticulate formulation: 6) Sustained Action Arthropod Repellent (41.8% deet) Biotek Corp., Woburn, MA (Biotek). The composition of the six prototype EDTIAR formulations is confidential.

Test Procedure: The test method was a modification of the American Society for Testing and Materials (ASTM) Standard E951-83, (1983). The six EDTIAR formulations were applied at random to the flexor region of the forearms of three volunteers according to the label instructions. A fourth volunteer served as the control. Control subjects were rotated daily in random order. At the start of the test, a 4 × 5 × 18 cm plastic cage containing 15 mosquitoes was bound to the forearm with Velcro® tape, and a slide was withdrawn to expose the EDTIAR-treated skin (Fig. 1). The number of mosquitoes

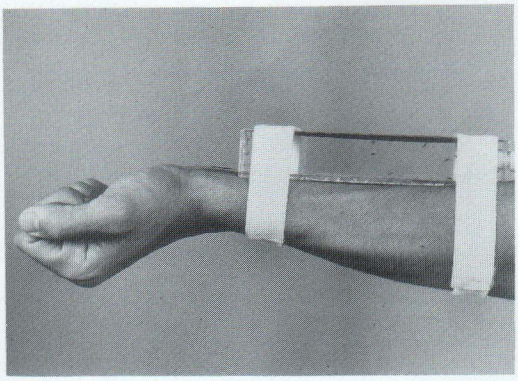

Fig. 1. Test cage on the volunteer's forearm.

biting in the test cage was recorded at the end of 90 sec. New mosquitoes were used in each test and the cages were removed after 90 sec. This test procedure was repeated every 2 hr for 12 hr. Thus, seven tests of each species of mosquito were conducted on each EDTIAR formulation at 0, 2, 4, 6, 8, 10 and 12 hr after application on the skin. The above procedure was repeated six times. The test volunteers stayed in the environmental chamber for the entire 12 hr. Each EDTIAR formulation was tested under three climatic regimens using average values of the 24-hr test cycles. The average temperature and relative humidity (RH) recorded for various climatic regimens were: basic variable high humidity, 30°C for entire 12 hr with 78% RH for first 6 hr and 98% RH for the last 6 hr; basic constant high humidity, 24°C with 97.5% RH; and basic hot, 37°C with 31% RH for the entire 12 hr.

Statistical Analysis: The biting counts recorded under different treatments and climatic conditions were analyzed by analysis of variance. The analyses were performed on a Data General MV/8000 computer using the BMDP2V (Biomedical Programs) computer package (Dixon et al. 1983). Differences were considered to be significant at $P \leq 0.05$. Also, the percentage repellency was determined from the total number of bites on control and EDTIAR-treated test volunteers by Abbott's formula (Abbott 1925).

RESULTS AND DISCUSSION

All six EDTIAR formulations effectively provided 95% or better repellency for 6 hr as shown in the test results (Fig. 2) when tested against *Ae. aegypti* in the basic constant high humidity environment. The Biotek repellent formulation had significantly greater repellency at 8, 10 and 12 hr after application than the rest of the formulations. Repellency of SRI and Bend formulations declined rapidly after 6 hr as compared to Hercon, Javelin or 3M. This decline in repellency was steady except in Hercon and SRI formulations, where the repellency increased at

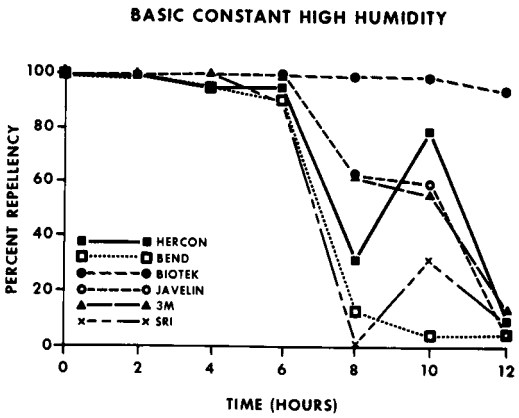

Fig. 2. Performance of repellent formulations in forested/wet environment against *Aedes aegypti.*

10 hr and then declined again. These increases may be attributed to the fluctuations in the repellent release rates at and after that time. The analysis of variance indicated that the EDTIAR formulations provided significantly different repellencies. The interactions between EDTIAR formulation and time (interval after application) was significant at 5% level of confidence, implying that EDTIAR formulations provided significantly different repellencies after different time intervals.

Figure 3 shows the results from tests against *Ae. aegypti* under the basic variable high humidity environment. All except 3M and Javelin formulations provided 100% protection from bites for 6 hr. The Biotek formulation provided the best protection under this climatic regimen. The analysis of variance indicated that there was significant difference in the duration of protection provided by various EDTIAR formulations. Bend, SRI and 3M repellent formulations were attractant to mosquitoes at 12 hr. This effect occurred only in the 12th hr of testing when the repellent residues were presumably lowest and only under this climatic regimen. The attractancy of such residues has also been observed in other studies (Gupta et al. 1987, Mehr et al. 1985, Potapov et al. 1977). This effect may be attributed to the greater perspiration induced by the increased temperature and humidity to which the volunteers were exposed in the last 6 hr of testing, which reduced the residue of the repellent on the skin to a level at which attraction occurs.

In the hot-dry environment, all the EDTIAR formulations except javelin provided 95% protection or better for 4 hr against *Ae. aegypti* as shown in Fig. 4. There were statistically significant differences among the EDTIAR formulations and among the time intervals after application. The interaction between the EDTIAR formulations and the test days was also significant, implying that the protection provided by the repellent formulations was different on the different days of testing.

Against *Ae. taeniorhynchus,* all the EDTIAR formulations provided 95% or better protection for 6 or more hr except that the protection provided by the Javelin formulation was lower at 4 hr. The protection under the three climatic regimens is summarized in Table 1. Under the basic variable high humidity climatic regimen, *Ae. taeniorhynchus* were attracted by the 3M and SRI formulations at 10 hr but were repelled again at 12 hr.

Attempts to extend the persistence of repellents with natural polymers such as shellac and gum tragacanth and powders such as zinc oxide, talcum powder, bentonite, and china clay were made as early as 1947 by Christophers. In 1970,

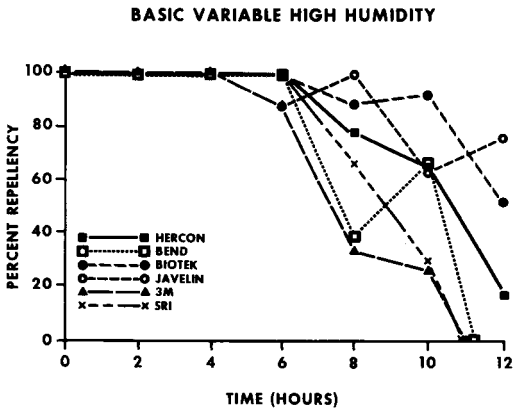

Fig. 3. Performance of repellent formulations in tropical environment against *Aedes aegypti.*

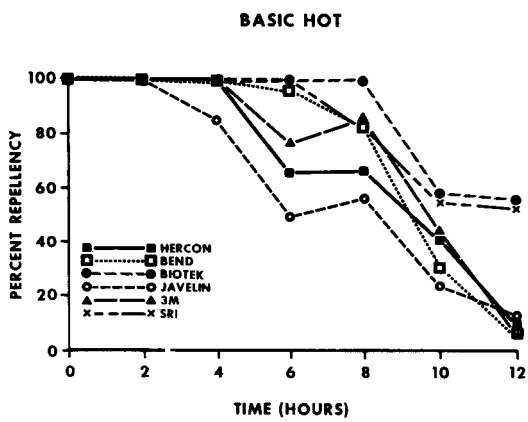

Fig. 4. Performance of repellent formulations in hot-dry environment against *Aedes aegypti.*

Markina et al. associated the loss of repellent by evaporation directly to physical exertion and the presence or absence of film-forming materials in the formulation. A year later, Dremova et al. (1971) reported increased persistence and

Table 1. Effectiveness of EDTIAR formulations against *Aedes taeniorhynchus* in various climatic regimens.

EDTIAR formulations	Hrs of 95% or better protection		
	Forested/wet environment	Tropical environment	Hot-dry environment
Bend	8	8	10
SRI	6	6	6
Hercon	10	12[a]	8
Javelin	12[b]	10	8
3M	12	6	10
Biotek	12	8	12

[a] 50% protection at 10 hr.
[b] 83% protection at 4 hr.

less loss of repellent with the fat-based creams containing such film-forming agents as ethyl cellulose and liquid silicone. In 1983, Reifenrath and Rutledge evaluated several film-forming formulations containing silicone and acrylate polymers and observed significant improvement in duration of protection. Mehr et al. (1985) also demonstrated that the protection period of deet can be effectively extended through controlled-release techniques.

In the present study, all of the six EDTIAR formulations provided extended repellency against mosquitoes as compared to simple deet formulation in ethanol which provided repellency from 2 to 4 hr (Buescher et al. 1983). This study also showed that the repellency was not directly related to the deet concentration in the various EDTIAR formulations, thus supporting the report of Smith (1970) that several controlled-release formulations containing less repellent were as persistent as the higher strength repellent.

Until recently, the controlled-release technology had not been applied to the problem of preventing arthropod/insect-borne diseases by increasing the effectiveness and persistence of the arthropod/insect repellents. This technology may play an important role in future since an increasing number of arthropods/insects are becoming resistant to pesticides. The present repellent formulations, as a form of personal protection, are an inexpensive and practical means of reducing the biting activity and preventing arthropod-borne disease transmission. These controlled-release formulations provide longer protection and, according to other data not included in this report, are compatible with modern-day plastics and are easily accepted by people as compared to the full/higher strength repellents. EDTIAR formulations may be an excellent alternative to present-day chemical vector control strategies.

ACKNOWLEDGMENT

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