LABORATORY EVALUATION OF CONTROLLED-RELEASE INSECT REPELLENT FORMULATIONS[1, 2]

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ABSTRACT. Seven microcapsule formulations and two polymer formulations of deet were tested on white rabbits for their repellency against the mosquito, *Aedes aegypti*. Two microcapsule formulations and one polymer formulation provided more than 80% protection for 12 hours. Results demonstrated that the protection period of deet can be extended through controlled-release techniques.

INTRODUCTION

Historically, insect repellents have been selected on the basis of their persistence on the skin. Adding adjuvant materials to increase persistence was reported as early as 1928 when Freeborn recommended a formula consisting of oil of citronella, spirits of camphor, oil of tar, oil of pennyroyal and castor oil. Dover (1930) suggested a similar formula and reported that one application usually lasted for an entire night. Recently, Skinner and Johnson (1980) suggested the use of film-forming or polymer-fixative technology to produce the ideal repellent formulation. Daily application of these formulations would provide long-lasting protection even under difficult field conditions.

During the past several years, the persistence of several controlled-release formulations of diethyl toluamide (deet) has been tested at Letterman Army Institute of Research (LAIR). This paper presents the results of testing nine formulations of deet on white rabbits against the yellow fever mosquito, *Aedes aegypti* (Linn.).

MATERIALS AND METHODS

TEST INSECTS. The strain of *Aedes aegypti* used in these experiments was obtained from Dr. A. A. Khan of the University of California at San Francisco (UCSF). Mosquitoes were reared and maintained at 27 ± 3°C and 80 ± 10% relative humidity (RH) under a 12:12 hr photoperiod. Larvae were reared on a diet of floating catfish food (Continental Grain, Chicago, IL). Adults were maintained on 10% sucrose solution. All formulation tests were conducted with nulliparous females 7 to 14 days old.

TEST ANIMALS. Sixteen New Zealand adult white rabbits, 1–2 years old and weighing 6–9 kg, were the test animals.

REPELLENT FORMULATIONS. The active ingredient of all formulations tested was *N,N*-diethyl-3-methylbenzamide (deet). K. L. Smith, Bend Research, Inc., Bend, OR, provided three microcapsule and free-repellent formulations, 186-79A, 186-79B, and 186-79C containing 27.0, 31.0 and 28.7% deet, respectively. The formulation additives and processes of Bend Research, Inc., are confidential. Spray Control Systems, University of Georgia, Athens, GA, provided four microcapsule formulations, samples 1, 2, 3 and 4, containing 15, 10, 10 and 20% deet, respectively. These formulations were based on hydrophilic vinyl polymers. H. Libby, Libby Laboratories, Inc., Berkeley, CA, provided a film-forming polymer formulation, UX-179B, containing 20% deet. The polymer component of formulation UX-179B was polyvinylpyrrolidone (PVP). R. Harryman, Javelin Corporation, Redwood City, CA, provided a film-forming polymer formulation, formulation X, containing 2.9% deet. The composition of the Javelin Corporation formulation is confidential.

TEST PROCEDURE. Before treatment, rabbits were anesthetized with 1 ml of ketamine and 1 ml of acepromazine injected intramuscularly into the thigh. The rabbit was then put in a restrainer, and its abdomen was shaved. The abdomen was marked with six circular areas (each 29 mm diam) using a plastic template designed for the purpose. Two circular areas were treated at random with 0.025 ml of ethanol as the control, two were treated at random with 0.025 ml of deet in the same concentration as the test formulation, and two were treated at random with 0.025 ml of the test formulation. The rabbit was kept in a restrainer

[1] Opinions and assertions contained herein are the private views of the authors and should not be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. Use of a trade name does not imply official approval or indorsement of the product mentioned.

[2] In conducting this study, the authors adhered to the "Guide for Care and Use of Laboratory Animals," published by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

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for 4, 8, 12, 16, 20 or 24 hr, depending upon the length of persistence being evaluated. However, rabbits treated with formulation X were tested for periods ranging from 4 to 24 hr at 2-hr intervals. After the holding period, the rabbit was again anesthetized. A plastic cage (4 x 5 x 21 cm) containing 25 female mosquitoes was placed on the rabbit's abdomen so that 6 circular cutouts in the floor of the cage coincided with the 6 treated areas on the rabbit. Once positioned, the cage was attached to the rabbit's abdomen with pressure-sensitive adhesive tape. A slide in the cage floor was removed to admit the mosquitoes to the treated areas. Mosquitoes were given a free choice to feed on any of the six areas. After 90 sec, the number of mosquitoes feeding on each area was counted and recorded. The mosquitoes were then killed with a jet of carbon dioxide and discarded. This procedure was repeated five times for each rabbit. Two rabbits were evaluated simultaneously so that a total of 10 counts was obtained at the end of each experiment. Twenty to 40 counts were obtained for each formulation to insure reproducibility of the data. Sixteen rabbits were used in rotation.

STATISTICAL ANALYSIS. The percentage of repellency was determined from the total number of bites on the control, deet, and formulation-treated areas by converting to percentages of the total for the control and subtracting from 100:

$$\% \text{ Repellency} = 100 - \frac{\text{Total no. of bites on treatment}}{\text{Total no. of bites on control}} \times 100$$

The analyses were performed on a Data General MV/8000 computer using the BMDP (Biomedical Programs) computer package (Dixon et al. 1983). A two-way analysis of variance was done on the percent repellency of simple deet and the formulations with time periods taken as the second factor. Differences were considered to be significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Test results on the three microcapsule formulations from the 186–79 series are given in Fig. 1. Although formulation 186–79A showed greater repellency than simple deet at 4, 8, 12 and 16 hours, it showed no repellency at 20 and 24 hr and was not significantly different from deet over the full 24-hr period. Formulations 186–79B and 186–79C were significantly more repellent than simple deet throughout the test period ($p = 0.004$ for 186–79B and $p = 0.004$ for 186–79C).

Test results on microcapsule formulations

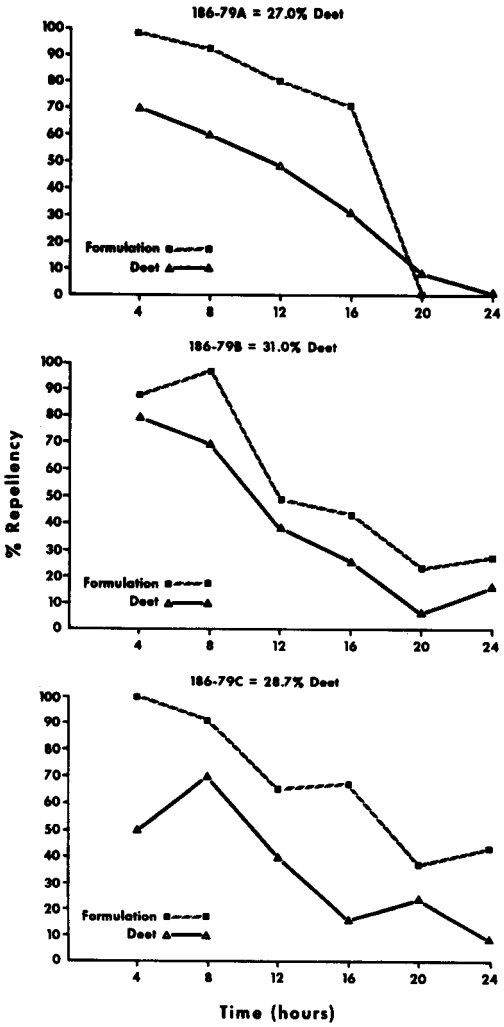

Fig. 1. Performance of formulations of 186–79 series

1–4 are given in Fig. 2. Formulation 1 had greater repellency than simple deet at the 4, 8 and 12-hr tests; however, its repellency was not significantly different from deet through the rest of the test period. Formulations 2 and 4 were significantly more repellent than simple deet for 24 hr ($p = 0.03$ for formulation 2 and $p = 0.002$ for formulation No. 4). Formulation 3 was not significantly different from simple deet.

Generally, the microcapsule formulations of deet displayed greater persistence as determined by the number of bites recorded than deet alone under the same conditions and at the same concentration. Deet provided 70% protection at 4 hr, and 60% protection at 8 hr, while formulation 186–79A provided 98% and 93% protection at the same intervals, a 1.4–1.6

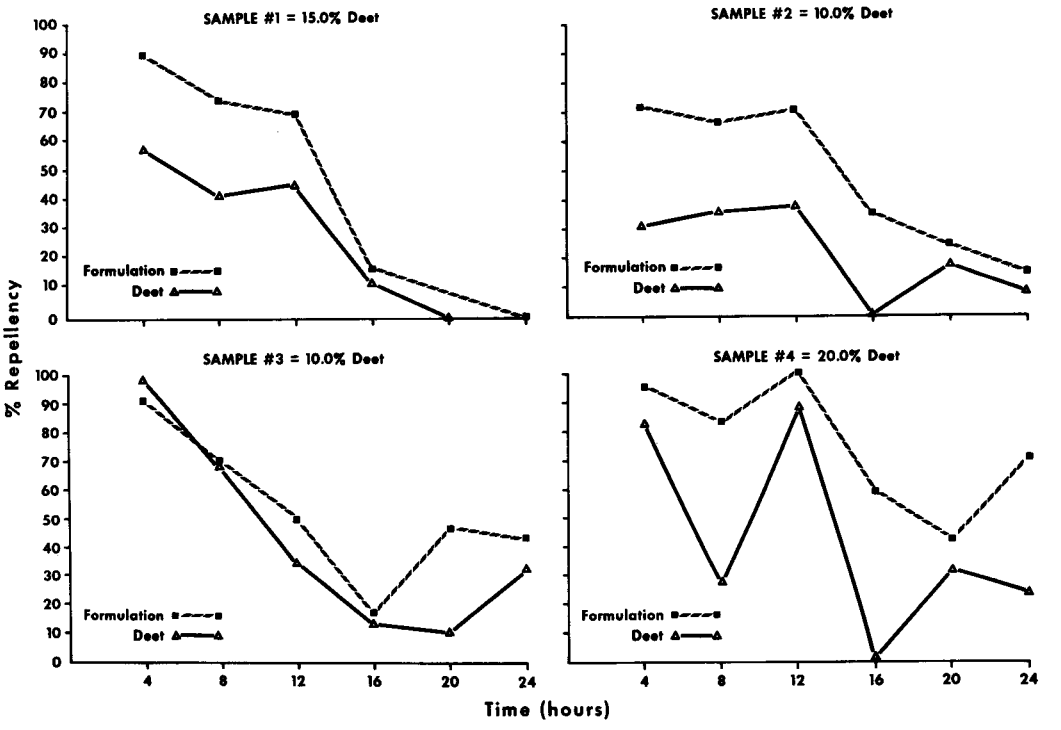

Fig. 2. Performance of formulations 1 to 4

fold increase in repellency. Galun et al. (1980) reported that microencapsulated formulations of natural pyrethrum at 0.52 mg/cm² protected guinea pigs against *Glossina morsitans* Westwood for 8 to 11 days and against *Ornithodoros tholozani* (Laboulbene and Megnin) for 3 days. Non-encapsulated pyrethrum at the same dosage protected against *G. morsitans* for 6 days and against *O. tholozani* for less than 2 days.

Results from tests of the two film-forming polymer formulations, UX-179B and X, are presented in Fig. 3. Formulation X with only 2.9% deet, exhibited a more complete and consistent repellency than simple deet in all of the test periods ($p < 0.00005$). The gain in repellency obtained from formulation X was calculated by subtracting percent repellency of simple deet from percent repellency of formulation X and is presented in Fig. 3.

Natural polymers such as shellac and gum tragacanth, and powders such as zinc oxide, talcum powder, bentonite, china clay, etc., were used by Christophers (1947) to extend the persistence of dimethyl phthalate (DMP). More recently, several film-forming formulations containing silicone or acrylate polymers and deet were evaluated by Reifenrath and Rutledge (1983). They observed significant improvement in persistence for several formulations tested. Koshkina and Kharitonova (1976) found that polymers did not prolong the persistence of deet and DMP when used on fabric. However, synthetic fixatives increased the persistence of deet and DMP on fabric from 1.6 to 2.7-fold compared to the reference preparations, alcohol solutions of the test repellent.

In addition to improving persistence, polymers also increase the wash-resistance of insect repellents. Khan et al. (1977) formulated copolymers of hydrovinyl chloride acetate and sebacic acid, maleic rosin ester and glycolate plastizer with ethyl hexanediol, DMP, Indalone and deet to improve persistency and wash-resistance. They demonstrated that the wash-resistance of deet was improved 11-fold and that the formulation remained effective for 24 hr. However, the cosmetic features of the formulation required improvement.

Study of the mechanisms of repellent loss from the skin is important for development of an improved insect repellent. Markina et al. (1970) reported that adding film-forming agents or stabilizers such as hydroxypropyl cellulose, silicone fluid and ethyl cellulose, to deet significantly retarded both its evaporation and absorption. Loss of repellent by evaporation was directly related to physical exertion and the nature of film-forming agent. In a 6-hr study, losses through evaporation were greater than absorption through the skin. The lowest

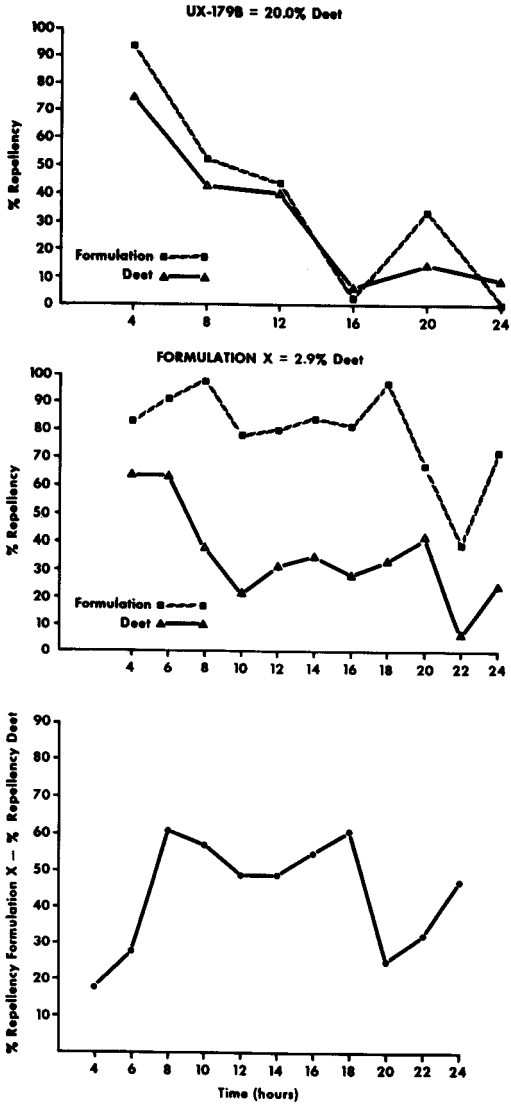

Fig. 3. Performance of formulation UX-179B, formulation X, and the gain in repellency obtained from formulation X.

rate of deet evaporation was observed for 20% creams prepared by using silicone liquid no. 5 and propylcellulose oxide as the film-forming agent on an emulsion base and 40% creams prepared with ethyl cellulose as stabilizer in a soap base. Dremova et al. (1971) also evaluated various repellents for loss from the skin. Repellent loss was least with the fat-based creams containing film-forming agents such as ethyl cellulose and silicone liquid. Repellent loss from the body was found to be directly dependent upon the amount of physical exertion by the subject and the nature of the repellent. Of the repellents evaluated, deet had the highest loss and carboxide (dihexamethylenecarbamide) the lowest.

Our study of nine different deet formulations has demonstrated that the protection provided by deet can be prolonged by controlled-release techniques. Formulation X, which contained only 2.9% deet, provided 80% protection for 12 hr compared to 30% for unformulated deet at the same strength. This shows that persistence is not directly related to the concentration of active ingredient in controlled-release formulations. This conclusion is supported by reports from the Smith, Kline and French Laboratories that several formulations containing 40% repellent were as persistent as the full strength repellent (Smith 1970).

It may be that insect repellents can be even further improved by controlled-release techniques. Possible improvements include lower toxicity through reduced absorption, more uniform spreading, reduction of odor and greater economy.

ACKNOWLEDGMENTS

The authors would like to express their appreciation to Dr. Virginia Gildengorin for statistical advice and analysis and Ms. Ann Wilkinson for the secretarial services provided in typing this manuscript.

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