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EVALUATION OF CONTROLLED-RELEASE MOSQUITO REPELLENT FORMULATIONS[1]

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ABSTRACT. Eight polymer and 9 microcapsule formulations of deet were tested on laboratory rabbits against *Aedes aegypti* and *Anopheles albimanus.* Several formulations were significantly more effective than simple (unformulated) deet at the same strength for periods up to 24 h. Best results were obtained with a polymer formulation containing a high molecular weight fatty acid and 3 microcapsule formulations containing lanolin, gum arabic, gelatin, tannic acid, stearic acid, polypropylene glycol, water, and a commercial lotion in the microcapsule and carrier fractions.

INTRODUCTION

Additives have been used for many years to extend the period of effectiveness of mosquito repellents. Materials recommended include olive oil (MacNay 1939), mineral oil (Weaving and Sylvester 1967), and perfume fixatives (2,6-dinitro-3,4,5-triethyl-t-butyl benzene, 2,4-dinitro-3-methyl-6-t-butyl anisole, 2,4-di-t-butyl-5-methoxybenzaldehyde, 5-t-butyl-2,4,6-dinitro-m-xylene) (Khan et al. 1975). It is thought that such materials act by inhibiting loss of active ingredient by evaporation, absorption, and abrasion.

Polymeric additives act by forming thin films that incorporate the active ingredient and release it slowly in the vapor phase. Polymers recommended include polyacrylic and polymethacrylic esters, vinylidine polymers, cellulose acetate butyrate, and ethyl cellulose (Skinner et al. 1975, Spencer et al. 1977).

Recently, several microcapsule and microparticle systems were evaluated as controlled-release repellent formulations (Galun et al. 1980, Mehr et al. 1985, Gupta and Rutledge 1989). In microcapsule and microparticle delivery systems, the active ingredient is released slowly from the surface and/or interior spaces of the capsules or particles. Microcapsule and microparticle materials reported include waxes, polyamides, polyvinylpyrrolidone, and other vinyl polymers.

From 1984 to 1989, the U.S. Army sponsored an extensive program of research and development leading to development of an extended duration polymer formulation of deet by the 3M Corporation. This formulation is now standard issue in the U.S. armed forces and is sold commercially by the developer. The present paper reports previously unpublished information on alternative approaches and materials obtained from 1981 to 1984 at the former Letterman Army Institute of Research, Presidio of San Francisco, CA, in connection with the development program.

MATERIALS AND METHODS

Active ingredient: The active ingredient of the formulations tested was technical grade deet (*N,N*-diethyl-3-methylbenzamide).

Polymer formulations: Formulations 15 and 16, containing 6.1% deet, and formulations 24, 25, 26, 27, 28, and 69, containing 3.9% deet, were prepared at the Letterman Army Institute of Research. Formulations 15 and 24 contained a silicone polymer, 200 fluid, 350 centistoke, obtained from Dow Corning Corp., Midland, MI. (The stoke is the unit of kinematic viscosity in the centimeter-gram-second system, obtained by dividing viscosity by density.) Formulation 16 contained 200 fluid, 1,000 centistoke. Formulations 25, 26, 27, and 28 contained acrylate polymers, Carboset® 526, 525, 514, and 515, respectively, obtained from B. F. Goodrich Chemical Co., Cleveland, OH. Formulation 69 contained a high molecular weight fatty acid, 1010 dimer acid, obtained from Emery Industries, Cincinnati, OH. Additional information on the composition and characteristics of the polymer formulations was given by Reifenrath and Rutledge (1983) (formulations 15–28) and Reifenrath et al. (1989) (formulation 69).

Microcapsule formulations: Formulations A, B, and C, containing 16.5, 11.1, and 8.5% deet in microcapsules, and formulations 225-44A to 225-44F, containing 43.0, 43.0, 31.8, 31.8, 30.8, and 30.8% deet in microcapsules and as free deet, were prepared by Bend Research, Inc.,

[1] Opinions and assertions herein should not be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. Use of trade names does not imply official endorsement or approval of the products named.

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Table 1. Effectiveness of polymer and microcapsule formulations of deet against *Aedes aegypti* and *Anopheles albimanus*.

Test species	N[1]	Number of bites[2]			Percentage of effect[3]		
		Control	Standard	Formulation	Standard	Formulation	Gain[4]
			Formulation 15				
Ae. aegypti	60	226	38	97	83.2	57.1	−26.1
An. albimanus	20	37	36	16	2.7	56.8	54.1*
			Formulation 16				
Ae. aegypti	20	127	118	108	7.1	15.0	7.9
An. albimanus	20	38	37	19	2.6	50.0	47.4
			Formulation 24				
Ae. aegypti	20	47	36	3	23.4	93.6	70.2*
An. albimanus	20	67	44	32	34.3	52.2	17.9
			Formulation 25				
Ae. aegypti	20	49	21	5	57.1	89.8	32.7
An. albimanus	20	85	11	17	87.1	80.0	−7.1
			Formulation 26				
Ae. aegypti	20	36	26	27	27.8	25.0	−2.8
An. albimanus	20	31	22	7	29.0	77.4	48.4
			Formulation 27				
Ae. aegypti	20	47	24	29	48.9	38.3	−10.6
An. albimanus	20	24	32	53	−33.3	−120.8	−87.5
			Formulation 28				
An. albimanus	20	57	25	6	56.1	89.5	33.4
			Formulation 69				
Ae. aegypti	20	55	53	5	3.6	90.9	87.3*
An. albimanus	20	62	59	21	4.8	66.1	61.3*
			Formulation A				
Ae. aegypti	20[5]	872	493	251	43.5	71.2	27.7*
An. albimanus	20	19	5	1	73.7	94.7	21.0
			Formulation B				
Ae. aegypti	12[6]	138	64	12	53.6	91.3	37.7*
An. albimanus	20	28	6	2	78.6	92.9	14.3
			Formulation C				
Ae. aegypti	38	78	17	1	78.2	98.7	20.5
An. albimanus	22	27	11	1	59.3	96.3	37.0
			Formulation 225-44A				
Ae. aegypti	40[7]	300	207	181	31.0	39.7	8.7
			Formulation 225-44B				
Ae. aegypti	40[7]	375	224	201	40.3	46.4	6.1
			Formulation 225-44C				
Ae. aegypti	40[7]	388	231	214	40.5	44.8	4.3
			Formulation 225-44D				
Ae. aegypti	40[7]	334	254	162	24.0	51.5	27.5*

Table 1. Continued.

Test species	N[1]	Number of bites[2] Control	Standard	Formulation	Percentage of effect[3] Standard	Formulation	Gain[4]
			Formulation 225-44E				
Ae. aegypti	40[7]	351	166	184	52.7	47.6	−5.1
			Formulation 225-44F				
Ae. aegypti	40[7]	353	171	120	51.6	66.0	14.4*

[1] Number of replications.
[2] Totals over all replicates and test periods.
[3] Abbott (1925).
[4] Percentage of effect of formulation minus percentage of effect of standard. Asterisk indicates that stated value is significant at the 5% level.
[5] Forty replications at 24 h.
[6] Thirty-eight replications at 6 h.
[7] Twenty replications at 6 h.

Bend, OR. These preparations contained varying proprietary combinations and proportions of lanolin, gum arabic, gelatin, tannic acid, stearic acid, polypropylene glycol, ethanol, water, and a commercial lotion, Cocoa Butter® (Scholle Corp., College Park, GA), in the microcapsule and carrier fractions.

Test insects: Formulations were tested against *Aedes aegypti* (Linn.) or *Anopheles albimanus* Wiedemann or both (Table 1). Laboratory colonies were obtained from the University of California at San Francisco and the Medical and Veterinary Entomology Research Laboratory, U.S. Department of Agriculture, Gainesville, FL, respectively. Colonies were maintained as described by Mehr et al. (1985). Tests were conducted against nulliparous females 7–14 days old.

Test animals: Formulations were tested on New Zealand strain white laboratory rabbits, 1–2 years old, 6–9 kg in weight. In conducting the research, the investigators adhered to National Institutes of Health Publication 85-23, *Guide for the care and use of laboratory animals.* For an evaluation of the laboratory rabbit model for screening topical mosquito repellents, see Rutledge et al. (1996).

Test method: Rabbits were anesthetized by intramuscular injection of 1 ml of ketamine and 1 ml of acepromazine, secured in the supine position in a V-shaped wooden rabbit restrainer, and shaved over the abdomen. Six circular (29-mm-diam) treatment areas were marked on the shaved abdomen with a plastic template and a felt-tipped pen. The 6 circular areas were treated at random with 2 treatments of the test formulation (0.025 ml; formulation), 2 treatments of deet in ethanol at the same strength as the test formulation (0.025 ml; standard), and 2 treatments of ethanol only (0.025 ml; control).

All formulations were tested against both *Ae. aegypti* and *An. albimanus* except formulation 28 (*An. albimanus* only) and formulations 225-44A to 225-44F (*Ae. aegypti* only). Initial tests against *Ae. aegypti* were conducted 4 h after application of treatments. Selected formulations were then retested at additional intervals up to 24 h: Formulation 16 was tested at 4 and 18 h; formulation A was tested at 4, 6, 8, 12, 16, 18, 20, and 24 h; formulation B was tested at 4 and 6 h; and formulations 225-44A to 225-44F were tested at 4, 6, 8, 12, 16, 20, and 24 h. Formulations were tested against *An. albimanus* at 4 h only, except for formulations A and B (6 h only).

At the end of the test period, the rabbit was reanesthetized and a 4 × 5 × 21-cm acrylic plastic test cage containing 25 mosquitoes was placed on its abdomen and secured with adhesive tape. A slide was then withdrawn to allow the mosquitoes access to the treatment areas through matching holes in the floor of the cage, and the number of mosquitoes biting each treatment area was recorded after 90 sec. The slide was then replaced, and the cage was removed. Normally, 5 cages of mosquitoes were tested in succession.

Because 2 applications of each treatment (formulation, standard, and control) were tested against each cage of mosquitoes, the foregoing procedure provided 10 replications of the test on the same rabbit. The entire procedure, from beginning to end, was usually repeated one or 4 times on additional rabbits to obtain 20 or 40 replications of the test for each test period. However, the exact number of replications obtained ranged from 12 to 60 with a mean of 31.2 (Table 1).

Statistical analysis: The data obtained were analyzed separately for *Ae. aegypti* and *An. al-*

bimanus by one-way (treatments) or 2-way (treatments × test periods) analyses of variance. Interaction of treatments with test periods was tested in 2-way analyses of variance. Fisher's (protected) least significant difference test (Steel and Torrie 1980) was employed in multiple comparisons of treatment means. The 5% level of significance was employed in tests of significance.

Formulations were considered to be effective if the mean number of mosquitoes biting on the formulation was significantly smaller than the mean number of mosquitoes biting on the standard. Formulations were considered to be ineffective if the mean number of mosquitoes biting on the formulation did not differ significantly from, or was significantly greater than, the mean number of mosquitoes biting on the standard.

In addition, the observed variation of effectiveness of simple (unformulated) deet was evaluated in response to comments of an anonymous reviewer. Observed values of the percentage of effect of the deet standards in tests against *Ae. aegypti* and *An. albimanus* (Table 1) were transformed to probits, and the standard deviations were obtained from the 2-way (test periods × concentrations) analyses of variance. These were compared with the estimated means from the analysis of variance to obtain the respective coefficients of variation of the percentage of effect of simple deet (Steel and Torrie 1980).

RESULTS

Polymer formulations: Polymer formulations 15, 24, and 69 were significantly more effective than the respective standards (simple deet) in tests against one or both test species (Table 1). Formulation 24 was significantly more effective than simple deet in tests against *Ae. aegypti* but not in tests against *An. albimanus.* Observed protection against *Ae. aegypti* was 93.6%, compared with 23.4% for simple deet, for a net gain in effectiveness attributable to formulation of 70.2%. Observed protection against *An. albimanus* was 52.2%, compared with 34.3% for simple deet, for a net gain of 17.9%, which was not statistically significant. The gain in tests against *Ae. aegypti* (70.2%) was greater than the gain in tests against *An. albimanus* (17.9%).

Conversely, formulation 15, containing the same silicone polymer as formulation 24, was significantly more effective than simple deet in tests against *An. albimanus* but not in tests against *Ae. aegypti* (Table 1). Observed protection against *An. albimanus* was 56.8%, compared with 2.7% for simple deet, for a net gain of 54.1%. Observed protection against *Ae. aegypti* was 57.1%, compared with 83.2% for simple deet, for a net gain of −26.1% (net loss of 26.1%).

Formulation 69 was significantly more effective than simple deet in tests against both species (Table 1). Observed protection against *Ae. aegypti* was 90.9%, compared with 3.6% for simple deet, for a net gain of 87.3%. Observed protection against *An. albimanus* was 66.1%, compared with 4.8% for simple deet, for a net gain of 61.3%. The gain in tests against *Ae. aegypti* (87.3%) was greater than the gain in tests against *An. albimanus* (61.3%).

Formulation 16 was tested against *Ae. aegypti* at 4 and 18 h and was the only polymer formulation tested beyond 4 h. The average protection over 18 h was 15.0%, compared with 7.1% for simple deet, for an average gain of 7.9%, which was not statistically significant (Table 1). At 4 h the formulation provided 83.6% protection, compared with 61.8% for simple deet, for a net gain of 21.8%, but at 18 h both the formulation (−37.5%) and the standard (−34.7%) allowed more bites than the control and the observed gain was negative (−2.8%).

Microcapsule formulations: Microcapsule formulations A and B were significantly more effective than simple deet in tests against *Ae. aegypti* but not in tests against *An. albimanus* (Table 1). Formulation C was not significantly more effective than simple deet against either species. Formulation A provided 71.2% protection against *Ae. aegypti,* compared with 43.5% for simple deet, for a net gain of 27.7%. Protection against *An. albimanus* was 94.7%, compared with 73.7% for simple deet, for a net gain of 21.0%, which was not statistically significant. The gain in tests against *Ae. aegypti* (27.7%) was greater than the gain in tests against *An. albimanus* (21.0%).

Formulation B provided 91.3% protection against *Ae. aegypti,* compared with 53.6% for simple deet, for a net gain of 37.7% (Table 1). Protection against *An. albimanus* was 92.9%, compared with 78.6% for simple deet, for a net gain of 14.3%, which was not statistically significant. The gain in tests against *Ae. aegypti* (37.7%) was greater than the gain in tests against *An. albimanus* (14.3%).

Formulations A, B, and 225-44A to 225-44F were tested at intervals up to 24 h against *Ae. aegypti.* Formulations A, B, 225-44D, and 225-44F were significantly more effective than simple deet over the 24-h period (Table 1). Formulation A provided an average 71.2% protection against *Ae. aegypti,* compared with 43.5% for simple deet, for an average gain in effectiveness of 27.7%. The observed gain varied during the 24-h period of testing but was positive and nonzero throughout.

Formulation B was tested against *Ae. aegypti* at 4 and 6 h. The average protection over 6 h was 91.3%, compared with 53.6% for simple deet, for an average gain of 37.7% (Table 1). At 4 h both the formulation and simple deet provided 100% protection, and the observed gain was zero. At 6 h the formulation provided 89.3% protection, compared with 42.9% for simple deet, for a net gain of 46.4%.

Formulation 225-44D provided an average 51.5% protection, compared with 24.0% for simple deet, for an average gain of 27.5% (Table 1). The observed gain varied during the 24-h period of testing and was negative at the 8- and 16-h test periods.

Formulation 225-44F provided an average 66.0% protection, compared with 51.6% for simple deet, for an average gain of 14.4% (Table 1). The observed gain varied during the 24-h period of testing and was zero at the 4-h test period and negative at the 8-h test period.

DISCUSSION

Polymer formulations: In this study, positive results were obtained with formulations 15 and 24, based on a silicone polymer, Dow Corning 200 fluid, 350 centistoke, and formulation 69, based on a fatty acid, Emery Industries 1010 dimer acid (Table 1). Negative results were obtained with formulation 16, based on a different silicone polymer, Dow Corning 200 fluid, 1,000 centistoke, and formulations 25, 26, 27, and 28, based on four acrylate polymers, B. F. Goodrich Chemical Co. Carboset® 526, 525, 514, and 515.

The silicone polymers employed in the study differed primarily in having dynamic viscosities of 350 and 1,000 centistokes, respectively. Positive results were obtained only with formulations based on the 350-centistoke polymer. Formulation 24 was significantly more effective than simple deet in tests against *An. aegypti,* and formulation 15 was significantly more effective than simple deet in tests against *An. albimanus.*

Formulation 69, based on a high molecular weight fatty acid, was significantly more effective than simple deet in tests against both *An. aegypti* and *An. albimanus.* On this basis, formulation 69 was superior to formulations 15 and 24.

Microcapsule formulations: Microcapsule formulations tested in the study contained varying combinations and proportions of lanolin, gum arabic, gelatin, tannic acid, stearic acid, polypropylene glycol, ethanol, water, and a commercial lotion in the microcapsule and carrier fractions. Positive results were obtained only with formulations A, B, 225-44D, and 225-44F.

Qualitative chemical composition did not determine biological effectiveness: No single ingredient was present in all the effective formulations and at the same time absent from all the ineffective formulations; conversely, no single ingredient was absent from all the effective compounds and at the same time present in all the ineffective compounds. The possibility of quantitative chemical effects on biological effectiveness cannot be excluded.

However, microcapsule formulations have important physical dimensions not found in other preparations, including the composition, number, and size of the microcapsules and the thickness of the capsule walls. These characteristics are determined by details of manufacture that are highly technical and usually proprietary in nature. The present study has, at least, established that microencapsulation is a viable approach to the formulation of repellents for increased effectiveness and persistence. Four of 9 microcapsule formulations tested were significantly more effective than simple deet (Table 1).

Spectrum of activity: Polymer formulation 69 was significantly more effective than simple deet in tests against both *An. albimanus* and *Ae. aegypti,* whereas polymer formulation 24 and microcapsule formulations A and B were significantly more effective than simple deet in tests against *Ae. aegypti* only. Because *An. albimanus* is more tolerant of repellents than is *Ae. aegypti* (Rutledge et al. 1978, 1983; Schreck 1985), this result can be understood in terms of threshold levels. Controlled-release formulations that release deet at rates above the threshold level of response of *Ae. aegypti* but below the threshold level of response of *An. albimanus* will necessarily be effective against the former but not the latter. For this reason, we believe that the opposite result obtained in tests of polymer formulation 15 (Table 1) may be in error.

Statistical interaction: Microcapsule formulations A, B, 225-44D, and 225-44F were significantly more effective than simple deet against *Ae. aegypti* for up to 24 h (Table 1). However, the gain in effectiveness attributable to formulation was variable over time, being zero or negative in some cases. In the case of formulation 225-44D, the interaction of treatments (control, standard, and formulation) with test periods (4, 6, 8, . . . 24 h) was not statistically significant ($F = 1.56$, df = 12,759, $P > 0.05$), indicating that the observed variation in gain was statistically random. This was also true of formulation B, which was tested at 4 and 6 h only ($F = 1.52$, df = 2,144, $P > 0.05$).

However, the interaction of treatments with test periods was statistically significant for formulation A ($F = 4.83$, df = 14,516, $P < 0.05$) and formulation 225-44F ($F = 2.11$, df =

12,759, $P < 0.05$). Fisher's (protected) least significant difference test indicated that formulation A was significantly more effective than simple deet in tests at 4, 8, 12, 16, and 20 h, whereas formulation 225-44F was significantly more effective than simple deet in tests at 6 and 16 h only. On this basis, formulations A, B, and 225-44D were more consistently effective than formulation 225-44F.

Coefficient of variation: In the context of the present study, the coefficient of variation is a function of the cell means, which are determined by the concentration of deet applied and the elapsed time from the time of application. The largest value obtained in tests against *Ae. aegypti* was 25.2% for tests of the 6.1% concentration at 18 h, and the largest value obtained in tests against *An. albimanus* was 34.6% for tests of the 6.1% concentration at 4 h. These values are well within the range expected for biological data in general (Simpson et al. 1960) and repellent data in particular (Busvine 1971).

CONCLUSIONS

This study confirms prior reports of enhanced performance of deet in both polymer and microcapsule formulations. Increased effectiveness was demonstrated in tests against both *Ae. aegypti* and *An. albimanus* (polymer formulation 69) and in tests extending up to 24 h (microcapsule formulations A and 225-44D).

Intensive testing of controlled-release formulations of deet is necessary to ensure that the spectrum of efficacy of deet is not compromised and to ensure that the gain in effectiveness attributable to formulation is continuous throughout the test period.

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