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Evaluation of Mosquito Repellent Formulations

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Abstract □ *N,N*-Diethyl-*m*-toluamide was formulated with several acrylate polymers in ethanol solution and various silicone polymers in 2-propanol suspension; the ratio of polymer to *N,N*-diethyl-*m*-toluamide (I) was varied. Formulations that had drying times of <10 min were evaluated for film hardness and elasticity. Contact angles made by water on films cast from the formulations were measured when such films were uniform. For the acrylate formulations, containing polymers that are solid at room temperature, the presence of I increased drying times; decreased film hardness and elasticity resulted from decreasing the ratio of polymers to I. Lower contact angle with water resulted from decreasing the ratio of acrylate polymer to I. However, this effect was less pronounced with the lower molecular weight acrylate polymer formulations. Films cast from the silicone formulations had low contact angles with water. In addition, formulations of repellents, ethohexadiol and *N,N*-diethyl-*p*-toluamide, each in combination with a silicone polymer, were evaluated. Films with short drying times, high contact angle, and measurable hardness could be cast from the *N,N*-diethyl-*p*-toluamide–silicone formulations due to the film-forming ability of the repellent itself. The physical properties of the ethohexadiol–silicone formulations were similar to the I–silicone formulations. Selected formulations received preliminary evaluation for duration of effectiveness against *Aedes aegypti* mosquitoes *in vitro* and in animal test systems. Except for one formulation of I with a lower molecular weight acrylate polymer, these formulations did not enhance the duration of effectiveness of I on hairless dogs. The *in vitro* ED_{50} of the test repellent for *A. aegypti* was significantly enhanced in 5 of 15 formulations tested. The 4-hr ED_{50} of the test repellent on white mice was significantly enhanced in 6 of 15 formulations tested.

Keyphrases □ Repellents, mosquito—film hardness and elasticity evaluation for *N,N*-diethyl-*m*-toluamide–acrylate polymer formulations □ *N,N*-Diethyl-*m*-toluamide—formulation with acrylate polymer, evaluation for film hardness and elasticity in mosquito repellents □ Polymers—*N,N*-diethyl-*m*-toluamide formulations, evaluation for film hardness and elasticity in mosquito repellents

The duration of protection afforded by a mosquito repellent is limited by the ways it can be lost from the skin surface, such as abrasion and removal by water immersion (1) and excessive evaporation and penetration into the skin (2, 3). Many efforts have been made to improve the persistance of mosquito repellents by incorporating the active ingredient (usually *N,N*-diethyl-*m*-toluamide, I) with a variety of materials such as polysaccharide esters or silicone and acrylic polymers (4), clay (5), zinc oxide (6), vanillin (7), and others. However, there remains to be found a repellent formulation which has acceptable cosmetic and toxicologic properties and has significantly

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Table I—Composition and Water Contact Angle Measurement of Mosquito Repellent–Acrylate Polymer Formulations

Formulation No.	*N,N*-Diethyl-*m*-toluamide concentration, % (w/v)	Polymer concentration, % (w/v)	Contact angle, degree[a]
N,N-Diethyl-*m*-toluamide–Polymer I Formulations			
36	3.9	0.4	10 ± 1
28	3.9	2.0	17 ± 1
37	3.9	3.9	31 ± 2
38	3.9	7.8	33 ± 2
39	3.9	11.8	—[b]
63	0.0	3.9	46 ± 1
N,N-Diethyl-*m*-toluamide–Polymer II Formulations			
32	3.9	0.4	10 ± 1
27	3.9	0.6	12 ± 2
33	3.9	3.9	12 ± 1
34	3.9	7.8	19 ± 1
35	3.9	11.8	25 ± 1
64	0.0	3.9	14 ± 4
N,N-Diethyl-*m*-toluamide–Polymer III Formulations			
11	6.1	3.1	32 ± 1
44	3.9	0.4	25 ± 1
25	3.9	2.0	42 ± 4
17	3.9	3.9	51 ± 1(2)
18	3.9	7.8	65 ± 2
19	3.9	11.8	70 ± 4(2)
61	0.0	3.9	71 ± 1
N,N-Diethyl-*m*-toluamide–Polymer IV Formulations			
40	3.9	0.4	14 ± 1
26	3.9	2.0	41 ± 4
41	3.9	3.9	47 ± 0
42	3.9	7.8	79 ± 4
43	3.9	11.8	71 ± 3
62	0.0	3.9	75 ± 1

[a] Contact angle measurements were replicated three times, except where number of replicates are indicated in parentheses following value, mean ± *SD*. [b] Film surface was too irregular to allow measurement.

Table II—Composition and Water Contact Angle Measurement of Mosquito Repellent–Silicone Polymer Formulations

Formulation No.	Repellent concentration, % (w/v)	Polymer concentration, % (w/v)	Contact angle, degree[a]
N,N-Diethyl-*m*-toluamide–Polymer V Formulations			
15	6.1	3.1	8 ± 2
50	3.9	0.4	10 ± 1
30	3.9	2.0	8 ± 1
24	3.9	3.9	—[b]
N,N-Diethyl-*m*-toluamide–Polymer VI Formulations			
16	6.1	3.1	—[b]
53	3.9	0.4	11 ± 1
31	3.9	2.0	—[b]
N,N-Diethyl-*m*-toluamide–Polymer VII Formulations			
14	6.1	3.1	7 ± 2
47	3.9	0.4	13 ± 1
29	3.9	2.0	10 ± 1
23	3.9	3.9	23 ± 4(5)
N,N-Diethyl-*m*-toluamide–Polymer VIII Formulations			
57	3.9	0.4	10 ± 1
58	3.9	2.0	16 ± 1
22	3.9	3.9	18 ± 1
Ethohexadiol–Polymer VIII Formulation			
12	3.9	2.0	19 ± 1
N,N-Diethyl-*p*-toluamide–Polymer VIII Formulation			
13	3.9	2.0	72 ± 1

[a] Contact angle measurements were replicated three times, except where the number of replicates are indicated in parentheses following value, mean ± *SD*. [b] Film surface was too irregular for determination of contact angle with water.

better duration of protection than unformulated I. In an effort to understand better the results obtained by adding I to various polymers, I was formulated in varying ratios to several commercially available silicone and acrylate polymers, some of which have been used extensively by the cosmetic industry.

EXPERIMENTAL

Materials—The materials used for the preparation of mosquito repellent formulations were acrylate polymers[1] I–IV, silicone polymers[2] V–VIII, and the mosquito repellents *N,N*-diethyl-*m*-toluamide[3], *N,N*-diethyl-*p*-toluamide[4], and ethohexadiol[5]. The composition of the formulations is listed in Tables I and II. Acrylate polymers III and IV required heating to effect solution in ethanol.

Determination of Drying Times—Films were cast on glass microscope slides by using a mechanical drive[6] and an applicator[7] with 0.19-mm wet film thickness and allowed to dry at ambient conditions (40% relative humidity and 23°). Using a cotton ball, the slide was checked for a dry tack-free surface every 2 min up to 15 min, every 10 min up to 1 hr, and then every hour up to 8 hr. Drying times were done in duplicate.

Film Hardness, Film Thickness, and Modulus of Elasticity—These were determined by published procedures (8). Films were cast as for drying time determinations; however, the substrate was a polished aluminum plate. Films were tested 4 hr after they were cast. The modulus of elasticity (E) was calculated by:

$$E = \frac{KR^3}{T^3}$$

where R is the Sward hardness (number of rocks) and K is a constant for thickness (T) determined by extrapolation from data in the literature (9).

Contact Angle—Films were cast on a glass microscope slide cut in half lengthwise, by the same procedure as for drying time determinations. Fifteen minutes after the film was cast, the slide was inserted in the contact angle viewer[8]. A drop of distilled water was placed on the film, and the angles, formed by the base of the drop on its left and right sides, were read. The volume of the drop was then increased by small increments until the left and right side angles were the same and remained constant. This final angle is reported as the contact angle. The determination was replicated three times.

Protection Time—The protection time of the formulations was determined on hairless dogs using a published procedure (10). After all the application sites on the dog's skin had failed, the skin was scrubbed with soap[9] and water to remove residual polymer. Dogs were usually rested 3–4 days between tests.

Median Effective Dosage—The median effective doses (ED_{50}) of 19 formulations for the yellow fever mosquito, *Aedes aegypti*, were determined by a previous method (11). This method utilizes an *in vitro* mosquito blood-feeding system having test surfaces of goldbeater's skin (the prepared outside membrane of the large intestine of cattle used for separating the leaves of metal in goldbeating).

Four-Hour Median Effective Dose—Four-hour ED_{50} values of 19 formulations for *A. aegypti* were determined on 7–10-day-old white mice. Four mice were wetted with serial dilutions of the test formulation, and a fifth (the control) was wetted with a corresponding solvent (ethanol or 2-propanol). The mice were held at 27° for 4 hr, after which they were transferred to a 30 × 30 × 30-cm mosquito cage containing 100 nulliparous, 5–15-day-old female *A. aegypti*. The number of mosquitoes feeding on each mouse was recorded at 2 min intervals for a period of 30 min. The totals of the 10 feeding counts obtained for each of the mice were combined with the corresponding totals from subsequent replicates of the test, and the totals for each dose were then converted to percentages of

[1] Acrylate polymer I is Carboset 515, II is Carboset 514, III is Carboset 526, and IV is Carboset 525. Polymers were obtained from B. F. Goodrich Chemical Co., Cleveland, Ohio.
[2] Silicone polymer V is 200 fluid, 350 centistoke; VI is 200 fluid, 1000 centistoke; VII is QF13593A; and VIII is MDX 4-4142. Polymers were obtained from Dow Corning Corp., Midland, Mich.
[3] Eastman Kodak Co., Rochester, N.Y.
[4] Hercules, Inc., Wilmington, Del.
[5] Niagara Chemical Division, FMC, Middleport, N.Y.
[6] Gardner mechanical drive, Gardner Laboratory, Bethesda, Md.
[7] Gardner film casting knife, Gardner Laboratory, Bethesda, Md.
[8] Contact angle viewer (No. D-1060), Gardner Laboratory, Bethesda, Md.
[9] Ivory soap, Proctor & Gamble Corp., Cincinnati, Ohio.

Table III—Film Characteristics of Polymers and Mosquito Repellent Formulations with Short Drying Times [a]

Formulation No.	Repellent–Additive ratio	Drying time, min	Film thickness, μm	Sward hardness, No. of rocks	Modulus of elasticity, ×10⁴ dyne/cm²
		Polymer II			
64	0	9	3	6	430
	N,N-Diethyl-*m*-toluamide–Polymer III Formulations				
18	1/2	7	15	5	590
19	1/3	7	18	9	3700
61	0	5	9	11	4800
		Polymer IV			
62	0	6	7	11	4100
	N,N-Diethyl-*p*-toluamide–Polymer VIII Formulation				
13	2/1	5	4	4	149

[a] Film characteristics were not measured for formulations with drying times >10 min.

the total for the control. The 4-hr ED_{50} was computed from the percentages and the logarithm doses by probit analysis.

RESULTS AND DISCUSSION

The composition and contact angle measurements of the acrylate formulations are given in Table I and of the silicone formulations in Table II. Film thickness, Sward hardness, and modulus of elasticity of the acrylate and silicone formulations with drying times <10 min are given in Table III. The protection times determined on the hairless dog of the acrylate formulations are given in Table IV and of the silicone formulations in Table V. The ED_{50}s and 4-hr ED_{50}s are given in Tables VI and VII and VIII and IX, respectively.

The acrylate polymers are derived from acrylic acid esters, methacrylic acid esters, and α,β-unsaturated carboxylic acids. The mean molecular weight of polymer I is 7000, II is 30,000, III is 200,000, and IV is 260,000. Polymer I is a viscous liquid at room temperature, while polymer II was obtained as a 30% solution of polymer in ammonia water with a final pH of 7.5; percentages of polymer II in Table I are based on the weight of polymer in solution.

For the I–acrylate formulations containing the higher molecular weight polymers, III and IV, increasing the ratio of polymer to a constant amount of I resulted in higher contact angles (Table I). The same trend was observed with Compound I–polymer I and II formulations, although it was less pronounced. The low contact angles for polymer II formulations were probably the result of the presence of water in polymer II and salt formation between the ammonia present and carboxylic acid functional groups.

Table IV—Protection Times Against *A. aegypti* Mosquitoes for Selected Repellent–Acrylate Polymer Formulations Tested on the Hairless Dog

Formulation No.	Repellent dose, mg/cm²[a]	Protection time, hr mean ± *SD*	*N*[b]	Significance[c]
	N,N-Diethyl-*m*-toluamide–Polymer I Formulations			
28	0.32	10.6 ± 1.1	7	S
Control[d]	0.32	6.4 ± 1.6	7	—
	N,N-Diethyl-*m*-toluamide–Polymer II Formulations			
27	0.32	6.5 ± 1.9	16	NS
Control[d]	0.32	6.5 ± 1.9	16	—
	N,N-Diethyl-*m*-toluamide–Polymer III Formulation			
11	0.5	9.6 ± 2.9	16	NS
Control[d]	0.5	8.5 ± 1.8	16	—
25	0.32	7.5 ± 2.4	16	NS
Control[d]	0.32	8.2 ± 2.1	16	—
17	0.32	9.8 ± 1.6	8	NS
Control[d]	0.32	9.4 ± 1.8	8	—
	N,N-Diethyl-*m*-toluamide–Polymer IV Formulations			
26	0.32	7.3 ± 1.8	16	NS
Control[d]	0.32	6.9 ± 1.9	16	—

[a] Dose obtained when 0.4 ml of formulation is spread over a 49-cm² skin area. [b] Number of replicates. [c] Significance was tested at the 95% confidence level using Dunnett's test for comparing *K* means with a control. [d] *N,N*-Diethyl-*m*-toluamide, dissolved in alcohol, was applied to the skin using the same volume as was used for application of the formulation to give the same dose of *N,N*-diethyl-*m*-toluamide.

Table V—Protection Time Against *A. aegypti* Mosquitoes for Selected Repellent–silicone Polymer Formulations Tested on the Hairless Dog

Formulation No.	Repellent, mg/cm²[a]	Protection time, hr mean ± *SD*	*N*[b]	Significance[c]
	N,N-Diethyl-*m*-toluamide–Polymer V Formulations			
15	0.50	4.8 ± 2.4	8	NS
Control[d]	0.50	6.8 ± 3.0	8	—
30	0.32	6.0 ± 0.8	8	NS
Control[d]	0.32	5.4 ± 0.8	8	—
24	0.32	8.1 ± 3.9	16	NS
Control[d]	0.32	8.3 ± 2.4	16	—
	N,N-Diethyl-*m*-toluamide–Polymer VI Formulations			
16	0.50	4.8 ± 1.5	8	NS
Control[d]	0.50	6.8 ± 3.0	8	—
31	0.32	6.0 ± 0.8	8	NS
Control[d]	0.32	5.4 ± 0.8	8	—
	N,N-Diethyl-*m*-toluamide–Polymer VII Formulations			
14	0.50	6.1 ± 2.3	8	NS
Control[d]	0.50	6.8 ± 3.0	8	—
29	0.32	5.9 ± 1.2	8	NS
Control[d]	0.32	5.4 ± 0.8	8	—
23	0.32	8.8 ± 2.9	16	NS
Control[d]	0.32	8.3 ± 2.9	16	—
	N,N-Diethyl-*m*-toluamide–Polymer VIII Formulation			
22	0.32	9.2 ± 2.8	16	NS
Control[d]	0.32	8.3 ± 2.4	16	—
	Ethohexadiol–Polymer VIII Formulation			
12	0.50	5.5 ± 2.2	16	S
Control[d]	0.50	8.5 ± 1.8	16	—
	N,N-Diethyl-*p*-toluamide–Polymer VIII Formulation			
13	0.50	5.4 ± 2.9	16	S
Control[d]	0.50	8.5 ± 1.8	16	—

[a] Dose obtained when 0.4 ml of formulation is spread over a 49-cm² skin area. [b] Number of replicates. [c] Significance was tested at the 95% confidence level using Dunnett's test for comparing *K* means with a control. [d] *N,N*-Diethyl-*m*-toluamide, dissolved in alcohol, was applied to the skin using the same volume as was used for application of the formulation to give the same dose of *N,N*-diethyl-*m*-toluamide.

The silicone polymers, V and VI, are linear polydimethylsiloxanes. Polymer V has ~200 dimethylsiloxane units and a molecular weight of ~15,000. Polymer VI has a molecular weight of ~25,000, which corresponds to 336 dimethylsiloxane units. Polymer VII is composed of a linear polydimethylsiloxane (~100 dimethylsiloxane units) and a highly crosslinked trimethylsiloxysilicate resin. Polymer VIII is related to polymer VII and is made by a slightly different manufacturing procedure[10].

Contact angles of water with the silicone formulations were generally low relative to those of the acrylate formulations and bore little apparent relationship to the ratio of repellent to polymer. The exception was formulation 13. In this case the *N,N*-diethyl-*p*-toluamide (which is a solid at room temperature, in contrast to the *meta* isomer) itself forms a film (visible films of *N,N*-diethyl-*p*-toluamide sometimes can be observed after the repellent is applied in alcoholic solution to human skin).

For each silicone polymer tested, formulations containing higher ratios of polymer to I than those in Table II were prepared along with suspensions of silicone polymers alone in 2-propanol. Films cast from these suspensions did not dry, and the surface of the films were too irregular for measurement of the contact angle with water.

With the exception of formulations 13, 18, and 19 (Table III), all of the mosquito repellent polymer formulations had drying times >8 hr (formulations containing silicone polymers or acrylate polymer I were not expected to dry, because the polymers are liquid at room temperature), and films cast from these formulations were not tested for hardness because of a probable change in component ratios due to evaporation of the repellent during the drying period required. The plasticizing effect of I on the acrylate polymers III and IV was shown by comparing formulation

[10] Personal communication, Mike Starch, Dow Corning Corp., Midland, Mich.

Table VI—*In Vitro* ED_{50}s of Selected Repellent–Acrylate Polymer Formulations for *A. aegypti*

Formulation No.	N[a]	ED_{50}, mg/cm^2[b]	95% Confidence interval	Significance[c]
N,N-Diethyl-*m*-toluamide–Polymer I Formulation				
28	2	0.013	0.006–0.019	S
N,N-Diethyl-*m*-toluamide–Polymer II Formulation				
27	2	0.030	0.020–0.046	NS
N,N-Diethyl-*m*-toluamide–Polymer III Formulations				
11/25[d]	4	0.017	0.012–0.022	NS
17	2	0.031	0.024–0.041	NS
18	2	0.009	0.004–0.013	S
19	2	0.019	0.011–0.027	NS
N,N-Diethyl-*m*-toluamide–Polymer IV Formulation				
26	2	0.021	0.013–0.029	NS
N,N-Diethyl-*m*-toluamide in Ethanol				
—	6	0.031	0.020–0.041	—

[a] Number of replicates. [b] Refers to the dosage of active ingredient (*N,N*-diethyl-*m*-toluamide). [c] The ED_{50}s of formulated and unformulated *N,N*-diethyl-*m*-toluamide are significantly different if their respective confidence intervals do not overlap. [d] Formulations having the same active ingredient–polymer ratio (Tables I and II) are equivalent in the ED_{50} test.

Table VIII—Four-hour ED_{50}s of Selected Repellent–Acrylate Polymer Formulations on White Mice Against *A. aegypti*

Formulation No.	N[a]	4-hr ED_{50}, % concentration[b]	95% Confidence interval	Significance[c]
N,N-Diethyl-*m*-toluamide–Polymer I Formulation				
28	8	0.05	0.002–0.12	NS
N,N-Diethyl-*m*-toluamide–Polymer II Formulation				
27	6	0.03	0.006–0.06	S
N,N-Diethyl-*m*-toluamide–Polymer III Formulations				
11/25[d]	14	0.03	0.002–0.06	S
17	3	0.06	0.000–0.12	NS
18	3	0.07	0.000–0.15	NS
19	6	0.20	0.000–0.47	NS
N,N-Diethyl-*m*-toluamide–Polymer IV Formulation				
26	6	0.03	0.010–0.06	S
N,N-Diethyl-*m*-toluamide in Ethanol				
—	6	0.16	0.108–0.22	—

[a] Number of replicates. [b] Refers to the concentration of active ingredient (*N,N*-diethyl-*m*-toluamide). [c] The 4-hr ED_{50}s of formulated and unformulated *N,N*-diethyl-*m*-toluamide are significantly different if their respective confidence intervals do not overlap. [d] Formulations having the same active ingredient–polymer ratio (Tables I and II) are equivalent in the 4-hr ED_{50} test.

61 with 17 for polymer III and comparing formulation 62 with 41 for polymer IV. The contact angle with water was higher, drying time was shorter, and films cast from the formulations were harder when I was absent. A similar observation can be made for polymer II (compare formulation 64 with 33); however, in this case, the presence of ammonia and water in the polymer obscures the effect of I on the contact angle. Since polymer I is a liquid, the effects of Compound I on drying time and film hardness are irrelevant. The plasticizing effect of I is not limited to the acrylate polymers. This effect occurs with a variety of polymers, which causes user acceptability problems (*e.g.*, softening or marring of plastics and painted surfaces).

The effect of the increasing modulus of elasticity (Table III) with higher ratios of polymer III to constant levels of I is illustrated in the following observations. Formulations 17–19 were applied to hairless dogs along with a I–ethanol control which gave the same dose of I per unit area (0.32 mg/cm^2). Four hours after application, the sites were observed. As expected, the I–ethanol treated sites were not noted to be any different than the surrounding nonapplication areas of skin. A film was visible on five of the eight sites to which formulation 17 was applied; however, there was no evidence of cracking or peeling. With formulation 18, a film had peeled from two of the application sites. With formulation 19, six sites were peeling, and on another site the film had cracked.

The results of the longer drying times associated with lower ratios of polymer III to compound I were the production of sticky, cosmetically unacceptable films when formulation 25 was tested on human subjects (4).

If the contact angle to water by films cast from the formulations is an indication of the wash resistance of the formulation, then the acrylate

Table VII—*In Vitro* ED_{50}s of Selected Repellent–Silicone Polymer Formulations for *A. aegypti*

Formulation No.	N[a]	ED_{50}, mg/cm^2[b]	95% Confidence interval	Significance[c]
N,N-Diethyl-*m*-toluamide–Polymer V Formulations				
15/30[d]	4	0.019	0.014–0.025	NS
24	2	0.027	0.020–0.037	NS
N,N-Diethyl-*m*-toluamide–Polymer VI Formulation				
16/31[d]	4	0.015	0.011–0.019	
N,N-Diethyl-*m*-toluamide–Polymer VII Formulations				
14/29[d]	4	0.017	0.013–0.020	NS
23	2	0.005	0.002–0.008	S
N,N-Diethyl-*m*-toluamide–Polymer VIII Formulation				
22	2	0.021	0.013–0.028	NS
Ethohexadiol–Polymer VIII Formulation				
12	2	0.027	0.019–0.036	S
N,N-Diethyl-*p*-toluamide–Polymer VIII Formulation				
13	2	0.015	0.010–0.020	[e]
N,N-Diethyl-*m*-toluamide in Ethanol				
—	6	0.031	0.020–0.041	—
Ethohexadiol in Ethanol				
—	4	0.113	0.081–0.197	—

[a] Number of replicates. [b] Refers to the dosage of active ingredient (*N,N*-diethyl-*m*-toluamide, ethohexadiol, or *N,N*-diethyl-*p*-toluamide). [c] The ED_{50}s of formulated and unformulated *N,N*-diethyl-*m*-toluamide or ethohexadiol are significantly different if their respective confidence intervals do not overlap. [d] Formulations having the same active ingredient–polymer ratio (Tables I and II) are equivalent in the ED_{50} test. [e] Unformulated *N,N*-diethyl-*p*-toluamide not tested.

Table IX—Four-hour ED_{50}s of Selected Repellent–Silicone Polymer Formulations on White Mice Against *A. aegypti*

Formulation No.	N[a]	4-hr ED_{50}, % concentration[b]	95% Confidence interval	Significance[c]
N,N-Diethyl-*m*-toluamide–Polymer V Formulations				
15/30[d]	9	0.03	0.000–0.06	S
24	9	0.02	0.001–0.05	S
N,N-Diethyl-*m*-toluamide–Polymer VI Formulation				
16/31[d]	15	0.02	0.002–0.05	S
N,N-Diethyl-*m*-toluamide–Polymer VII Formulations				
14/29[d]	11	0.01	(not determined)	NS
23	4	0.08	0.003–0.16	NS
N,N-Diethyl-*m*-toluamide–Polymer VIII Formulation				
22	6	0.04	0.000–0.11	NS
Ethohexadiol–Polymer VIII Formulation				
12	3	0.08	0.061–0.10	NS
N,N-Diethyl-*p*-toluamide–Polymer VIII Formulation				
13	9	0.05	0.004–0.10	—[e]
N,N-Diethyl-*m*-toluamide in Ethanol				
—	6	0.16	0.108–0.22	—
Ethohexadiol in Ethanol				
—	12	0.15	0.030–0.26	—

[a] Number of replicates. [b] Refers to the concentration of active ingredient (*N,N*-diethyl-*m*-toluamide, ethohexadiol, or *N,N*-diethyl-*p*-toluamide). [c] The ED_{50}s of formulated and unformulated *N,N*-diethyl-*m*-toluamide or ethohexadiol are significantly different if their respective confidence intervals do not overlap. [d] Formulations having the same active ingredient–polymer ratio (Tables I and II) are equivalent in the 4-hr ED_{50} test. [e] Unformulated *N,N*-diethyl-*p*-toluamide not tested.

formulations such as those made from polymers III or IV would be expected to provide greater wash resistance than the I–silicone formulations. In tests on human subjects, acrylate formulations such as 25, but not silicone formulations, enhanced I wash resistance (4). However, the contact angles would not necessarily reflect a difference in wash resistance due to a difference in film adhesion to the skin or a difference between a solid flexible film and a liquid film with similar contact angles.

In a preliminary test on hairless dogs, only one of the I formulations (28) appeared to provide greater duration of protection against *A. aegypti* mosquitoes than unformulated I. Results obtained by the ED_{50} and 4-hr test methods were more promising. The ED_{50} of the test repellent was significantly enhanced in two of the seven repellent–acrylate polymer formulations and in three of the eight repellent–silicone polymer formulations (Tables VI and VII). The 4-hr ED_{50}, which measures the combined effects of repellency and persistence on the skin of the test animal, was enhanced significantly in three of the seven repellent–acrylate polymer formulations and in three of the eight repellent–silicone polymer formulations (Tables VIII and IX). Both ED_{50} and 4-hr ED_{50} were enhanced in only one formulation (16, Tables VII and IX). This demonstrates that repellency and persistence on the skin are fundamentally different properties of the repellent formulation. As has been pointed out (12), these two properties of topical repellents have usually been confounded in the past.

In future studies with mosquito repellent formulations, the release kinetics of the repellent (either to evaporation or penetration) will be examined using an *in vitro* skin evaporation–penetration apparatus. Additional knowledge will permit more than an empirical approach to the design of longer lasting mosquito repellent formulations.

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Vaginal Absorption of a Potent Luteinizing Hormone–Releasing Hormone Analogue (Leuprolide) in Rats III: Effect of Estrous Cycle on Vaginal Absorption of Hydrophilic Model Compounds

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Abstract □ The effect of estrous cycle stages on vaginal absorption was determined by the use of insulin, phenolsulfonphthalein, and salicylic acid as hydrophilic model compounds. Absorption of these compounds was markedly affected by the stage, possibly due to the change of transport rate through the pore-like pathways. The absorption of phenolsulfonphthalein during proestrus and estrus is roughly one-tenth of that during metestrus and diestrus. An increase of the nonionized form of salicylic acid, produced by a lowered pH, resulted in an enhancement of absorption during proestrus and diestrus; higher contribution of the transport through the cell membrane possibly reduced an effect of the estrous cycle. However, consecutive daily administration of leuprolide halted the cycle at diestrus and reduced the cycle effect on the vaginal absorption of phenolsulfonphthalein; when the treatment was started at any of the four stages of the cycle, vaginal absorption was enhanced ~20%, with less variance than that observed in normal diestrous rats.

Keyphrases □ Absorption, vaginal—luteinizing hormone–releasing hormone, leuprolide, effect of estrous cycle on vaginal absorption of hydrophilic model compounds □ Leuprolide—effect of estrous cycle on vaginal absorption of hydrophilic model compounds □ Luteinizing hormone–releasing hormone analogue—effect of estrous cycle on vaginal absorption of hydrophilic model compounds □ Releasing hormone analogue—luteinizing hormone, effect of estrous cycle on vaginal absorption of hydrophilic model compounds

In previous studies (1, 2), vaginal application was proposed as a rational dosage method for long-term self-administration of hydrophilic drugs, because leuprolide (I), a potent luteinizing hormone–releasing hormone (II) analogue, and several hydrophilic compounds (phenolsulfonphthalein, insulin, and II) are well absorbed through the vaginal membrane of diestrous rats.

(Pyro)Glu-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NH-CH_2CH_3

I

(Pyro)Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2

II

In those studies, vaginal absorbability was estimated at the diestrus only, since the ovulation-inducing activity of leuprolide could be examined during other stages.

The estrous cycle of the rat is completed in 4–5 days, and during this cycle changes in the vaginal mucosal membrane, the ovaries, and the uterus occur (3). Similar, but not as remarkable, changes of the vaginal mucosa occur in women during the menstrual cycle (4).

In the present study, the effect of estrous cycle stages on vaginal absorption was determined with phenolsulfonphthalein, insulin, and salicylic acid in rats. Furthermore, as continuous administration of leuprolide halts the estrous cycle of rats at diestrus (5), the vaginal absorption of phenolsulfonphthalein following consecutive subcutaneous injection of the analogue over a 10-day period was also estimated.

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