EVALUATION OF MOSQUITO REPELLENTS

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The purpose of the present study was to evaluate the repellent effect of various compounds against a number of blood-sucking insects. The compounds used were synthesized by and obtained from the U.S. Dept. of Agriculture. This paper is concerned with the tests conducted on mosquitoes.

The mosquito *Culex pipiens molestus* Forskal is the most common domestic mosquito in Israel. It is a fierce biter, and is abundant in various parts of the country, especially in the spring and summer. A method has been developed for evaluating candidate repellents on rabbits. The best repellents thus obtained will later be evaluated against this species of mosquito on humans in the field. In order to test the validity of this method, 87 compounds were also evaluated against the mosquito *Aedes aegypti* (L). The compounds used by us have already been evaluated by the U.S. Dept. of Agriculture against *A. aegypti* (as well as against other blood-sucking insects). They all belong to their best category, i.e. class 4 or 4A (King, 1954; Anonymous, 1967) and represent a selection from thousands of compounds. These compounds include well known repellents developed by the U.S. Dept. of Agriculture. Products of this research are now widely used throughout the world. (Altman and Smith, 1955; Gilbert, 1955; 1957; Gilbert, Gouck and Smith, 1957; Smith, 1965).

MATERIALS AND METHOD. The belly of a rabbit, which had been immobolized, dorsal side down in a holder, was shaven with an electric clipper, and then smoothed by a depilatory paste.[1] A rubber sheet (17.5 x 10 cm. and 0.6 cm. thick) with two circular holes (7 cm in diameter), 1 cm apart from each other, was firmly attached to the shaven belly by three rubber bands which were tightened around the belly to the desired tension. Inside each hole of the rubber a glass ring (7 cm outside diameter, 6.3 cm inside diameter and 2 cm high) was inserted. Thus, any possible movement by the tied rabbit will not move the rings from their fixed position. (Fig. 1).

Half a ml of a 5 percent solution of a candidate repellent in ethanol was spread with a hypodermic syringe and rubbed evenly with a glass rod inside the area of one of the above mentioned rings (31.1 cm^2). A similar aliquot of another compound was applied inside the area of the second ring. A small glass ring (0.5 cm. thick and 0.5 cm. high) fitting exactly into the above mentioned ring was then placed inside it (see Fig. 1). The purpose of the inner ring is to prevent any possible contamination of the testing cage by the repellent. The testing cage (16 x 25 x 25 cm), containing several hundreds of 5–7 day old *C.p. molestus* was then placed on the rabbit. The bottom of the cage has in its center a round opening enclosed by a glass ring, which fits inside the ring attached to the belly of the rabbit, but cannot touch the treated area because of the smaller inside ring. The treated area of the belly was then exposed to the mosquitoes, by removing a temporary cover from the round opening of the cage. During this time the room was completely darkened. At the end of the exposure period (5 minutes) light was turned on, and the number of mosquitoes engorging was observed through the glass top of the cage. The testing cage was then removed from the rabbit. Preliminary experiments have shown that the feeding process of this mosquito including search, alighting, feeding etc., usually lasted more than the 5 minutes of the test, and that mosquitoes which have started to engorge were not interrupted by light. More

[1] Mishi Manufacturer, P.O. Box 626, Tel-Aviv.

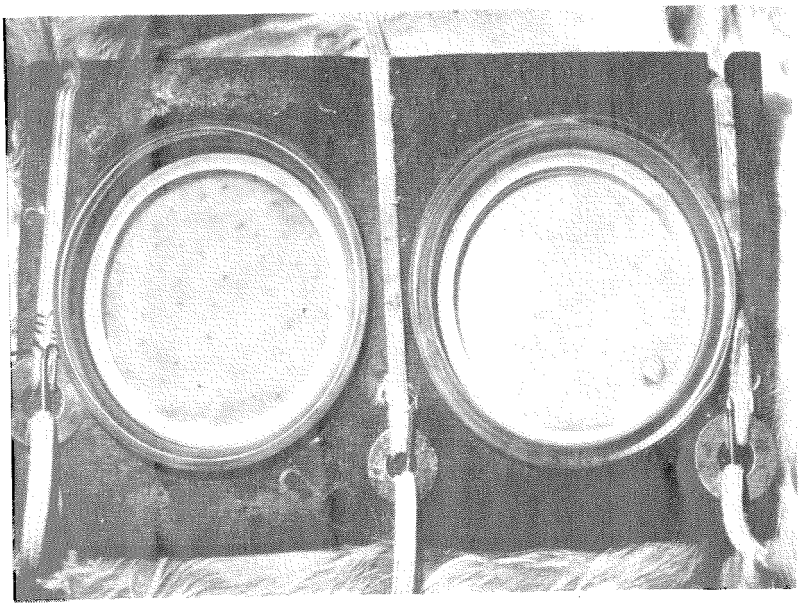

FIG. 1.—Shaved belly of rabbit exposed to mosquitoes.

than 30 mosquitoes, out of a few hundred in the testing cage, fed on an untreated belly during this period. The first test was carried out 1 hour after the application of the repellent, followed by successive tests at hourly intervals, for a length of time depending on the quality of the repellent.

The candidate repellents were classified into the following categories according to the number of mosquitoes feeding after various periods of time after treatment:

A. Five or more mosquitoes fed 1 hour after application of the repellent in at least 2 replicates.
B. A total of 5 or more mosquitoes fed 1 and 2 hours after application of the repellent in at least 2 replicates.
C. As above after 3 hours.
D. As above after 4 hours.
E. As above after 5 hours.
F. Better results than those mentioned above.

Compounds falling into the above best category (F) were then re-evaluated in a 1 percent solution in ethanol and divided into four categories, similar to categories A to D mentioned above (except that category D included also compounds which gave better results). Compounds falling into the best category (D) were then either compared in paired tests with deet (*N,N*-diethyl-*m*-toluamide), used as a standard, or evaluated in balanced-incomplete block tests. These latter tests consisted of groups of 5 repellents each, and deet; each repellent was paired twice against each other repellent. A statistical analysis was made according to Kempthorne (1952), as suggested by I. H. Gilbert of the U.S.D.A. From an analysis of variance the least significant difference (LSD) at the 5 percent level, and 1 percent level between any two repellents was determined. The protection period was taken as the time (hrs.) at which no more than 5 feedings were obtained. The relative effectiveness of the repellent to deet was expressed as the ratio of the protection period.

Tests against *Aedes aegypti* were carried out in a similar manner, except that

there was no need to darken the room during the test, because this mosquito also bites in daylight. The belly of the rabbit was exposed to the mosquitoes for 3 minutes instead of 5.

RESULTS AND DISCUSSION. A total of 279 compounds were evaluated against *C.p. molestus*. Twenty-four out of 151 compounds, which fell into the best category were evaluated in paired tests with deet. The results (Table 1) indicate that seven compounds (5796, 18014, 6275, 6390, 23497, 23772, 20297) were superior to deet, seven other compounds (6269, 152, 6388, 6458, 6365, 5913, 17586) about equal to deet and all the others inferior to deet.

Fifteen out of 128 compounds, which fell into the best category, were divided into three equal groups. To each group deet was added and the group was evaluated in round robin tests. Results are given in Table 2. It can be seen that in compounds of group 1 there was no significant difference between the following repellents: deet, 6373, 12166, 31641 and 12149. Repellent 1395 was highly significantly inferior to the other 5 repellents. In group II, repellents 1000 and 10548 were significantly superior to deet. There was no significant difference between deet and the other 3 repellents (7783, 1492, 6315). In group III, repellents 3393 and 15130 were highly significantly superior to deet, but no significant difference was found betwen deet and the other 3 repellents (6496, 2220, 14913).

As stated previously, 87 compounds were also evaluated against the mosquito *Aedes aegypti*. Twenty-five of these compounds fell into the best category. These were

TABLE 1.—Relative effectiveness of repellents in paired tests with deet as skin application on rabbits against *Culex pipiens molestus*.[a]

Compound	U.S.D.A. code No.	Protection period (hrs.) Experimental repellent	Deet	Ratio to deet
Deet	22542	....	....	1.00
Dimethyl phthalate	262	2.00	3.66	0.54
N,N-dipropyl-O-ethoxybenzamide	19083	3.00	4.33	0.69
Benzyl-ethyl-acetoacetate	7790	2.43	3.28	0.74
Isopropyl ester of *N,N*-diisopropylsuccinamic acid	6350-b	2.33	3.00	0.77
4-(*p*-methoxyphenyl)-5-methyl-*m*-dioxane	5533	4.00	5.10	0.78
N-Ethyl-*N*-(2-butyroxyethyl)butyramide	6312	3.50	4.33	0.81
Ethyl ester of N,N-dipropylsuccinamic acid	6252	3.66	4.50	0.81
o-Cyclohexylphenol	9047	2.33	2.83	0.82
N-Propionyl-1,2,3,4-tetrahydroquinoline	5973-b	3.00	3.50	0.85
4-Methyl-3,5-nonanediol	5947-E	4.12	4.75	0.86
Isopropyl *N,N*-dipropylsuccinamate	6269	4.00	4.33	0.92
Hexachlorophenol (Hexachloro-2,5-cyclohexadien-1-one)	152	4.00	4.16	0.96
sec-Butyl ester of *N,N*-diethylsuccinamic acid	6388-b	5.25	5.33	0.98
Cyclohexaneacetamide, N,N-diethyl-1-hydroxy	6456	4.16	4.16	1.00
Ethyl *N,N*-dipropylglutaramate	6365	4.00	3.83	1.04
N-Propionyl-N-methylanthranilic acid, methyl ester	5913-a	4.80	4.20	1.14
O-chloro-N,N-diethylbenzamide	17586	4.41	3.83	1.15
N-Butylbutyranilide	5796-a	4.60	3.80	1.21
Reduced alcohol of butadiene-furfural copolymer	18014	5.41	4.41	1.22
Isobutyl ester of *N,N*-diethylsuccinamic acid	6275-a	5.16	4.16	1.24
sec-Butyl *N,N*-diisopropylsuccinamate	6390-c	5.83	4.50	1.29
1-Ethylpropyl m-chlorocarbanilate	23497	5.08	3.66	1.38
1,1-Dimethylpropyl m-chlorocarbanilate	23772	5.58	3.66	1.52
2-ethoxy-N,N-diethylbenzamide	20297	6.17	2.83	2.18

[a] Repellents used at 1% concentration in ethanol. Six replicates.

TABLE 2.—Relative effectiveness of repellents in round robin tests as skin application against *Culex pipiens molestus*.[a]

Compound	U.S.D.A. code No.	Protection period (hrs.) Range	Adjusted average	Ratio to deet
Group I				
Deet	22542	2–4	2.99	1.00
Phthalimide, *N*-propyl-	1395-c	1–2	1.37	0.46
Piperidine, 1-(*o*-toluoyl)-	31641-aGa	2–4	3.06	1.02
2,4-Nonanediol, 5-ethyl-	12149-a	1–5	3.54	1.18
Glutaramic acid, *N*,*N*-dipropyl-, methyl ester	6373-Gc	2–6	3.63	1.21
Spiro [cyclohexane-1,3′-indan]-1′-one, 4,5,6,7(or 7a)-tetrahydro-	12166-B	2–5	3.70	1.24
		LSD (5 percent)	0.85	
		LSD (1 percent)	1.15	
Group II				
Deet	22542	2–5	2.85	1.00
o-Chloro-alpha-(trichloromethyl)benzyl alcohol	7783	2–4	2.74	0.96
N-(mixed Monoamyl) imide of 3,6-endomethylene-4-cyclohexene-1,2-dicarboxylic acid	1492	2–5	3.44	1.21
Butyramide, N-butyl-N-(2-butyroxyethyl)-	6315	2–6	3.52	1.24
N-(n-Amyl)imide of 1,2-dicarboxy-3,6-endomethylene-4-cyclohexene	1000	3–6	3.75	1.32
Ethyl beta-benzoylacrylate	10548	2–6	4.00	1.40
		LSD (5 percent)	0.89	
		LSD (1 percent)	1.21	
Group III				
Deet	22542	2–4	3.13	1.00
cyclopentanecarboxylic acid, 1-acetoxy-, tetrahydrofurfuryl ester	6496	2–6	3.38	1.08
2-(diethylphenoxy)ethanol	2220-a	2–5	3.54	1.13
N,*N*-diethylbenzenesulfonamide	14913-Gb	2–6	3.76	1.20
2-dicyclohexylaminoethanol	3393-a	3–6	4.54	1.45
N-hexyl butyramide	15130-b	3–6	4.76	1.52
		LSD (5 percent)	0.89	
		LSD (1 percent)	1.20	

[a] Each repellent was paired twice against each other repellent of the same group. Repellents used at 1% concentration in ethanol.

compared in paired tests with deet. The chemical names with their corresponding code number are given in the tables, and for brevity, the code numbers only are given in the test. Results are given in Table 3. Some repellents (20297, 17586, 6456, 152, 6252) gave somewhat similar results against both species of mosquitoes. Of these the most effective one was 20297, which is a well known repellent against mosquitoes (Altman and Smith, 1955; Gilbert, Gouck and Smith 1957). Other compounds, however, gave different results against the two species. The following compounds (mentioned in Table 3): 375, 2531, 3775, 6369, 6478, 6500, 7039, 7409, 7627, 9485, 14825, 14896, 16769, 20626, 20828, 22388, which fell into our best category against *A. aegypti,* did not pass the first or second screening against *C.p. molestus*. On the other hand, two compounds 23772 and 23497 which were very effective against *C.p. molestus* (see Table 1) were quite ineffective against *A. aegypti* (belonging to categories B and A respectively). These results confirm the fact that some compounds may have a different effect on different species of mosquitoes (Gilbert, Gouck and Smith, 1957).

TABLE 3.—Relative effectiveness of repellents in paired tests with deet as skin application on rabbits against *A. aegypti*.[a]

Compound	U.S.D.A. code No.	Protection period		Ratio to deet
		Experimental repellent	Deet	
Deet	22542	....	....	1.00
N-3-methoxypropylbenzamide	22388	0.83	3.82	0.21
Methyl ester of N,N-diethylglutaramic acid	6369	0.66	2.83	0.23
N-alkyl(C_{12}–C_{14})cyclohexylamine	16769	1.00	3.70	0.27
diallyl maleate	2531	0.83	2.83	0.29
N-n-Butyl salicylamide	9485	0.83	2.83	0.29
alpha-Tetralol	7039	1.00	3.16	0.31
Tribromophenol	14896	1.00	3.00	0.33
Cyclopentanecarboxylic acid, 1-hydroxy-, 2-butoxyethyl ester	6500	1.16	3.00	0.38
Diisobutyl di-malate	20828	1.32	3.33	0.39
3-(p-chlorophenyl)-1-propanol	20626	2.00	3.83	0.52
1,3-Propanediol, monobenzoate	6378	1.16	2.16	0.53
Reduced alcohol of butadienefurfural copolymer	18014	1.83	3.00	0.61
Ethyl ester of N,N-dipropylsuccinamic acid	6252	1.33	2.16	0.61
Hexachlorophenol (Hexachloro-2,5-cyclo-hexadien-1-one)	152	1.30	1.80	0.72
Dimethyl phthalate	262	2.30	3.10	0.74
N-Butyl hexahydrophthalimide	7409	2.16	2.66	0.81
o-chloro-N,N-diethylbenzamide	17586	2.40	2.90	0.82
Cyclohexaneacetamide, N,N-diethyl-1-hydroxy-	6456	2.16	2.16	1.00
2-Ethyl-1,3-hexanediol	375	3.32	3.11	1.06
1,3-bis(Butoxymethyl)-2-imidazolidone	14825	4.33	4.00	1.08
2-Butyl-2-ethyl-1,3,-propanediol	3775	3.90	3.52	1.10
N,N-dipropyl-o-ethoxybenzamide	19083	3.80	3.00	1.26
1-Butyl-4-methyl-carbostyril	7627	4.32	3.31	1.30
2-(benzyloxy)-N,N-dipropyl acetamide	20571	4.00	3.00	1.33
2-ethoxy-N,N-diethylbenzamide	20297	5.44	2.91	1.86

[a] Repellents used at 0.5% concentration in ethanol. Six replicates.

SUMMARY. The repellent effect of 279 compounds, selected from several thousands evaluated by the U.S. Dept. of Agriculture against a number of blood-sucking insects, was evaluated against a local mosquito *Culex pipiens molestus* Forsk. Eighty-seven compounds were also evaluated against the mosquito *Aedes aegypti* L. A method for evaluating the repellents in the laboratory on rabbits is described. Twenty-eight repellents were more effective than, or similar to, deet (N,N-diethyl-m-toluamide), which served as a standard repellent, against *Culex pipiens molestus*. The eight most effective repellents against this mosquito, given in an increasing order of effectiveness, were *sec*-butyl *N,N*-diisopropylsuccinamate (6390-c), N-(n-Amyl) imide of 1,2-dicarboxy-3,6-endomethylene-4-cyclohexene(1000), 1-ethylpropyl m-chlorocarbanilate (23497), ethyl beta-benzoylacrylate (10548), 2-dicyclohexylaminoethanol (3393-a), 1,1-dimethylpropyl m-chlorocarbanilate (23772), N-hexyl butyramide (15130-b), 2-ethoxy-N,N-diethylbenzamide (20297). Some repellents gave somewhat similar results against both species of mosquitoes. Of these the most effective was 2-ethoxy-N,N-diethyl-benzamide (20297). Some repellents were effective only against one species. Of these 1,1-dimethylpropyl m-chlorocarbanilate (23772) and 1-ethylpropyl m-chlorocarbanilate (23497) which were highly effective against *C.p. molestus* were ineffective against *A. aegypti*.

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ARBOVIRUS ISOLATIONS FROM MOSQUITOES COLLECTED AT CALLAO, UTAH 1966 AND 1967

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Crane *et al.* (1970) reported arbovirus isolations from mosquitoes collected in Central Utah. Further isolations are reported here from mosquitoes collected in and near Callao, Utah, a small farming community east of the Deep Creek Mountains in western Utah. Mosquitoes were collected in 1966 by light traps and livestock-baited stable traps at eight sites (Olson *et al.*, 1968); in 1967 light traps alone were used at five of the 1966 sites. Light-trapped mosquitoes and arbovirus isolates are compared from the five sites used both years. Light traps were operated from May to October each year, 5 nights a week in 1966 and 3 nights a week in 1967. Collections were made in 1966 by military personnel of the Dugway Ecology and Epidemiology Division and in 1967 by the David C. Bagley family of Callao.

At each site two to four light traps were located at least 100 feet apart where vegetation was heaviest. Traps were placed at the same location each year, but not all locations were used both years. The elevation of all sites was about 4,300 feet. At Callao three sites were on the Bagley farm separated by ½ to 1 mile. At Redden (=Redding) Springs (Horne, 1945), two sites were about 1 mile apart. South Redden Springs is 7 air miles from Callao. Two trap locations at Callao North Marsh were on the border of a sedge (*Carex* spp.) marsh and a field of greasewood (*Sarcobatus vermiculatus*) and saltgrass (*Distichlis stricta*). The marsh was lined with wild rose (*Rosa* spp.) and willows (*Salix* spp.). Adjacent to one trap was a stream channel covered with cattail (*Typha angustifolia*). Four trap locations at Callao West Field were alongside irrigation ditches lined with white poplar (*Populus alba*), rabbitbrush (*Chrysothamnus* spp.), clematis (*Clematis* spp.), wild rose, mixed grasses, and greasewood. An irrigated alfalfa field was adjacent to three traps. At Callao Center Meadow, four trap locations were along a fence separating irrigated meadows of mixed grasses.