EVALUATION OF MOSQUITO REPELLENTS ON THE HAIRLESS DOG[1]

JENNIFER A. HILL, PETER B. ROBINSON, DAVID L. McVEY, WILLIAM A. AKERS AND WILLIAM G. REIFENRATH

Department of Dermatology Research, Letterman Army Institute of Research, Presidio of San Francisco, CA 94129.

ABSTRACT. Protection times and minimum effective dose determinations were made on the hairless dog for several mosquito repellents against *Aedes aegypti* mosquitoes. Good agreement was found between the results from man and from the hairless dog. It is proposed that the hairless dog can be used as an efficient animal model for screening new repellents prior to toxicology testing and application to man.

INTRODUCTION

The development of more effective mosquito repellents presently requires the screening of a large number of compounds. Since testing on humans requires extensive toxicity tests of candidate compounds, *in vitro* and animal tests are necessary, initially, to select only the most promising compounds for testing on humans. In particular, an animal model is needed which can screen rapidly the duration of effectiveness of new repellents. The guinea pig is useful for repellent protection time determination (Bayles et al. 1973, Kasman et al. 1953); however, the animal has to be shaved and restrained during the test period.

Because of its larger size (four repellents can be tested simultaneously), ease of handling, and lack of hair, the hairless dog was investigated as an animal model for determination of mosquito repellent efficacy. Two major types of tests were conducted with the dog. One was the minimum effective dose (MED) test or the minimum amount of repellent needed to repel mosquitoes. The second was the protection time (PT) test, which is a measure of repellent duration. Analogous tests were performed with human volunteers.

MATERIALS AND METHODS

Repellents used were N,N-diethyl-m-toluamide (m-deet), Eastman Chemical Co.; N,N-diethyl-p-toluamide (p-deet), Hercules, Inc.; 2-ethyl-1,3-hexanediol (Rutgers 612) and dimethyl phthalate, Niagara Chemical Division, FMC; triethylene glycol monohexyl ether (SRI-6), Stanford Research Institute (Johnson et al. 1975); n-butane-hexamethylene-iminesulfonamide (sulfonamide) (Pervomaiskii et al. 1963); cyclohexamethylene-carbamide (Stephanov et al. 1969); and Indalone, K & K Laboratories.

REPELLENT PROTECTION TIME. Protection times (PT) were determined in human volunteers by using a 4-site technique (Gabel et al. 1976). This test was adapted to the dogs as follows: two 7 × 7 cm sites were drawn on both sides of the dog's back and known weights of the repellents were applied with a 1 cc syringe

[1] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Department of the Army or the Department of Defense

Human subjects participated in these studies after giving their free and informed consent. Investigators adhered to AR 70–25 and USAMRDC Reg 70–25 on use of volunteers in research

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

and spread with a clean glass rod. A 0.4 cc volume of ethanolic repellent solution was used for each site. Four repellents (three experimental repellents with m-deet as a control) were tested simultaneously. Application of repellents was rotated among the 4 sites so that each repellent appeared twice on each different site, and every repellent was paired with every other repellent on the same side of the dog at least twice in a group of 8 dogs. Testing began 1 hr after application. An adhesive-backed foam pad (Reston^R, 3M Company) was affixed around the perimeter of 2 trap door openings of a twin compartment cage (each compartment is 7.5 × 7.5 × 15 cm and contains 50 female mosquitoes). Two of these twin compartment cages were placed over the 4 sites with the foam pad touching the perimeter of the repellent treated sides. The trap doors were opened simultaneously for 3 min periods. If one of the sites received 2 bites in one test period or 2 bites in consecutive test periods, the site was graded "failed," and the time (in hours) from application until failure (i.e., PT) was recorded as the protection time. Testing was continued until all the sites had failed, or to the point where only 1 experimental repellent had test sites remaining.

ALTERNATE DURATION EVALUATION. As a variation of the usual PT test, repellents were applied at 0600, 0900, 1200 and 1400 hr on the test day (4 dogs per treatment time). All of the sites were tested within a 1 hr period starting at 1600 (10 hr after the first repellents were applied). One dog from each group was tested until all the sites were tested. The criterion for site failure was 2 bites per site.

REPELLENT MINIMAL EFFECTIVE DOSE. Minimum effective dose (MED) determinations were made in human volunteers by using established techniques (Gabel et al. 1976). This test was adapted to the dogs as in the following example: A repellent solution is applied at 0.10 mg/cm^2 to one of four 7 × 7 cm square sites on the dog's back and is allowed to dry for 15 min. Again, a 0.4 cc volume of ethanolic repellent solution is used for each site. This site is then exposed to a mosquito test cage (7.5 × 7.5 × 15 cm) containing 50 female mosquitoes for 3 min. If the site receives 2 bites, a 0.15 mg/cm^2 concentration of the same repellent will be applied to another test site, allowed to dry and tested again. Depending on the success or failure of this test, a lower or higher (respectively) concentration for the repellent will be tested in order to bracket the MED. If the initial application does not receive 2 bites, a 0.05 mg/cm^2 repellent concentration will be applied and tested. Again, depending on the success or failure of this test, the last 2 sites will bracket the MED.

MOSQUITOES. *Aedes aegypti* mosquitoes, 6 to 12 days old, were maintained at 27°C and 80 percent relative humidity under constant light for volunteer testing. Sugar solution from cotton was available *ad libitum*. For dog testing, the mosquitoes were maintained at 27°C and 50% RH during the test periods.

VOLUNTEERS. Healthy active-duty military personnel were selected. All volunteers gave written informed consent prior to participation in the tests.

DOGS. Hairless dogs were selected at random from a colony of 25 healthy dogs maintained at Letterman Army Institute of Research.

RESULTS AND DISCUSSION

Eight different mosquito repellents were tested for duration in the hairless dog and man. With the exception of SR1-6, the dogs ranked the repellents in the same order as man (See Table 1 and Figure 1). Many factors affect the duration of repellent protection, but one of most importance was the minimum effective dose (MED). MED tests were done on 4 compounds in both hairless dogs and man (Table 2). Both the dog and man ranked the repellents similarly. Comparing Tables 1 and 2, compounds with lower MEDs last longer. Carbamide was found to have a MED greater than 0.32 mg/cm^2 in the dog, so its duration tests at

Table1. Repellent protection time (PT) in the hairless dog and man.

	Dog				Man			
Repellent	Dose (mg/cm²)	PT±S.D.	N	SIG[a]	Dose (mg/cm²)	PT±S.D.	N	SIG[a]
Indalone	0.32	0.69±0.54	16	S	0.32	2.2±1.8[b]	8	S
Carbamide	0.32	1.0 ±0.75	40	S	0.32	2.1±1.7	23	S
Dimethyl phthalate	0.32	2.7±1.6	16	S	0.32	2.9±0.9[b]	8	S
Ethyl hexanediol	0.32	3.3±2.3	24	S	0.32	3.6±1.2[c]	40	S
SRI-6	0.32	4.7±3.1	72	S	0.32	2.5±2.4	23	S
p-deet	0.32	5.2±2.7	25	S	0.25	4.8±1.4[d]	20	S
Carbamide	0.96	5.5±3.0	32	S	—	—	—	—
m-deet	0.32	7.2±2.7	72	—	0.32	5.7±1.8	23	—
Sulfonamide[e]	0.32	8.3±4.3	72	S	0.32	7.9±4.4	23	S

[a] Significance at the 95% confidence level, Student's two tailed t test, paired comparison with m-deet. S=significant, NS=non-significant.
[b] Brodel et al. 1974.
[c] Gabel et al. 1976.
[d] Kurtz et al. 1973.
[e] In both man and dog, 40% of test applications lasted longer than 10 hours.

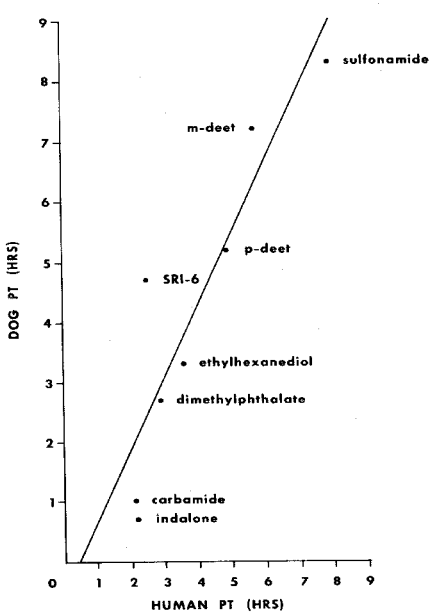

Fig. 1. Repellent protection time (PT) in the hairless dog and man (linear regression analysis: $r=0.91$, $r^2=0.83$).

this standard dosage failed during the first hour of testing. At a dose above the MED (0.96 mg/cm²), carbamide provided on the average 5.5 hours of protection for the dog.

As a variation of the usual PT method, we applied repellents at staggered pretreatment intervals (4 dogs per interval) to dogs and tested all of the animals (one-time challenge) with mosquitoes at the end of the test day. As can be seen from Table 3, sulfonamide provided at least 75% protection in each of the 4 test groups, whereas m-deet provided equivalent protection only through the 4.5 hr period. SRI-6 appeared to provide borderline protection after the 2.5 hr pretreatment interval and carbamide, at a dose of 0.32 mg/cm², provided no useful protection throughout the entire test period. These results are in agreement with the duration test results reported in Table I.

CONCLUSION. The hairless dog is a good model for insect repellent studies. The animal ranks repellents similarly to man in both MED and duration tests. Percutaneous absorption studies with radiolabeled repellents suggest the permeability of the dog's skin does not differ markedly from man (W. G. Reifenrath,

Table 2. Minimum effective dose (MED) in the hairless dog and man.

	Dog			Man		
Repellent	MED(mg/cm²)±S.D.	N	SIG[a]	MED(mg/cm²)±S.D.	N	SIG[a]
Sulfonamide	0.024±0.022	24	S	.021±.021	14	NS
m-deet	0.066±0.047	24	—	0.026±0.02	24	—
SRI-6	0.12 ±0.08	24	NS	0.064±0.06	24	S
Carbamide	0.61 ±0.18	24	S	0.156±0.06	24	S

[a] Significance at the 95% confidence level, student's two tailed t test, paired comparison with m-deet. S=significant, NS=Not significant.

Table 3. Alternate duration test for repellents using the hairless dog. Number of dogs protected in each experimental group.

		Pretreatment Interval			
Repellent	Dose (mg/cm²)	2.5 hr	4.5 hr	7.5 hr	10.5 hr
Sulfonamide	0.32	7/8	8/8	7/8	6/8
m-deet	0.32	8/8	7/8	2/8	5/8
SRI-6	0.32	5/8	3/8	1/8	3/8
Carbamide	0.32	1/8	0/8	2/8	1/8

unpublished data). The hairlessness, ease of handling, and large size of the dog allow rapid screening of simple repellents and formulations.

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