TESTING SPACE REPELLENTS AGAINST MOSQUITOES

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The U. S. Dept. of Agriculture has synthesized and evaluated thousands of chemicals against a number of blood sucking insects (King, 1954). Products of this research are now widely used throughout the world. Several years ago the U. S. Dept. of Agriculture decided to search for repellents that would be effective at a distance, and called them "space repellents." There are a number of advantages in the use of space repellents as pointed out by Gouck *et al.* (1967) and McGovern *et al.* (1967). The suggested mode of application allows for their greater persistence, greater comfort for the user and the lower cost of protection. The pharmacological requirements should be less stringent for a chemical that is not used directly on the skin. Such repellents, as they pointed out, have to be sufficiently volatile so that their vapor envelops some portion of the body, thereby deterring the approach of insects to the area thus protected.

The term "space repellents" has also been used by Ginsburg (1935), but in this case it meant repellents to be sprayed over an outdoor area in the form of a fine mist, aimed at providing a temporary protection for outdoor gatherings. Gouck *et al.* (1967) and McGovern *et al.* (1967) used an olfactometer to test space repellents. Mosquitoes (*A. aegypti*) were attracted to a human arm which could be reached only after passage through treated cotton 4-mesh netting (holes about 0.25 in. square). The parameter used by them was the time (in days) in which less than 10 percent of the mosquitoes passed through the treated netting.

In the present investigation the purpose was to determine the distance from a focus of a repellent that will deter mosquitoes from biting. The duration of effectivity of the repellent was not taken into consideration. It is assumed that the higher volatility of a chemical can be compensated for by using an absorbent that will take up a relatively large amount of the material on each application.

MATERIALS AND METHODS. A rectangular piece of rubber (17 cm x 13 cm in area, 0.6 cm in thickness) having at its center a hole 11 cm in dia., was firmly attached by rubber bands to the shaved belly of a tied rabbit. A glass ring, 2.5 cm and 3 mm thick, was inserted into the hole, and into it was fitted a metal disk with a circular hole at its center. Disks having holes of: 3 cm, 4.8 cm, 6.6 cm, or 8.5 cm in diameter were used as required, so as to vary the exposed area of skin (maximum 10.4 cm in diameter when no disk was used). At the center of the exposed area was placed a small circular metal disk, 1.0 cm in diameter to which was attached a ball of cotton wool (100 mg) treated with 0.5 ml of the candidate repellent. In some tests, the treated cotton ball was placed at the

rim of the exposed area, instead of at the center, giving a maximal exposure distance of 9.4 cm from the treated cotton ball.

A cage (16 x 25 x 25 cm) containing about 2,000 six-day old female mosquitoes (weighed in the cold) was placed on the rabbit. A round aperture at the center of the bottom of the cage, enclosed by a glass ring, fits the outside rim of the glass ring inserted in the rectangular piece of rubber attached to the rabbit. The hole at the bottom of the cage was temporarily closed with a petri dish to prevent the escape of mosquitoes. When the cage was *in situ* the petri dish was removed for 5 minutes. A variable skin area depending on the disk used was thus exposed to the mosquitoes. The number of mosquitoes engorging during this period could be observed from the top of the cage made of glass. At the end of the test, the petri dish was replaced and the cage removed. Thus, it was possible to determine up to what distance (maximum 9.4 cm) the treated cotton ball deters the mosquitoes from biting. Each compound was tested at first at the least distance (1 cm) from the focus of the repellent. If more than 10 mosquitoes fed in any one test the particular compound was discarded. If an average of 5 or less mosquitoes fed in three replicated tests, the next distance was similarly tested. The procedure was repeated at greater distances (2.8, 3.7, 4.7, and 9.4 cm) when appropriate from the results of the preceding tests.

Three groups of compounds were tested:

1. Compounds of relatively high volatility. These were as follows: chloral, diisopropylamine, diethyl amine, acetic acid, γ-dimethyl amino-1-propanol, diethyl amino ethanol, ethyl chloroformate, benzylamine, carbon tetrachloride, cyclohexane, fluorobenzene, 4-fluoroaniline, ethyl isonipecotate, ethyl ether, dioxane, triethyl phosphate, benzene, acetonitrile, acrylonitrile, allyl bromide, acetone, pyrethrum extract, 1,2-dichloroethane, toluene, 3-picolylamine, terpinol, kerosene, nitrobenzene, aniline, tributile phosphite, 4-picolylamine, p-fluorobromobenzene, n-butyl bromide, dibenzylamine, dimethyl sulfaoxide, diphenyl sulfide and 2-chloromethylpyridine hydrochloride.
2. Candidate space repellents obtained from the U.S.D.A. (by courtesy of Dr. Carroll N. Smith). These were as follows: chrysanthemumic acid (phenethyl ester), butyl tartrate, crotonic acid, 3-methyl-, 2-[2-(2 butoxy=ethoxy) ethoxy] -ethyl ester, mandelic acid (p-methoxy-,propyl ester), 1-methylbutyl malate, fumaric acid (bis (2-ethyl hexyl) ester), mandelic acid (p-isopropyl-,hexyl ester), pentyl malate.
3. Known repellents applied to the skin. These were as follows: Dimethyl phthalate (DMP), diethyl toluamide (deet), 2-ethoxy-N,N-diethyl-benzamide, 2-Butyl-2 ethyl-1,3-propanediol, dimethyl carbate (bicyclo [2,2,1]-5-heptene-2, 3-dicarboxylic acid, cis-, demethyl ester), 1,3-bis(butoxymethyl)-2-imidazolidone, indalone, succinamic acid-(*N,N*-diethyl-, propyl ester), propyl anisate, benzyl benzoate.

RESULTS AND DISCUSSION. Of the first group, three compounds (chloral, diisopropylamine, diethyl amine) were effective (an average of less than 1 mosquito fed in three replicates) to a distance of 9.4 cm, the largest distance that could be tested under the experimental conditions. Acetic acid to a distance of 4.7 cm. Ethyl chloroformate to a distance of 3.7 cm, γ-dimethyl amino-1-propanol and diethyl amino ethanol to a distance of 2.8 cm. All other compounds in this group were ineffective at distances of 1.0 or 1.9 cm.

Of the second group, all compounds were ineffective at distances of 1.0 or 1.9 cm. Of the third group, 2-butyl-2-ethyl-1, 3-propanediol, was effective only at 1.0 cm. All the others were ineffective when at this distance.

These results show that the third group of compounds when applied to the skin, are effective only on the treated area and its very close proximity (usually less than 1 cm). The same is true of the second group, although they were very effective and persistent (62–145 days) in preventing mosquitoes from passing through treated

4-mesh cotton netting i.e., holes about 0.25 in. square (Gouck *et al.*, 1967; McGovern *et al.*, 1967). This group of compounds may be very useful, as pointed out by these investigators, in treatment of nets on windows to prevent entry by insects small enough to pass through ordinary screens. Some of the first group of compounds were effective, especially the three mentioned above. Compounds of this group were selected for their relatively high volatility. However, factors such as irritating odor, toxicity, flammability, etc., were not taken into consideration. The purpose was to find out if in principle this mode of application is workable. Furthermore, finding effective compounds, even not of practical use, can give us a lead to search for compounds that will be both effective and of practical use. In view of the fact that a small number of compounds of the third group were tested, and a few gave encouraging results, indicate that this line of research is promising.

SUMMARY. Various compounds were tested as to their effectiveness at a distance in inhibiting mosquitoes from biting a shaved belly of a rabbit. Known skin repellents were ineffective in repelling mosquitoes at a distance, as were also "space repellents" obtained from the USDA; some compounds of higher volatility were relatively effective. Chloral, diisopropylamine and diethylamine were effective to a distance of 9.4 cm (the maximal distance tested) and acetic acid to 4.7 cm. Other compounds were variously less effective.

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