A METHOD FOR BIOASSAY OF THE EFFECTIVENESS OF RESIDUAL SPRAYING [1]

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INTRODUCTION. The effectiveness of house spraying with contact insecticides of persistent action (DDT and other chlorinated hydrocarbons) depends on a variety of physical factors: the kind of sprayed surfaces, the quality of the insecticide, the technique of application, age of spray deposit, changes of room temperature and humidity, dust and smoke covering the sprayed surfaces, etc., and also on the different response of the insect populations to the toxicant (behaviour, susceptibility, irritability, flight reactions, etc.), such responses depending on many factors and conditions which are beyond the scope of this paper.

Taking into consideration that residual house spraying constitutes a method of insect control in certain sanitary operations, such as antimalaria campaigns, it is of great interest to be able to determine, at any given moment, the amount of insecticide which persists on the sprayed surface after a certain lapse of time—weeks or months—since the house has been sprayed, and to what degree the sprayed surfaces preserve their effectiveness. Chemical tests in some ways answer the first question more or less satisfactorily, but not the second, which from a practical viewpoint is more important. The biological method which we shall describe is not entirely new; however, it combines the characteristics of some methods already in use with the personal experience of the authors and some general ideas which came up in discussions with other members of the C.N.E.P. of Mexico.

MATERIAL. The living material for the proposed bioassay is obtained in or near the village or hamlet where the testing is required, but in neither case in the interior of sprayed houses. The anophelines, for example, can be caught with human or animal bait, or obtained from pupae collected in not too distant breeding places; but preferably, all specimens used in the test and controls should be of the same sex and the same physiologic condition. The most desirable are females, blood-fed some hours before (for instance, baiting them at night and using them for the experiments next morning). Only in special cases can material taken from another locality be used, and this should be pointed out in the reports.

TECHNIQUE. A number of test boxes are located on the sprayed walls or other sprayed surfaces (or on sheets of various materials which had been treated with the insecticide for experimental purposes). We use round boxes similar to Petri dishes, made of transparent plastic material (plastic sheets "cristal acetate" No. 1500). The upper diameter measures 70 mm, the diameter of the lower, open side, which will be put against the surface to be tested, measures 80 mm, and the height is 13 mm (fig. 1). The boxes can be molded in the laboratory, out of a plastic disk of approximately 105 mm, while still hot. On the upper side a circular orifice has been cut, through which the mosquitoes (for instance 10 anophelines) can be introduced and which is closed by a disk of the same plastic material, fixed to the box with two small strips of adhesive tape (fig. 1, B). Numerous small perforations are made on the upper side with a slightly warmed pin to provide an outlet for the air stream with which the mosquitoes are blown into the box from the suction tube used for catching them. Furthermore, they serve

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to avoid the accumulation of humidity in the box and to prevent the concentration of gas, in the case of testing the effectiveness (by contact) of a surface treated with an insecticide (e.g., dieldrin) which may have a certain fumigation effect also.

The edge of the box has a brim, approximately 3 mm wide (fig. 1, A); with this brim the box can be fixed to the surface which is to be tested, by pins or thumbtacks. The boxes used for the controls can be fastened by rubber bands (fig. 1, E) to pieces of cardboard, which in the case of "experimental controls" (see below, The Testing Method) have previously been lined with disks of paper or cloth impregnated with a definite amount of insecticide, and in the case of "net controls" with disks impregnated only with the solvent used for the insecticide of the experimental control.

The disks treated with insecticide for

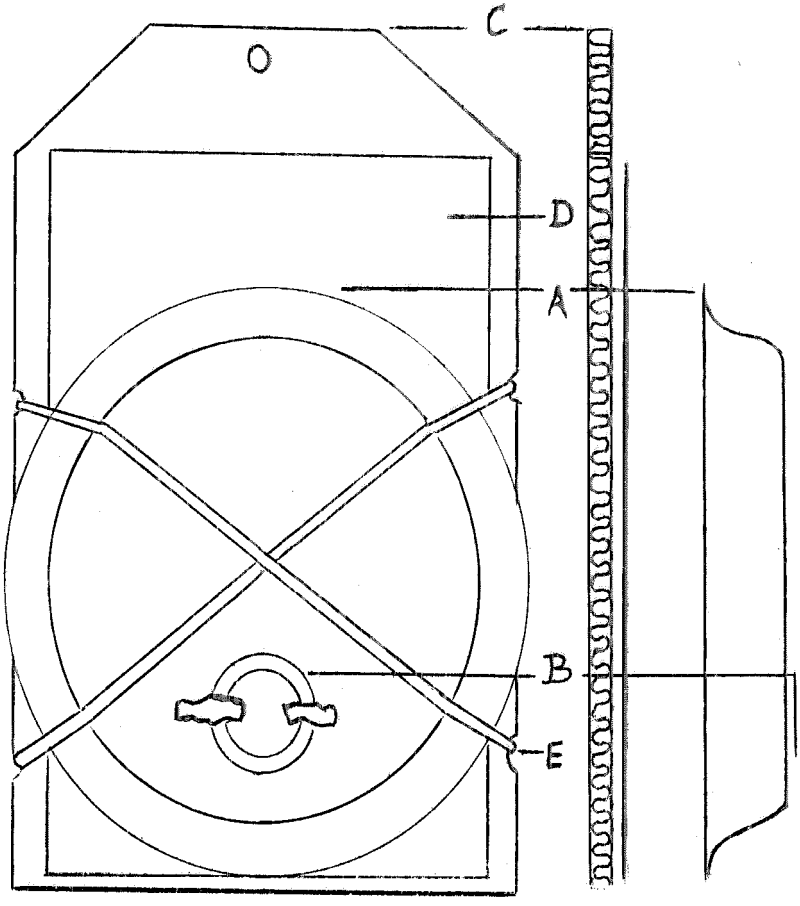

FIG. 1.—Frontal and lateral views of a testing box fixed onto a piece of corrugated pasteboard. A, brim of the box of transparent plastic material. B, hole for putting in mosquitoes and circular plastic cap fastened with two strips of adhesive tape. C, piece of corrugated pasteboard. D, disk of cloth or paper, which is prepared for the net control without insecticide, for the experimental control with insecticide. E, rubber bands.

the experimental control may be sheets of cotton cloth or of slightly absorbent (mimeograph) paper impregnated with a dose of insecticide smaller (e.g. 50 percent) than that which theoretically should have been applied to the wall or similar surface to be tested, in order to make the experiment under sufficiently severe conditions.

THE TESTING METHOD. The test is a triple test. Following Downs, Bordas and Navarro (1951) and Gómez Mendoza (1958), adequate insect samples, in our case anophelines, are exposed: (a) to the action of the insecticide on the sprayed wall or similar surface the efficiency of which is being investigated (accurately speaking: the *test*), (b) to the action of the insecticide on a surface (piece of paper or cloth) purposely treated with a known amount of insecticide (*experimental control*), and (c) to all conditions of the test except the action of the insecticide (*net control*).

The exposure time for the mosquitoes is not an arbitrarily fixed one as commonly used in other methods. In the tests as well as in the experimental controls, the exposure time will be the time necessary to produce the knock-down of the individuals, (at which time the specimens tested on vertical surfaces lose the contact with the insecticide). However, when a 100 percent knock-down has taken place only in the experimental control, the time of exposure in the test must be prolonged for such a lapse of time as will be considered sufficient, according to the species, to come to the conclusion that the insecticide is no longer working.

But we do not limit our observations to stating the time when, for instance, all 10 specimens which had been introduced into the test boxes had been knocked down (*total knock-down*). During the experiments the number of insects knocked down in each box is counted at regular intervals which are established in accordance with the species, the insecticide used and the particular conditions of the experiment. Working with *Anopheles albimanus, A. aztecus* and *A. quadrimaculatus* we found it practical to count the mosquitoes which were knocked out every 20 minutes during three hours. Thanks to these counts at regular intervals, the exposure time as well as the proportion of the insects which are knocked down more quickly or more slowly, can be determined more accurately. This is necessary because the observation of a total (100 percent) knock-down within two equal periods can conceal significant differences; for examples, the fact that 90 percent of all individuals should be knocked down in the first minutes of exposure and the remaining 10 percent should go on living for a long time without any sign of intoxication, and the other fact that only 10 percent should be knocked down quickly and 90 percent go on without being affected for a long time.

When exposure time is terminated, that is, usually when total knock-down has taken place, the mosquitoes are kept for possible recovery in cages, glass jars lined with a thin layer of gypsum, or drinking cups made of stiff waxed cardboard with a sleeve or surgical gauze, provided with the usual sugar water nourishment; in these containers the mortality count is made 24 hours later, in order to state an eventual recovery rate of knocked down individuals, or a retarded lethal effect on a specimen which had not been knocked down previously.

The number of test boxes to be collected on the sprayed surfaces and of controls can vary with the living material at our disposal, the number of dwellings or other rooms to be tested, and other circumstances; but it is advisable to have a number of experimental and net controls which should add up to the number of tests. A minimum of four tests should be made in each room. Every box, regardless of either being destined for the test itself or the experimental or the net control, should contain 10 specimens.

DISCUSSION. The method will be useful to estimate the persistency of the effect of the insecticide on the mosquitoes of the respective locality and under the environ-

mental conditions of that place. The simultaneous arrangement of test and experimental control will enable us to detect any decrease of the effectiveness of the insecticide deposit (survival in the test; mortality in the experimental control) and, furthermore, to discover differences which would indicate a decreasing susceptibility (considerable survival not only in the test but also in the experimental control).

However, the observation of exposure times to provoke a knock-down, longer than usually necessary for the same species, and of low mortality rates, both in the tests and in the experimental controls, can be valued only as circumstantial evidence that a decrease of susceptibility might have developed in the insect population. Such evidence will require subjecting the insects of that locality to the precise susceptibility tests which have been established for investigating the "resistance problem"; in the case of mosquitoes, for example, the WHO test.

SUMMARY. The method presented in this paper has been intended for the study of the residual effect of insecticide applications under the local conditions of the sprayed houses. The method will be useful to detect any failure of effectiveness of the insecticide and may also afford a rough estimation of the grade of susceptibility of the local insect population which is supposed to be controlled.

Circular boxes of 80 mm of diameter, made of transparent plastic material, were used to expose at the same time in each box groups of ten specimens of insects gathered from the same source: (*a*) to the action of a surface which had received the routine spraying ("test"), (*b*) to the action of a piece of paper or cotton cloth which had been treated with a definite amount of the insecticide ("experimental control"), and (*c*) to the conditions of the test without the toxicant ("net control").

The observations are made by registering at regular intervals the time in which knock-down takes place and recording the corresponding mortality rates within the following 24 hours. In the tests made with *Anopheles albimanus*, *A. aztecus* and *A. quadrimaculatus* it was found suitable to observe the knock-down rate every 20 minutes.

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