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EXPERIENCES WITH THE EXCITO-REPELLENCY TEST BOX - MODEL OPS[1]

by

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The recognition of the fact that populations of vector species of anopheles mosquitos, in several different parts of the world, now possess "evasive" habits in respect of DDT-sprayed wall surfaces makes it desirable to have some procedure by which the behaviour of populations of wild anopheles mosquitos can be characterized and compared. This paper will describe and discuss the Excito-Repellency Test Box - Model OPS, which has been developed and used in preliminary field tests by the staff of the Pan American Health Organization, Malaria Eradication Branch (PAHO/ME) and is considered to be useful for the above-mentioned purpose.

The WHO susceptibility test for adult mosquitos and the various irritability tests of mosquitos, even when used together, do not provide a very good picture of what free-flying anopheles will do when they enter houses, the walls of which have been sprayed with a residual insecticide. It is very difficult indeed to study the reactions of wild mosquitos in occupied human habitations, because of the large number of variables that is involved. This has long since led to the use of test huts of many kinds. These huts eliminate many variables, but they have the disadvantage of being either immobile or movable with difficulty.

A truly portable device was, therefore, greatly to be desired - portable enough for two or more of them to be taken into the heart of Amazonia by aeroplane.

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[1] Paper presented at the joint meeting of the New Jersey Mosquito Extermination Association and the American Mosquito Control Association, Atlantic City, New Jersey, 12-15 March 1963. (The full text of this paper will be published at a later date.)

The prototype Excito-Repellency Test Box was constructed in El Salvador and is a cube 50 x 50 x 50 cm, made of plywood. The E-R Test Box is considered to be a miniature house, into which mosquitos are introduced, and allowed to leave as fast as they wish to.[1] The window-trap covers a relatively large opening in the wall of the box, 15 x 30 cm, and is not merely a narrow slit. This means that any mosquito that does not want to stay in the window-trap can fly back into the interior of the box. The provision of two sliding doors for each window-trap, one for the box itself and one for its window-trap, makes it easy to count the number of mosquitos that leave in each of the six observation periods of 10 minutes each. Including an initial "conditioning" period of five minutes, the total length of the observation period is 65 minutes, after which all mosquitos are held for 20-24 hours to observe their mortality.

At least two identical boxes are always used simultaneously, 50 mosquitos from the same source being placed in each box at the same time. One of the boxes is lined with kraft-paper which has previously been sprayed with insecticide, and the other with identical paper that has not been sprayed. The unsprayed box serves as the control on the sprayed box. More than one box with insecticide can be used at the same time, provided that there is one person available to handle each box, and the illumination of all the window-traps is equal. Tests have been done with four boxes simultaneously, to compare the effect of DDT in three different amounts per square metre. In this manner, variables of temperature and relative humidity, time of day and illumination, can be compensated for, even if not standardized. Tests are best done at night.

If results of test by different persons are to be comparable it is vitally important that the paper used to line the boxes be sprayed with known quantities of insecticides per unit area. This makes it necessary to have a spraying device with which to apply measured amounts of insecticides on sheets of paper that measure 50 x 100 cm, or enough to line two of the inside surfaces on one box.

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[1] Details of construction of the E-R Test Box are shown in figures 4, 5, 6 and 7.

A suitable device for this purpose is a small nasal atomizer, after discarding its rubber bulb. A supply of air under 10-20 p.s.i. is needed to aspirate a previously measured portion of insecticide out of the reservoir on to the kraft-paper. With a little practice an adequately uniform deposit of insecticide can be applied to each separate sheet of paper. Inasmuch as it is desirable to keep the insecticide on the surface of the paper, a suspension of the insecticide in water is preferable to a solution or an emulsion. The treated and dried paper can be fastened to the inside of the box with tacks, staples, or two-faced adhesive tape.

Our main interest in the E-R Test Box is for the detection of "evasive" behaviour by the vector species of anopheles in a malaria eradication "problem area" - one in which the interruption of the transmission of malaria has not been attained after a suitable number of years of efficient house spraying. In such an area it is important to ascertain whether the reason lies in some defect in the spraying operations or in the behaviour of the vector species of anopheles.

It has been found feasible to take a pair of E-R Test Boxes to a locality in the problem area, make captures of the local anopheles with human or animal bait, and test them the same night they are collected.

A pair of the E-R Test Boxes is shown in Figure 1. The left-hand box is ready to be loaded with mosquitos through the sleeve in the screened door of the box. On the top of the box is one of the two window-traps for the box. The box on the right has been loaded with mosquitos after which it was rotated 90°, so that the screened door has become the floor of the box, which does not need spraying. The window-trap is in position, with both shutters closed, so that the mosquitos can enjoy their five-minute conditioning period.

There are two window-traps for each box, so that one can be removed and immediately replaced by the second. The first window-trap is then taken to a lighted room and the mosquitos in it removed to a gauze-covered paper cup to be held 20-24 hours to observe mortality. The window-trap is changed every ten minutes, so that there are seven cups containing mosquitos: six from the ten-minute intervals, and the seventh with the mosquitos that remained in the box at the end of the sixth period.

The results of the test are summarized by calculating two simple indices: the percentage of mosquitos that escaped in the 60-minute period; and the percentage that survived 24 hours, after a variable length of stay inside the box. This is done for both the control and the insecticide-treated boxes. In addition, the cumulative percentage of mosquitos that escaped from the box is plotted.

The insecticide susceptibility of the population of mosquitos being studied is determined by means of the WHO susceptibility test for adult mosquitos.

Summaries and plots of some E-R tests done by PAHO/ME staff in El Salvador on A. albimanus and A. pseudopunctipennis are shown in Figure 2. The A. albimanus from La Canoa are highly irritable and highly resistant while those from Metalio are perhaps even more irritable, but susceptible. A. albimanus from Moncagua are somewhat less irritable but equally resistant, while those from Guajoyo are so susceptible that their potential irritability does not protect them. Lastly, it is evident that a population of A. pseudopunctipennis is not irritable and is highly susceptible.

In Figure 3 the results of some tests on A. albimanus from four places in Nicaragua are summarized. The marked spread in results is apparent. The transmission of malaria has not been interrupted in the Posintepe area represented by the top line, with highly irritable and highly resistant A. albimanus, while it has been interrupted in the area El Carmen (bottom line), where the A. albimanus are much less irritable and much more susceptible.

We have called the device the Excito-Repellency Test Box, on the assumption that it would permit the study of the combined effect of irritation and repellency of free-flying mosquitos, even if it did not measure the two phenomena separately. The E-R Test Box described is the prototype device. It has not yet been subjected to thorough study in a search for possible improvements. Among the many things that are not known are: the optimum size and shape of the box and its outlet window; the optimum number of mosquitos to release in the box in any one test; the effect of the time of day and illumination upon the behaviour of the mosquitos; the effect of the nutritional state (i.e. the time elapsed since the last blood meal) of the females being tested; the age and condition of the ovaries of the females; and last, but not least, the general applicability of the observations that have been made with A. albimanus in El Salvador.

It would appear to be possible to use the E-R Test Box to compare insecticides in various formulations, applied to various types of wall surfaces. DDT technical grade could be compared with pure p-p DDT. Combinations of insecticides could also be tested, e.g. does malathion "neutralize" the irritant effect of DDT on anopheles mosquitos?

Line drawings of the Excito-Repellency Test Box, and detailed instructions for its use may be obtained upon request from the Malaria Eradication Branch of the Pan American Health Organization, 1501 New Hampshire Avenue, N.W., Washington, D.C.

Editor's comments

The development of the OPS Excito-Repellency Test Box represents a further step towards the ideal goal of designing standard laboratory equipment in which the movements of mosquitos in the presence of insecticide are sufficiently unrestricted as to provide some indication of natural behaviour in the field. It would be instructive to refer briefly to earlier examples of apparatus designed with the same object.

Fay & Sheppard (1949) used an experimental chamber in which single mosquitos were allowed free access to both DDT-treated and untreated resting surfaces. Although the main object of their work was to find exactly what period of contact with treated surfaces is necessary to produce high mortality, many of their techniques and experiments have a direct bearing on principles used in the E-R Test Box.

Davidson (1953) used a simple apparatus consisting of two one-foot cube chambers, lined with white cardboard, and joined together by a perspex chamber of similar dimensions. Mosquitos blown into the middle had the option of flying into one chamber or the other, one of these having its internal surface in the form of insecticide-treated panels. Mosquitos entering the treated chamber and becoming irritated could escape naturally into the perspex chamber.

The same general idea and design of apparatus - which allows the irritated mosquito to escape from a cage with treated panels and settle in an untreated cage - was used by Coluzzi (1958), and by Hecht and his colleagues (1958-1960).

More recently Elliott (1961) has given a great deal of attention to the design of an "excito-repellency" box based on that used to study blowflies by Brown & Evans (1960). This consists of a wooden box subdivided into four boxes, each 12 x 12 x 15 centimetres, the partitions between the boxes being pierced with a rectangular opening fitted with a card baffle. The activity or irritability of insects exposed to the treated compartment is assessed by the extent to which their subsequent activities carry them progressively into the second, third or fourth untreated compartment. These experiments were carried out in the dark, and the penetration of activated mosquitos to the untreated compartments was not assisted in any way by light stimuli.

It should be noted that all these methods - including the present OPS box - allow free movement of irritated mosquitos away from the irritant insecticide-treated surface. At the same time, they all involve a point of technique which might possibly prove to be a complicating factor, namely that mosquitos have to be artificially introduced into the apparatus - usually in batches. In this connexion the apparatus designed by Reid (1951) appears to have some advantage in that mosquitos are allowed free access to the experimental box or cage from a reservoir cage in which they are first allowed a period of quiescence. When mosquitos enter the main insecticide-treated chamber and are irritated or activated, they have free access to a light opening or window cage as in the OPS box. The original idea of Reid's apparatus was to have the large (treated) cage provided with animal bait so that the whole apparatus resembled an occupied habitation in miniature. In practice, however, most mosquitos moved spontaneously into the dimly-lit treated cage at the period of their natural increased activity at dusk.

In the present method, it is stated that with some practice a fairly uniform deposit of insecticide can be obtained on each sheet of paper. It is likely that for wider use of the OPS [illegible] accuracy [illegible] standardization of this deposit might be necessary to obtain comparable results from different workers. Discussions on the method of using pre-impregnated papers or standard concentrations of DDT for ad hoc impregnation of sheets of paper are now in progress.

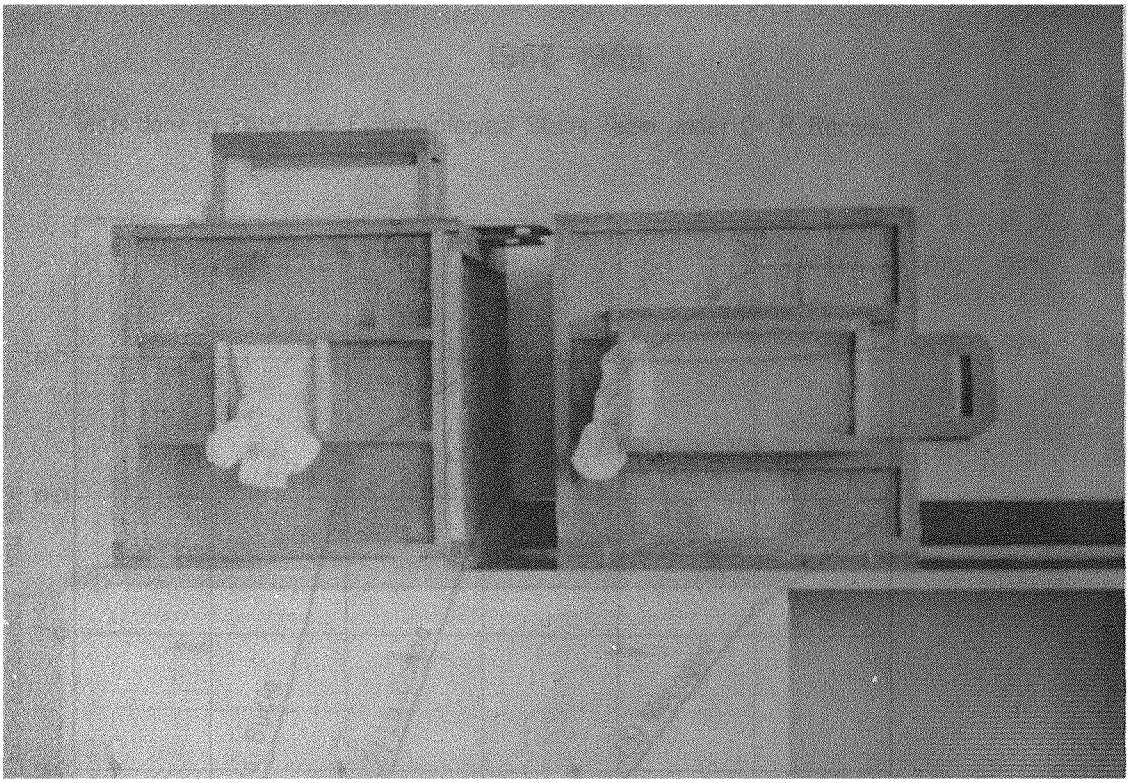
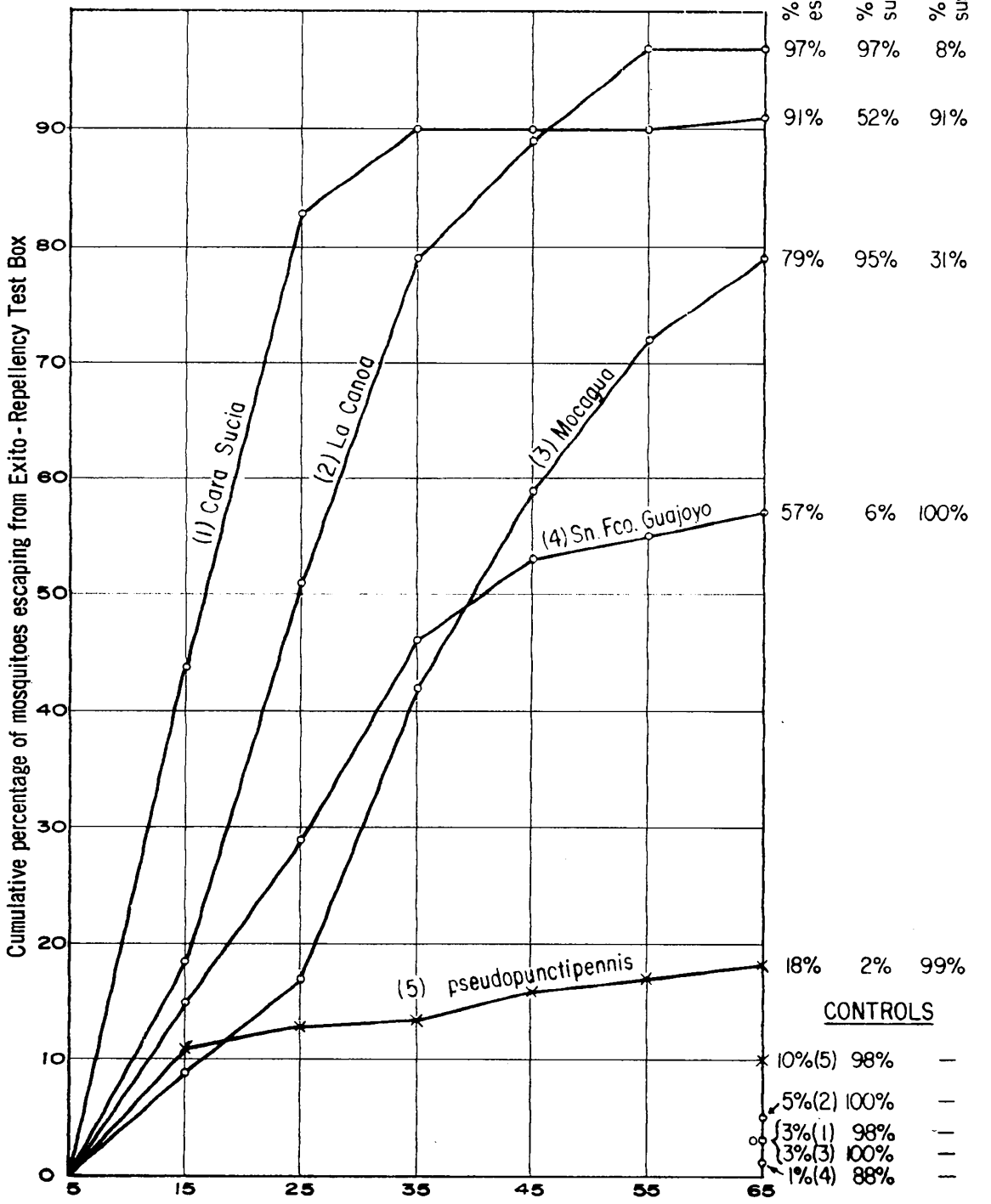


Fig. 1. A pair of identical Excito-Repellency Test Boxes (Model OPS), one with insecticide and the other without. The boxes are made of plywood, with inside dimensions of 50 x 50 x 50 cm. The left hand box is ready to be loaded with 50 mosquitoes through the sleeve in the hinged and screened front of the box. The right hand box has been loaded and then rotated 90 degrees so that the screened door is now the floor of the box. Each E-R box has a single window, and two interchangeable window traps, one of which is in place at all times during the test.

FIG. 2

RESULTS OF EXITO-REPELLENCY TESTS (OPS) - DDT AT ONE GRAM/m^2 OF *Anopheles albimanus* and *A. pseudopunctipennis* IN EL SALVADOR, 1962-1963

Tests done by PAHO/ME Epidemiological Study Team under Dr. R. Rachou.



	% that escaped	% that survived	% DDT-susceptible (*)
(1) Cara Sucia	97%	97%	8%
(2) La Canoa	91%	52%	91%
(3) Mocagua	79%	95%	31%
(4) Sn. Fco. Guajoyo	57%	6%	100%
(5) pseudopunctipennis	18%	2%	99%
CONTROLS			
(5)	10%	98%	—
(2)	5%	100%	—
(1)	3%	98%	—
(3)	3%	100%	—
(4)	1%	88%	—

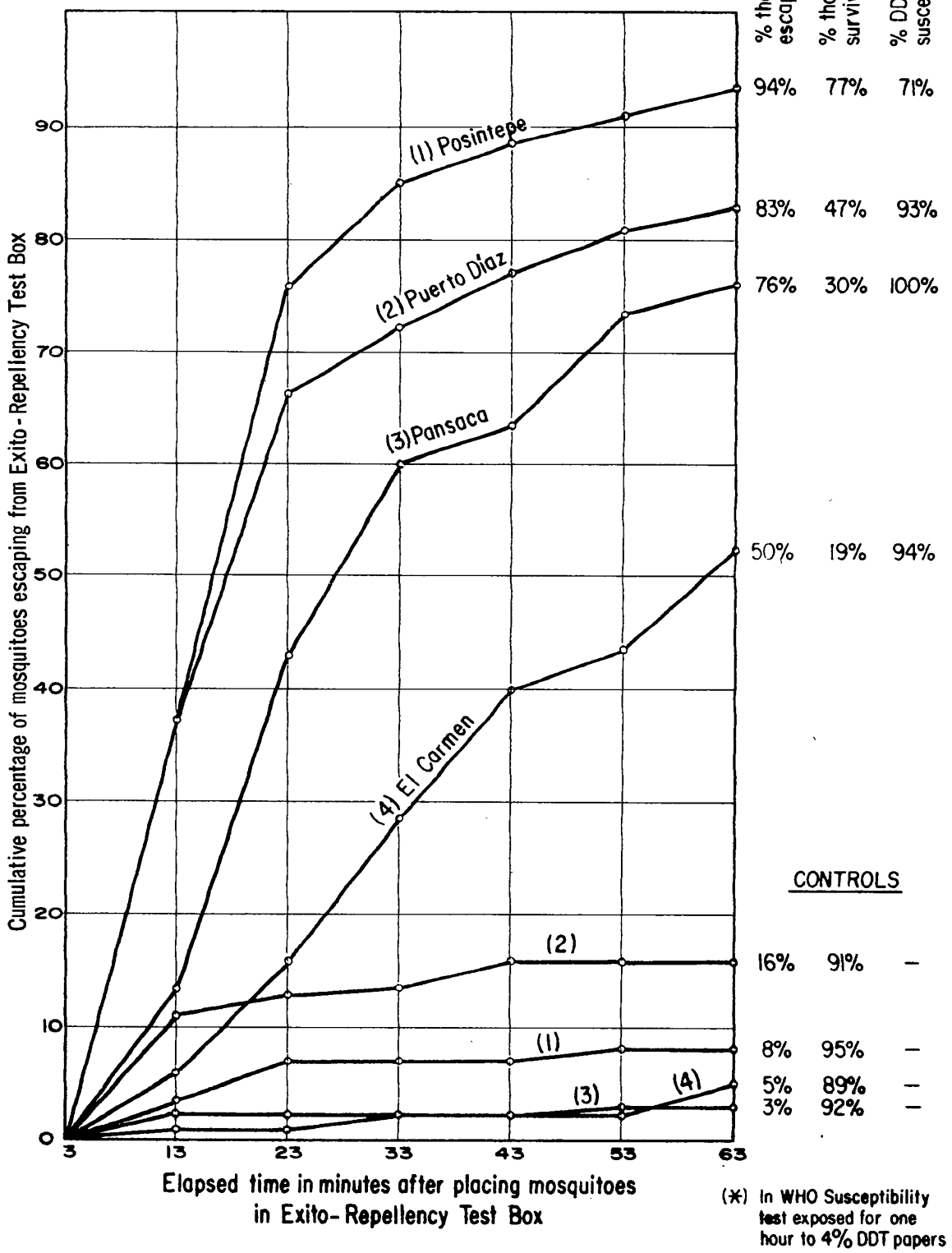
(*) In WHO Susceptibility test exposed for one hour to 4% DDT papers

WHO 3479

FIG. 3

RESULTS OF EXITO-REPELLENCY TESTS (OPS) - DDT AT ONE GRAM/m^2 OF FOUR POPULATIONS OF *Anopheles albimanus* IN NICARAGUA, 1962

Tests done by Dr. J. P. Duret, of PAHO/ME Staff.



(*) In WHO Susceptibility test exposed for one hour to 4% DDT papers

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FIG. 4

DETAILS OF CONSTRUCTION OF THE O.P.S. EXITO-REPELLENCY BOX

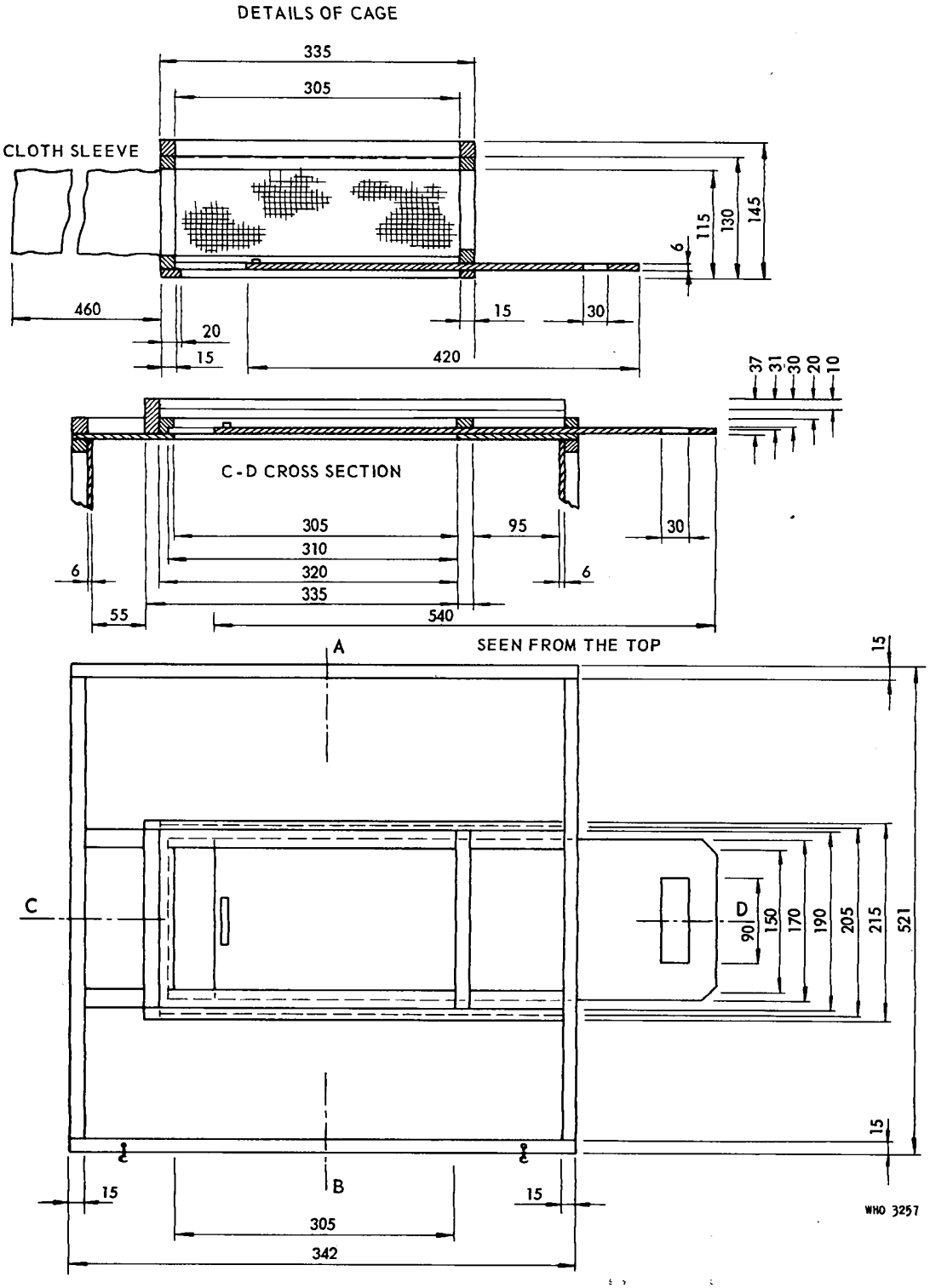


FIG. 5

FURTHER DETAILS OF CONSTRUCTION OF THE O.P.S. EXITO-REPELLENCY BOX

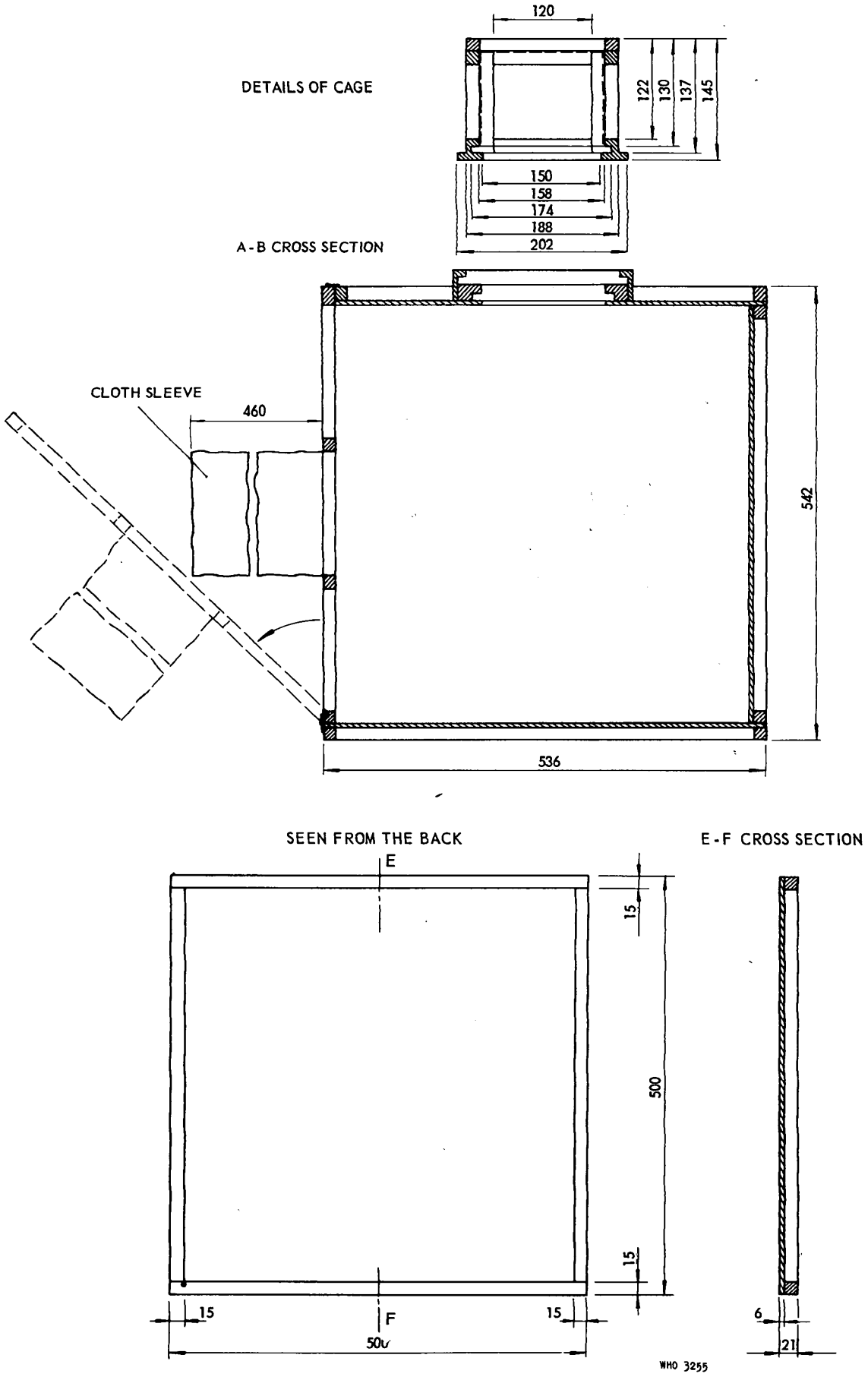


FIG. 6

DETAILS OF CONSTRUCTION OF WALLS AND FLOOR OF THE O.P.S. EXITO-REPELLENCY BOX

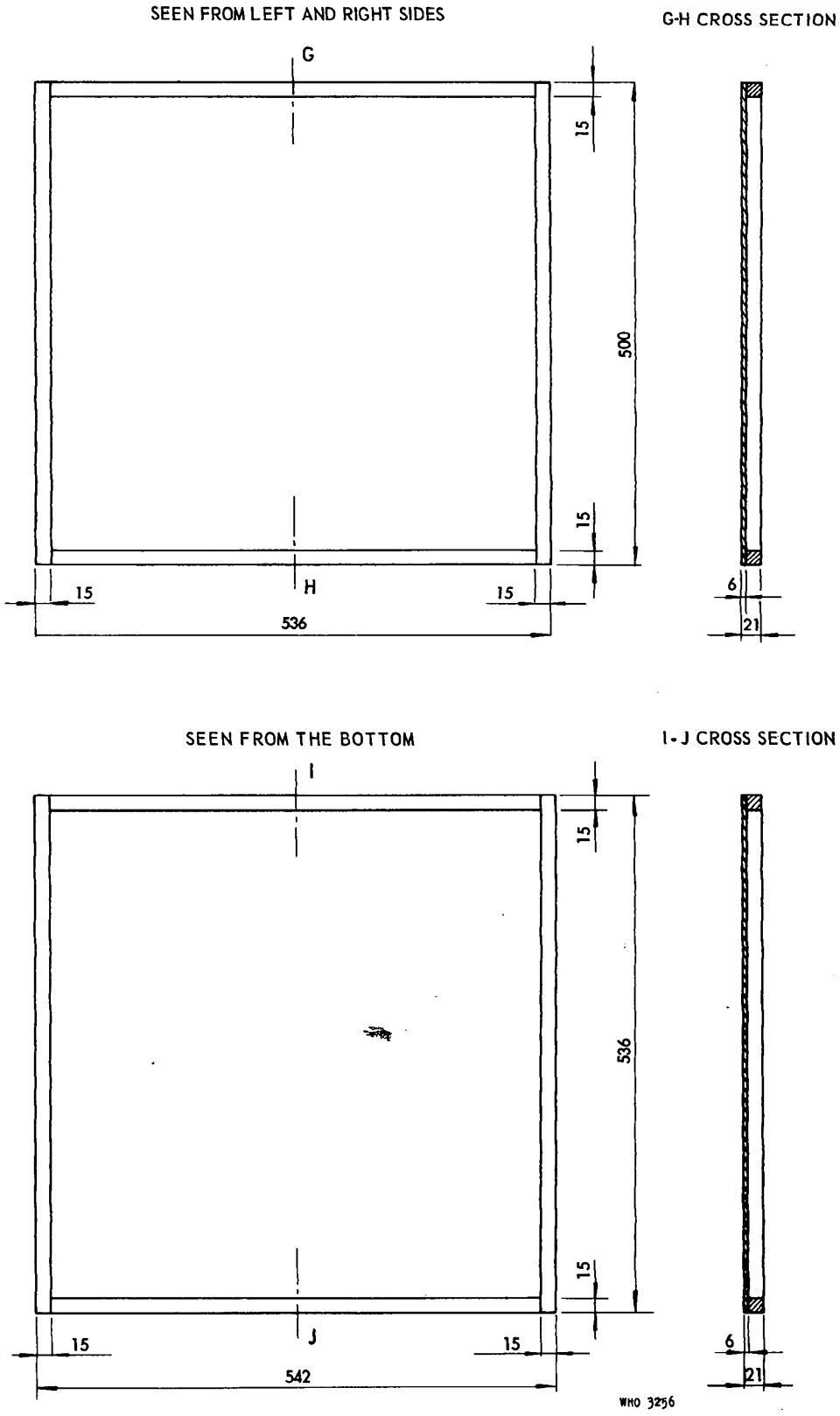
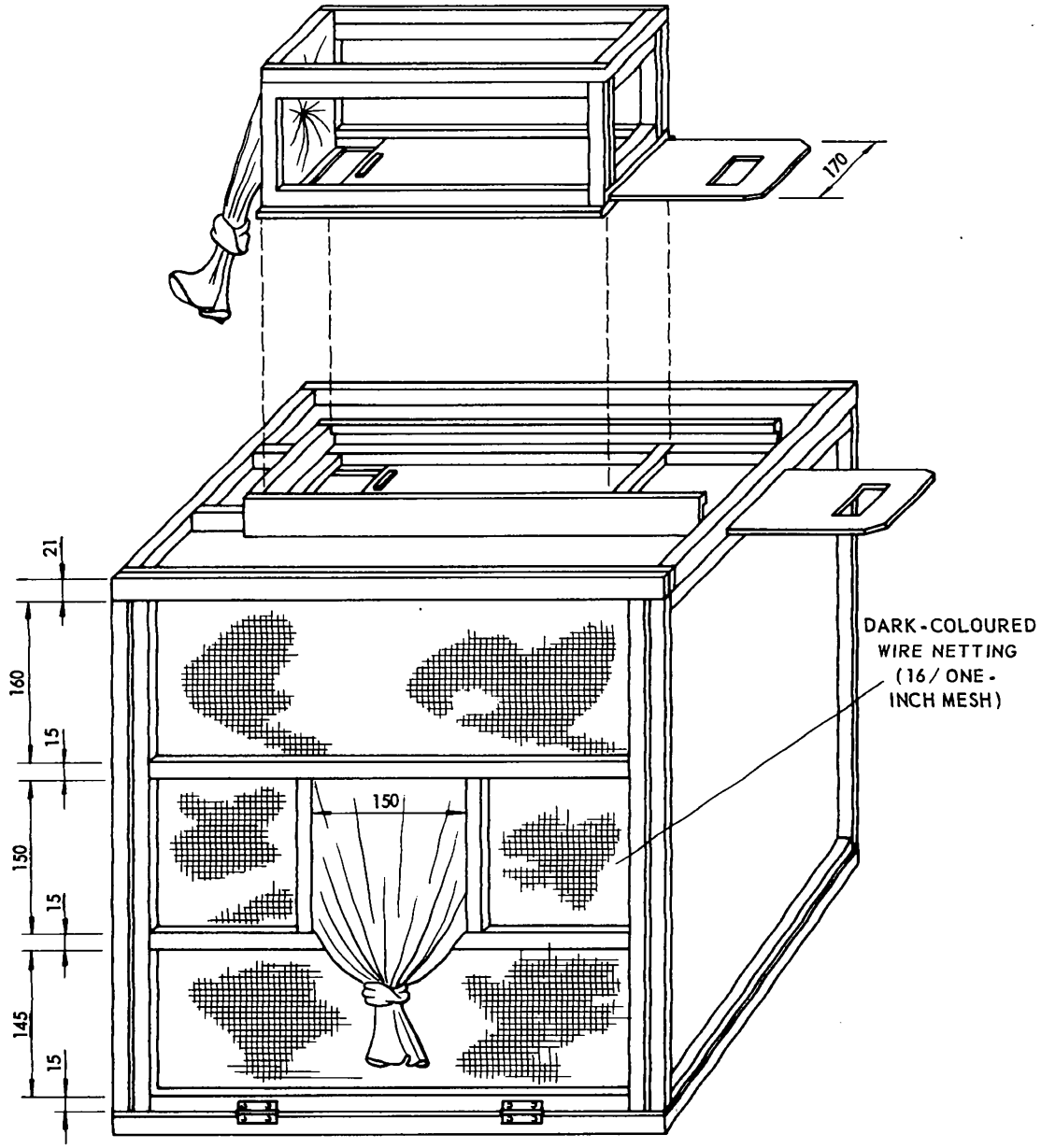


FIG. 7

THE O.P.S. EXITO-REPELLENCY BOX ASSEMBLED



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ALL MEASUREMENTS IN MILLIMETRES

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