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ENTOMOLOGICAL FIELD-TRIALS FOR ASSESSING THE EFFECTS OF DIFFERENT DOSAGES OF DDT

The document (Appendix 1) attached to this note is entitled "Tentative operational method for assessing the entomological effects of different dosages of DDT in the field". It has been prepared at WHO Headquarters by the Divisions of Malaria Eradication and of Environmental Sanitation, in response to an urgent demand for a method permitting re-examination in the field of the most economical DDT-dosage and frequency of application which can be used effectively against the vector-mosquitos in malaria eradication programmes.[1]

It has not yet been possible to submit the method to testing under field-conditions in order to improve it in the light of experience thus gained. The draft proposals were, however, commented on by WHO Regional Offices and by entomologists experienced in operating similar methods, and the tentative method has been modified after consideration of their views. The description of the method is issued in this series to make it available to all concerned in malaria eradication programmes and projects and to obtain the consensus of opinion of the readers of WHO/Mal documents with experience of the problem involved.

Ever since the introduction of residual insecticides, technical difficulties have been encountered by those attempting to assess their effects in entomological terms. We believe it is for this reason that no standard method was evolved for the purpose at an earlier stage. It is recognized that the ultimate criterion of strategic effectiveness in malaria eradication, whatever the method of attack, is the interruption of transmission, assessed epidemiologically. But that depends on many factors and cannot be applied to evaluate a field-trial which is concerned solely with insecticide-dosage and frequency of application. In such a trial an attempt must be made to observe the direct entomological effects of a given dosage

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[1] Field-trials for this purpose were recommended by the Eighth Session of the WHO Expert Committee on Malaria, held in Geneva in July 1960: see Wld Hlth Org. techn. Rep. Ser., 1961, 205

in a sample of huts or rooms to be sprayed. It is considered that the information obtainable by applying the WHO bio-assay test cannot provide the chief criterion of evaluation, though it may serve as accessory evidence of effectiveness. The bio-assay test confirms the potency (and later the loss of potency) of a residual deposit on a particular surface, against mosquitos exposed to it for an arbitrary fixed period. It does not tell us the insecticidal effectiveness of the sprayed room as a whole against all the vector mosquitos which enter, and which are free to move about and to leave it any time.

The assessment of the insecticidal effectiveness of the sprayed internal surface of the room is the object of the index emphasized in the tentative method: the room-kill (see Appendix 1, paragraph 5.2.1 and Annex 1). The average 24-hour room-kill of a vector is the over-all per cent. mortality resulting among all the females found to visit sprayed rooms during a day and a night, corrected for the mortality among those which visit similar rooms in an unsprayed locality in the same period. The operational aim in field-trials employing the method is to find out for how long a given dosage continues to give satisfactory room-kills of all the vector-species, in those types of building most commonly entered by them. This information will serve the strategic purpose stated in paragraph 1.3 of the Appendix.

In paragraph 5.3.2 of the Tentative Operational Method a "satisfactory" level of room-kill is suggested. This is a rough guide only, since the room-kill necessary to prevent malaria-transmission by a particular vector depends on that vector's longevity in nature, and particularly on the frequency of its visits to sprayed rooms: the less the house-visiting propensity of the mosquito, the greater must be the room-kill to secure adequate vector-control.

The task of drafting a procedure for the trials was far from easy. A field-trial of scientific validity could best be carried out by a team of research-workers able to devote all their time to the trial and possessing the facilities and research-funds of a well-established field-research station. Moreover, such a group should work in an area uncontaminated by massive applications of residual insecticides. Instead the present operational method is modified in view of the very limited facilities

usually available to the entomologist engaged on routine duties in one district or zone of a malaria eradication programme already in full swing. It is recognized that he may have few trained assistants and may have difficulty in obtaining special funds for hiring premises, making window-traps and so forth. At the same time the entire area may already have been treated with residual insecticide. In such circumstances even this tentative method, or any other that might be put forward, may raise operational problems; yet the economizing of insecticide without loss of effectiveness of the attack is of such importance in every malaria eradication campaign that any expense involved in these field-trials should be regarded as a trifle, in comparison to the major economies which their results may justify.

While the test method has been designed with DDT primarily in view, this being the most widely-used insecticide in malaria eradication campaigns, it might equally well be applied to other residual insecticides used against mosquitos in buildings.

APPENDIX 1

WHO tentative test method

TENTATIVE OPERATIONAL METHOD FOR ASSESSING THE ENTOMOLOGICAL EFFECTS OF DIFFERENT DOSAGES OF DDT IN THE FIELD

1. Purpose

1.1 It has become necessary to re-examine the dosage and frequency of application of some insecticides, particularly DDT.

1.2 An entomological test method is required which will supply data on the effective duration of different dosages in the field. The method described here, while not perfect, aims at providing an expedient tool for use by the entomologist who works at district level with limited trained staff and resources, often in an area already entirely sprayed with DDT. A fuller field trial might only be undertaken where there are facilities such as a substantial unsprayed area with high densities of anophelines, a field research team, and special funds for the research equipment.

1.3 The purpose of the present tentative method (the details of which will undoubtedly be improved in the light of practical experience gained in applying it) is the following:- to determine for how long each of two dosages of DDT (usually 2 g - 1 g per m^2) will continue to give what is deemed to be satisfactory control of the local malaria vectors visiting their chief indoor daytime and night-time haunts, and to estimate how many applications of either dosage may be required in a year in order to maintain satisfactory vector-control through the transmission season.

2. Selection of village or area for test

2.1 Where a typical village is available which has not been sprayed in the current or the preceding year, the whole test should take place in one village. Elsewhere it may be necessary to spread the work over an area of several villages in order to find sufficient suitable untreated capture-stations. The villages must be surveyed before the start of the spraying-programme for the year, and must be withdrawn from the ordinary spraying-programme for the duration of the test.

2.2 The village or area must be a fair sample of the wider area for which estimation of the appropriate dosage and cycle is required. It should however be chosen to provide the maximum density of vector-anophelines throughout the transmission season.

2.3 The test is run in the two types of rooms (prevalent type A and B) mainly visited by the vector-mosquitos by day or by night. The most important types to distinguish are those presenting different combinations of sprayable surfaces; but differences in usage (human and animal-occupation, respectively) should also be taken into account, since the control of even an anthropophilic vector may depend on the kill obtainable in cattle-sheds if these happen to be its chief daytime haunts. The test-village or area must be one possessing not less than 25 unsprayed huts or rooms of each type to be tested, and these should be in groups of 5 or more per village if the test has to be spread over several villages.

2.4 If an area with sufficient unsprayed rooms suitable for the test cannot be found because of total insecticidal coverage of the whole country or district, there will be no alternative but to erect a group of experimental huts. These should be of two types corresponding closely (as to their indoor surfaces and night-time occupants) to the prevalent types for the area, and they should be placed close to a good source of vector-mosquitos if the site of such a breeding place is known.

2.5 An uncontaminated building should be hired or built in the area to serve as a field-laboratory, where cages of live mosquitos can be kept and the collections be identified and counted.

3. Selection of houses and rooms

3.1 Each capture-station will be an unsprayed one-room building or a room in an unsprayed house. In the first instance not less than 20 capture-stations of each type indicated in 2.3 should be found, numbered, and marked on a sketch-map. An assurance must be obtained from the owner that the room will be available through six months or longer for a once-weekly evaluation lasting a day and a night, and will remain occupied in the normal way especially at night. In some conditions payment will have to be offered for this privilege.

3.2 The capture-stations must be suitable for the efficient capture of mosquitos by means of outlet window-traps and space-spraying. On the one hand they must have a window-opening capable of being fitted with a rectangular trap (or it must be permissible to make such an opening when the room is selected). On the other hand it must be possible to close or mask all other openings so that mosquitos, while not prevented from entering, will not readily find an alternative way out. For the space-spraying the room, including its door and window, must be well-closed.

3.3 It will much increase the value of the test if the furniture and usage of at least some capture-stations are such that floor-sheets can be laid down overnight, to permit collection in the morning of the mosquitos killed during the night.

4. Operating procedure

4.1 Pre-treatment period

4.1.1 Once-a-week for three weeks make space-spray collections in not less than 40 rooms (20 of each type) to find out the average densities of resting mosquitos. An aerosol space-spray containing pyrethrins and piperonyl butoxide (but no persistent insecticide) will be supplied by WHO on request. Record if possible the number of people or animals occupying each room the previous night, the numbers of females (by species) in the catch, and the numbers of blood-fed females. The detailed technique of space-spraying is given in Annex 2.

4.1.2 Select for further work at least 10 rooms of each type[1] showing the highest densities of the vectors. (Exclude any rooms unoccupied at night, for in those the night-densities will probably be low even if the daytime densities are high). Allot two window-traps to each room selected, mark them with the number given to the room and with an indication to show which trap is for daytime use and which for night-use. A design for the construction of a window-trap is appended (fig. 1).

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[1] These are minimal figures. Where conditions and resources permit, 15 - 20 capture-stations of each type should be included in phases 2 and 3 of the schedule.

Schedule of Capture-stations

Phase	Prevalent type A	Prevalent type B
1. Period of spray-catches only (3 weeks)	20	20
2. Pre-treatment period of spray-catches and trapping (3 weeks)	10	10
3. Spraying and evaluation	10:	10:
(a) Higher dosage	4	4
(b) Lower dosage	4	4
(c) Controls	2	2

4.1.3 Once-a-week for a further three weeks make day and night window-trap catches, morning space-spray catches and (where possible) overnight floor-sheet collections in the selected capture-stations. The first trap is placed in position on each room as soon as possible after sunrise and is removed to the field-laboratory about sunset. It is at once replaced by the second trap, which remains in position through the following night so that the total capture-period is 24 hours. Overnight floor-sheets should be laid down at a regular time, e.g. when the night-trap is installed. Early the next morning the floor-collections are carefully transferred to a suitable container. After removal of the trap the window is closed and the room is space-sprayed to collect the mosquitos still resting in it.

4.1.4 After its removal each window-trap is held in the field laboratory for 24 hours under the best conditions obtainable for ensuring survival of the trapped mosquitos. At the end of this holding-period any dead mosquitos are removed by extracting the sliding tray from the trap. The living mosquitos are then collected by suction-tube for identification.

4.1.5 The categories to be recorded are shown on the record-form attached. It should be noted that the form covers the catches of one species only, in one capture-station and in one 24-hour period, in order that the results may be easily combined and reviewed from any desired point of reference.

4.2 Application of residual DDT

4.2.1 Where there are 10 capture-stations of each type, four will be sprayed with the higher dosage, four with the lower, and two will be controls. The controls should be those found to have the median average densities of vector-mosquitos. Of the four stations having the highest densities, the first and fourth should be sprayed with one dosage, the second and third with the other, thus avoiding bias. The same should be done in the group having the lowest densities.

4.2.2 The control-stations should be sprayed with water, as this will help the investigator to retain the confidence of the occupants.

4.2.3 The residual spraying should be carried out by experienced men and be directly supervised by some officer of professional status in the malaria eradication service. Care should be taken to see that the operation conforms closely to the standard demanded in an eradication campaign.

4.2.4 The whole internal surface of ceiling and walls of the test-station should be sprayed, and that of the roof if it is not entirely closed off from the room. Where the room is part of a larger building the whole house should be sprayed. If any portion cannot be sprayed, the fact should be recorded. Surfaces behind or under heavy furniture should be sprayed also. Outside walls, eaves and verandahs should not be sprayed for the purposes of this test.

4.3 Evaluation

4.3.1 The procedure described in 4.1.3 is repeated throughout the evaluation period, the collections being made once-weekly in each capture-station[1]. The preferable plan of work is to evaluate 10 capture-stations per day, spreading the work over the week. On any one day the evaluation should cover five stations of each type, viz:-

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[1] It is anticipated that a proportion of the vector-mosquitos will enter the traps on unsprayed stations during daytime, and the proportion may be increased by the presence of an irritant insecticide. But if it is found in practice that insignificant numbers reach all the traps by day, the daytime trapping may be discontinued.

Evaluation schedule for 20 capture-stations

Day	Type A			Type B		
	2 g/m^2	1 g/m^2	Control	2 g/m^2	1 g/m^2	Control
1 - 2	2	2	1	2	2	1
3 - 4	2	2	1	2	2	1

4.3.2 The daily record-form is used throughout, a copy being used for each station and for each species considered of importance in vector-control. Figures for other species may be valuable too, for example where there is likelihood of the numbers of vectors falling away during the test. It is clear therefore that the initial recording of a week's work in 20 capture-stations may entail the filling of 20, 40 or 80 record-forms.

4.3.3 In principle the evaluation period lasts while either dosage in either type of station continues to exercise measurable effect on the vectors. The test can be terminated six months after the spray-application if it is known that a longer cycle is not under consideration, or if the transmission-season has then ended. On the other hand it may be desired to study the duration of effectiveness of two applications of the lower dosage (e g. of 1g per m2). The second application in the stations treated with this dosage should be given only when there is definite evidence of failure of vector-control following the first application. Comparative evaluation of DDT at "one gramme twice" and "two grammes once" should be continued beyond the sixth month if useful.

4.3.4 A given dosage may last for a longer or a shorter time according to the wetness or dryness of the season. To cope with this special case it would be necessary to repeat the test in the wet and the dry seasons, using different capture-stations.

4.3.5 The daily record-form provides space for the crude per-cent mortalities among the mosquitos visiting the capture-stations. When the entomologist compiles the results, however, he should first add the total numbers (not the rates of mortality) for all the capture-stations of one type sprayed with one dosage, and calculate the average uncorrected mortalities for this group of stations. He should then correct them for the average mortalities found in the group of control-stations of the same type, if the latter are between 5% and 20%. For this purpose Abbott's formula is used, viz:-

$$\frac{\%\ \text{test-mortality} - \%\ \text{control-mortality}}{100 - \%\ \text{control-mortality}} \times 100$$

4.3.6 It would be meaningless to work out an overall rate of mortality combining the results from the different dosages. On the other hand a combination of results from any one dosage in the different types of capture-station has value, since it will indicate the general level of room-kill of the vector to be foreseen at a given interval after application of that dosage throughout the area. Where there is more than one vector a safe guiding principle is that the level of vector-control obtained is that of the worst-controlled vector.

4.3.7 In addition to the quantitative evaluation so far described it is advantageous that the entomologist should personally visit the capture-stations by day and by night in order to observe the normal behaviour of the vector-species and their reactions to the presence of DDT. Points particularly to be watched for are movements of the mosquitos from one type of resting-place or one part of the room to another, contact with sprayable surfaces before and after feeding, preference for non-sprayable types of surface such as clothing, spider-webs etc., and repeated flight of irritated mosquitos in search of an unsprayed surface. It will be of value if these observations can be correlated with differences in the room-kills of associated species

5. Interpretation

5.1 Assessments required. The test-procedure described is designed to provide a basis for the following assessments in the area concerned:-

(i) the relative duration of entomological effect of two dosages in houses or rooms of a given type of structure and usage,

(ii) the relative duration of a given dosage in houses or rooms of the two prevalent types,

(iii) the duration of entomological effect of each dosage in relation to the type of area studied and the season of transmission.

5.2 Indices employed

5.2.1 Where overnight floor-collections are possible the chief index employed is the average 24-hour room-kill of the vector, defined as:-

$$\frac{\text{number dead on floor + numbers dying in day and night traps}}{\text{number dead on floor + total catches in traps}}$$

5.2.2 Where floor-collections are not possible it is necessary to use instead the average 24-hour window-trap mortality. Either of these indices must be corrected for the mortality in the controls.

5.2.3 Other indices used for the assessments are the 24-hour total catch of the vector per sprayed room ÷ the catch per control-room (i.e. on the floor and in traps, or in traps alone); the space-spray catch per sprayed room ÷ the catch per control-room; the proportions of blood-fed and gravid vector-females in the catches; and the percentage survivals in the blood-fed fraction. The entomologist should base his assessments on a consideration of all these factors and not on one factor only.

5.3 Critical levels of indices

5.3.1 Total catch: Measurement of the room-kill will only be valid if based on the collection of a substantial proportion of all the vector-mosquitos visiting the sprayed stations in the 24-hour period. The average total catch per sprayed test-station (dead on floor + dead and alive in traps) should be at least half the average per corresponding control-station. If it is less, the reason and remedy should be sought.

5.3.2 Room-kill: 70% or over for the two types of capture-station together may be regarded as a satisfactory room-kill under all but the severest conditions of malaria endemicity.[1]

[1] Macdonald, G. and Davidson, G. (1953) Bull. Wld. Hlth Org., 9, 785, state:- "Though workers will adjust their standards to local conditions, it is suggested that the normal criterion of efficacy in an insecticide should be the attainment of a 65%-mortality rate within the day among all mosquitos entering a treated shelter. One producing an 85%-mortality rate might be rated as suitable for use under the most severe conditions, and one not exceeding 50%-mortality as suitable for the control of moderate transmission by endophilic mosquitos only."

5.3.3 Window-trap mortality: 50% or over may be regarded as satisfactory so long as there is reason to think that a substantial proportion of the vectors are dying within the rooms. At other times a mortality of 70% should be the criterion. (Alternatively, the average number of blood-fed vectors surviving in the traps from the sprayed rooms may be considered in relation to those from the controls. This ratio should be not higher than 1:5.)

5.3.4 Daytime resting-density: The space-spraying should reveal a density per sprayed station not higher than one tenth the density per control-station of the same type.

5.3.5 Blood-fed vectors: Except where the total catch (including overnight floor-collections) of blood-fed vectors is sharply reduced by the insecticide, the average corrected mortality in this fraction should be at least 80%.

5.3.6 Gravid veetors: The average total catch of this fraction should be a ratio not exceeding 1:5 in the sprayed stations compared with the control-staions. Room-kill or window-trap mortality of the gravid females should be at least 80%.

WHO Insecticide-Dosage Trial Appendix 1 - Annex 1

DAILY RECORD-FORM FOR ONE ROOM, ONE SPECIES

A. General

1. House number _____ 2. Date _____

3. Night-occupants of room (a) human _____; (b) animal _____

4. Anopheles species: A. _____

B. Results of day-catch: numbers caught Trap number _____

		Males	Females			
			empty	blood-fed*	gravid	Total
1	Dead in room (if collected)					
2	Dead in trap (after holding-period)					
3	Alive in trap (after holding-period)					
4	Total catch (1 + 2 + 3, or 2 + 3)					
5	per cent mortality (by day)	X	X			

C. Results of night-catch: numbers caught Trap number _____

		Males	Females			
			empty	blood-fed*	gravid	Total
1	Dead in room (if collected)					
2	Dead in trap (after holding period)					
3	Alive in trap (after holding-period)					
4	Total catch (1 + 2 + 3, or 2 + 3)					
5	per cent mortality (by night)	X	X			

D. Uncorrected 24-hour mortality (in room or traps)

	blood-fed females	all females
1 Total catch, day + night		
2 Total dead, day + night		
3 24-hour mortality (room-kill or traps only)		

E. Results of spray-catch in room

1. males _____ 2. females: (a) empty _____ (b) blood-fed _____
(c) gravid _____ (d) all _____

3. Index of resting females ÷ total catch, day + night _____

F. Remarks (note carefully any disturbance of room or trap which may have influenced the results: e.g. change of use of room, replastering etc.)

Name of investigator _____

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* Classify as "blood-fed" all mosquitos judged to have fed during the night preceding capture. Those surviving through the holding-period will be semi-gravid when counted, but they should if possible be distinguished from the fully-gravid.

Note: supplies of this form may be obtained from Regional Office of WHO

Procedure for collection of resting mosquitos by space-spraying

1. The operator is equipped with the following:

A corked 3 x 1-inch tube (or other container) for each capture-station to be space-sprayed, the tubes being labelled by the entomologist with numbers corresponding to those of the capture-stations, and with the date and name of the operator.

Sheeting sufficient to cover the floor and the upper surfaces of beds, tables, etc., in the largest capture-station to be space-sprayed.[1]

An electric torch (with a spare bulb and battery).

A pair of entomological forceps.

either

An aerosol-bomb containing a quick-acting volatile insecticide solution, but with no addition of any persistent insecticide.

or

A hand-operated spray-gun filled with a 0.1% or 0.2% solution of pyrethrins in kerosene, together with a supply of the same solution sufficient to fulfil his programme of space-spraying.

2. The operator visits all the rooms to be space-sprayed and performs the work between sunrise and 8.30 a.m. (In some countries collections later in the morning are unreliable as too many mosquitos are disturbed by the activity of the people.)

3. On reaching the capture-station the operator asks the people to vacate the room. It is an advantage if he is able at this time to record the number of people who slept in the room overnight.

4. He closes up the room as completely as possible, particularly by using blankets or other suitable material to close doorways and windows. He then lays down the sheeting, covering the whole floor and other horizontal surfaces.

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[1] This sheeting may be made of cheap material such as half-white (unbleached) calico preferably in 2 or 3 pieces of 9 x 12 ft.

5. If the room has spaces under the eaves, or other spaces by which mosquitos might escape, the operator sprays these spaces from the outside before spraying inside the capture-station.

6. To space-spray the station he stands in the middle and turns about, directing the spray upwards. The cubic capacity of the station should have been assessed beforehand by the entomologist, and the operator should know how much insecticide to spray in each capture-station. When using an aerosol-bomb the dose of spray should be gauged at ten seconds per 1000 cu. ft. When using 0.2% pyrethrum-kerosene solution, the operator should give 75 strokes of the spray gun per 1000 cu.ft. (which is equivalent to 15 to 20 cc of insecticide solution). The operator should be instructed how to count seconds when using an aerosol-bomb.

7. After finishing the spray operation he must wait at least ten minutes, within the room or outside, during which time no-one must enter.

8. For collection of the catch, if the day is windless, the sheets are carefully gathered up and carried outside. But if a breeze is blowing the catch must be collected while the sheets are in place in the station, and a torch must be used to find all the mosquitos.

9. The mosquitos are picked up by the wing or the leg with forceps, and placed in the corked tube bearing the number of the capture-station. They are taken to the laboratory for identification as soon as the space-spraying programme for the morning is completed.

Explanatory notes on outlet window-trap

1. Materials

In the perspective drawing the dotted surfaces (shown by a double line in the cross-section) are of mosquito-netting or plastic or wire-gauze. The other surfaces (with the exception of the tray which should be of metal) are of wood or other suitable material obtainable locally. In the framework are four holes through which are passed the strings to hold the gauze flaps forming the entry-slit.

2. Dimensions

The drawings are not to scale. The dimensions marked should be regarded as standard ones, but they may be modified to suit local conditions.[1]

3. Fitting of trap to window

Where windows are small, of regular shape, and have wooden frames the trap may be hooked directly on to the frame, the hooks being screwed into the special wooden frame built on to the trap. But for window-outlets of irregular shape or in very uneven walls it is advisable to provide a large board in which is a central square hole slightly smaller than the special frame round the entry of the trap. This board should be screwed over the window-opening, and be left always in position if that is acceptable to the householders.

4. Ant-barrier

Creosote may be painted round the square entry of the trap or (if acceptable) on the walls round the window. It will be noted that a trap of this design does not touch the building except at the hooks and round the edge of the special wooden frame.

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[1] It is estimated that a trap of the standard size will require the following quantities of materials:-

sheet-plywood	- 0.61 m^2 (7 ft^2)	carrying-handle (top of trap)	- 1
gauze or netting	- 0.76 m^2 (8.8 ft^2)	knob-handle (for hinged flap)	- 1
sheet-metal for tray	- 0.16 m^2 (1.85 ft^2)	hinges	- 2
wood battens for frame	- 6.2 m (20.5 ft)	screw-hooks (large)	- 2
string or tape	- 1 m (40 in)	screw-eyes	- 2
attachment board (if used)	- 0.55 m^2 (6.4 ft^2)	screws	- 24

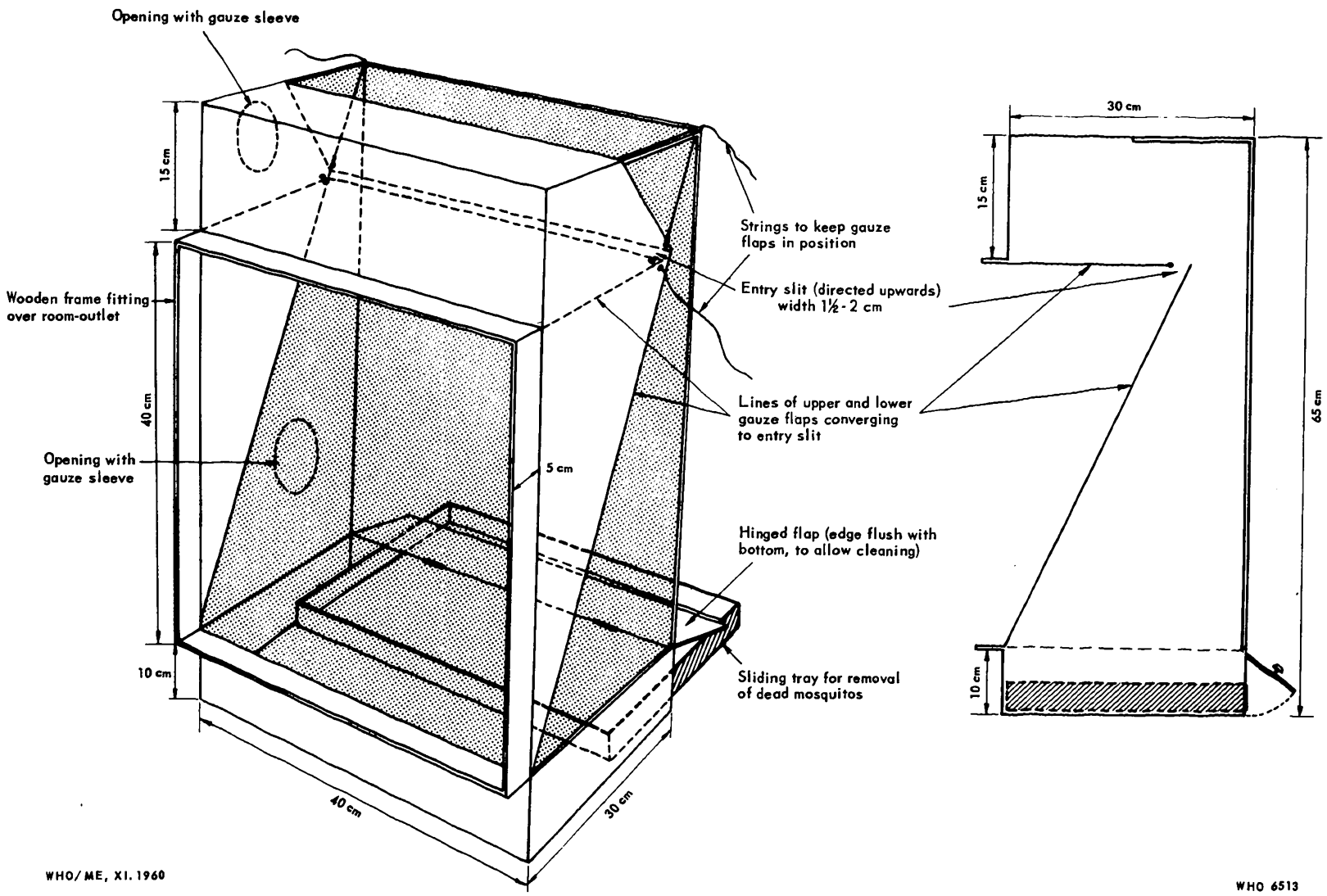
5. Collection of dead mosquitos

When the sliding tray is removed to collect dead mosquitos, the floor of the trap should be examined and cleared also. A search should be made for any specimens that have fallen on to the upper gauze flap.

6. Contamination of traps

All possible precautions must be taken to prevent any contamination of the window-traps by residual insecticide, to detect it promptly if it should nevertheless occur, and to clean the contaminated traps thoroughly before they are re-used.

FIG. 1 OUTLET WINDOW TRAP



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