# OBSERVATIONS ON THE WORLD HEALTH ORGANIZATION ADULT TEST KIT FOR BIOASSAY OF INSECTICIDE DEPOSITS ON WALL SURFACES

J. A. ARMSTRONG, B.Sc.
Senior Scientific Officer
AND
W. R. BRANSBY-WILLIAMS, B.A., D.A.P. & E.
Senior Field Officer, Colonial Pesticides Research Unit, Arusha, Tanganyika

INTRODUCTION. The use of bioassay tests in insecticide trials and in control schemes has enabled much extra and extremely useful information to be obtained. However, up to the present there has been no standard technique for carrying out such tests, and each entomologist has either developed his own methods or made use of previously known methods. As with the adult mosquito test kit, the Expert Committee on Insecticides (1957) felt that much more information could be made available if a standard wall test kit was put into use. The majority of bioassay tests had been carried out with apparatus based on either a three-inch diameter glass funnel or an eleven-centimeter petri dish (Burnett, 1957; Davidson, 1950; Hocking *et al.*, 1959); the two chambers supplied by the World Health Organization were of approximately the same dimensions. These chambers were supplied to several different research organizations with the request that their merits and faults should be assessed.

The conical exposure chamber (Figure 1) was constructed of plastic with a flange around the mouth and in place of the stem of the funnel there was an inverted conical orifice through which the mosquitoes could be introduced. With the funnels was supplied adhesive-backed plastic strip to be used to plug gaps between the funnel and the wall surface. When in use the chamber was to be fixed to the test surface and the mosquitoes blown in through the orifice by means of a straight tube. The purpose of the inverted conical orifice was to reduce the chances of an insect flying back out of the opening. At the end of the exposure time a bent sucking tube could then be reinserted through this opening and the mosquitoes removed to a holding cage.

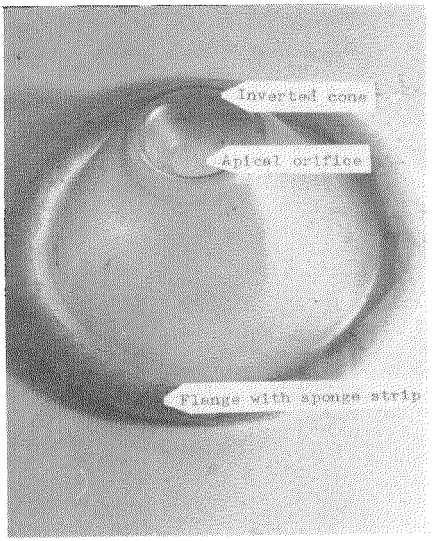


FIG. 1.—The World Health Organization Cone exposure chamber.

The dish exposure chamber (Figure 2) also of plastic, was in two sections. The base section had a turned lip along the top and bottom edges and a centrally placed opening eight centimeters in diameter. The top section was an eight-centimeter dish approximately one centimeter deep with a squared flange so made that it would slide into the base under the two

turned lips. A set of cardboard shutters was also provided. To assemble the apparatus, the dish was slid into the base and a cardboard shutter inserted between the dish and the base. A small hole towards one end of the shutter permitted mosquitoes to be blown into the chamber by means of a straight tube, after which the shutter was then adjusted to block the hole. The complete exposure chamber was then fastened to the wall surface and at the start of the exposure time the shutter was removed, at the end of the exposure time the shutter was reinserted and the insects isolated within the chamber. The complete apparatus was then removed and the mosquitoes transferred to a suitable holding cage.

A sucking tube was also provided with the kit; it consisted of a rubber tube with a mouthpiece, a one-by-three-inch chamber and a one-quarter-inch diameter tube with an angled tip.

The wall exposure chambers were received with a request to carry out an investigation along the following lines:—

(1) The relative convenience of handling the two types in the field.
(2) The consistency of results obtained with each type of exposure chamber when replicate exposures are made (i.e. more than one test performed on the same wall surface).
(3) A comparison of results obtained with the two types of exposure chamber when applied to the same type of wall surface. (In the evaluation of results, control as well as test mortalities should be taken into account.)
(4) Which of the two methods indicate the aging of insecticide deposits with the greater degree of accuracy.

It was also suggested that, "observations be made over a period of six months and

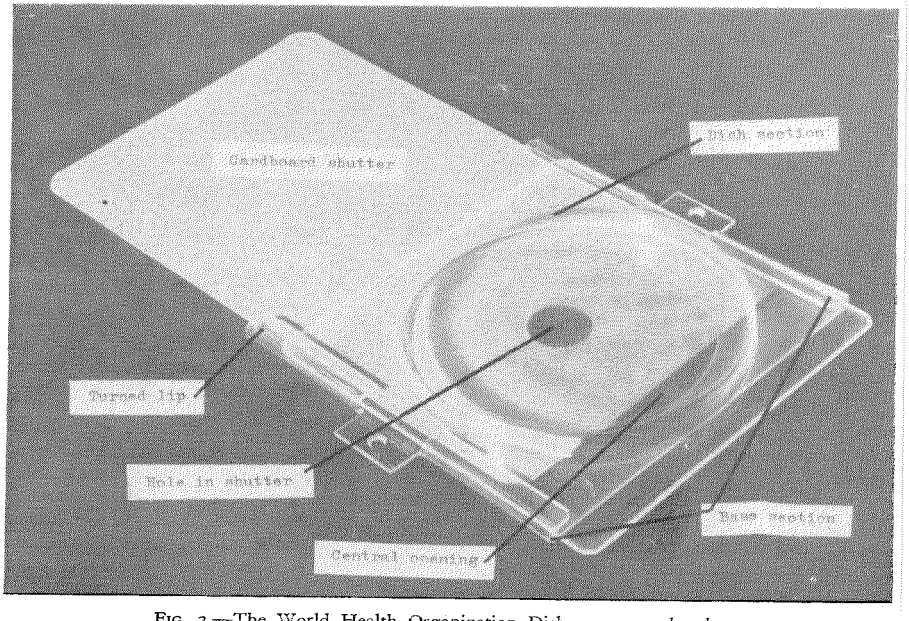


FIG. 2.—The World Health Organization Dish exposure chamber.

that exposures be carried out (a) twice weekly for the first three months, and (b) monthly for the second three months on walls sprayed with DDT at a dosage of 2 gm. per sq.m. and dieldrin at 0.5 gm. per sq.m. Both sorbtive (mud) and non-sorbtive surfaces (material to be stated) to be used if possible and data on the sorbtive character of the wall to be provided with the report."

METHODS. In order to carry out all phases of the work a series of tests were carried out both at the Colonial Pesticides Research Unit laboratory in Arusha, and at the field station at Magugu, 93 miles southwest of Arusha. The observations for section (1) were obtained from tests carried out in the experimental houses and in native houses at Magugu. To provide a wall surface on which to carry out tests for sections (2), (3) and (4) a brick wall plastered with sorbtive and non-sorbtive mud was constructed at Arusha. The wall was built around the inside of a circular aluminum house (Uniport Altent). The wall, 5 feet 9 inches (178 cm) high and about 20 feet (5 metres) long was divided into two sections, one plastered with a sorbtive mud (from an area near Magugu) and the other with a non-sorbtive mud from Njoro Nkubwa near Taveta used previously by Burnett (1957). Before being sprayed, each section was divided into two identical panels each 2.15 square metres in area which were marked on a grid system and coded so that each square possessed a reference letter and number. The space between each pair of panels was to be left unsprayed and to be used as a control surface. When the wall was plastered it was intended to have the muds about one inch thick, but unfortunately this was not possible and the plaster was only about half an inch thick.

The panels were sprayed with an Oxford Precision sprayer, fitted with a number zero flat-fan nozzle, at a nozzle pressure of 30 lbs per square inch. One panel of each type of mud was sprayed with DDT at an intended dosage of 2.0 gms per sq. metre, and the other panel of each type of mud was sprayed with dieldrin at an intended dosage of 0.5 gm per sq. metre. During the spraying, each control panel was shielded to prevent contamination. The required weight of the wettable powder was mixed with one litre of water and each panel was sprayed using vertical swathes. When a panel had been coated once (three swathes), the operator paused to permit the water to soak into the mud, and then repeated the cycle until the whole of the spray mixture was applied to the wall. The spray apparatus was rinsed with water between the application of the DDT and the dieldrin wettable powders. For chemical estimation of the spray deposit three sample papers were fixed to each panel prior to the application of the insecticide. These papers were analysed by the chemistry department using the Stepanow reduction method for total chlorine estimation. The mean deposit on each panel was found to be: DDT on sorbtive mud, 1.98 gms per sq. m.; DDT on non-sorbtive mud, 1.69 gms per sq. m.; dieldrin on sorbtive mud 0.63 gm per sq. m.; and on non-sorbtive mud 0.59 gm per sq. m.

Various methods of fastening the exposure chambers to wall surfaces were tried and it was found that for exposures on solid wall surfaces four pieces of cellotape ⅝ inch (1.5 cm) wide by 6 inches (15 cm) long were sufficient to hold the conical chamber in place and two such pieces would hold the dish chamber in place.

The test insects were adult female *Anopheles gambiae* and adult female *Aedes aegypti*, each maintained as a colony at the laboratory. The *A. gambiae* colony originated from the East African Institute of Malaria at Amani (Shute, 1956). The *A. aegypti* colony is a mixed strain which has been maintained at this Unit for several years. In all tests the insects were exposed for a thirty-minute period, three hours after the blood-meal. Whenever possible only three-day-old mosquitoes were used in the tests, but unfortunately it was at times necessary to use mosquitoes up to six days old that might be gravid or unfed. When the insects were exposed to the

DDT-treated surface, it was noticed that they became excited after a few minutes (Kennedy, 1947; Davidson, 1953) and if more than 20 insects were inserted in the conical chamber the exposure time was frequently lengthened in the attempts to remove the last few. For this reason all tests with the cone and dish chambers on DDT and dieldrin-treated surfaces were set up with an average of 15 mosquitoes per chamber.

The holding cages were 6" x 3" x 3" (15 x 7.5 x 7.5 cm.) wire frame cages covered with mosquito net. To transfer the mosquitoes from the conical exposure chamber to the holding cage, a long, straight sucking tube with an angled tip, but without a central chamber, was found to be most efficient. The mosquitoes were sucked out of the chamber and blown gently into the holding cage. With the dish exposure chamber, the method suggested in the instructions was employed, "the exposure chamber is taken from the wall and placed over the top of a half-pint disposable paper cup. The cardboard slide is then removed carefully, thus letting the mosquitoes pass into the paper cup which will serve as recovery cage during the following 24 hours," except that in place of the cup a wire holding cage was used. It was found that although one person could do the job, it was more easily done if there was an assistant. A piece of cotton wool soaked in sugar solution was placed on top of the net of each cage, and the cages were placed in a darkened cupboard at a relative humidity of 70–90 percent and a temperature of 21 ± 3° C. for a twenty-four hour holding period after which the mortality count was taken. Any mosquito that could not fly up off the bottom and rest on the sides or top of the cage was counted as dead. After the mortalities were recorded the cages were dismantled, washed in soap and water, rinsed and then dried; at no time was there any sign of contamination of the frames or nets. On completion of each series of tests the exposure chambers were also washed in the same manner and the cardboard slides were wiped with a damp cloth.

The apical hole of the conical exposure chamber became torn and enlarged after repeated use, and when mosquitoes were observed flying out of the hole a group of tests were set up with 20 *A. aegypti* in each of ten cone chambers. The exposure chambers were set in a vertical position against filter papers treated with a 0.05 percent dieldrin-risella oil solution. At the end of a thirty-minute exposure time the mosquitoes remaining in the cones were counted. In only one cone were there still twenty mosquitoes and in two of the chambers half the mosquitoes had escaped. In the control chamber (non-insecticidal oil-treated paper) 30 percent of the insects had escaped. The average number escaped from the ten chambers was 33 percent. With practice, it was possible to insert enough wool to block the hole and yet provide the minimum non-lethal surface on which the insects might rest. The first series of comparison tests with the dish and the cone showed that there was no difference between the two, and in order to check the effect of plugging the orifice of the cone, a test was set up in which *A. aegypti* were exposed, in the plugged cone, and the dish, to filter papers impregnated with an 0.05 percent dieldrin-risella oil solution. The mosquitoes were exposed for a thirty-minute period and twenty-four hours later the mortality counts were taken. There was no difference in the mortalities obtained with the plugged cone and the dish, and since the unplugged cone and the dish were already known to give similar results, all further tests with the cone were carried out with the orifice plugged.

## OBSERVATIONS

### 1. The Convenience of Handling the Two Types of Exposure Chamber in the Field

The main observations from the field experiments were that the wall of the average native house was so badly cracked and uneven that it was impossible to carry out a test. In the case of the dish exposure

chamber, if the wall surface was rough or uneven, any method of plugging the gaps between the chamber and the wall surface resulted in the chamber being so far from the wall that on reinserting the shutter it passed over the insects and trapped them between the shutter and the wall rather than within the chamber. On a smooth surface, when a series of exposures were required, the dish chamber had the advantage that it could be closed on schedule and removed at leisure for transfer of the insects to the recovery cages. Thus any problems in transferring the insects from the chamber to the holding cage did not result in an increased exposure time. The conical exposure chamber could be affixed to any type of surface and plugging the gaps between the chamber and the surface did not interfere with the test. Exposure tests have been carried out using the conical chamber to confine insects on treated thatch. The conical chambers could be loaded readily, a straight tube was found to be the most efficient method of getting the insects into the chambers. For removing the insects, the angled end section of the sucking tube provided with the kit was used, and with this the mosquitoes could be removed 5–10 at a time to the holding cage.

In the first series of experiments it was not necessary to save the wall surface for future tests and the chambers were nailed to the wall; later, when it was desirable to save the surfaces for future tests cellotape was used. When using cellotape it is essential to place the replicates on exactly the same spot since the cellotape will have stripped insecticide off the mud.

2. The Consistency of Results Obtained with Each Type of Exposure Chamber When Replicate Exposures Are Made

The results of this series of tests are shown in Tables 1 and 2. In each series there are eight replicates, plus a control where shown, with an average of 15 insects each. The mortalities recorded are the control mortalities, and mean experimental mortalities corrected for control deaths by application of Abbott's formula (Finney, 1952), with their standard deviations. These tests were carried out six months after treatment of the mud surfaces (at the completion of the wall exposure series), and for this reason the *A. aegypti* exposure time was extended to 60 minutes while the *A. gambiae* exposure time remained unchanged at 30 minutes. It is the authors' opinion that there would have been less variations among the replicates if the exposure time had been extended even more (2–3 hours for *A. aegypti* and 1 hour for *A. gambiae*).

With the small number of insects per replicate (an average of 15), there was a wide variation in the mortalities and with the too short exposure period this resulted in a correspondingly high variance among replicates. Thus we have a range of mortalities of from zero to 25 percent in one

TABLE 1.—*A. aegypti* exposed for 60 minutes to DDT and dieldrin on sorbtive and nonsorbtive surfaces

| Insecticide | Type of Mud | Exposure chamber | Control mortality | Corrected mean mort. with s.d. | t |
|---|---|---|---|---|---|
| DDT | Sorbtive | Cone | 0 | 4.98 ± 4.42 | t = 0.25 |
| | | Dish | 0 | 4.50 ± 3.02 | |
| | Nonsorbtive | Cone | — | 3.71 ± 5.61 | t = 0.31 |
| | | Dish | — | 4.55 ± 5.34 | |
| Dieldrin | Sorbtive | Cone | 0 | 13.53 ± 9.50 | t = 2.87 |
| | | Dish | 0 | 2.87 ± 4.40 | |
| | Nonsorbtive | Cone | — | 4.43 ± 5.31 | t = 0.02 |
| | | Dish | — | 4.48 ± 4.43 | |

TABLE 2.—*A. gambiae* exposed for 30 minutes to DDT and dieldrin on sorbtive and nonsorbtive surfaces

| Insecticide | Type of Mud | Exposure chamber | Control mortality | Corrected mean mort. with s.d. | t |
|---|---|---|---|---|---|
| DDT | Sorbtive | Cone | 0 | 5.93 ± 4.31 | t = 1.79 |
| | | Dish | 23.1 | 17.89 ± 17.40 | |
| | Nonsorbtive | Cone | — | 21.06 ± 17.90 | t = 0.07 |
| | | Dish | 6.7 | 21.67 ± 9.67 | |
| Dieldrin | Sorbtive | Cone | 6.7 | 10.96 ± 7.17 | t = 0.49 |
| | | Dish | — | 13.02 ± 9.14 | |
| | Nonsorbtive | Cone | 10 | 16.33 ± 7.25 | t = 0.99 |
| | | Dish | 0 | 21.57 ± 12.36 | |

of the series and a similar range in the others. It is for this reason that the standard deviation is frequently equal to, or in some cases larger than, the mean mortality. The tests with *A. gambiae* suffered in a like manner, although with the slightly higher general mortality, not quite to the same extent. The actual mortalities for each replicate may be found in Tables I and II of the mimeographed report circulated by the World Health Organization (Armstrong and Bransby-Williams, 1959).

3. A Comparison of Results Obtained with the Two Types of Exposure Chamber When Applied to the Same Wall Surface

A comparison of the mean mortalities with their standard deviations using the method for determination of significance of difference between means of small samples (Paterson, 1939) was carried out on the data of the cone and dish exposures on each type of treated surface (Tables 1 and 2). The values of "t" obtained are shown in the last column of the tables. For seven degrees of freedom, a value of "t" greater than 2.365 indicates a significant difference at the P = 0.05 level. In Table 1 the only significant difference is in the series on dieldrin-treated sorbtive mud (t = 2.87). Since one of the standard deviations is of the order of three-quarters of the mean and the other is almost twice the mean, and none of the other tests approach significance we consider this result is due to chance and that there is no real difference in the results obtained with the two types of exposure chambers. Although the other tests all show a large standard deviation when compared to the mean mortality (see previous paragraph), the mean mortalities by themselves are in very close agreement. In Table 2, there is no value of "t" which indicates a difference between the dish and the cone. The very small values of "t," in spite of the large difference in some of the mean mortalities, may be accounted for by the large standard deviations, i.e. in Table 2, for cone versus dish on DDT-treated sorbtive mud, mean mortalities were 5.93 and 17.89 but the standard deviations were 4.31 and 17.40 respectively. As discussed in section 2 above, the variances in the tests were exaggerated by the small number of insects used in each replicate.

4. Comparison of the Two Methods for Demonstrating the Aging of Insecticide Deposits

A series of exposures were carried out comparing the dish and the cone exposure chambers on the different surfaces, over the full twenty-four week period. The results are not shown in this report but they do show that the two methods are equally good in showing the aging of insecticide deposits. The graphs of this series are to

be found in the previous report by Armstrong and Bransby-Williams (1959). These findings were also confirmed by the observations in section 3.

5. Comparison of the Two Methods for Demonstrating the Sorbtive Character of the Walls

In the twice weekly wall exposure series, *A. aegypti* and *A. gambiae* were exposed under cone and dish exposure chambers to DDT- and dieldrin-treated sorbtive and non-sorbtive muds. The weekly mortalities obtained were plotted against time after treatment and graphs drawn to show the loss of efficiency of insecticide deposits. To conserve space only one graph (Figure 3) is shown. This illustrates the mortalities recorded when *A. gambiae* were exposed for thirty minutes to dieldrin-treated sorbtive and non-sorbtive muds. The full series of graphs are shown in the preliminary report (Armstrong and Bransby-Williams, 1959).

Since there was shown to be no difference in the results obtained with the cone and dish exposure chambers, these mortalities were combined, and for the period of weeks 1–12 each point on the graph represents eight replicates of approximately 15 mosquitoes each. For the tests at weeks 16, 20 and 24 there were four replicates of 15 insects each. Throughout the wall exposure series daily temperature and humidity records were kept and the daily rainfall measured. For the whole of this period the mean temperature (degrees Centigrade) was 22.4 with a maximum of 27.2 and a minimum of 18.3, and the mean relative humidity was 75.5 percent

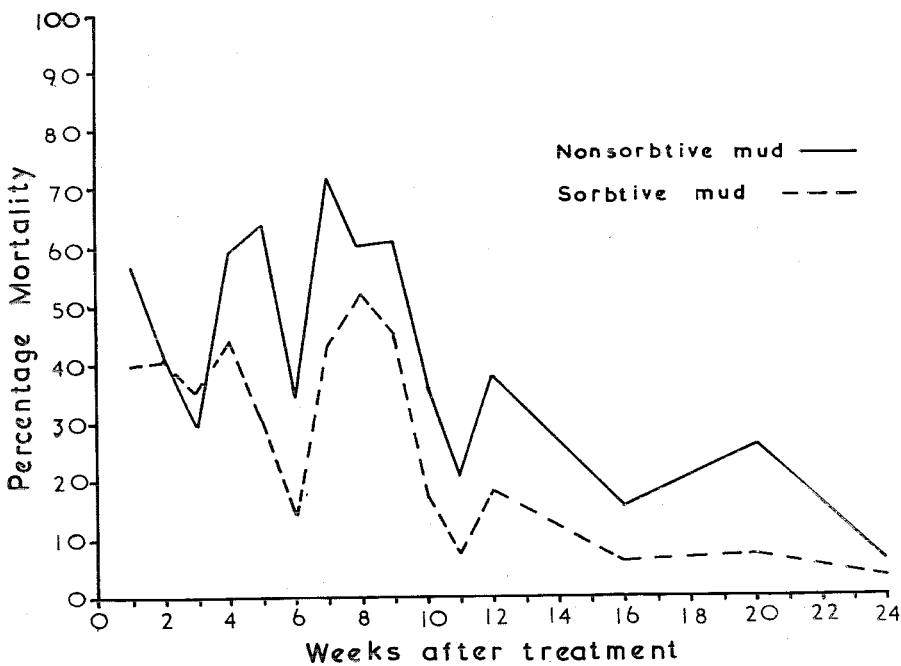


FIG. 3.—Percentage mortalities of adult female *A. gambiae* exposed for 30 minutes to dieldrin treated nonsorbtive and sorbtive muds.

with a maximum of 90.9 and a minimum of 58.1 percent. There was rain at weeks 2 and 3, 6–9 and weeks 17 and 18.

From the graph we see that there is a consistently higher kill by dieldrin on non-sorbtive mud than on sorbtive mud. This difference showed in all exposure tests but was not as apparent with the *A. aegypti* exposures on the dieldrin-treated surfaces nor with the exposures of the insects on the DDT-treated surfaces. The graph shows the general decrease in mortality over the twenty-four week period and the increase in mortality which occurs following an increased humidity (Burnett, 1956) although it is surprising that this effect was not shown to a greater extent on the sorbtive mud than on the non-sorbtive mud. The Njoro Nkubwa mud was found to be not completely non-sorbtive, and has been described by Burnett (1957) as less active than the active (sorbtive) mud which he used in the insecticide trials at Taveta, and this, together with the fact that the mud plaster on the walls was not as thick as it was intended, may explain why there was not a marked difference in mortalities recorded on the two surfaces, and why kills recorded on the sorbtive mud did not show the effect of changing humidity to a greater degree.

## CONCLUSIONS

1. The Relative Convenience of Handling the Two Types in the Field

The dish chamber was found to be convenient to use on a smooth surface and it was possible to set up a series of tests at closely timed intervals, but it could not be used if the surface was irregular. The conical exposure chamber could be used on all types of surfaces, and has been used successfully in the exposure of mosquitoes to treated thatch.

2. The Consistency of Results Obtained with Each Type of Exposure Chamber When Replicate Exposures Are Made

The main cause of the variations observed in this series of tests was the small numbers of insects per replicate; if about thirty insects had been placed in each chamber the variation would have been much smaller. The two chambers produced remarkably consistent results, which would be even more consistent if the chambers were large enough to contain 30 insects.

3. A Comparison of Results Obtained with the Two Types of Exposure Chamber When Applied to the Same Wall Surface

There is no difference in the results obtained with the two types of exposure chamber and it is considered that the results obtained with the two are fully interchangeable.

4. Which of the Two Types Indicate the Aging of Insecticide Deposits with the Greater Degree of Accuracy

As stated in section 3, there is no difference in the results obtained using the two types of exposure chamber, and they are equally good in showing the aging of insecticide deposits.

5. The Sorbtive Character of the Wall

The test technique is accurate enough to show the different mortalities produced when mosquitoes are exposed to dieldrin on non-sorbtive and sorbtive mud. The method also shows the effect of a changing humidity on the potency of the insecticide deposits and the general falling-off of the efficiency of the treated surface.

## ACKNOWLEDGMENTS

The authors are grateful to Dr. R. A. E. Galley and Mr. K. S. Hocking for permission to carry out this program of work, and to the World Health Organization for providing the wall exposure test kits. We also wish to thank the members of this Unit who assisted in the construction and chemical analysis of the test surfaces and for their many helpful comments.

SUMMARY

With the aim to standardize all tests associated with assessing the efficiency of insecticide deposits, the World Health Organization provided two types of wall exposure chambers for a series of calibration tests. The chambers were both made of plastic, one basically a funnel and the other basically a petri dish.

The field trials revealed a major problem in carrying out bioassay tests; namely that the wall of the average native house is so broken and irregular that it is impossible to carry out these tests. The dish exposure chamber could only be used on a very smooth surface such as is found in experimental houses constructed for field trials, and for this type of experimental work it proved to be very good. The cone exposure chamber could be used on much rougher and more irregular surfaces, and has in fact been used by this Unit for bioassay tests on treated thatch. For general bioassay tests in the field the cone exposure chamber has many advantages over the dish exposure chamber.

Each chamber was shown to produce equally reliable and reproduceable results. The main factor responsible for the variation between replicates was the small number of insects in each chamber, and it is considered that 25–30 insects per replicate would result in a much closer agreement between replicates. There was no difference in the results obtained with the cone and the dish exposure chambers and in a series of tests to show the loss in efficiency of insecticide deposits the cone and dish results were combined. It was also shown that bioassay tests will give a picture of the general loss of the insecticide and also the effect of variations in humidity on insecticidal deposits on sorbtive and non-sorbtive surfaces.

*References*

ARMSTRONG, J. A. and BRANSBY-WILLIAMS, W. R. 1959. A report on the W.H.O. adult test kit for bioassay of insecticide deposits on wall surfaces. World Health Organization, Expert Committee on Insecticides. Working Paper No. 10 Add 1.

BURNETT, G. F. 1956. Variation in mortality with differences in humidity among mosquitoes exposed to BHC, dieldrin and DDT. Nature (Lond.) 177, 663–664.

BURNETT, G. F. 1957. Trials on residual insecticides against anophelines in African-type huts. Bull. ent. Res. 48(3):631–668.

DAVIDSON, G. 1950. A field study on gammexane and malaria control in the Belgian Congo. II The effect of spraying of houses with gammexane on the mosquito population and on malaria incidence in children. Ann. Trop. Med. & Parasit. 44(1):1–26.

DAVIDSON, G. 1953. Experiments on the effect of residual insecticides in houses against *Anopheles gambiae* and *Anopheles funestus*. Bull. ent. Res. 44:231–254.

EXPERT COMMITTEE ON INSECTICIDE RESISTANCE AND VECTOR CONTROL. 1957. WHO/Insecticides/72, Section 3, pp. 14–16.

FINNEY, D. J. 1952. Probit Analysis. 2nd Edition, Cambridge University Press.

HOCKING, K. S., ARMSTRONG, J. A., and DOWNING, F. S. 1959. BHC/Cereclor, A new long-acting lindane formulation for malaria control. (In press.)

KENNEDY, J. S. 1947. The excitant and repellant effects on mosquitoes of sublethal contacts with DDT. Bull. ent. Res. 37:593.

PATERSON, D. D. 1939. Statistical Technique in Agricultural Research. McGraw-Hill Company, Inc.

SHUTE, G. T. 1956. A method of maintaining colonies of E. African strains of *Anopheles gambiae*. Ann. Trop. Med. & Parasit. 50(1):92–94.