DIELDRIN FORMULATIONS ON MUD WALLS IN MEXICO

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In the worldwide malaria eradication programs, the usual method of attack is the application of a residual insecticide to the interior surfaces of the homes. In many areas, mud is the predominant building material. Recently in several countries where malaria eradication campaigns are under way, it has been reported that when the insecticides are applied to mud walled houses, sometimes the effectiveness of the insecticide is lost at a very rapid rate. It is believed that this phenomenon may be due in part to the adsorption of the insecticide on the mud walls. If this adsorption problem proves to be serious and if it becomes widespread it could seriously affect the malaria eradication programs unless some means are found to prevent this reaction.

In 1956 the Pan American Sanitary Bureau, the Mexican Government and the Shell Companies joined in a project known as the Dieldrin Study Project with the purpose of evaluating dieldrin as an insecticide for use in malaria eradication programs, particularly on mud walls. The study was to be made in Mexico in conjunction with their National Malaria Eradication Program which was getting under way, and where most of the homes in the rural area are made of mud; bricks, blocks or plaster.

The chief objectives of the study are:

(a) To evaluate the biological effectiveness of test pesticides against the important vectors of malaria under various climatic conditions in different areas.

(b) To determine the susceptibility levels of the malaria vectors to test pesticides.

There are other objectives but the two given above are the major ones and will be the ones discussed in this paper.

Three areas were selected in which to conduct these studies. They contained a total of 20 villages, most of them small, as we preferred villages of approximately 200 homes. In selecting an area, there are several criteria which had to be met such as: unsprayed area, many small villages to select from, plenty of mosquitoes, home owners willing to allow their homes to be used, a high percentage of the homes constructed of mud, all-weather roads, fair accessibility from headquarters, an area where a government malaria eradication project was operating, a town where the personnel could live, and each area had to be in a different geographical zone. The three areas selected were: Acapulco, where studies are conducted in eight villages; Oaxaca, where five villages are in use; and Puebla, where seven villages are in the tests.

Acapulco is on the west coast, an area where malaria is well known and where the two chief Mexican malaria vectors (*Anopheles albimanus* and *Anopheles pseudopunctipennis*) are found. Oaxaca is located in the southern part of Mexico and is a state that has been noted for its high malarial rate. The chief malaria vector here (Oaxaca) is *A. pseudopunctipennis* which breeds in the streams so common in this section. Grassy pools, roadside ditches, and some irrigated fields also produce this mosquito in fairly large numbers. Puebla is located in the central section of the country at an elevation of approximately 7,000 feet above sea level. This is an extensively irrigated rice and cane growing area. This results in a large population of *A. pseudopunctipennis*.

The majority of the houses in the small villages where we work are made of mud and most of them are about the same size. Along the coast, where it is warm, sticks and canes are often used to make a frame over which the mud is plastered, while in

the areas of greater altitude, mud or adobe brick are used to build the home and mud plaster may or may not be applied. Most of the homes are of one room; if two rooms are needed a second room with a separate roof is built. If the roof is of grass, and many of them are, it is built on the ground and then lifted to the top of the walls. This tends to limit the size of the houses which probably average 10 feet wide and 16 feet long. Most of the cooking is done outside or in a small shed attached to the main room.

In making the experiments to determine the lasting qualities of the test insecticide, dieldrin was applied at the rate of 25, 50 and 100 mg. per sq. ft. to the interior walls of the homes as a residual spray. Each village was sprayed at one application rate, and the test was repeated in each of the three areas. An exception was that a few houses were selected for special tests such as using different rates of application on separate walls and/or using special types of mud on the separate walls and then treating all walls at the same application rate. In these villages are found many types of soil and even within a village or within a house adobe of several colors can be found. Samples of these different building muds were taken from the villages in the test areas and sent to the Shell Chemical Corp. where chemical and biological tests were made of each type of mud.

We obtained some of our dieldrin from the Mexican Malaria Eradication Commission and in the tests this dieldrin is called Regular Dieldrin in order to distinguish it from a special formulation of dieldrin supplied to us by the Shell Chemical Corp. which is classed as Experimental Dieldrin. This Experimental Dieldrin was made specially for use on mud walls that are deactivating to residual insecticides. We received shipments from several "batches" of this experimental dieldrin, and they differ from Regular Dieldrin mainly by having been treated by special resins. This Experimental Dieldrin is composed of 50 percent dieldrin, 25 percent resin, plus regular wetting agents and inerts. We also have the 75 percent water wettable dieldrin under test.

The first application of insecticide in our tests was applied on October 4, 1956. Three villages were treated at this time, two villages with dieldrin using 25 mg. per sq. ft. and one village with DDT using 200 mg. per sq. ft. In one of the villages Experimental Dieldrin No. 232 was used and in the other village Regular Dieldrin was used. The 25 mg. rate of application is approximately 40 percent of the WHO recommended rate, and was used in order to reach the "breaking-point" of effectiveness of the insecticides quickly. The last of the 20 villages were sprayed in May of 1957. There are at present in the experiment three villages treated with DDT at 200 mg. per sq. ft. and 17 villages treated with Dieldrin (Experimental and Regular) at rates from 25 mg. to 100 mg. per sq. ft.

All of the villages used in the study were sprayed by the Malaria Eradication Teams of the National Government. A typical spraying was sought, the type that was being done nation-wide and we believe that a representative spraying was obtained. We weighed the insecticide, enough in a paper bag to charge one spray can, and delivered it to the foreman in the village to be sprayed. A different colored bag was used in each village and thus we could prevent the wrong material or a different amount from being used in a village where it was not intended for use.

In evaluating the results of these tests we used five methods which are as follows:

1. Wall Tests.
2. House Inspection.
3. Larval Inspection.
4. Trapping or releasing of mosquitoes in sprayed houses.
5. Chemical analyses made of samples from the walls of sprayed houses.

In the wall tests the mosquitoes are confined in petri dishes, 15 in each dish, and held against the wall for one hour. The final readings are made 24 hours after being removed from the wall.

The mosquitoes used in the wall tests are collected locally, are held overnight and the strongest specimens used the next morning. The mosquitoes are carried to the field in small cages and are removed with a suction tube and placed in a petri dish that has a bristol board cover. This petri dish is placed against the wall with the bristol board against the wall and the paper is then removed. The petri dish is fastened to the wall using either tape or pins and rubber bands. Thus the mosquitoes are caged against the treated surface, the petri dish confining them to the area selected. After an exposure period of one hour the bristol board is slipped back under the petri dish and the mosquitoes are removed and released in a holding cage where they are kept for 24 hours at which time the mortality is determined. Controls are given the same handling except that they are exposed to untreated surfaces —we seldom have any specimens die in the controls as great care is taken to prevent insecticide contamination and the mosquitoes are carefully handled.

In the house inspection, 15 houses in each village are inspected for 20 man-minutes twice a month. If mosquitoes are found in sizable numbers they are held in cages for 48 hours to determine if they have received a lethal exposure to the insecticide. Larval inspections are conducted in mosquito breeding places located within or adjacent to the sprayed village. Ten stations were selected and 100 dips are made in each station per month. The larvae are recorded as to species, number and size. Traps were placed in a few vacant houses (we rented these houses and had the people move) and mosquitoes are allowed to enter or are released and are captured later in door traps or found dead on the paper-covered floor. It required a large mosquito population to conduct this type of experiment and we feel that we did not have enough mosquitoes to receive the maximum benefits from these houses.

Wall scrapings and cellulose tape samples were taken from the sprayed walls, the first samples being taken one hour after spraying and at regular intervals thereafter. These samples were sent to the Shell Chemical Laboratories in Denver where chemical analyses were made. The chemical analyses showed that the spraying was very uniform. Five samples taken one hour after spraying in a room showed little variation; however, samples taken at monthly intervals showed a greater percentage of loss in the insecticide by chemical analyses than by biological tests. Often the chemical analyses show no insecticide while the biological tests produced a very good kill.

The first villages which were sprayed in October 1956 using 25 mg. of dieldrin per sq. ft. showed, as the insecticide aged, a very slow decline in the percentage of mortality by wall tests and also the number of mosquitoes found at inspection slowly increased until 335 days after spraying when some of the tests were giving a percentage of mortality as low as 29 percent, others were as high as 77 percent. We had set a 40 percent mortality by wall tests, as the percentage to be reached when we would declare that the insecticide had lost enough of its effectiveness that respraying should be done.

This is based on the fact that a female mosquito must visit a house two or more times and probably rest on the treated walls three or more times in order to take blood from one person and then 10 or more days later return to a treated house and bite a person. If 40 percent of the mosquitoes are killed each time they rest on the treated wall, by the time the third resting period has passed there will be few if any mosquitoes alive. No consideration is taken of natural death during the 10-day period or of the fact that the mosquitoes will feed several times during the 10-day interval following the first feeding and thus may enter and rest on the wall many times more than the three times that we consider when determining the effectiveness of the residual spray.

Using this criterion we received satisfactory mosquito mortality for over 300

days with the 25 mg. rate of application applied to mud surfaces. In fact, in a few houses the insecticide was active (65 percent kill) at 386 days. The tests were discontinued after 335 days and the villages were returned to the National Malaria Eradication Commission for inclusion in their annual spraying campaign.

There are four other villages in the test sprayed at the 25 mg. rate using either the Experimental Dieldrin or the Regular Dieldrin. However, these villages were sprayed in April of 1957 and are now approaching the 300th day since spraying and the data indicated that satisfactory mortality (40 percent or above) will be obtained for over 300 days.

In the experiment there are nine villages treated with either Experimental Dieldrin or Regular Dieldrin at the rate of 50 mg. per sq. ft. These villages have been treated for less than 300 days and it appears that it will be 50 to 100 days more before observations are terminated. Four of these villages are in the Acapulco area and these villages are showing a rapid loss in the effectiveness of the residual insecticide, and the data indicate that satisfactory results may not be obtained over a longer period of time than that received from the 25 mg. rate. The above is not true in either the Puebla area or the Oaxaca area where somewhat better results are being obtained with the heavier application than with the 25 mg. rate.

There are several tests under way in which each wall of the house is treated at a different application rate (25, 50, 75 and 100 mg. per sq. ft.). This type of experiment gives us data on the four rates under the same conditions. There is a series of houses in a test where a different type of soil (adobe) is used on each wall of a house and all walls are then sprayed alike. In another experiment a group of houses are used in which the same type of mud is used in all houses, but two walls in each house have the mud plaster finished very smoothly, while the other two walls are left with a very rough finish. All of the walls are sprayed with the same material and at the same rate. These rough and smooth walls have been treated for less than 200 days and at present there is no difference in the killing effect of the insecticide on either wall. Observations will be continued.

The data from these village-wide tests that have been completed, show that the Experimental Dieldrin and the Regular Dieldrin gave satisfactory mortality of mosquitoes when applied at the rate of 25 mg. or more per sq. ft. to mud walls for 300 or more days, and that longer effectiveness is obtained with the heavier dosages but not in the same ratio as the application rate.

The mud used in home construction in the Puebla area and the Oaxaca area shows no deactivating qualities towards the insecticides used. However, in the Acapulco area there is a deposit of yellow mud that is used to plaster some of the homes and tests made in houses plastered with this mud gave a low percent of mortality, by wall tests, at the end of 60 days. After 120 days the percent of mortality based on "wall tests" increased and at the end of 250 days after spraying the insecticides are producing satisfactory kills. We cannot explain why the percent of mortality was low at 60 days and later showed an increase in effectiveness. Additional tests are under way in this area using houses treated with the yellow adobe. There is also found a red mud in this same area that is used in house construction. The earlier tests indicate that the effectiveness of the insecticides was reduced by 15 to 20 percent when applied to this Red Acapulco Mud. We have several tests now under way to determine if it is the mud that causes the reduction in effectiveness or some outside factor such as dust, smoke or housekeeping methods.

The World Health Organization's kit for testing susceptibility of mosquitoes to insecticides was used in the Puebla and Acapulco areas to determine the susceptibility level of the species of mosquitoes that we are working with. We used DDT and dieldrin impregnated papers at the

recommended concentrations with *A. albimanus* and *A. pseudopunctipennis*. These tests are to be repeated every six months and a comparison made of the data collected at each testing to see if the susceptibility of the species that we are working with changes during the time that we are making the study. At present we have only the first readings; other readings will be made at 6-month intervals.

In the tests made to date we found that *A. albimanus* and *A. pseudopunctipennis* were very susceptible to both insecticides to about the same degree; however, *A. albimanus* did show a slight bit more susceptibility to the test materials.

CONCLUSIONS. Tests were made in Mexico to determine the lasting qualities of several formulations of dieldrin and also of DDT when applied as a residual insecticide in malaria eradication work. It was found from tests made in three areas in Mexico that the insecticides when applied to mud surfaces gave over 300 days of effectiveness using 25 mg. of dieldrin per sq. ft. in two of the three areas and slightly less in the third area. Higher application rates, 50 mg. to 100 mg. of dieldrin per sq. ft., gave a longer lasting period of effectiveness but not in the same ratio as the quantity of insecticide used. Tests are still under way in the area where the red mud gave a shorter period of effectiveness.

Tests made to date using the World Health Organization's kit for testing the susceptibility of mosquitoes to insecticides showed that the *Anopheles* in the areas where we are working are very susceptible to dieldrin and DDT.

A SAMPLING PROCEDURE FOR *CULICOIDES MELLEUS* (COQ.) (DIPTERA: HELEIDAE) WITH OBSERVATIONS ON THE LIFE HISTORIES OF TWO COASTAL *CULICOIDES* [1]

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Culicoides melleus (Coq.). The unusual breeding habitat of *Culicoides melleus* (Coq.), sandy beaches along the coastline, was apparently first recorded by Goulding *et al.* (1953) in Florida. On Long Island, N. Y., small numbers of *Culicoides melleus* larvae were collected from samples of intertidal sand examined under a wide-field dissecting microscope. The larval sampling methods described by Dove *et al.* (1932), Carpenter (1951), Kettle and Lawson (1952) and Wirth (1952) were tried with only limited success.

LARVAL SAMPLING. In the spring of 1957, a more useful method of sampling was developed after observing the behavior of *C. melleus* larvae in the laboratory. Larvae placed in a 50 ml. graduate containing about 10 ml. of sand and 30 ml. of water stayed beneath the surface of the sand. When the graduate was inverted and returned to an upright position the sand settled in a few seconds and the larvae could be seen swimming in the water. A few swam upward for a short time before sinking slowly to the bottom but most swam downward. In less than two minutes almost all of the larvae disappeared again beneath the sand. These

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