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EVALUATION OF CHLORPHOXIM USED AGAINST *ANOPHELES ALBIMANUS* ON THE SOUTH COAST OF MEXICO: 2. USE OF TWO CURTAIN-TRAP TECHNIQUES IN A VILLAGE-SCALE EVALUATION TRIAL[1]

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Curtain-trap methods, in which a house is completely curtained off, have been devised for the purpose of assessing mosquito vector behavior patterns. This account describes the use of two curtain-trap methods to evaluate the impact of chlorphoxim on the malaria vector Anopheles albimanus.

Introduction

Mexico's malaria campaign, initiated in 1955, has had considerable success in many malarious parts of the country. The initial technique used, like that employed in most other Central and South American countries, involved applying DDT at a dosage of two grams per square meter to the indoor walls, interior roofs, and exterior eaves of houses.

It was subsequently reported, on the basis of four years' spraying, that malaria transmission had been interrupted in 77% of the country's malarious areas *(1)*. At about the same time, however, it was suggested that the main reason for persistence of transmission in other parts of Mexico was the behavior of two vectors, *Anopheles albimanus* and *An. pseudopunctipennis*, relative to DDT *(2)*. In this same vein, the authors of the former report *(1)* stated that despite unchanged susceptibility to the chemical, prolonged use of DDT had caused *An. pseudopunctipennis* to avoid the lethal effects of DDT better in the early 1960s than this species did in 1950. Thus, both sets of authors agreed that because of insecticide pressure, a change in vector behavior had taken place; and malaria transmission had persisted as a result of the vector being able to escape lethal contact with the insecticide.

At present we are aware that even short-term use of an insecticide may affect mosquito behavior in such a way as to determine whether the mosquito will come into contact with the insecticide and will receive a lethal dose. Since indoor spraying programs are directed against a very limited portion of the overall mosquito population, an evident need exists to more carefully examine mosquito activity, including the behavior of target species once they enter houses.

We have previously reported the results of work along this line at one test village (La Victoria) and two control villages (El Gancho and San Francisco Palo Blanco) near Tapechula in the state of Chiapas *(3)*. That work, performed in 1981–1982 to assess the effects of chlorphoxim on local *An. albimanus*, in-

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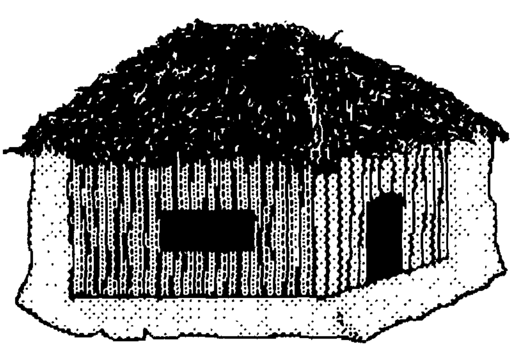
cluded use of mark-recapture and curtain-trap techniques to assess target mosquito behavior. The data from the mark-recapture tests were presented in the earlier report. However, it was felt that the special nature of the curtain-trap techniques made it appropriate to report the results obtained with them (regarding target mosquito mortality, repulsion by chlorphoxim, and procurement of blood meals) in a separate article. Therefore, those results are being reported here.

Curtain-Trap Tests

The curtain-trap technique, first used by Elliott in Colombia *(4)*, involves encircling the exterior of a house from the ground to the roof with a nylon mosquito-net (Figure 1). Since house walls in many parts of Latin America are commonly constructed with loose-fitting poles of bamboo or other material, this technique (in contrast to trapping techniques that cover only windows) permits collection of mosquitoes that are naturally entering or leaving the house.

During our study, two types of curtain-trap collection techniques were evaluated twice-weekly, following three applications of chlorphoxim, as previously described.[7] The original technique described by Elliott *(4)*, which keeps the curtain raised for the first half of each hour and lowered for the second half, was employed at three houses in the sprayed village of La Victoria and at three houses in the unsprayed village of San Francisco Palo Blanco, beginning eight weeks after the second (September 1981) spray application. A modified technique, described later by PAHO, that was developed as a result of work carried out in 1972 in El Salvador and designated "901" was employed at three La Victoria houses and three San Francisco Palo Blanco houses, beginning with the first (June 1981) spray application. The houses used in these tests were selected at random, and people living in the villages remained inside the houses during the experiments, serving as bait. (People were free to enter and leave the curtained houses through an overlapping fold in the curtain.)

Figure 1. Typical house suitable for use of curtain-trap, with curtain-trap in place.



In using the unmodified method, mosquitoes were collected hourly from the interior of the periodically raised curtains, and were then classified and placed in cups to provide a basis for estimating mortality after 24 hours. In those cases where the curtain was not raised periodically (where the 901 technique was used), *An. albimanus* were collected alternatively from the curtain's two surfaces (from the outside of the curtain for an hour and then from the inside of the curtain for an hour) continuously from 6 p.m. to 6 a.m. Those collected from the exterior were counted, classified as being fed or unfed, and released immediately inside the house. Those collected from the inside (on alternate hours) were placed in net-covered cups containing moist cotton for the purpose of estimating mortality after 24 hours.

[7] Three applications of 5% chlorphoxim in a 50% water-dispersible powder formulation were made in June and September 1981 and in February 1982. The insecticide was sprayed onto all interior walls, and also onto the lower third of roofs and exterior eaves, at a target dosage of 2.0g of active ingredient per square meter using Hudson X-Pert sprayers *(3)*.

Results

A comparison of mortality following each spray application is presented in Figure 2. Mortality rates are expressed triweekly in terms of the percentage of mosquitoes dead within 24 hours of being collected using the 901 (unraised curtain) technique. The original (periodically raised curtain) technique was not used for purposes of this evaluation.

The first triweekly means indicated 91% mortality in the period following the first spray application. However, a sharp drop in mortality was observed for a prolonged time thereafter, so that overall mortality observed during the 12 weeks following the first spray application was only 47%. Mortality during the first three weeks following the second spray application was very low, a fact that can probably be attributed to the small sample size (less than 12 captured mosquitoes). However, average overall mortality for 20 weeks after the second spray application was also fairly low, averaging around 55%. The highest and most stable mortality, as indicated by the 901 technique, occurred after the third spray application. This mortality appeared to average 82%. Regression or "trend" lines were calculated in order to compare changes in mortality patterns following the second and third applications.

Hourly or bihourly *An. albimanus* exit densities and mortality were calculated using data from both curtain-trap techniques after the second and third spray applications in the sprayed and unsprayed villages. These data are shown in Figure 3. In general, the 901 method showed peak exit activity in both villages occurring around 2200 hours, while the unmodified method showed no consistent peaks and instead indicated roughly the same level of exit activity throughout the collection period.

A statistical comparison of exit densities (as indicated by both collection methods) revealed no significant difference ($P < 0.02$) between the hourly exit patterns in the sprayed and unsprayed villages. In contrast, both curtain-trap methods showed significant differences in mortality. In general, hourly mortality fluctuated considerably in the

Figure 2. Changes in *An. albimanus* mortality observed by using the "901" (modified) curtain-trap technique. The data shown represent three-week means of percent mortality for *An. albimanus* collected during the three spray rounds. The broken lines show general mortality trends calculated from data obtained following the second and third spray applications. Each point in the graph represents a mean for a three-week period. These means were calculated for three spray rounds.

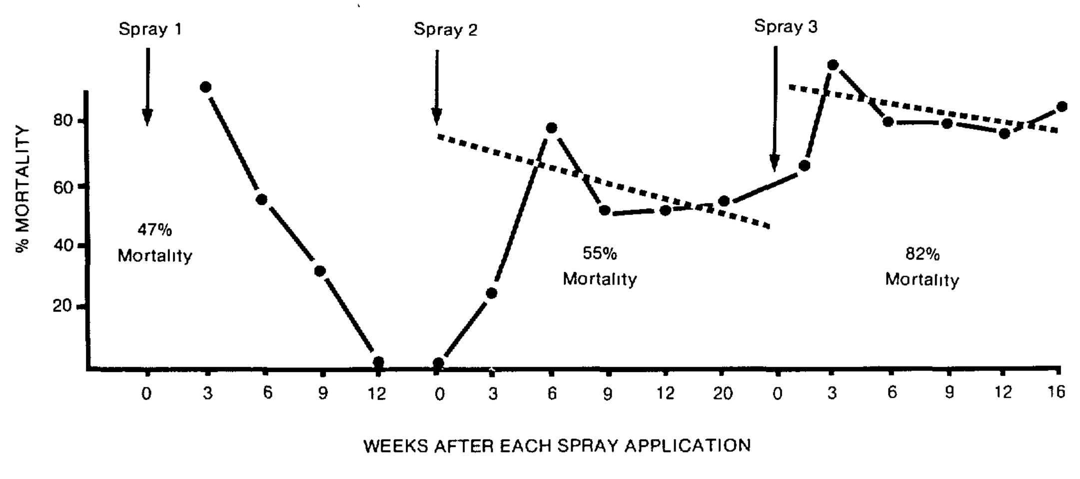
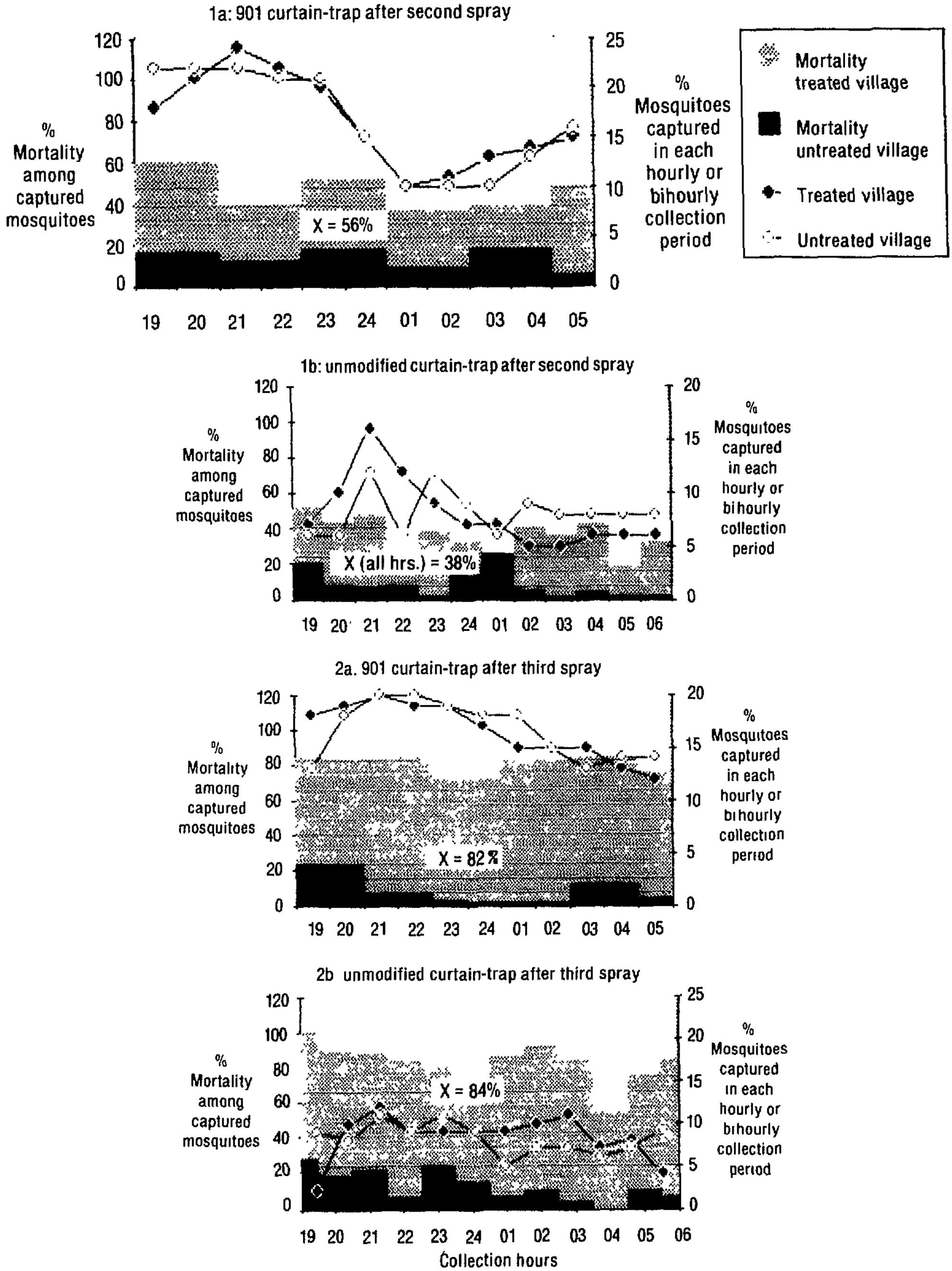


Figure 3. Average hourly and bihourly exit densities and mortality among *An. albimanus* collected by both curtain-trap techniques at the sprayed and unsprayed villages. From top to bottom: (1) results obtained after the second spray with (a) the modified (901) curtain-trap and (b) the unmodified curtain-trap; (2) results obtained after the third spray with (a) the modified (901) curtain-trap and (b) the unmodified curtain-trap. The lines in each chart relate to the index at the right and show the percentage of captured mosquitoes that were collected at each of the hours indicated at the bottom. The bar graphs in each chart relate to the index at the left and show the percentage mortality occurring among the mosquitoes captured at the hours indicated.



treated village following the second spray application. However, this fluctuation was less marked after the third spray application and average mortality was higher, generally amounting to 75% or more of the collected insects. By comparison, average hourly mortality in the untreated village was much lower, never exceeding 26%.

The four charts in Figure 4 show cumulative hourly data for collection of fed and unfed mosquitoes after the second spray application, and again after the third spray application. Cumulative mortality data for the fed and unfed mosquitoes are also shown. It should be noted that the data shown for the second spraying are for all mosquitoes captured with 901 curtain-traps over a twenty-one week period following the second spray application, while the data shown for the third spraying are for all mosquitoes captured with 901 curtain-traps over a sixteen week period following the third spray application.

As the charts show, a cumulative 58% of all the exiting mosquitoes captured after the second spray application became engorged within the curtained area in the untreated village, while a smaller percentage of the exiting mosquitoes captured within the curtained areas of the treated village (41%) became engorged. Cumulative mortality among the exiting mosquitoes captured at the treated village after the second spray application was 35% among the mosquitoes that had fed and 55% among those that had not fed.

After the third spray application, the modified (901) curtain-trap method captured an average of 55 mosquitoes per curtain-night, while the unmodified method captured an average of 67 mosquitoes per curtain-night. Of the mosquitoes captured with the 901 method, 72% in the untreated village were found to have become engorged; in contrast, only 41% of the exiting mosquitoes captured in the treated village were engorged (43% if dead mosquitoes collected from the floor are included). Cumulative mortality was 82% among those engorged mosquitoes in the treated village, and was likewise 82% among the captured exiting mosquitoes that were not engorged.

Discussion

The loose types of home construction commonly found in much of Latin America make it impractical to employ the standard window-traps generally recommended for determining the density of exiting mosquitoes for purposes of insecticide evaluation. Under these circumstances, use of a curtain that completely surrounds the outside of a house permits a more complete study of mosquito dynamics—including entry and exit patterns, feeding behavior, the length of time spent inside the house, and mortality data that can be correlated with similar data obtained from mark-recapture studies and bioassay tests.

Regarding the two curtain-trap methods used in the study reported here, the modified (901) method offered two advantages. First, since it used a stationary curtain it eliminated the unknown number of entries and exits that occurred when the curtain was periodically raised for 30 minutes; and second, it permitted the number of entering mosquitoes (resting on the outside of the curtain) to be counted. These advantages made it possible not only to determine the numbers of entering and exiting mosquitoes, but also to relate those numbers to feeding patterns and to mortality resulting from contact with the insecticide.

Exit Patterns

Regarding exits, the 901 curtain-trap method appeared to detect slightly higher than average exit activity around 2200 hours, followed by a slight relative decline in exit activity that lasted the rest of the night. The other (unmodified) curtain-trap method did not register a consistent peak at this hour, but the differences in the results obtained (see

Figure 4. Cumulative percentages, by collection hour, of exiting mosquitoes that were captured engorged and unengorged in the sprayed and unsprayed villages after the second and third sprayings; and the cumulative mortality, by collection hour, of the mosquitoes collected from curtain-traps in the treated village.

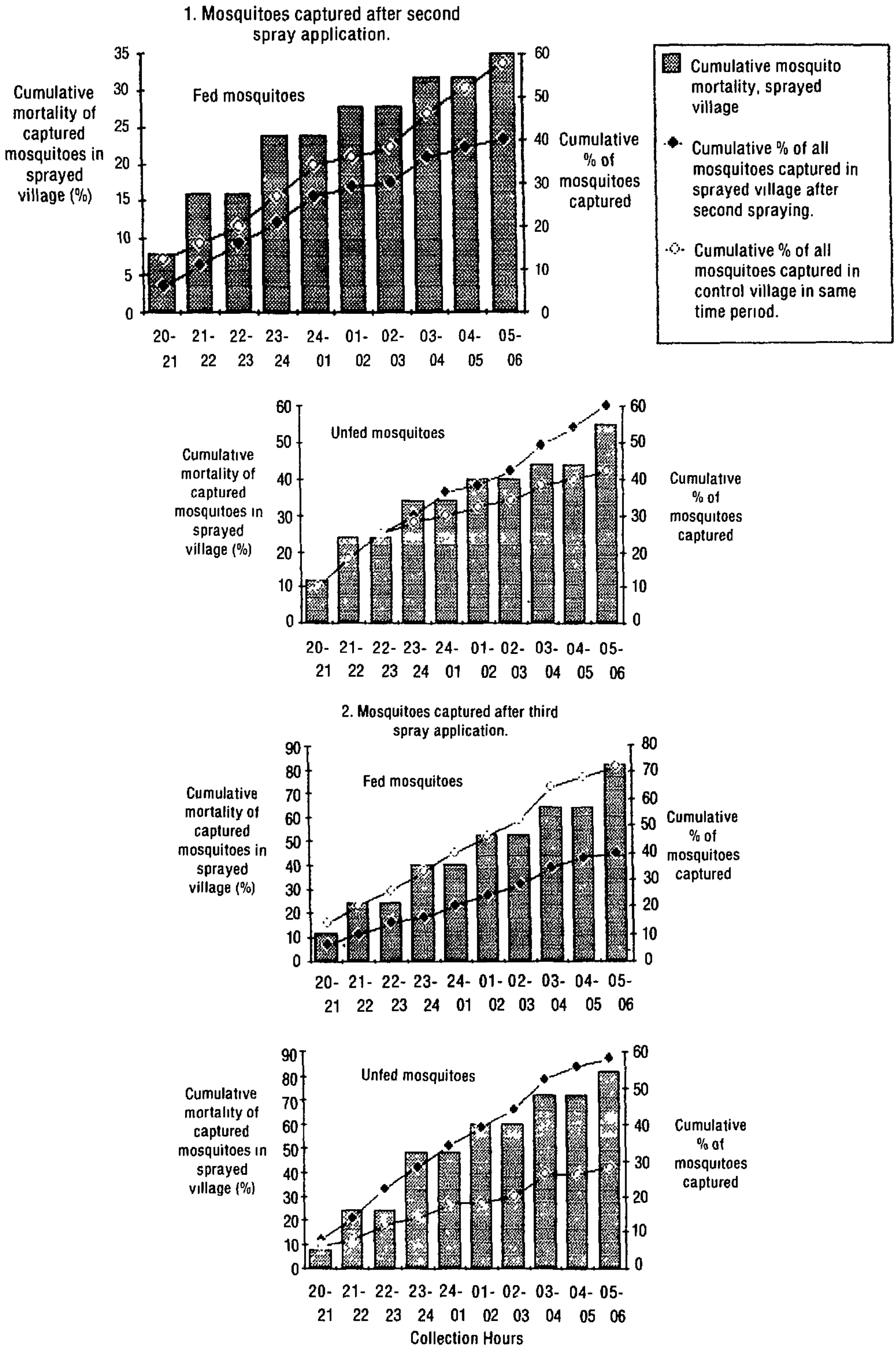


Figure 3) were not statistically significant. Overall, both methods yielded results indicating that mosquito exit patterns from sprayed and unsprayed dwellings were very similar, thereby indicating that the mosquitoes were not being repelled from the treated dwellings by the insecticide.

However, the mark-recapture studies reported earlier *(3)* indicated that although the numbers of marked mosquito landings on treated and untreated surfaces were similar, the mosquitoes were found to rest for an average of seven minutes less time (29 versus 22 minutes) on treated surfaces in treated houses than they did on similar but untreated surfaces in untreated houses. In addition, the time spent resting by marked mosquitoes in houses treated with chlorphoxim (31 minutes) was 12 minutes less, on the average, than marked mosquitoes spent resting in untreated houses (43 minutes). These differences could simply reflect the sensitivity of the mark-recapture technique, since any repulsion caused by sublethal intoxication was apparently not strong enough to significantly influence the overall daily exit pattern.

Feeding Patterns

As already noted, an important reason for conducting a curtain-trap evaluation was to determine the rate of conversion of the indoor mosquito populations from unfed to fed, and to assess corresponding mortality produced by the insecticide during the peak 12-hour activity period. The rate of conversion of the exiting mosquitoes resting on the inside of the curtain was found to be generally stable at around 40% (see Figure 4) following the second and third spray applications.

In Colombia, Elliott *(5)* observed a conversion rate of 44% before applications of DDT-HCH against *An. darlingi*. After these applications, he noted reduction of the proportions obtaining blood meals and a dramatic decrease in the numbers of mosquitoes leaving the house. This was associated with mortality inside the house, including observation of dead mosquitoes on the floor.

In the study reported here, we found a marked difference (20 to 30%) between the rate of engorgement in treated and untreated houses—indicating that mosquitoes became intoxicated by landing or resting on chlorphoxim-treated surfaces and were partially inhibited from biting. While the percentage conversion from unfed to fed was similar after the second and third spray applications, 24-hour mortality among engorged exiting mosquitoes resting on the curtain doubled after the third spray application, indicating an accumulation of the insecticide and a higher degree of intoxication among the engorged mosquitoes. Overall, after the third application 82% of both engorged and unfed mosquitoes collected indoors (including dead mosquitoes collected on the floor) died as a result of their contact with treated surfaces.

Mortality

As was pointed out in our previous report *(3)*, surface bioassays only provide indexes of residual insecticide activity; they do not measure natural mosquito contact time with treated and untreated surfaces. A more reliable assessment of an insecticide's impact can be made by determining mortality (after exposure to treated homes) among exiting fed and unfed mosquitoes captured from the curtain and dead mosquitoes collected from the floor. This combined mortality reflects the total indoor vector-insecticide contact time during the peak 12-hour activity period.

In our specific case, there was a sharp decline in mortality during the weeks following the first application; but mortality fell more gradually after the second spray application and remained practically unchanged for a long time after the third application—with three-week averages of 78% mortality or greater for 16 weeks. Similarly high mortality (80% or greater) was found following the second and third applications among indoor resting mosquitoes collected during the morning hours, further demonstrating that chlorphoxim has a strong residual indoor killing potential after two spray applications made within the time-frame employed here. These findings contrast with results of a stage VII evaluation[8] of deltamethrin made recently in Guatemala *(6)*, in which the 901 curtain was used. That evaluation found a considerably lower mortality (20%), which was attributed to repulsion of the target mosquitoes by the product.

It has been demonstrated in this trial that it is possible to evaluate the efficacy of an insecticide on a small scale in order to detect changes in mosquito mortality and behavior—including repellence and other effects resulting from the presence of residual insecticide. This technique could also be used to evaluate more than one insecticide in the same village, an approach with considerable cost-saving potential.

[8] A district-wide operational trial performed to test the efficacy of an insecticide by means of an epidemiologic malaria assessment.

SUMMARY

Effects of the insecticide chlorphoxim upon the malaria vector *Anopheles albimanus* were investigated by means of village-scale trials conducted in southern Mexico in 1981 and 1982. The houses of a test village, La Victoria in the state of Chiapas, were sprayed three times—in June and September 1981 and in February 1982. During each application the insecticide was applied to the treated dwellings' interior walls, eaves, and roofs (the lower third) at a target strength of 2g active ingredient per square meter.

To assess the results, a variety of procedures were performed. These included bioassay tests, weekly human bait and resting collections, a mark-recapture study, and collection of mosquitoes in the treated and untreated villages by means of curtain-traps surrounding individual homes. The purpose of this article is to report the results obtained with the curtain-traps, the results of the other procedures having been reported previously *(3)*.

Two curtain-trap methods were employed. Most of the reported data were obtained by the "901" method in which the curtain is left in place (rather than raised periodically), entering mosquitoes captured on the outside of the curtain are transferred inside, and exiting mosquitoes captured on the inside of the curtain are collected for evaluation.

Overall, the results indicated that the chlorphoxim on treated surfaces did not repel *An. albimanus* mosquitoes sufficiently to disturb normal departure patterns, but that many mosquitoes in the treated area were intoxicated to the extent that feeding rates declined, and (especially after the third spray application) a large share of the intoxicated mosquitoes perished.

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