EVALUATION OF CHLORPHOXIM USED AGAINST *ANOPHELES ALBIMANUS* ON THE SOUTH COAST OF MEXICO: 1. RESULTS OF INDOOR CHLORPHOXIM APPLICATIONS AND ASSESSMENT OF THE METHODOLOGY EMPLOYED[1]

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Village-scale trials were conducted in southern Mexico in 1981 and 1982 to assess the effects of chlorphoxim upon the malaria vector Anopheles albimanus. *This article reports the results of human bait collections, resting collections, bioassay tests, and procedures using marked mosquitoes that were employed in the course of this evaluation.*

Introduction

While the malaria outlook in recent years has either remained the same or shown improvement in some parts of South America, the number of reported malaria cases in certain parts of Middle America has continued to increase *(1)*. The eighteenth report of the WHO Expert Committee on Malaria, published in 1980 *(2)*, lists twelve constraints impeding the progress of antimalaria activities. These include administrative and operational problems, difficulties posed by prevailing social patterns of behavior, parasite resistance, vector resistance, the degree of vector-human contact, changes in vector behavior leading to avoidance of treated surfaces, and a poor understanding of vector behavior.

In the past, the strategy of indoor malaria spray programs has depended upon the efficiency of the insecticides used to control vector populations with divergent behaviors and resistance patterns. The behaviors involved have usually included a wide range of biting and resting habits that influence the insecticide contact time needed to effectively control those members of the vector population involved in the transmission cycle. However, with the mounting resistance of malaria vectors to insecticides and the continuing alteration of environmental and ecological behavior patterns, it is becoming increasingly evident that there is a need to modify this vector control strategy.

Specifically, it is necessary to develop a strategy that includes thorough study of the target vector's bahavior, especially as it relates to transmission of the parasite. In this vein, a primary aim of the Malaria Research Center in Tapachula, Mexico, has been to develop an entomologic and epidemiologic strategy appropriate for Mexico's malaria problems, one that may also prove relevant for dealing with malaria problems in other portions of the hemisphere.

The specific work reported here, involving trial applications of chlorphoxim, was directed at determining that insecticide's efficacy in controlling *Anopheles albimanus* and at further investigating this susceptible vector's indoor behavior.

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Materials and Methods

The Trial Area

The areas around Tapachula in the state of Chiapas, Mexico, contain three distinct agricultural zones. The first, located along the border with Guatemala, is dominated by large banana plantations with extensive irrigation systems. The second, immediately to the north, is dominated by cotton plantations; despite limited irrigation systems, this zone has a long history of heavy agricultural spraying. The third zone, containing the study village of La Victoria, is located further to the north; its agricultural areas consist mainly of grazing lands and small farms raising corn, cacao, and beans.

Extensive susceptibility studies reported by Ríos et al. *(3)* found generally higher levels of insecticide resistance in mosquitoes from the banana and cotton areas than in those from the mixed farming area further north. Respective levels of resistance recorded in the banana and cotton zones were 31% and 39% to DDT, 72% and 66% to malathion, 64% and 52% to fenitrothion, and 61% and 74% to propoxur. However, chlorphoxim susceptibility tests conducted on mosquitoes from all three zones found 97% of the insects susceptible to that insecticide.

The study area, the village of La Victoria, is situated at 92^0 10' North, 92^0 30' West, some 2.5 km from the Pacific Coast and about 30 km northwest of Tapachula. At the time of the study, this village contained 196 houses adjacent to small farms and a large cotton-growing area. One control village, El Gancho, contained 119 houses and was located approximately 37 km to the south of La Victoria, near banana plantations on the Pacific Coast. Another control village, San Francisco Palo Blanco, contained 55 houses and was located 15 km south of La Victoria in the cotton-growing zone. (This latter village was used as a control for curtain-trap and marked mosquito surveys after the third spray application in La Victoria.)

The houses of the three localities are predominantly small multiroom dwellings with palm-thatch roofs and with walls constructed mainly of split bamboo. The climate of the region is tropical, the annual rainfall averaging 2,152.7 mm and the humidity ranging between 61 and 95%. The wet season extends from May to October, but mosquito breeding occurs year-round.

Treatment Procedures

Three applications of 5% chlorphoxim in a 50% water-dispersible powder formulation were made in June and September 1981 and in February 1982. In each case the insecticide was sprayed onto all interior walls of the village homes, and also onto the lower third of the roofs and the exterior eaves, at a target dosage of 2.0g of active ingredient per square meter using Hudson X-Pert sprayers. Each spraying operation was completed within two days by spray teams furnished by the National Malaria Eradication Service. The spraymen were provided with protective clothing, and each sprayman's exposure was limited to a maximum of six hours per day. Cholinesterase levels were measured before and after each application.

Evaluation Procedures

Human bait collections. Captures were made weekly during a twelve-hour period (from 1800 to 0600 hours) by one collector indoors and one outdoors. The collectors changed places (the indoor collector going outdoors and vice versa) every three hours, and each collected for a total of six hours. Prespray mosquito densities in the treatment and control villages were determined by making human bait collections three weeks before the first spray was applied. Post-treatment densities were determined by making human bait collections weekly from early July through the second week of December for a period of 23 weeks.

Indoor and outdoor resting densities. Weekly indoor resting collections were made between 0900 and 1100 hours at 32 houses selected at random in the treatment and control villages. Living and dead *Anopheles albimanus* were collected with an aspirator from the walls, floors, and furniture of each house for a fifteen-minute period. Live mosquitoes were observed 24 hours for the purpose of obtaining mortality information. Houses were considered positive if live mosquitoes were found inside during the capture period. Using the same technique, weekly collections of resting *An. albimanus* were made outdoors in adjacent animal corrals between 2000 and 2100 hours and in vegetation between 2100 and 2200 hours.

Marked mosquito studies. Biweekly marked mosquito studies were made in the treatment and control villages, using two houses per night. Between 1900 and 2300 hours a person serving as human bait was seated near the inside door of a selected house. Engorging *An. albimanus* were then dusted with fluorescent powder and were followed inside the house with the aid of an ultraviolet lamp for one hour. During this time, the activities of each marked mosquito (number of landings, resting time, and type of resting surfaces chosen) were recorded. At the end of this hour, the marked mosquito was collected and placed in a cup in order to determine whether it would survive 24 hours.

Bioassays. Wild captured *An. albimanus* were bioassayed weekly using cone-shaped cups. Four cones, each containing 10 blood-fed females, were exposed to four different wall surfaces for intervals of five, 30, and 60 minutes. The mosquitoes were then placed in holding cups for 24 hours.

Results

Indoor and Outdoor Bite Rates

The average biting rates of *An. albimanus* were found to increase during the first seven weeks after the first application at the evaluation village (La Victoria), the peak biting rates recorded being 115.7 bites per hour indoors and 137.5 outdoors (Figure 1). While biting rates in the untreated village of El Gancho increased proportionally, the rates were barely a quarter as high as those in the treated village.

During weeks eight to 11 after the first treatment (in August and September 1981), indoor and outdoor bite rates declined in both villages in response to reduced rainfall. However, during the first two weeks following the September 1981 treatment, bite rates in the treated village increased. This was followed by a gradual decline in those rates that continued through the end of the wet season and on through the end of November.

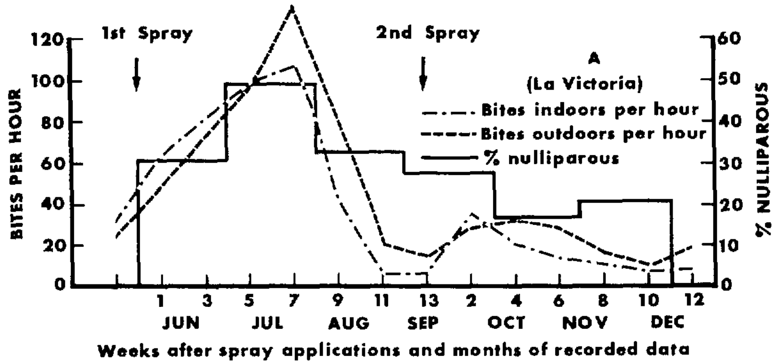
Nulliparous rates, determined from the human bait collections, increased by nearly 40% in the treated village between the fourth and eighth weeks after the first spray (Figure 1). Following the second spray, however, a gradual decline in the nulliparous rate was observed in both the treated and control villages.

Table 1 and Figure 2 show variations in *An. albimanus* bite rates in La Victoria over a twelve-hour period (from 1800 to 0600 hours between June and November), and Table 1 provides parity data for the same period. The highest indoor biting activity was recorded between 1900 and 2300 hours, while the peak outdoor biting activity began around 1900 and extended through 0100 hours. Somewhat lower indoor and outdoor parity rates were recorded for mosquitoes captured during the first two hours of the biting

Table 1. Variations in *Anopheles albimanus* bite rates and parity at La Victoria from 1800 to 0600 hours between June and November, as indicated by indoor and outdoor human bait collections.

Time (hours)	Average mosquitoes captured per man-hour		% parous mosquitoes	
	Indoors	Outdoors	Indoors	Outdoors
1800-1900	7.9	13.9	55	78
1900-2000	29.8	50.9	64	75
2000-2100	44.1	55.8	81	85
2100-2200	36.1	56.0	85	84
2200-2300	31.5	54.5	86	84
2300-2400	17.2	42.3	69	81
2400-0100	13.6	46.1	73	83
0100-0200	12.0	32.2	90	78
0200-0300	6.4	19.5	81	74
0300-0400	4.4	15.4	90	72
0400-0500	6.4	18.6	72	75
0500-0600	4.6	6.7	59	66
Hourly average	17.8	34.3	75	78

Figure 1. Indoor and outdoor bite rates and parity recorded for *Anopheles albimanus* collected in the treated village of La Victoria (above) and the control village of El Gancho (below) during the period June-December 1981.



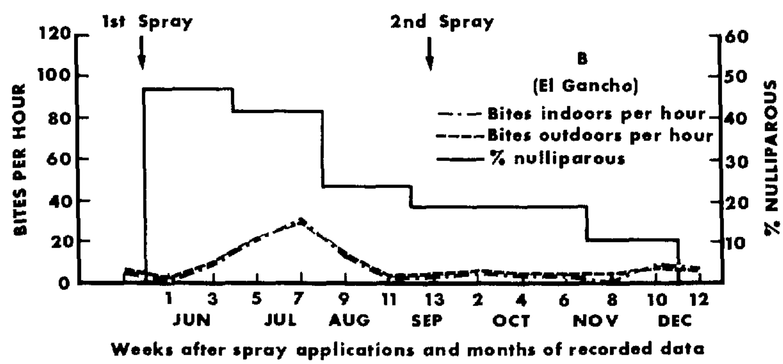
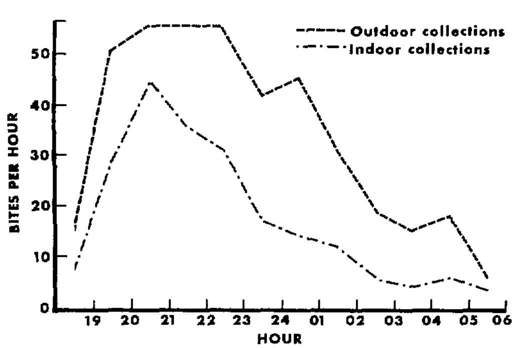


Figure 2. Indoor and outdoor human bait catches of *Anopheles albimanus* made at La Victoria over a twelve-hour period (from 1800 to 0600 hours) between June and November.

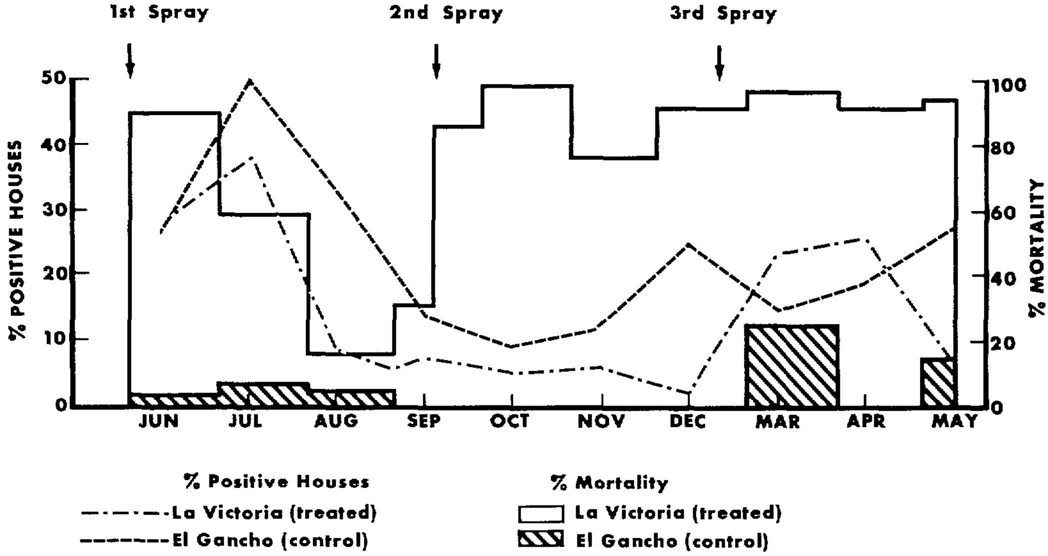


period. In general, the parity rates of mosquitoes captured indoors and outdoors were similar. The susceptibility status of the indoor/outdoor collected mosquitoes did not change through the post-spray period.

Indoor Resting Densities

Data regarding the numbers of resting and dead *An. albimanus* collected in houses during the morning hours were used to determine the percentage of houses positive and mosquito mortality (Figure 3). Density levels during the first two application periods remained low (less than

Figure 3. Percentages of houses infested with *Anopheles albimanus* and mosquito mortality, as indicated by morning resting collections made in La Victoria and the control village of El Gancho from June 1981 to May 1982.



7.0 captures per man-hour) in both villages. However, density levels of 16 captures per man-hour were recorded during April 1982. In June, following the first application, mortality in the treated village increased sharply to 90% but declined to less than 20% in August. Higher and more persistent mortality was recorded following the second and third applications. In the control village, mortality remained at less than 5% through most of the study period and then increased temporarily to 22% in March 1982.

The proportion of sprayed houses positive for *An. albimanus* actually increased in July, following the first spray application, but remained at less than 10% between August and December of 1981. As compared to houses in the untreated village of El Gancho, this meant that 68% fewer of the La Victoria houses were positive.

Marked Mosquito Studies

A total of 562 marked and engorged *An. albimanus* were followed in treated houses after the second and third chlorphoxim applications. The highest average total contact time on the different surfaces involved was 13.4 minutes per mosquito on roofs with treated surfaces (Table 2). Less time, on the average, was spent in contact with untreated surfaces (8.1 minutes per mosquito) and treated walls (8.8 minutes per mosquito). The average number of landings per mosquito was also highest on the treated roof surfaces (1.7 per mosquito), as compared to treated walls (1.1) and untreated surfaces (0.7). In all, during the one-hour "following" period, the marked mosquitoes spent an average of 30.3 minutes resting in treated houses and landed an average of 3.6 times. All but two of the 562 marked mosquitoes made at least one landing before leaving the house. Among the 427 marked mosquitoes that were followed for an hour and recaptured, the highest mortality (95%) was recorded after the third spray application; however, the combined mortality of marked mosquitoes captured after both the second and third applications was also relatively high (87%).

In the untreated control village (San Francisco

Table 2. Landing and resting behavior of marked and followed mosquitoes on treated and untreated surfaces in La Victoria and the control village of San Francisco Palo Blanco, and mortality among the marked mosquitoes that were recaptured.

Village, spray round, and date	No of mosquitoes marked and followed	Avg. No. of landings per mosquito	Avg. minutes resting per mosquito	Avg No. of landings on indicated surfaces				Avg. minutes resting on indicated surfaces				Mortality among mosquitoes recaptured after resting on indicated surfaces					
				Treated				Treated				Treated surfaces			Untreated surfaces		
				Walls	Roofs	Combined Avg	Untreated (various)	Walls	Roofs	Combined Avg.	Untreated (various)	No. alive	No. dead	% dead	No. alive	No. dead	% dead
La Victoria (treated):																	
Second spray round (10 September 1981)	324	3.1	26.9	0.8	1.6		0.7	7.4	11.8		7.7	31	154	83.2	17	24	58.5
Third spray round (3 February 1982)	238	4.2	37.1	1.4	1.9		0 7	10.8	15.6		8.6	5	181	97.3	4	11	73.3
Total	562	3.6	31.2	1.1	1.7	2.8	0.7	8.8	13.4	22.2	8.1	36	335	90.3	21	35	62.5
San Francisco Palo Blanco (untreated):																	
10 September 1981	73	3.9	36.9	0 8	1 6		1.4	7.5	15.4		14.1	28	11	28.2	5	1	16.7
3 February 1982	102	3.4	47.2	0.8	1.8		0.8	8.5	24.9		13.2	73	3	3.9	11	0	0
Total	180	3.6	43.1	0.8	1 8	2.6	1.1	8.1	21.0	29.1	13.5	101	14	12.2	15	1	6.3

Palo Blanco), the 180 marked mosquitoes that were followed were found to spend more time in contact with portions of the roof treated in La Victoria homes (an average of 21 minutes per mosquito) than they spent in contact with surfaces not sprayed in La Victoria (13.5 minutes per mosquito) or with wall surfaces (8.1 minutes per mosquito). Likewise, roof areas that would have been treated in La Victoria received the highest number of landings (an average of 1.8 per mosquito) in the untreated houses of San Francisco Palo Blanco, with the average number of landings on surfaces that would not have been treated (1.1 per mosquito) and on walls (0.8 per mosquito) being somewhat lower. Overall, the average number of landings (3.6 per mosquito) was about equal in La Victoria and San Francisco Palo Blanco. However, the mosquitoes rested an average of 12 minutes longer in the untreated houses of the control village.

On the other hand, mosquitoes in the treated villages showed no obvious preference for untreated surfaces, making four times as many landings on treated as compared to untreated surfaces and spending three times as much time on the treated surfaces. The average resting time on both treated and untreated surfaces was found to be less in the treated village than in the control village (being an average of 6.9 minutes less per mosquito on treated surfaces and 5.4 minutes less per mosquito on untreated surfaces).

Bioassays

Residual chlorphoxim activity was measured by exposing lots of 10 engorged *An. albimanus* to four different types of treated wall surfaces after each round of spraying for 60, 30, or 5 minutes (Table 3). Among those exposed to bamboo, palm, or wood surfaces for 60 minutes, the resulting mortality was generally 90% or greater. After the first application, such exposure generally yielded at least 90% mortality for 10 to 12 weeks; after the second application, exposure to treated bamboo and palm surfaces for five minutes yielded at least 90% mortality for 11 weeks, and after the third application, exposure for 30 or 60 minutes to treated bamboo, palm, or wood surfaces generally yielded at least 90% mortality for 16 weeks. By and large, less residual effect was observed when the treated surfaces were made of cement; and a decline in mortality on all surfaces was observed after 11 weeks when the mosquitoes were exposed for only five minutes.

Table 3. Results of bioassay tests, showing mortality among groups of 10 mosquitoes exposed to different treated wall surfaces various weeks after the indicated spray application.

Spray round	Exposure time (minutes)	Mortality (%)	Results obtained on the indicated weeks after the spray round by exposing 10 test mosquitoes to the indicated surface			
			Bamboo	Palm	Wood	Cement
First	60	90-100	Weeks 1-12	Weeks 1-10	Weeks 1-12	Weeks 1-8
"	60	≥ 90	–	Week 1	–	Weeks 1-4
"	30	90-100	Weeks 1-3	Weeks 1-2	Weeks 1-3	Week 1
"	30	≥ 90	–	Week 1	–	Weeks 1-2
Second	60	90-100	Weeks 1-7	Weeks 1-7	Weeks 1-7	Weeks 1-5
"	60	≥ 90	–	–	–	Weeks 1-2
"	5	90-100	Weeks 1-11	Weeks 1-11	Weeks 1-9	Weeks 1-4
"	5	≥ 90	Weeks 1-2	Weeks 1-4	Weeks 1-2	—
Third	60	90-100	Weeks 1-16	Weeks 1-16	Weeks 1-16	Weeks 1-9
"	60	≥ 90	–	–	–	Weeks 1-2
"	30	90-100	Weeks 1-16	Weeks 1-16	Weeks 1-16	Weeks 1-13
"	30	≥ 90	Weeks 1-2	Weeks 1-3	–	Weeks 1-3
"	5	90-100	Weeks 1-12	Weeks 1-11	Weeks 1-13	Weeks 1-5
"	5	≥ 90	Weeks 1-4	Weeks 1-5	Weeks 1-2	Weeks 1-7

Discussion and Conclusions

Neither indoor nor outdoor human-bait bite rates were notably reduced by the insecticide applications. Indeed, these rates indicated that mosquito densities increased after each of the first two applications. It was also observed that although considerably lower vector density levels occurred during early September, the second insecticide application appeared to have little abating effect. In general, similar density increases occurred in the comparison village, indicating that these were natural seasonal increases.

There is some evidence that the insecticide may have influenced the parity structure of the mosquito population. Specifically, a sharp increase was noted in the nulliparous rate following the first application in La Victoria, an increase not observed in the control village of El Gancho. However, no similar increase was observed after the second application.

In general, these findings appear compatible with those of Fanara et al. *(4)*, who reported that a village-scale trial of chlorphoxim in Indonesia against *Anopheles aconitus* only slightly affected man-biting rates and did not reduce mosquito parity. Similarly, Rishikesh et al. *(5)*, in reporting the results of an expanded field trial that compared *Anopheles funestus* and *Anopheles gambiae* hut resting densities with trap densities, stated that chlorphoxim had a less than satisfactory impact on biting densities, especially of *An. gambiae*. This was attributed to exophily and the possible existence of subpopulations that did not closely depend on villages but survived in an intervillage environment.

In our own work, the very high densities that were encountered, especially after the first application, suggest a strong potential for infiltration of adults from habitats surrounding the treated village. Although not reported elsewhere in the results, our rates of mosquito captures in animal corrals remained high (over 100 mosquitoes per man-hour each month) after the first and second applications, and this likewise pointed to infiltration pressure from areas outside the treated village that were unaffected by the insecticide.

Because of this intense infiltration pressure exerted by mosquitoes entering the village from surrounding breeding habitats, we should not expect that the spraying of interior house surfaces would dramatically reduce mosquito densities. Therefore, it is suggested that evaluation should be more directly focused on the target mosquito's behavior and how that behavior is affected when the insect attempts to enter houses.

In this context, certain aspects of mosquito behavior can be related to the types of surfaces contacted, the length of those contacts, and the degree of mortality exhibited by mosquitoes that are marked, followed, and recaptured. In particular, mosquito intoxication and subsequent mortality is influenced by a wide range of possible behavior patterns, some of which were observed in the present study. For example, the *An. albimanus* specimens followed were found to spend an average of 44% of their resting time on treated roof surfaces, 29% on treated walls, and 27% on untreated surfaces. This diversity could be expected to produce greater mortality than resting behavior more directed at untreated surfaces; and it could be expected to produce less mortality than resting behavior more directed at treated surfaces.

As in the present case, Lassen et al. *(6)*, evaluating propoxur in El Salvador, observed *An. albimanus* to exhibit a wide range of indoor flight and contact activities for up to four hours before taking a blood meal. During most of that period, the vector generally rested on wall and roof surfaces.

On the other hand, Damar et al. *(7)*, observing *An. aconitus* in Indonesia, found that nearly 80% of that species' indoor resting was limited to areas within 85 cm of the ground. Using this information, Bang et al. *(8)* carried out a small control study that effectively reduced *An. aconitus* indoor-outdoor landing and resting rates by spraying a single horizontal swath along the lower portion of indoor walls.

Mosquito intoxication may also be related to an increased concentration or accumulation of insecticide on treated surfaces. The effects of such insecticide accumulation (as well as insecticide degradation) may thus correlate with surface bioassay mortality. In our study, the results of tests exposing mosquitoes to treated palm and split bamboo surfaces demonstrated mortality equaling or exceeding 90% for up to 16 weeks after the third spray application.

It is also true, however, that surface bioassays of this kind only provide an index of the insecticide's residual activity. They do not measure the mosquitoes' contact time with treated and untreated surfaces, nor do they measure actual mortality. Therefore, a more reliable assessment of a given insecticide application's long-term impact can be made by capturing exiting mosquitoes (e.g., by means of a curtain-trap technique), collecting dead mosquitoes from the floor of test houses, and assessing actual mortality in the target mosquito population at different times. The results of this approach, which was employed in our study, will be reported in a subsequent article *(9)*.

In general, it is suggested that mosquito density levels should not be considered the predominant factor in evaluating the efficiency of an insecticide or a vector control program. This is especially true in areas where high-density vector populations persist, where the insecticides being assessed are only directed against indoor populations, or when only one limited locality is being treated. The methodologies employed in our study (only some of which are being reported here) were specifically designed to examine the dynamics of the target vector. The results showed that although biting rates were not significantly reduced, the insecticide produced high indoor mortality among both fed and unfed mosquitoes. As a result, a significant proportion of the potential indoor feeding population died from contact with the insecticide before or after feeding, or became intoxicated and was inhibited from taking a blood meal.

Of course, this ability of an insecticide to reduce or eliminate a target vector's participation in the transmission cycle depends in large measure upon the behavior of that vector. This matter was found to pose a particularly difficult problem in the state of Oaxaca, Mexico, in the 1960s. At that time, Zulueta and Garrett-Jones *(10)* showed that persistent malaria transmission in this region resulted primarily from the behavior of the two vectors, *An. albimanus* and *An. pseudopunctipennis*, relative to DDT. That is, the females of both species were able to penetrate DDT-sprayed houses, bite the inhabitants, and escape without picking up a lethal dose of the insecticide.

However, behavior variability is common. For example, in our study the target *An. albimanus* mosquitoes were observed to rest an average of seven minutes longer on treated surfaces after the third spray than on treated surfaces after the second spray. In a similar vein, general observations of mosquito resting patterns reported by Elliott *(11)* indicated that *An. darlingi* rested for a relatively short time (six minutes) on DDT-sprayed surfaces and a relatively long time (12 minutes) inside unsprayed houses. Also, Lassen *(6)* found that over 80% of the *An. albimanus* that bit inside houses treated with propoxur stayed inside for more than five hours.

In the present case, our findings regarding *An. albimanus* behavior and mortality upon exposure to chlorphoxim indicate that similar applications of this insecticide should produce over 80% indoor mortality among fed and unfed mosquitoes following three spray rounds for at least 16 weeks after the last application.

However, it is also necessary to consider that the vector demonstrated a strong exophagic tendency (65% as determined by human bait collections), so that at any given time the insecticide was directed at less than 50% of the anthropophilic population. This clearly demonstrates a need to further investigate the indoor and outdoor behavior patterns of *An. albimanus* and to devise a way of delivering the insecticide that will more effectively abate the target species. For it is only after such studies have been given high priority and have been used to more clearly define the transmission cycle with regard to time and place that control programs aimed at this vector can be expected to prove effective and to achieve a good measure of success.

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, both human bait and resting collections of *An. albimanus* were made weekly for extended periods. Also, bioassay tests were performed by exposing wild-caught *An. albimanus* specimens to various treated surfaces in order to determine the insecticide's residual activity; individual mosquitoes were marked, followed, and recaptured in order to better assess behavior patterns and mortality; and curtain-trap collections were made to assess behavior and mortality in larger mosquito populations. Findings derived from these curtain-trap collections are to be reported separately *(9)*.

In general, the collection data indicated that *An. albimanus* densities were not notably reduced by the insecticide applications because of intense infiltration into treated homes by mosquitoes from untreated outlying areas. However, exposure of wild-caught *An. albimanus* to treated palm and split bamboo surfaces demonstrated mortality equaling or exceeding 90% for up to 16 weeks after the third spray application; and mortality among the marked and recaptured mosquitoes was also high. These data, when combined with those obtained from the curtain-trap collections, indicate that the chlorphoxim applications produced over 80% indoor mortality among fed and unfed *An. albimanus* mosquitoes for at least 16 weeks after the last application. However, the fact that the mosquito also showed a strong exophagic tendency clearly demonstrated a need for further investigation.

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