

House-scale Evaluation of Bifenthrin Indoor Residual Spraying for Malaria Vector Control in India

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J. Med. Entomol. 40(1): 58-63 (2003)

ABSTRACT In an area of India where the main rural malaria vector, *Anopheles culicifacies* Giles, has developed triple resistance to DDT, HCH, and malathion sprayed indoors in antimalaria program, bifenthrin (10% wettable powder) was evaluated in a randomized house-scale trial between July 1999 and March 2000. Entomological impact of four serial doses of bifenthrin (25, 50, 100, and 200 mg/m²) sprayed in rooms in five villages was compared with malathion (2 g/m²) and unsprayed control. *An. culicifacies* was 100% susceptible to bifenthrin (0.1%), but only 57% to malathion (5%) test papers. Contact bioassays were carried out on sprayed surfaces for 24 wk, and 24 h mortality in *An. culicifacies* was recorded. Bifenthrin 100- and 200-mg doses caused $\geq 80\%$ mortality until 24 wk. The 50-mg dose caused $\geq 80\%$ mortality on tin, wood, and mud surfaces for 24 wk, and on brick walls for 16 wk. Bifenthrin 25-mg dose produced $\geq 80\%$ mortality for 24 wk on tin, 20 wk on mud walls, 16 wk on brick walls, and 8 wk on wood surfaces. Persistence of $\geq 80\%$ mortality did not differ for 25- and 50-mg doses on any surface except on wood ($P < 0.05$). Malathion sprayed in three rounds of 6 wk apart caused $\geq 80\%$ mortality for 16 wk on the brick and mud walls, and for 20 wk on the tin and wood surfaces. Bifenthrin 25- and 50-mg doses produced a similar impact on the densities of *An. culicifacies* and other mosquitoes but a superior one to malathion or control. Bifenthrin 25-mg dose caused least excito-repellency. Overall, efficacy of bifenthrin was superior to malathion. Considering the duration of the persistence of significant insecticidal action of bifenthrin on the most common surfaces (mud and brick walls), least excito-repellency and a relative impact on the mosquito densities, the 25-mg dose was the most superior among all the four doses evaluated.

KEY WORDS bifenthrin, malathion, spraying, *Anopheles culicifacies*, excito-repellency, vector density

INDOOR RESIDUAL SPRAYING OF DDT was introduced in India for malaria control in the late 1940s. The use of HCH was later introduced. In the very early phase, first reports on tolerance to DDT in the main rural malaria vector, *Anopheles culicifacies* Giles, came from Panchmahal in Gujarat, a part of the then Maharashtra State (Rahman et al. 1959). In subsequent years, resistance to DDT became widespread, and it was replaced by HCH, and later by malathion. Although in the forested areas of India, these insecticides are still effective against sylvan vector species such as *Anoph-*

les fluviatilis James and *Anopheles minimus* Theobald, in parts of the Gujarat and Maharashtra States *An. culicifacies* has developed triple resistance to all of the three above insecticides. The use of HCH has been banned in public health in India since 1997 because of environmental concerns. Pyrethroids, such as deltamethrin and cyfluthrin, sprayed indoors have been found effective in control of malaria or mosquitoes in India (Ansari et al. 1986, Singh et al. 1989, Yadav et al. 1996) and are now in use in Gujarat for malaria control. Mosquito nets treated with pyrethroids have also been found effective against malaria (Sharma and Yadav 1995; Yadav et al. 1998, 2001).

The antimalaria program of India has continuously been in need of better insecticides or their formulations. Among the new pyrethroids developed recently, bifenthrin has not been evaluated in India for malaria vector control. Hence, in association with the World Health Organization Pesticide Evaluation Scheme (WHOPES) a house-scale randomized trial was carried out from July 1999 to March 2000 in an area of Gujarat where *An. culicifacies* is the major malaria

This study has been carried out as part of the World Health Organization Pesticide Evaluation Scheme (WHOPES). The mention of specific companies and/or products does not in any way imply that they are recommended or endorsed by WHO in preference over others that are not mentioned.

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vector and is triple-resistant. The trial compared the persistence of the insecticidal action of bifenthrin on common indoor surfaces, and its impact on the vector population with the currently used insecticide malathion or with unsprayed control.

Materials and Methods

Study Villages and Climate. Entomological studies were conducted in five villages of *taluka* Thasra in district Kheda, Gujarat State. The villages were Rania (pop 5,226), Bhadrasa (pop 3,481), Aklacha (pop 807), and Umba (pop 2,139) under the Primary Health Center (PHC) Nes, and Vamali (pop 521) under PHC Sevalia. The villages are located along the perennial river Mahi and are well connected by all-weather roads. The climate is tropical and broadly the seasons are monsoon (mid-June to mid-October), winter (mid-October to mid-February), and summer (mid-February to mid-June).

Malaria Vector and Baseline Insecticide Susceptibility. The major malaria vector in the study area is *An. culicifacies*. It breeds in riverbed pools, irrigation canals and channels, ponds, and freshly inundated paddy fields (Yadav et al. 1989); and its bionomics in the area has been studied in detail (Bhatt et al. 1989, 1994). Its baseline susceptibility to bifenthrin and malathion was tested by using WHO diagnostic test papers (bifenthrin 0.1%; malathion 5%) following the standard method (WHO 1998). Five replicates of 20 mosquitoes each were exposed for 1 h, and mortality was recorded at 24 h. Concurrent controls were also run.

House Selection. Most of the houses in the villages are made up of mud or brick walls with or without mud plastering, and the roofs are of earthen tiles or corrugated tin-sheets supported by wooden frames. The houses generally have 30-cm wide eaves. Cattle sheds are adjacent enclosures. In each of the five villages, six comparable dwelling houses were selected. Based on the baseline entomological surveys conducted for 6 wk during July and August 1999, the houses were randomized into the following six comparisons: bifenthrin, 10% wettable powder (WP): 25, 50, 100, and 200 mg/m², single round of spraying; malathion, 25% WP: 2 g/m², three rounds of spraying 6 wk apart; unsprayed control.

In each house, a living room was chosen, and eave-curtains made of black cloth were hung inside. Two prefabricated exit traps (35 × 35 × 35 cm) were fitted either on the existing windows, or the walls were modified for this purpose in the selected rooms. As far as possible, position of the traps faced the rising sun.

Indoor Residual Spraying. The gap between the last indoor residual spraying with malathion in 1998 and the beginning of the study in July 1999 was nearly 11 mo. Within the study villages, all the houses except for those included in study were kept unsprayed during the trial period. However, the multipurpose health workers continued with the routine active malaria surveillance in all the villages for early diagnosis and treatment of malaria. Trained sprayers, provided with protective clothing, were employed for insecticide

spraying. Hudson X-Pert compression sprayers (H.D. Hudson Manufacturing, Chicago, IL) fitted with pressure gauze were used after calibrating the discharge rate. The accuracy of doses was confirmed by considering the sprayable surface areas of the target rooms and the total amount of insecticide used. The chemical assays were also conducted but with rather high variation, which in part might be due to sampling from the sprayed surfaces.

The householders were informed well in advance about the spraying, and their informed consent was obtained. To compare the persistence of insecticides on different local surfaces, various surfaces in different houses such as mud-plastered walls, unpainted wood panels, brick walls, and flat tin-sheets were sprayed after careful selection. Bifenthrin [IUPAC: 2-methyl-biphenyl-3-ylmethyl(Z)-(1RS)-cis-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl cyclopropanecarboxylate] was supplied on gratis by FMC Corporation, Philadelphia, PA.

Contact and Airborne Bioassays. To determine persistence of the biological efficacy of bifenthrin and malathion on different local surfaces such as tin-sheet, wood, brick walls, and mud walls, we carried out contact bioassays using WHO-supplied bioassay cones following standard guidelines (WHO 1981). Cones were fixed on the selected surfaces, and bioassays were carried out each time on these surfaces except in malathion-sprayed houses where cones had to be reattached at the adjacent new sites on the same surfaces after second and third round of spraying. The householders were asked not to disturb, mud-plaster, or mutilate the cones.

Contact bioassays were performed on day 1, and weeks 2, 4, 8, 12, 16, 20, and 24 after spraying using laboratory reared, sugar-fed, 48–72 h old females of *An. culicifacies* sibling species C. A bioassay of five replicates (20 mosquitoes per dose/surface) was run each time. Knockdown of mosquitoes was recorded 30 min after releasing them in cones. Mosquitoes were transferred to cups provided with cotton wool moistened with 10% glucose solution and brought to the insectary in Nadiad to check mortality 24 h after exposure. Mortality was corrected as per Abbott's formula when mortality in control was between 5 and 20%. Indoor relative humidity and temperature were recorded using digital thermometer and hygrometer, respectively, each time contact bioassays were carried out.

For airborne bioassays, five cubical cages with a metal-rod frame (12 × 12 × 12 cm) covered with nylon netting were hung 50 cm below the ceiling and 50 cm from the wall. Twenty sugar-fed female mosquitoes were released in each cage. Knockdown was recorded after 1 h of exposure and all the mosquitoes were transferred to cups provided with cotton wool moistened with 10% glucose solution to check overall mortality after 24 h. The frequency of airborne bioassays was the same as for the contact bioassays.

Mosquito Collections and Processing. In the evening before the day of mosquito collections, the exit traps were cleaned, and cotton sheets were spread

on the floor. The next morning, mosquitoes found dead on the floor-sheets were collected using forceps and placed in the cups for identification of the species. Thereafter, using a suction tube, a collector searched for mosquitoes resting in the room for 15 min. All dead or live mosquitoes found in the exit traps were then collected. The live mosquitoes were kept in cups provided with cotton wool moistened with 10% glucose solution to record mortality after 24 h. Employing two collectors, the room was then sprayed with pyrethrum solution (0.2% in kerosene) using hand sprayers. After 15 min, all mosquitoes found dead on the floor-sheets were collected. All activities were carried out weekly during the intervention phase ending the third week of March 2000.

Sibling species of *An. culicifacies* were identified by examining ovarian polytene chromosomes (Subbarao et al. 1988a). The mosquito blood meals, collected on Whatman paper, were identified by the gel diffusion method (Collins et al. 1986).

Statistical Methods. Significance of the differences in the mosquito densities in comparison groups was tested by Kruskal-Wallis test, and that in the mortalities was analyzed by *t*-test. Differences in the excitorepellency effect of insecticides on mosquitoes, as determined by exit rate, were analyzed by chi-square test. The data of all the four doses of bifenthrin were pooled and compared with malathion and control. To assess the impact of the insecticides on the indoor resting mosquito populations, geometric mean densities were calculated and compared.

Results and Discussion

Climate. Between January 1999 and March 2000, the minimum temperature in the study area ranged from 10 to 28°C, and the maximum temperature from 26 to 42°C. The relative humidity ranged from 43 to 90%. During contact bioassays, mean daily indoor temperature ranged from 25.5 to 33°C and mean relative humidity ranged from 32 to 57%.

Baseline Susceptibility. In the susceptibility tests, there was 100% knockdown of *An. culicifacies* exposed for 3 min to bifenthrin (0.1%). Mortality after 24 h was also 100%. Mortality with malathion (5%) impregnated test papers was 57%. Thus, the baseline susceptibility tests showed that the local population of *An. culicifacies* was fully susceptible to bifenthrin but largely resistant to malathion.

Sibling Species and Anthropophily. Among the 686 blood meals of *An. culicifacies* tested, 17 (2.5%) had human blood, 435 (63.4%) had bovine blood, and 234 (34.1%) had blood of other hosts. Out of 100 semi-gravid ovaries of *An. culicifacies* examined for sibling species, 10 showed species A, 74 species B, and 16 species C. Species B is not a malaria vector (Subbarao et al. 1988b), thus, vector species A and C together accounted for 26% in the trial villages, and the overall anthropophilic index was 2.5%.

Persistence of Insecticidal Action. Summary of the weeks with $\geq 80\%$ mortality in *An. culicifacies* exposed on four different surfaces by contact bioassays is given

Table 1. Weeks with mortality $\geq 80\%$ of *Anopheles culicifacies* exposed to the walls sprayed with bifenthrin once or malathion three times 6 wk apart

Surfaces	Bifenthrin doses in mg/m ²				Malathion 2 g/m ²
	25	50	100	200	
Tin	24	24	24	24	4 ^a
Wood	8	24	24	24	20
Brick walls	16	16	24	24	2 ^b
Mud-plastered walls	20	24	24	24	16

^a Mortality increased to 100% after second round of spraying, and after 24 wk, it decreased to 87%.

^b Mortality declined to 60% by wk 4, but increased to 100% up to 16 wk after second and third rounds.

in Table 1. The lowest dose of 25 mg/m² produced 100% mortality until week 16 and 95% mortality up to 24 wk on the tin surface. Bifenthrin at 50, 100, and 200 mg/m² produced 100% mortality up to 24 wk, when the bioassays were terminated. The first round of spraying with malathion at 2 g/m² produced 100% mortality up to 4 wk. Mortality declined to 78% by week 8, but improved to 100% after the second round of spraying, and lasted until 20 wk. By week 24, mortality declined to 87%.

On wood surfaces sprayed with 25 mg/m² bifenthrin, bioassays showed 100% mortality up to 4 wk and >70% up to week 20 postspraying. With 50 mg, the residual effect was 100% until 8 wk, and >80% until 24 wk when the tests were terminated. The dose of 100 and 200 mg caused 100% mortality throughout. The knockdown effect of malathion dropped below 45% by the fourth week. Two subsequent spray rounds maintained 100% mortality up to 20 wk, but the mortality declined to <42% by week 24.

On brick walls, the 25- and 50-mg doses produced 100% mortality of *An. culicifacies* for 4 wk, and >80% up to week 16 after spraying. Mortality was almost 100% with 100 and 200 mg doses. Knockdown after exposure on malathion sprayed surfaces was low, and 24 h mortality was below 92% even on day 1. After two subsequent rounds of spraying, insecticidal action improved, and toward the end, by week 24, mortality reached 43%.

On mud walls sprayed with the 25-mg dose, mortality was 100% until week 8, >80% until week 20, and >66% until the end. With 50 mg, mortality was 100% until week 8, and >80% until the end. The doses of 100 and 200 mg caused 100% mortality throughout. On malathion-sprayed surfaces, mortality was 100% until week 4, thereafter declined to 96% by week 8, improved again to 100% following two subsequent rounds of spraying, and again declined to <50% after week 24.

The airborne effect of 25- and 50-mg doses of bifenthrin, and of malathion was between 89 and 100% on day 1, which declined significantly by the second week. At 100- and 200-mg doses, the effect remained high up to 4 wk only.

In contact bioassays, except for the difference in persistence of residual insecticidal action between 25 and 50 mg doses on the wood surface ($P = 0.049$),

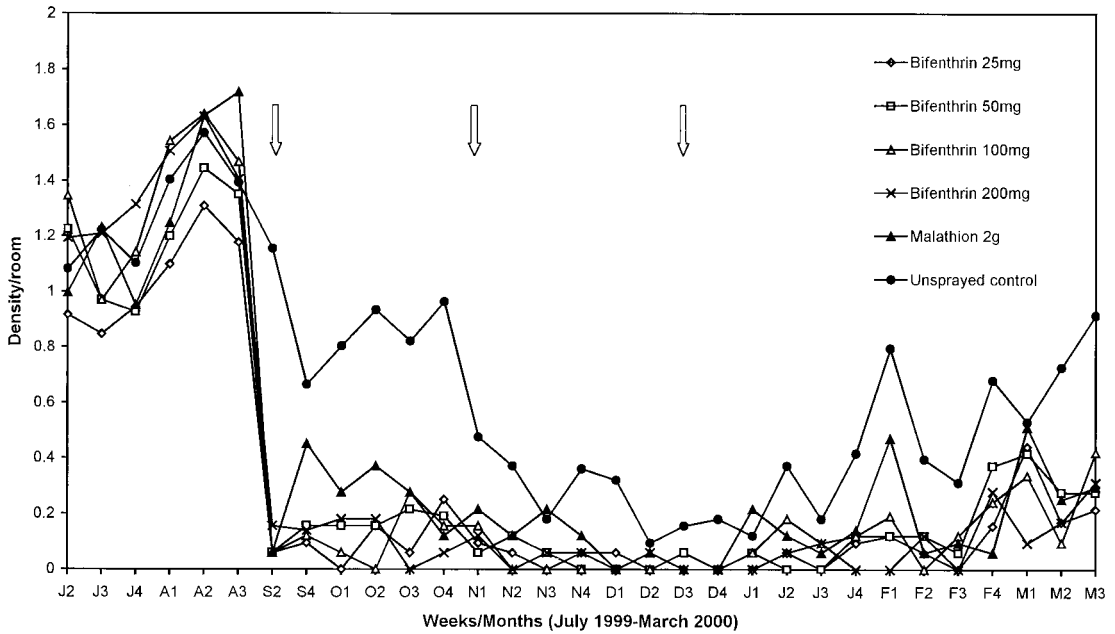


Fig. 1. Geometric mean densities of *Anopheles culicifacies*. The first arrow indicates the first round of spraying of bifenthrin or malathion, whereas second and third arrows indicate, respectively, second and third rounds of spraying of malathion in the respective rooms.

there was no significant difference in persistence on tin surfaces, brick walls, and mud walls. Airborne effect of these two doses also did not differ significantly ($P > 0.05$).

Vector Population. From the unsprayed control rooms, 1,384 female mosquitoes were collected. These comprised *An. culicifacies* (48.8%), *An. annularis* (12.9%), *An. subpictus* (21%), *An. stephensi* (2.1%), *An. nigerrimus* (0.2%), *An. fluviatilis* (0.1%), *Culex* spp. (14.3%), and *Aedes* spp. (0.6%). The geometric mean densities (GMD) of *An. culicifacies* in rooms sprayed with various doses of bifenthrin compared with those in the malathion-sprayed and unsprayed control rooms are given in Fig. 1. During the baseline period, that is, before spraying, the densities were comparable in all the groups, and there was no statistical difference between them ($H = 8.47$, $df = 5$, $P > 0.1$). After spraying, the densities declined in rooms sprayed with bifenthrin single round as compared with the unsprayed control. In malathion-sprayed rooms, densities declined initially but reappeared intermittently. Densities in malathion-sprayed rooms and control rooms increased markedly from the fourth week of January.

The GMD during the post-treatment period in rooms sprayed with 25, 50, 100, and 200 mg bifenthrin were 0.09, 0.12, 0.11, and 0.08, respectively. In malathion-sprayed and unsprayed control rooms, the GMD were 0.18 and 0.52, respectively. The densities in rooms sprayed with any of the four doses of bifenthrin did not differ statistically ($H = 1.53$, $df = 3$, $P > 0.6$). Therefore, the densities in the rooms sprayed with 25 mg dose of bifenthrin were compared with those in the

malathion sprayed and unsprayed control rooms. These were significantly lower in the rooms sprayed with bifenthrin 25 mg compared with malathion ($H = 6.38$, $df = 1$, $P < 0.02$) or control ($H = 29$, $df = 1$, $P < 0.0001$). Densities of other mosquitoes (excluding *An. culicifacies*) are shown in Fig. 2. The pattern was similar to that observed for *An. culicifacies*.

Excito-repellent Action and Delayed Mortality. The exit rate of *An. culicifacies* (proportion of mosquitoes collected in exit traps to total entry) in bifenthrin sprayed rooms was 0.31 (21/67) with 25 mg dose, 0.46 (59/127) with 50 mg, 0.50 (56/111) with 100 mg, and 0.41 (32/78) with 200 mg, as compared with 0.12 (20/161) in rooms sprayed with malathion, and 0.13 (86/669) in unsprayed control rooms. Thus, the exit rate was lowest with 25 mg dose, though the difference among the four doses was not significant ($P > 0.05$), and the overall exit rate with bifenthrin was significantly higher than that observed with malathion or control ($P < 0.001$). No significant difference was seen between malathion and the unsprayed control ($P > 0.05$). Similar results were also observed with the remaining mosquito species.

The proportions of fed to total entry of *An. culicifacies* in rooms with bifenthrin 25, 50, 100, and 200 mg doses were 0.40, 0.30, 0.33, and 0.38, respectively, compared with 0.40 in rooms with malathion and 0.31 in control rooms. The differences were statistically not significant ($P > 0.05$, $df = 5$). The proportions of fed to total entry of other mosquitoes in rooms with bifenthrin 25, 50, 100, and 200 mg doses were 0.41, 0.34, 0.26, and 0.29, respectively, compared with 0.38 in malathion sprayed rooms and 0.34 in control room.

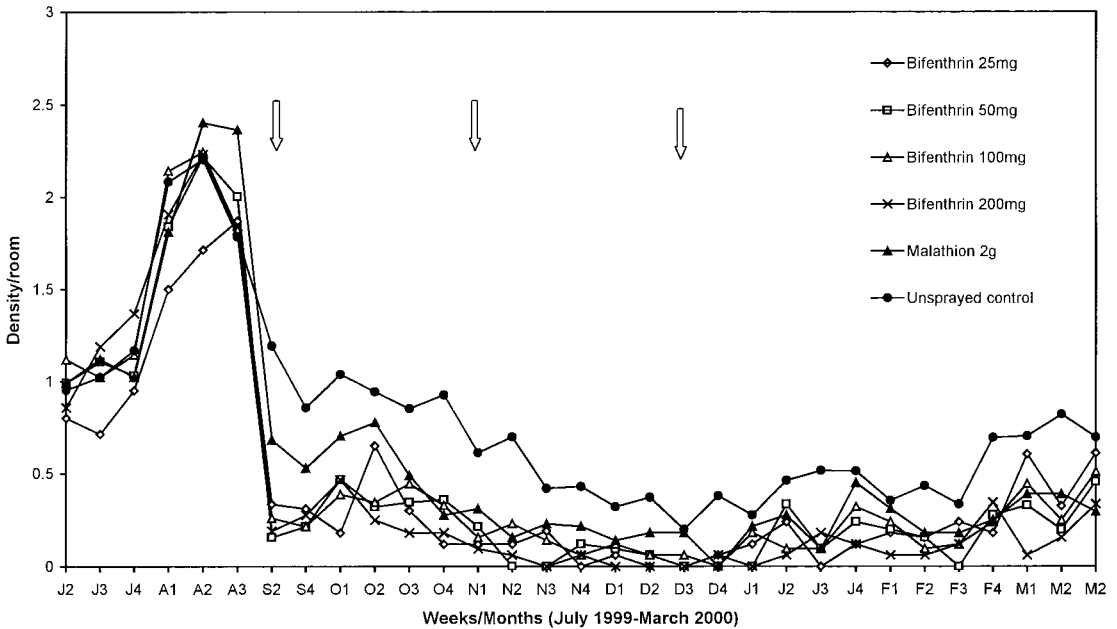


Fig. 2. Geometric mean densities of mosquitoes excluding *Anopheles culicifacies*. The first arrow indicates the first round of spraying of bifenthrin or malathion, whereas second and third arrows indicate, respectively, second and third rounds of spraying of malathion in the respective rooms.

The difference between bifenthrin, malathion, and control was statistically significant ($P < 0.01$, $df = 5$). The difference among the proportions for all four doses of bifenthrin was also significant ($P < 0.01$, $df = 3$). Thus, at the higher doses, the proportion of fed mosquitoes among all entered declined.

The proportions of fed to gravid in total entry of *An. culicifacies* in rooms with bifenthrin 25, 50, 100, and 200 mg doses were 3.38 (27/8), 2.0 (38/19), 3.08 (37/12), and 4.29 (30/7), respectively, compared with 2.5 (65/26) in malathion-sprayed rooms and 1.35 (206/153) in unsprayed control rooms. The differences were statistically significant ($P < 0.01$, $df = 5$). However, the difference was not significant among the four doses of bifenthrin ($P > 0.05$, $df = 3$), or between bifenthrin and malathion ($P > 0.05$, $df = 1$). The differences among proportions of fed to gravid of other mosquitoes were also similar to that of *An. culicifacies*.

The delayed mortality (number of mosquitoes dead after 24 h holding/number caught alive indoors and in the traps) among *An. culicifacies* or other mosquitoes caught from insecticide-sprayed rooms was higher than in those caught from the control, but the difference was not significant ($P > 0.05$).

Conclusions. In Kheda district where the trial was carried out, DDT, HCH, and malathion were sprayed indoors in different years under the antimalaria program during the past four decades. In this area, *An. culicifacies* has developed triple resistance to these insecticides, and there is a need for better insecticides or formulations. This trial has shown that the overall impact of bifenthrin on vector population was supe-

rior to that caused by malathion. The effect of all the four doses of bifenthrin on indoor resting densities was similar, thus on this count, the 25-mg dose emerged as the most preferred one. Further, the 25-mg dose caused least excito-repellency among all the doses evaluated, and its airborne effect was short lasting and nearly equivalent to 50-mg dose. A low excito-repellent action of an insecticide sprayed indoors is likely to produce a better mass killing effect on the mosquito population. From the results of the contact bioassays, we observed that on the most common surfaces there was no significant difference in the persistence of the residual action of 25-mg and 50-mg doses of bifenthrin. Therefore, we concluded that among all the four doses of bifenthrin evaluated, the 25-mg dose is a most favorable one, which produced $\geq 80\%$ kill at least for 16 wk on the most common surfaces. In earlier trials, cyfluthrin at 25-mg dose was found to be effective up to 12 wk on mud walls in Gujarat State and 15 wk in Maharashtra State against *An. culicifacies* (Yadav et al. 1996). In the State of Uttar Pradesh, 25-mg dose of deltamethrin persisted well up to 12 wk against this species on mud walls (Singh et al. 1989).

Acknowledgments

We are grateful to V. P. Sharma, M. K. K. Pillai, and N. N. Singh for their invaluable suggestions during the design of the protocol and the data analysis. We also thank the officials of the government of Gujarat (P. T. Joshi, G. Kurien, D. M. Patel, K. K. Kapadia, R. J. Dalwadi, and L. T. Gondalia) for their support during the trial. We appreciate dedicated efforts made by the staffs at the Malaria Research Center, and

the cooperation of the householders. M/s FMC Corporation, USA, supplied free bifenthrin.

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Received for publication 21 December 2001; accepted 2 June 2002.