INDOOR AND OUTDOOR ULV APPLICATIONS OF MALATHION FOR THE EXTENDED CONTROL OF *ANOPHELES* AND *AEDES* SPECIES IN WOODED RURAL COMMUNITIES IN EASTERN NIGERIA

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ABSTRACT. Indoor and outdoor ULV treatments for control of malaria and arbovirus vectors demonstrated that technical malathion applied by knapsack sprayers gave long-term control of both *Anopheles* and *Aedes* species. Post-treatment indoor landing rates of *Anopheles* species remained at 0.2/man-night for 14 weeks with a small increase recorded only in the 20th week. Cement walls retained a slightly higher insecticidal effect than mud walls. High droplet densities were also found on floors and ceilings. This technique was shown to be inexpensive, fast and highly flexible.

INTRODUCTION

As recently as 1976 malaria was rampant in Africa south of the Sahara (WHO 1978) and remains today the most important vector-borne disease in Africa. Problems encountered in combating the disease are manifold and range from resistance to insecticides to insufficient finances. Also important in any picture of endemic disease in Africa are the insidious flaviviruses, such as yellow fever, which are transmitted by sylvatic vectors of the *Aedes (Stegomyia)* complex. These vectors not only bite people near villages in forested areas but also frequently enter human dwellings for blood meals and oviposition.

Since 1948, indoor spraying of residual insecticides by conventional methods have been applied in tropical Africa with varying degrees of success (Kouznetsov 1977). The WHO Arbovirus Vector Research Unit (AVRU), Enugu, Nigeria has demonstrated that ground application of technical grade malathion can successfully control populations of *Ae. (Stegomyia)* species in densely vegetated habitats through a combination of direct exposure at the time of application and the longer-term residual effect of the insecticide. However, little is known about the efficacy of ground applications of insecticide against indoor-resting mosquitoes such as *Anopheles* and *Ae. (Stegomyia)* species. Indoor ULV application of insecticide is a new approach to malaria control, the success of which would depend upon the residual effect of the insecticide.

The main objective of the present trial now reported was to evaluate the efficacy of ground indoor and outdoor (within compounds) ULV applications of technical grade malathion against mosquito vectors with the interruption of disease transmission as the ultimate goal. Because of the prime importance of malaria, the principal malaria vectors were the main target species. The trial, which was a result of collaboration between the Anambra State Ministry of Health and AVRU, coincided with and complemented a field training program in medical entomology and ULV spray techniques for the State's malaria teams and was also the State's first step in the nation-wide Pilot Malaria Control Programme.

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MATERIALS AND METHODS

LOCATION. The trial was carried out at Amankanu (6°17'N-7°33'E) a village approximately 35 km southwest of Enugu, Anambra, Nigeria consisting of 247 compounds in an area of 190 ha. The village has a fluctuating population of over 1,700 inhabitants in 582 multi-roomed dwellings (7.1 people/2.4 dwellings/compound) situated in a broken forest relict. It is entirely surrounded by grassland and cassava farms and within individual compounds and adjacent clearings are farms of yam, cocoyam, cassava and banana/plantain. In the village are many chickens, goats and sheep and some dogs, cats, pigs and cows.

Amankanu is bisected by a secondary road which connects it with Agbani 2 km to the north and with Umuigbo-Amurri which was used as the comparison village, 2.5 km to the south.

Pre-treatment collections of adult mosquitoes over a period of 4 weeks yielded 3 *Anopheles* and 8 *Aedes* species. Ovitrap indices for *Aedes* spp. were high: indoor was 35.8% and outdoor was 73.3%. Anophelines were present at densities of 5.4 and 4.2 per man night indoors and outdoors respectively.

TREATMENT PROCEDURES. Two weeks before the 1st application 3 spray teams, each with 3 spraymen, were recruited from the mosquito scouts employed in the Anambra State Ministry of Health's Malaria Unit who were attending the field training course. Each individual was evaluated and coached according to his disposition and ability to carry the spray equipment and to employ correct spray techniques. During each application the spraymen alternated at 30 min intervals in order to minimize fatigue and exposure to insecticide. Each spray team was supervised by a member of the State Malaria Unit.

Two applications of technical grade malathion, 7 days apart, were made by using the Fontan R12 backpack sprayer with size 1.0 nozzle, during the last week of May and the 1st week of June 1978. As primary biting activities of target *Anopheles* and *Aedes (Stegomyia)* mosquitoes varied, it was decided that the applications would be most effective if made between 08.00 and 14.00 hr when wind velocity did not exceed 10 km/hr. Because of the large number of compounds, machine malfunctions, and a lack of cooperation on the part of some villagers, the 1st application took 3.5 days. With greater success, the 2nd application was completed in 2.5 days. Of a projected dosage of 350 ml/compound, overall usage for each application was 90 liters which was equivalent to 364.4 ml/compound. This gave an approximate total indoor dosage of 27.4 liters (46.2 ml/house) and a total outdoor dosage of 62.6 liters per application.

Each application was divided into 2 phases:

Indoor application. Before each application inhabitants were asked to remove light domestic articles (e.g. cooking utensils, etc.) and food from houses, and plastic sheets were used to cover furniture and appliances. To spray the interior walls of each room the sprayman took 2 steps inside the doorway of the room and directed the spray head at 30° up and down directing it only at the walls. While the sprayman moved to a different room the stopcock on the spray arm was closed. Because of the heavy blast from the machine, the throttle was reduced by 40%, and this gave a discharge rate of 4.0 liters/hr with a total elapsed mean spray time of 15 seconds/room.

Outdoor application. The outdoor area in each compound was treated by spraymen proceeding around the perimeter at a speed of 3 km/hr. The nozzle of the spray machine was always directed to the left-hand side at an angle of approximately 45°. This angle was altered to spray over buildings or high vegetation. The eaves of each house were also treated.

Spraymen were given 2 weeks' training in the safe use of pesticides and equipment as outlined by WHO (1973, 1974). Protective equipment and clothing, con-

sisting of an overall with a hood, goggles, a face mask, gloves and rubber boots, were provided and instructions in personal hygiene and safety precautions were given. Exposure was limited to a maximum of 6 hr per week for each of the 9 spraymen. Cholinesterase levels were measured before and after each spray round.

EVALUATION PROCEDURES. Landing rates of the vector population in Amankanu were measured by 8 mosquito scouts located in 4 compounds (1 scout indoors and 1 outdoors). Catches were made during a 12 hr period, 18.00–06.00 hr. Pre-treatment densities were calculated from 2 catches, 2 weeks apart, 2 and 4 weeks before treatment. Post-treatment densities were measured twice a week for 10 weeks and then weekly for a further 10 weeks. At Umigbo-Amurri, the check village, collections were carried out twice a month.

Morning indoor resting densities were determined in 10 compounds in Amankanu from pyrethrum spray sheet collections. Pre-treatment densities were calculated from 3 collections and post-treatment densities were measured twice a week for 10 weeks and thereafter until the 20th week. Weekly collections were made in the check village.

All the mosquitoes collected were identified in the laboratory at Enugu, and *Anopheles* species were dissected for sporozoites. Parity was determined according to the methods of Beklemishev et al. (1959).

Twenty-four ovitraps were installed in 12 compounds (12 indoors and 12 outdoors). The ovitrap surveys were carried out weekly in both the trial village, Amankanu, and the comparison village, Umuigbo-Amurri. Paddles were exposed for 2 days and egg-positive paddles were soaked 5 times, 1 week apart.

Bioassays were undertaken with laboratory-reared *Aedes aegypti* during the 2nd application. Paper cups netted along the top and sides were used for aerial bioassays, and cone-shaped cups were used for contact bioassays. Each cup contained 10 blood-fed females. Cups were placed in each of 10 compounds as follows: 1 cup at floor level indoors for the aerial bioassay and 1 cone attached to an indoor wall at a height of 1.5 m for the contact bioassay. In each of a further 10 compounds, 1 cup was placed outdoors at ground level for aerial bioassays. Ten cups were held as controls.

Mortality counts for the aerial bioassays were made when mosquitoes were transferred to new holding cups 1–2 hr after the application of insecticide, and final readings were taken after 24 hr. Mortality counts for the contact bioassays were made after 30 min exposure. Thereafter twice weekly post-treatment wall surface bioassays were carried out with exposure for 1 hr after which the mosquitoes were transferred to new holding cups and observed for 24 hr.

Droplet densities were measured in 10 compounds, indoors and outdoors, with Intek paper (7 × 10 cm). In a house in each of the 10 compounds, 8 cards were positioned indoors as follows: 2 horizontally on the floor; 4 vertically on interior walls; and 2 on the ceiling. Cards were also placed outdoors in each compound horizontally at ground level and attached, horizontally and vertically, to bamboo poles at a height of 7 m.

RESULTS

ADULT LANDING DENSITIES. Indoor and outdoor mean landing rates of *An. gambiae* at Amankanu were reduced from a pre-treatment density of 5.4 per man night (indoor) and 4.2 (outdoor) to nil in the 1st week after treatment (Table 1). Both the indoor and outdoor landing rates remained at about 0.3 female per man night, or less, up to week 20 after treatment. Although female densities of *Anopheles* species at the untreated village, Umuigbo-Amurri, began to decline as the dry season advanced, landing rates were 10-fold greater than those in the sprayed village 5 months following treatment. Suppressed landing rates for *An. funestus* were much higher than those for *An.*

Table 1. Mean landing rates per man night of *Anopheles gambiae, Aedes africanus* and other vectors before and after two ULV applications of malathion at Amankanu village, Nigeria.[1]

During weeks after treatments	*An. gambiae*		*Ae. africanus*		*Aedes* spp. Combined[2]	
	Indoors	Outdoors	Indoors	Outdoors	Indoors	Outdoors
before treatment	5.38(3.4)	4.19(4.4)	0.94(1.0)	3.63(2.8)	1.06(2.0)	4.81(7.7)
1 after	0	0	0	1.75	0.06	2.06
2	0.19	0.44	0.13	1.00	0.31	1.31
3	0.25(2.0)	0.25(3.5)	1.50(2.6)	2.50(7.0)	1.81(3.3)	3.75(11.2)
4–6	0.17(3.1)	0.11(1.3)	0.98(2.0)	2.09(3.6)	1.35(2.3)	3.17(5.9)
7–9	0.19(3.0)	0.17(1.8)	1.19(3.3)	2.62(3.9)	1.75(4.8)	3.75(6.8)
10–12	0.04(1.0)	0.13(0.3)	1.25(2.6)	2.84(4.9)	1.61(3.1)	3.94(6.6)
13–15	0.25(1.1)	0.04(1.0)	0.96(2.4)	2.75(4.3)	1.34(3.3)	3.58(4.0)
16–18	0.21(0.4)	0.17(0.6)	1.25(1.4)	5.79(4.3)	1.96(2.4)	8.25(5.7)
19–20	0.25(0.6)	0.19(0.4)	1.26(5.0)	5.82(9.0)	1.44(6.5)	8.97(11.6)

[1] In parentheses the landing rate in the control village is given.
[2] *Ae. africanus, Ae. luteocephalus* and *Ae. aegypti* are combined.

gambiae, which was also initially low. Evaluation of the changes in the fluctuating mosquito populations were expressed by percent reductions using the formula 100–100 (p/P).

Parous rate of all *An. gambiae* captured indoors and outdoors during the first 6 weeks of the treatment was 25%, about 50% lower than in the check area. Thereafter, the parity returned to the level (75%) of pre-treatment or of the check area.

Reduction in indoor landing rates of *Aedes* species was not as drastic as that for anophelines two weeks after spraying (Table 1), particularly in the outdoor landing rate of *Ae. africanus*. The reduction was about 50% for a period of 15 weeks as based in the check village during which indoor densities were below 2 females per man night and 3 in the outdoor catches. When 3 most common vector species of yellow fever *(Ae. africanus, Ae. luteocephalus* and *Ae. aegypti)* were combined and compared to those of the untreated village, the mean indoor landing rate for the post-treatment at 15 weeks was about 57% and 49% in the outdoor densities. In the treated village, among the *Aedes (Stegomyia)* species caught, *Ae. africanus* comprised 72% with 26% *Ae. luteocephalus* and 2% *Ae. aegypti*.

During the initial 2 weeks post-treatment, 42% of *Ae. africanus* captured outdoors were parous while that from untreated village was 73%. The parity determined after the 3rd week onward ranged from 72 to 91%, not significantly different from those of the comparison village.

RESTING DENSITIES. Table 2 shows the indoor resting densities of *An. gambiae* determined with pyrethrum spray catches before and after two ULV applications of malathion. The mean number of resting *An. gambiae* in the sprayed village dropped to nil from 10 females per room. During the 20-week period, a total of 12 females was collected from 483 rooms (0.025 female/room) as compared with 1,045 from 415 rooms (2.5/room) in the control area, with an overall reduction of 90%. As in the landing collections. *An. funestus* was almost absent in the pyrethrum spray catches in treated area. One *An. gambiae* collected during the 20th week was the 1st vector positive for sporozoites while in the untreated village the rates fluctuated between 5.3 and 20.1%, much higher than before the treatment (Table 2).

OVITRAP INDICES. The indoor and outdoor indices of ovitraps positive for *Ae. aegypti* were reduced by 94% and 69% respectively 2 weeks after treatment (Table 3). Ovitrap indices increased gradually, starting from the 6th week following treatment. Similar reductions

Table 2. Resting densities of *An. gambiae* as determined with indoor pyrethrum spray catches, and percentage of positive for sporozoites before and after two ULV applications of malathion.

Weeks before or after treatment	Treated village			Untreated village		
	No. rooms surveyed	Mean No. per room	Sporozoite %	No. rooms surveyed	Mean No. per room	Sporozoite %
before 2	36	10.10	3.3	19	16.4	3.2
after 2	34	0	—	30	7.5	8.8
4	50	0.02	0	32	5.0	20.1
6	48	0.04	0	36	8.8	11.1
8	65	0.08	0	38	4.3	6.7
10	56	0	—	44	1.4	10.0
12	50	0.02	0	47	0.7	8.8
14	47	0	—	44	0.3	13.3
16	48	0.04	0	50	0.5	12.0
18	40	0	—	49	0.6	10.7
20	45	0.02	1[1]	45	0.8	5.3

[1] One female dissected was positive.

were also recorded for *Ae. africanus* but the recovery rate was faster than for *Ae. aegypti* and returned to the pre-treatment level during the 3rd week. Ovitrap indices for both species were not clearly correlated with the reduction in and recovery of adult landing densities (Table 1).

BIOASSAYS. The results of contact bioassay tests are shown in Table 4. Mortalities of over 50% were maintained for 10 weeks on mud walls and 20 weeks on cement surfaces. Higher mortalities were obtained on mud walls than on cement walls for the first 10 weeks after treatment. However, during the second 10 weeks after treatment mortality rates on cement walls were generally higher and never below 56%.

There were more droplets on inside walls (24/cm²) than outside walls (7/cm²), on ground level (40–47/cm²) than at a height of 7 m or on the ceiling (Table 5). The cards positioned vertically received a lower density than on those placed horizontally.

DISCUSSION

The 1st outdoor ULV application of technical grade malathion against *Aedes* species in a wooded environment in West Africa was undertaken in 1977 by the Arbovirus Vector Research Unit. A combination of direct exposure and residual insecticidal activity reduced populations of *Ae. africanus* by 95% for a 2-week period,

Table 3. Percentage of ovitrap paddles positive for *Ae. aegypti* and *Ae. africanus* before and after malathion ULV treatment.

Week before or after treatment	Treated village				Untreated village			
	Ae. aegypti		*Ae. africanus*		*Ae. aegypti*		*Ae. africanus*	
	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
Before 2	35.8	73.3	7.5	8.0	32.3	79.7	3.1	8.7
After 1–2	8.3	12.5	4.2	0	—	—	—	—
3–4	9.1	10.6	2.4	8.5	55.0	71.4	5.0	4.8
5–6	8.7	27.3	4.3	18.2	30.0	40.9	15.0	9.1
7–8	23.3	27.1	6.8	16.7	63.2	40.9	10.5	13.6
9–10	12.5	29.2	12.5	4.2	25.0	47.6	0	14.3
11–12	26.1	12.8	4.4	14.5	15.8	35.0	0	5.0

Table 4. Percentage mortality of contact bioassays in houses sprayed with two ULV applications of malathion.[1]

Weeks after treatments	exposed on mud walls	exposed on cement walls
1–2	98[2]	92[2]
3–4	95	91
5–6	87	77
7–8	76	72
9–10	78	74
11–14	47	85
15–18	62	56
19–20	39	76

[1] Mean mortality for weekly tests using 10 blood-fed females of *Ae. aegypti* exposed in each of 10 houses for one hour. Mortality count made after a holding period of 24 hours.
[2] Exposed for 30 minutes.

and continued evaluation showed the technique to be highly effective in suppressing vector populations with an intent to interrupt virus transmission. In outdoor ground application of fenitrothion at rates of 520–580 ml/ha in an area of about 10 km² in Central Java, Pradhan et al. (1979) obtained adequate control of DDT-resistant *An. aconitus* for 10–14 days. This residual effectiveness of outdoor treatment appears to be much less than the indoor application of fenitrothion at 0.1 ml/m² of room space where perfect control of *Ae. aegypti* lasted for 6–7 months after treatment (Pant et al. 1974).

In the trial now reported, the technique was extended to include indoor and outdoor treatment for the control of vectors of both malaria and arboviruses and it was demonstrated that technical grade malathion applied by motorized knapsack sprayers, indoors and outdoors, as an aerosol mist in 2 sequential applications gave long-term control of both *Anopheles* and *Aedes* species. The highest percentage and longest lasting reduction was achieved indoors against *An. gambiae*. In addition, however, post-treatment indoor landing rates of *Anopheles* species remained at 0.2/man-night for 14 weeks with a small increase recorded only in the 20th week. This increase was also reflected in the percentage reduction and appears to be strongly correlated with a gradual decrease in the residual activity of the malathion on indoor walls. Further evidence of this was seen in pyrethrum spray catches where the 1st sporozoite positive *An. gambiae* was collected during the 20th week, while in the untreated village the rates fluctuated between 5.3 and 20.1%.

Table 5. Droplet density of technical malathion sprayed with Fontan R12 using 1.0 nozzle (mean for 20 cards).

Houses		Compound/outdoor	
Site	No./cm²	Site	No./cm²
Floor	47	Horizontal/ground	40
Ceiling	19	Horizontal/7 mh	17
Walls/inside	24	Vertical/7 mh	11
Walls/outside	7		

When considering a malaria control program, the behavioral changes due to insecticidal pressure within the *An. gambiae* complex, and to a lesser degree with *An. funestus*, as summarized by Brown and Pal (1971) must be borne in mind. Thus treatment both indoors and outdoors may be necessary. In addition, when dealing with vector species with a potential for prolonged man-vector contact and which may survive during dry periods the duration of insecticide activity is of primary importance.

In this trial the sustained indoor effect of the 2 applications was related to large droplet size (between 70 and 101 μm), substrate surface and absorption rate, and high droplet density. For example, residual activity on cement surfaces appeared to decrease more quickly immediately after spraying than it did on mud surfaces but was demonstrated to be more effective 20 weeks after treatment. Highest droplet density was recorded at floor level (465/cm²). Of cards placed vertically on inside walls directly opposite the door 45% received a direct blast of droplets and densities were too high to count. A surprisingly high density (200/cm²) was recorded on cards positioned horizontally on ceilings which were not directly ex-

posed to the spray. Accordingly it would seem reasonable to assume that effective, prolonged control of mosquito vectors and other ectoparasites can be achieved.

Backpack ULV spraying has been shown to be inexpensive (e.g., the cost of insecticide and shipping charges to Lagos, Nigeria in this trial worked out at $0.70/compound) and is fast and highly flexible under village conditions. It is feasible even in remote areas where roads may not exist.

Safety precautions, such as the removal or covering of food and furniture and advice to occupants to wait outside compounds during the spraying, are considered to be essential if this type of indoor spraying is included in a routine control program. Attention should also be given to domestic animals to avoid directly exposing them to blasts from the spray machine.

Further experimentation is essential if techniques for long-term control of vectors are to be devised. Dosage rates and droplet size as related to residual insecticide activity need to be examined more closely, as does the possibility of alternating insecticides. Further investigation is needed of the effectiveness of dry season spraying and residual activity. The effect of continuing to apply insecticidal pressure at the end of the wet season also needs to be determined.

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