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Reuse of experimental huts for indoor residual spraying is feasible

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Experimental huts are costly investments for programs evaluating insecticides and researching mosquito behaviour. These huts can be used to evaluate indoor residual sprays for malaria control but when the hut trials finish, contamination by the long lasting insecticide treatments might prevent further use of the hut. To see if experimental huts could be reused after indoor residual spraying tests, huts in Cotonou, Benin, were treated with a high dose of chlorpyrifos methyl (500 mg/m²). Bioassays with susceptible *Anopheles gambiae* indicated the treatment was successful. After this, untreated surfaces were washed and the inner surface of the cement walls was chipped away and replaced. Bioassays indicated that contamination was not present and that reuse of huts after indoor residual spraying is possible.

Key words: *Anopheles gambiae,* experimental huts, reuse, indoor residual spraying, Benin, chlorpyrifos methyl.

INTRODUCTION

Indoor Residual Spraying (IRS) has been an effective tool in the control of malaria vectors. In India, the number of cases of malaria was dramatically decreased, from 75,000,000 cases in 1947 to 100,000 cases in 1965, after 7 years of eradication operations, largely through IRS programs (Sharma, 1987). Significant reductions were also attributed to IRS programs in Kenya and Tanzania (Draper and Smith, 1962). The primary vector control intervention at the moment is insecticide treated nets (ITN), which are being widely distributed, especially for children and pregnant mothers. However, indoor residual spraying is also being rapidly scaled up; the number of people protected by IRS increased from 15 million in 2006 to 59 million in 2008 (WHO, 2009).

The World Health Organization (WHO) has provided guidelines for the testing of indoor residual sprays (WHO, 2006). These include three test phases. Phase I is composed of bioassays in the laboratory. Phase II trials are carried out in experimental huts, and Phase III evaluations are large-scale field trials. In addition to IRS treatments, evaluations of insecticides, repellents, and even behavioural studies can be tested in experimental huts (WHO, 2006; Suwonkerd et al., 2006).

Experimental huts are small houses with entrances allowing mosquitoes to enter but not easily leave. Thus all mosquitoes entering the hut can be counted and scored the following morning as alive or dead, and bloodfed or unfed. The addition of a veranda allows induced

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exophily to be measured as mosquitoes try to distance themselves from a treatment. Hougard et al. (2007) described the type of experimental hut used in this study.

As experimental huts are expensive to build and maintain, it is important to know whether or not the huts can be reused after treating the walls with a residual insecticide. Any contamination of the walls or other structures could result in misleading results in later tests. The WHO recommends a complete refurbishment of huts between trials to prevent this contamination (WHO, 2006). The feasibility of reusing huts by removing and replacing the inner surface of the walls was tested in Cotonou, Benin. Mortality of *An. gambiae* in cone bioassays was used as a measure of contamination.

MATERIALS AND METHODS

Three experimental huts were selected for use in Ladji, a neighbourhood in Cotonou, Benin (6°23'23N, 2°25'56E). Ladji is on the edge of Lake Nokoué and has experimental huts that have been in use, primarily for testing insecticide treated mosquito nets, since 2002 (N'Guessan et al., 2010).

An initial series of bioassays was conducted to determine the mortality of mosquitoes exposed to various surfaces in the huts before treatment. Bioassays were performed in WHO cones attached to the surfaces with masking tape. Surfaces tested included doors (2 cones), walls (10 cones), entry window slits (2 cones), ceiling (2 cones), floors (2 cones), and verandas (2 cones). The locations of the cones were in random locations for the treated walls and very near the areas treated for untreated surfaces. Mosquitoes used were female *An. gambiae* s.s. Kisumu (a pyrethroid-susceptible laboratory strain originally from Kenya) between two and three days old. Five mosquitoes were put into each cone for 30 min. After this period, they were removed from the cone and put into plastic cups covered with untreated mosquito netting and given access to 10% honey solution. Mortality was scored after 24 h.

After the initial survey, two huts were treated with the organophosphate chlorpyrifos methyl ('Reldan GF 1246', Dow AgroSciences) at a dose of 500 mg/m^2. This was intended to represent a highly effective dose for indoor residual treatments for this insecticide. Ansari and Razdan (2004) came to the conclusion that an IRS treatment of 500 mg/m^2 gave high efficacy in controlling *Anopheles culicifacies*. N'Guessan et al. (2010) found chlorpyrifos methyl (500 mg/m^2) to provide high levels of control for a longer period than DDT (2 g/m^2) and the pyrethroid, lambda-cyhalothrin (30 mg/m^2). It is estimated that this dose would give a good idea if any of the insecticide was remaining after the resurfacing of the cement walls. The window slits that allow mosquitoes to enter, the metal door, the plastic ceiling, the floor near the wall, and the veranda were not treated and these surfaces were covered with plastic sheeting in one treatment hut (Hut 1), and with cement bag paper in the other (Hut 2). Reed mats were treated and nailed to the ceiling. Treatment was performed using a Vexmorel 2000 Pro (Berthoud, Villefranche, France) backpack sprayer. Five days after the treatment, bioassays were repeated following the same procedures.

After these bioassays, the interior structures of the huts, including the floors, were washed using laundry detergent (Omo; Unilever Nigeria, Aba, Nigeria). The surface of inner walls of the treated rooms (1 to 2 cm, not including the veranda) was chipped away by a mason. They were then resurfaced using a cement and sand mixture.

The walls were left to dry for four days. After this period, the original bioassays were repeated using *An. gambiae* (Kisumu) of the same age (2 to 3 days).

The numbers of dead mosquitoes compared to total numbers were analysed using blocked logistic regression, using Stata 8.1 (Stata Co., College Station, Texas, USA).

RESULTS AND DISCUSSION

The mortality of mosquitoes on the different interior surfaces of the huts is shown in Table 1. Before the treatment, mortality was low in all huts, as expected. Huts 1 and 2 did not have any significant differences in overall mortality relative to the control hut ($p>0.05$).

All mosquitoes tested in the huts treated with chlorpyrifos methyl at 500 mg/m^2 died, whether they were exposed to treated surfaces or not. This was not expected as many of the surfaces tested had been covered during the treatment. It was unlikely that this contamination of untreated structures came from a faulty covering with the plastic sheeting or cement bags, as these were well secured. Mosquitoes that were held in cups in the room during testing but were not exposed to treated surfaces were also found dead after 24 h. This indicates there was probably another cause of the mortality of the mosquitoes than a direct contamination of these structures. Chlorpyrifos methyl has a low vapour pressure which may have contributed to the mortality of the mosquitoes, particularly as the tests were done only five days after the initial treatment. The huts were also closed after the treatments and not opened until the time of testing. A vapour effect in the veranda is worrying as it may result in an increased number of dead mosquitoes the verandas. Bar-Zeev and Self (1966) found greater mortality of mosquitoes in window traps on huts treated with propoxur and bromphos than in window traps on control huts, though in both cases the mosquitoes were not in contact with the treated surfaces indoors. As the main experimental hut models all use window traps or verandas to monitor mortality in exiting mosquitoes, this could lead to an overestimation of the insecticidal effect on mosquitoes that come into contact with treated walls and attempt to leave the hut.

There was little (less than 10%) mortality in mosquitoes tested on surfaces in the control hut was at the same time as the treated huts (shown in Table 1 as "after treatment").

After the removal of the inner surface of the walls and its replacement, the surfaces were tested again. There was some mortality (<15%) on the walls of Hut 2, and in the control, so these tests were repeated to see if this was mortality due to the treatment or not. In the end, no

Table 1. Results of WHO cone tests using *Anopheles gambiae* (Kisumu) in experimental huts in Ladji, Cotonou (Benin).

Test condition	Percentage mortality (number tested)					
	Walls	Doors	Window slits	Floor	Ceiling	Veranda
Hut 1 (plastic covering)						
Before treatment	2.0 (49)	0 (10)	0 (10)	10 (10)	-	0 (9)
After treatment	100 (50)	100 (10)	100 (10)	100 (10)	100 (9)	100 (10)
After refection of walls	1.0 (105)	0 (23)	0 (20)	5.0 (20)	0 (9)	4.2 (24)
Hut 2 (cement bag covering)						
Before treatment	0 (53)	0 (9)	0 (8)	12.5 (8)	0 (4)	11.1 (9)
After treatment	100 (47)	100 (10)	100 (8)	100 (10)	100 (9)	100 (10)
After refection of walls	3.7 (215)	5 (20)	4.2 (24)	4.2 (24)	0 (11)	4.3 (23)
Control						
Before treatment	0 (50)	0 (8)	0 (10)	0 (7)	0 (1)	0 (9)
After treatment	0 (46)	0 (12)	0 (11)	9 (11)	0 (10)	0 (10)
After refection of walls	1.9 (105)	9.1 (33)	4.5 (22)	0 (24)	4.8 (21)	0 (22)

hut had mortality greater than 10% on any surface. The results of mortality on covered structures for the hut with plastic coverings and the hut with cement bag coverings were not different before and after treatment (p=0.255). The plastic covering seemed to allow a better covering of surfaces to be left untreated. However, the run-off of the spray that happened to touch the plastic ran down the plastic sheeting more easily than on the cement bags, which absorbed some of the spray. It is important to keep this in mind while spraying to avoid having these drops touch surfaces that are not to be treated.

Before removing the inner layer of cement from the walls, the huts were vigorously cleaned. The plastic ceilings were not protected during spraying but were cleaned with soap and water. The fact that the ceilings caused no mortality in the final tests shows the impact of cleaning with soap and water.

Conclusions

The results from the treated huts indicate that reuse of experimental huts is possible, as the results of all the tests before the treatment and after the refection were not significantly different. The most important steps in reuse of experimental huts are: good covering of structures to be protected, vigorous cleaning of these structures before the refection of the walls (to avoid contaminating the new walls), a complete removal of the first layer of cement, and a proper refection of walls. The findings from this study provide conclusive evidence that proper refection of huts allows for their reuse, even with highly insecticidal and long lasting products.

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