Rev Saúde Pública 2007;41(2)

Roseli La Corte dos Santos[I]

Arnaldo da Silva Fayal[II]

Antonio Edvaldo F Aguiar[II]

Durval Bertran Rodrigues Vieira[II]

Marinete Marins Póvoa[III]

Evaluation of the residual effect of pyrethroids on *Anopheles* in the Brazilian Amazon

ABSTRACT

OBJECTIVE: To evaluate the residual effect of pyrethroids on the mortality rates of *Anopheles* in order to check their efficacy in indoor residual spraying in the Amazon Region, Brazil.

METHODS: The study was conducted in public housing unities in the city of Belem, Northern Brazil, in 2003. Twelve houses were randomly chosen, three in each of the four established areas. Pyrethroids cypermethrin wettable powder, deltamethrin suspension concentrate, lambda-cyhalothrin wettable powder, and etofenprox wettable powder, were sprayed on the indoor wall surface of local houses. Their effects on the mortality of *Anopheles* were assessed from July to November. Wall bioassay was performed using plastic cones attached to insecticide and wild mosquitoes from the town of Peixe Boi.

RESULTS: Mortality rate varied according to the type of wall that received the insecticide. Those inseticides applied to wood and non-plastered brick surfaces were more stable and lasted longer. Lambda-cyhalothrin presented shorter effect than the other insecticides, and Etofenprox had residual effects up to four months and was more effective in non-plastered brick surfaces. There was no statistical difference between the effect of deltamethrin and cypermethrin in all surfaces tested, and the duration of the residual effect was satisfactory up to three months after spraying.

CONCLUSIONS: Deltamethrin and Etofenprox presented grater performance when compared to the others. For these insecticides and formulations, a three-month interval between successive applications can be considered safe. In communities with predominance of houses with plastered brick surfaces, the smaller effectiveness of formulations should be considered, together with the importance of residual spraying as a vector control method in the area.

KEYWORDS: ***Anopheles.*** **Insecticides, administration & dosage. Vector control. Malaria. Pyrethroids. Residual effect. Insecticides.**

INTRODUCTION

Indoor spraying of insecticides is one of the main activities performed for vector control in areas with very high incidence of malaria, leishmaniasis, and Chagas' disease. Since the 90's, pyrethroids have been the choice to replace dichloro-diphenyl-trichloroethane (DDT) in spraying of houses in areas with high incidence of malaria in the Amazon.

Knowing how long a residual insecticide will last is important information for vector control, since it indicates the minimum interval between spraying to maintain the resistance of the insecticide. Regarding DDT, periodicity of application, called cycles, was six months. The lack of formal guidance on the duration of cycles of pyrethroids application raised issues on the possibility of a shorter persistence of these insecticides on the walls and the logistic ability of services to perform shorter cycles.

[I] Laboratório de Parasitologia. Departamento de Morfologia. Universidade Federal de Sergipe. São Cristovão, SE, Brasil

[II] Departamento de Controle de Endemias. Secretaria de Estado da Saúde do Pará. Belém, PA, Brasil

[III] Instituto Evandro Chagas. Ananindeua, PA, Brasil

Correspondence:
Roseli La Corte dos Santos
Laboratório de Parasitologia
Departamento de Morfologia da Universidade Federal de Sergipe
Av Marechal Rondon s/nº, Rosa Elze
49100-000 São Cristovão, SE, Brasil
E-mail: rlacorte@ufs.br

Received: 24/2/2006
Reviewed: 15/9/2006
Approved: 18/1/2007

Publications on insecticides recommended by World Health Organization (WHO) for spraying, indicates that the residual effects of pyrethroids last between two to six months. Such a variation of the time makes it difficult to plan field activities, including the amount of product to be bought and the need to better define the cycles.

Purchase of pyrethroids to control malaria by the Brazilian Ministry of Health started in 1996, and until 2003, cypermethrin wettable powder (WP) was most commonly used in residual applications.* In experiments conducted by the Fundação Nacional de Saúde (FUNASA – Management of Vector Control of National Health Foundation)** an 80% mortality was found after 60 days of cypermethrin application to unpainted wood surfaces. However, in treated surfaces, painted wood, mud, painted brick walls or lime coated, the mortality rates oscillated at 50% after 30 days, but this test was conducted in a few houses. This pilot essay, pointed out the need for repeating the experiment with a greater number of houses, to provide scientific support to the National Program on Malaria Control regarding the duration of spraying cycles of pyrethroids.

In this context, the present study was performed aiming at providing information to guide spraying actions. The objective was to assess the duration of residual effect of pyrethroids: cypermethrin, deltamethrin, etofenprox and lambda-cyhalothrin on the mortality of *Anopheles* in human houses in the urban area.

METHODS

The study was conducted in the public housing unity of Canarinho, in Belém, State of Pará, in 2003. This district had 829 houses, 85% were plastered with adobe, and 15% were made of wood.*** There was no history spraying in the area. We have chosen to perform the study in normal occupation conditions of the houses so the results would express the action of insecticides in real use condition. After mapping, the district was divided into quadrants, called A, B, C, and D. For each quadrant a type of insecticide was randomly chosen as follows: A= lambda-cyhalothrin, B=deltamethrin, C=etofenprox, and D=cypermethrin. For each of the four areas three plastered mud brick house, three unplastered mud-brick wall houses, and three wood houses were randomly chosen for follow-up. Over the study, some houses were replaced, because of refusal to take part in the study, or the house was closed or being redone. In these cases, that houses which was nearest and most similar to the excluded one was included for follow-up.

Pyrethroids such as cypermethrin WP 125 mg a.i./m^2, deltamethrin suspension concentrate (SC) 25 mg a.i./m^2, lambda-cyhalothrin WP 30 mg a.i./m^2, and etofenprox WP 200 mg a.i./m^2 were applied to internal walls of houses, each in one day, for 4 days in a row. Hudson X-Pert compression sprayer was used equipped with nozzle tip 8002-E. To ensure suitable application, eight technicians of the team received technical update one week prior to the study. Application was supervised by a technician of the State Health Secretariat of the State of Para, and for an engineer of the Brazilian Ministry of Health.

Bioassays were performed according to the procedure standardized by WHO.[9] Those responsible for bioassay proof were aware of the type of insecticide applied in each quadrant. Mosquitoes undergoing bioassay which were only *Anopheles* female mosquitoes, were collected in the night previous to the performance of the tests, in the city of Peixe Boi. Choosing the collection site was based on the knowledge of the high density of *Anopheles* all year round, and on the treatment of the site without vector control activities because they are low risk for malaria. Mosquitoes were captured from 18h to 21h, in corral of buffalos, using Castro mouth aspirator.

Female collected were carried in plastic wired cups, kept in a humid chamber. In the laboratory, they were fed through cotton soaked in sugared water and were at rest until the next morning. Tests were performed for four days in a row, in the first week of each month, starting as of 8h in the morning. Every day three houses from each wall of the quadrants type were assessed. Bioassays, in each house, had three plastic cones at 1.2m from the ground with approximately 20 mosquitoes each, standardized by the World Health Organization.[8] A fourth cone was used as control, for each replication, protected from the sprayed wall by a piece of cardboard wrapped in white paper, replaced daily.

After one hour of exposure to treated walls, mosquitoes were transferred from the cones to clean glasses, put into humid chambers and sent to the laboratory where they were fed by cotton soaked in sugared water. The temperature in the lab was kept at 22 to 25ºC. Mortality rate was calculated 24 hours after the end of the test. All mosquitoes that could fly were considered alive, after shaking the glass gently, regardless of the level of damage suffered. For mortality rates lower than 5% in the control group, no correction was applied. Mortality rates between 5% and 20% were corrected using Abbott's formula.[10]

Mortality rates were not higher than 20% in the control group. The first bioassay was performed 24h after initial application of the insecticides and the following with

* Sistema de Informação de Insumos Estratégicos. Secretaria de Vigilância em Saúde. Ministério da Saúde.
** Ministério da Saúde. Programa Nacional de Controle da Malária. Unpublished data.
*** Secretaria de Estado da Saúde do Pará. Relatórios do Programa de Agentes Comunitários de Saúde (PACS).

approximately 30-day intervals, totaling six bioassays in a five month follow up. For analysis effect, mortality rates higher than 70% were established as satisfactory in the exposed group. We decided to accept means lower than 70% whose confidence interval for the mean included this value.

Comparisons of survival rates, paired by insecticide and type of wall, were performed using Wilcoxon-Gehan's test to establish the significance level at 5% using the SPSS program.

The original project was submitted and approved by the Ethical Committee in Research of the Institute Evandro Chagas – Secretariat of Health Surveillance/Ministry of Health. Each participant was informed of the volunteer character of the research and doubts were settled. Inhabitants gave their written consent.

RESULTS

From June 2nd to November 7th, 2003, 11,342 mosquitoes underwent wall bioassays. Predominant specie was *Anopheles albitarsis* s.l., followed by *Anopheles triannulattus* and *Anopheles darlingi* (Table).

The effect of insecticides over time was compared considering surface in which the insecticides were applied and the type of insecticide. Criteria to define maximum duration of residual effect considered if the confidence intervals included or were above the line of 70% mortality of the exposed groups.

Table. Distribution of the number of mosquitoes used in wall bioassays, according to species. Belém, Northern Brazil, 2003.

Species	N	%
Anopheles albitarsis s.l.	7,271	64.1
Anopheles triannulattus	1,535	13.5
Anopheles darlingi	1,441	12.7
Anopheles aquasalis	328	2.9
Anopheles nuneztovari	140	1.2
Anopheles oswaldoi	108	0.9
Anopheles argyritarsis	36	0.3
Anopheles (Ano) sp.	4	0.0
Anopheles (Nys) sp.	479	4.2
Total	11,342	100.0

In wood surface, maximum duration of residual effect, considering confidence intervals for the average was two months for lambda-cyhalothrin WP, three months for deltamethrin CS, four months for etofenprox WP, and one month for cypermethrin WP. In the third and fifth month, the effect of cypermethrin increased, and it was higher than the 70%limit (Figure 1).

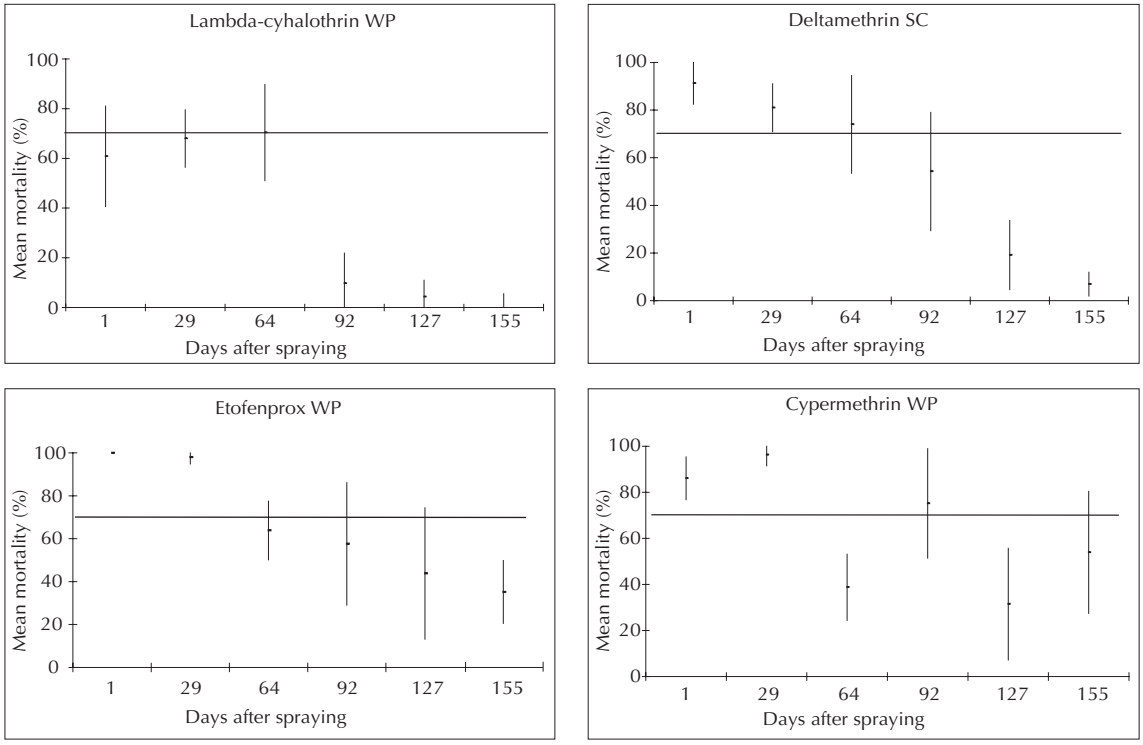

Figure 1. Mean mortality of *Anopheles* exposed to walls sprayed with pyrethroids. Vertical lines represent confidence interval. Belem, Northern Brazil, 2003.

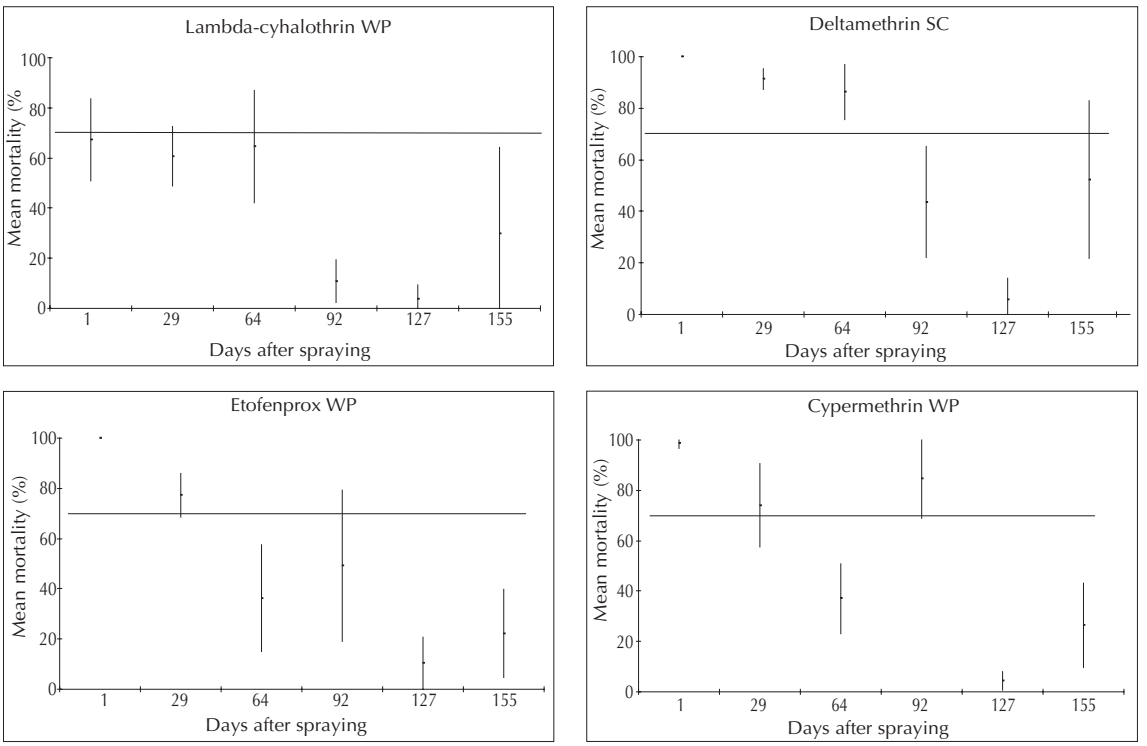

Figure 2. Mean mortality of *Anopheles* exposed to plastered brick walls sprayed with pyrethroids. Vertical lines represent confidence interval for the mean. Belem, Northern Brazil, 2003.

Comparing mortality through survival analysis, pyrethroids lambda-cyhalothrin WP, presented shorter effect, when compared to the other pyrethroids ($p<0.05$), and, unlike the others, mean mortality in 24h was lower than 80%. Mortality rates of deltamethrin suspension concentrate, cypermethrin WP, and etofenprox WP were not statistically different between bioassays over time. For these three last pyrethroids, confidence interval was huge after 60 days, when mortality reduced. Regarding lambda-cyhalothrin WP, confidence interval was great in the first bioassay and small for very low mortality rates.

In plastered brick wall houses, maximum duration of residual effect was two months for lambda-cyhalothrin WP, and deltamethrin suspension concentrate, and one month for etofenprox WP, and cypermethrin WP, considering confidence intervals for the average. Mortality rates presented wide variation in this type of wall. Deltamethrin SC presented satisfactory effect in the fifth month, etofenprox SC, and cypermethrin WP in the third. Lambda-cyhalothrin WP presented shorter effect, when compared to the others ($p<0.05$), whereas deltamethrin had longer effect (Figure 2).

In unplastered brick walls, duration of maximum residual effect was one month for lambda-cyhalothrin WP, and three months for deltamethrin SC, and etofenprox WP, considering confidence intervals for the average. Cypermethrin WP lasted one month, however, with variable effect, with satisfactory residuality in the third and fifth month (Figure 3). Lambda-cyhalothrin WP presented shorter effect, when compared to the others ($p<0.05$), whereas etofenprox PM had longer effect.

Regarding the type of insecticide, the effect of cypermethrin on mortality *of Anopheles* presented wide variation in all surfaces testes, as of the fifth month after application. There was statistical difference between the duration of the effect for the three types of wall ($p= 0.002$), and cypermethrin WP presented best performance in houses with unplastered brick walls.

Deltamethrin SC presented similar performance in all surfaces tested ($p=0.202$). Decrease of the effect was gradual and regular, and it lasted three months in wood, and unplastered brick surfaces.

Lambda-cyhalothrin WP presented shorter effectiveness, from three to six months, contrary to the indication of WHO. Statistical difference was not found in the surfaces tested ($p=0.085$). Low mortality and small amplitude of confidence intervals observed, generally after the second month, in the different houses, shows loss of insecticide activity, regardless of the surface.

Etofenprox WP was the insecticide that presented greater residuality, with mortality higher than 70% for up to four months after its application in wood surfaces. Statistical difference between mortality over time was

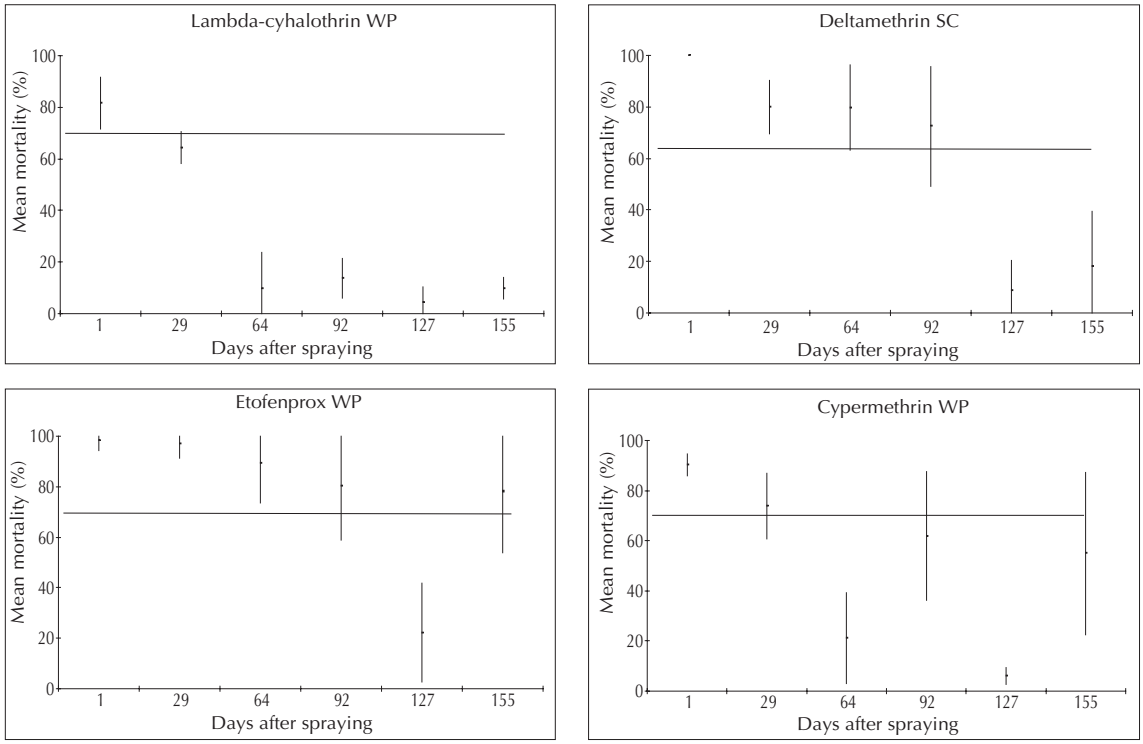

Figure 3. Mean mortality of *Anopheles* exposed to unplastered brick walls sprayed with pyrethroids. Vertical lines represent confidence interval for the mean. Belem, Northern Brazil, 2003.

seen, considering the several surfaces ($p<0.05$), and the best results were obtained for wood, and unplastered brick wall. Decrease in effect was gradual over time. In unplastered brick wall, this insecticide presented satisfactory effect again in the fifth month. However, the end of the study hindered from checking if the effect would be maintained in the following months, and residual effect could be longer than checked. In plastered brick walls, etofenprox WP had variable effect similarly to cypermethrin WP ($p=0.192$).

DISCUSSION

The first consideration to choose the insecticide to be used in a certain situation should be its proven effectiveness on the target species and its safety for inhabitants, workers, animals, and environment. Among the insecticides recommended by WHO for residual indoor spraying, because of its safety and effectiveness is the group of pyrethroids.[6]

Susceptibility of the Brazilian species of *Anopheles* to cypermethrin has been annually monitored by the Secretaria de Vigilância em Saúde (Secretariat of Health Surveillance) of the Ministry of Health. Results obtained up to the moment indicate that all monitored species, *An. darlingi, An. aquasalis, An. nuneztovari* and *An. albitarsis* sp. remained susceptible.* These species follow susceptibility standards for pyrethroids according to what is expected for the gender of *Anopheles,* according to Braogdon & McAllister.[2] The activity of entomologic surveillance gives support to the continuity of the use of pyrethroids in Brazil and assures that eventual loss of effect would be related to operational problems or degradation of the insecticide molecule rather than the resistance phenomenon.

Some factors hinder bioassays with *Anopheles*. The main factor is the difficulty to colonize, especially *An. darlingi*. The use of wild mosquitoes implies the non standardization of age of the species used and, consequently, in the possibility of interference of natural mortality in the result, as well as the different responses between young and old mosquitoes. On the other hand, populations maintained in insectaries may present alterations due to settling that may interfere in the answer of insecticides.

WHO recommends the use of wild mosquitoes in bioassays in walls because they represent better the response of field populations.[9] However, the duration of the study restricts its performance to places with high density of *Anopheles* during all year round and makes it difficult to select species to be tested, once there is change in density of each of them over time.

* Results presented at XXXIX Congresso da Sociedade Brasileira de Medicina Tropical, performed in Belém, PA, 16 to 21 March 2003.

In the present study, the capture area of mosquitoes was productive during all period for the subgenus *Nyssorhynchus.* Of the 11,342 mosquitoes used, only four belonged to another sub gender, and *An. albitarsis* s.l. was the predominant group. *An. darlingi* represented 12.7% of the total of mosquitoes undergoing tests. The factor that it was an animal and not human justifies the smaller frequency of *An. darlingi* in the captures. There are differences in the response between the species of *Anopheles* to very small concentrations of pyrethroids, in micrograms, as occurs, for example, in susceptibility test.[2] However, concentrations routinely used in spraying are above the level in which these variations of susceptibility manifested. Anyhow, the results presented must be interpreted regarding control of *Anopheles,* of the subgenus *Nyssorhynchus,* not from any species in particular.

In addition to the sensibility of target species to insecticide, duration of residual effect is essential information in the use of indoor spraying. Pyrethroids indicated by WHO to that end are: alphacypermethrine, benafentrine, cyfluthrin, deltamethrin, etofenprox, and lambda-cyhalothrin WP, all of them with residuality estimated between two and six months.[5]

The importance of a more precise definition of the duration of the residual effect is in the need for programming cycles so as the human population remains protected until a new spraying is conducted.

In the present study, we have established as a cutoff point for acceptable residual effect, mean mortality rate equal to or higher than 70%, although other works have considered as reasonable, rates higher than 50%.[1,4] However, we have chosen to consider satisfactory means inferior to 70%, whose confidence intervals reached this value. This decision took into account the fact that the study was performed in uncontrolled field conditions, where many external and particular factors of the houses may interfere in the activity of the insecticide.

The amplitude of confidence intervals for the average reflects the variation of the effects of pyrethroids in the several houses and types of walls studied. Apart from lambda-cyhalothrin WP, all insecticides were active until the first month after application, with confidence intervals of small amplitude. After this period, confidence intervals were higher, indicating variation in the effect depending on the house studied. This fact may reflect the action of other factors on the insecticide activity such as temperature and humidity of the house, cleaning of wall, lightening, and ventilation, among others.

Regarding the type of wall, apart from lambda-cyhalothrin WP, pyrethroids showed smaller and very variable effect when applied to plastered brick walls. One of the main reasons for the loss of pyrethroids activity is the fast absorption by porous surfaces. Concrete surfaces are also porous and the application of alkaline substances in addition to plaster may degrade the molecule of the insecticide faster.[3]

Among the insecticides compared, those which presented greater performance were deltamethrin suspension concentrate and etofenprox WP, both recommended by WHO.[6] Thus, these products may be considered safe and efficient to be used in wood walls and unplastered walls when applied in cycles every trimester.

Lambda-cyhalothrin WP presented one- or- two- month residual effect, lasting less than the other pyrethroids studied, therefore being a less favorable choice for the use in vector control programs because of the need for shorter application cycles.

In field conditions, cypermethrin presented unpredictable effect of decreasing and increasing residual effect in the three surfaces studied, which may hinder its use in residual application. Variation in the effects of pyrethroids was also noticed by other authors who studied mortality of triatominae exposed to treated surfaces.[1,3]

The effect of insecticides was not uniform, although spraying quality was assured by updating training of technicians, checking machines, and surveillance. Thus, in the routine of vector control where conditions may be more adverse, the effect of insecticides may be even shorter than the one recorded. Additionally, in communities with prevalence of plastered brick houses, the shorter effectivity of the formulations studied must be considered, as well as the importance of using residual spraying.

Assessment activities such as the one from the present study must be part of the routine of the services of vector control, as well as monitoring of the behavior of vector species. Considering that the amount of insecticides of the pyrethroids group is higher than those assessed in the present study, introducing other molecules and formulations will need new assessments so that the objective of decreasing the population of mosquitoes potentially infectious may be achieved.

AKNOWLEDMENTS

To Mr. Cláudio Cardoso Gonçalves of the Health Secretariat of the State of Para and Mr. Paulo Cesar da Silva of the Ministry of Health for the training and supervision of the spraying team; to Mr. Fabiano Geraldo Pimenta Jr. of the Ministry of Health for conceiving the study; to Mr. José Lázaro de Brito Ladislau for facilitating the exchange between the cooperating institutions; and to Mr. Raimundo Brandão Maia, of the Departamento de Controle de Endemias do Pará for coordinating the entomological team in field.

REFERENCES

1. Arias AR, Lehane MJ, Schofield CJ, Fournet A. Comparative evaluation of pyrethroid insecticide formulations against *Triatoma infestans* (Klug): residual efficacy on four substrates. *Mem Inst Oswaldo Cruz*. 2003;98:975-80.
2. Brogdon WG, Macaliister JC. Simplification of adult mosquito bioassays through use of time-mortality determination in glass bottles. *J Am Mosq Control Assoc*. 1998;14:159-64.
3. Diotaiuti L, Pinto CT. Suscetibilidade biológica do *Triatoma sordida* e *Triatoma infestans* à deltametrina e lambdacyhalotrina em condições de campo. *Rev Soc Bras Med Trop*. 1991;24:151-5.
4. Dorta DM, Vasuki V, Rajavel A. Evaluation of organophosphorus and synthetic pyrethroid insecticides against six vector mosquitoes species. *Rev Saúde Pública*. 1993;27:391-7.
5. Najera JA, Zaim M. Malaria vector control: insecticides for indoor residual spraying. Geneva: World Health Organization; 2001.
6. Najera JA, Zaim M. Malaria vector control: decision making criteria and procedures for judicious use of insecticides. Geneva: World Health Organization; 2003.
7. Passerat de Silans LNM, Dedet JP, Arias JR. Field monitoring of cypermethrin residual effect on the mortality rates of the phlebotomine sand fly *Lutzomyia longipalpis* in the State of Paraíba, Brazil. *Mem Inst Oswaldo Cruz*. 1998;93:339-44.
8. World Health Organization. Resistencia a los inseticidas y lucha contra los vectores. Ginebra; 1970. (Informes técnicos n.443).
9. World Health Organization. Instructions for the bioassay of insecticidal deposits on wall surfaces VBC/81.5. Geneva; 1981.
10. World Health Organization. Report of the WHO Informal Consultation. Test procedures for inseticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. Geneva; 1998.

Supported by Secretaria de Vigilância em Saúde/ Ministério da Saúde. Logistic support by Secretaria de Estado da Saúde do Pará, Secretaria Municipal de Saúde de Belém and Fundação Nacional de Saúde-PA.