Pyrethroid Insecticide Evaluation on Different House Structures in a Chagas Disease Endemic Area of the Paraguayan Chaco

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Insecticide effects of deltamethrin 2.5% SC (flowable solution) on different substrates and triatomine infestation rates in two indigenous villages (Estancia Salzar and Nueva Promesa) of the Paraguayan Chaco are reported. This field study was carried out to determine the extent to which variability in spray penetration may affect residual action of the insecticide. A total of 117 houses in the two villages were sprayed. Filter papers discs were placed on aluminium foil pinned to walls and roofs in selected houses and the applied insecticide concentration was determined by high pressure liquid chromatography (HPLC). The target dose rate was 25 mg a.i./m^2. The mean actual applied dose in Estancia Salazar was 11.2 ± 3.1 mg a.i./m^2 in walls and 11.9 ± 5.6 mg a.i./m^2 in roofs while in Nueva Promesa, where duplicates were carried out, the mean values were 19.9 ± 6.9 mg a.i./m^2 and 34.7 ± 10.4 mg a.i./m^2 in walls and 28.8 ± 19.2 mg a.i./m^2 and 24.9 ± 21.8 mg a.i./m^2 in roofs. This shows the unevenness and variability of applied doses during spraying campaigns, and also the reduced coverage over roof surfaces. However, wall bioassays with Triatoma infestans *nymphs in a 72 h exposure test showed that deposits of deltamethrin persisted in quantities sufficient to kill triatomines until three months post spraying. Knockdown by deltamethrin on both types of surfaces resulted in 100% final mortality. A lower insecticidal effect was observed on mud walls. However, three months after treatment, sprayed lime-coated mud surfaces displayed a twofold greater capacity (57.5%) to kill triatomines than mud sprayed surfaces (25%). Re-infestation was detected by manual capture only in one locality, six months after spraying,*

Key words: *Triatoma infestans* - deltamethrin - pyrethroid - substrates - persistence

Vector borne diseases can often be controlled through measures against vector insects. For Chagas disease this is the only practical intervention because there are neither drugs suitable for large-scale field use nor vaccines available. Control by elimination of vectors is effective because at least 80% of the transmission is attributable to domestic and peridomestic populations of triatomines (Dias & Pellegrino 1948, Pinchin et al. 1980, 1981, Dias 1987, Schofield 1994) which are accessible for control by insecticides, housing improvement, and health education (Dias 1987, CTA 1992).

Insecticide application in infested houses leads to high triatomine mortality rates. Early post-spraying evaluations generally reveal zero apparent infestation rates for the first few weeks, but this may be followed by a slow and steady rise in the number of bugs and the proportion of re-infested houses (Oliveira Filho 1984,1992,1999, Schofield 1985, Paulone et al. 1988, Gurtler et al. 1994). Recrudescence in the infestation rates is an important problem for control campaigns because retreatment costs can considerably increase the budget of control programmes.

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It was long argued that long-term control of domestic triatomines required the development of highly residual formulations of insecticides (WHO 2002). Wettable powder and highly residual slow-release formulations such as insecticidal paints based on various polymer matrices such as latex (Schofield & Pinchin 1979, Schofield 1982, Oliveira Filho 1984). Nevertheless, this assumption is difficult to sustain because organochlorines such as BHC and dieldrin are more stable than pyrethroids (Leahey 1985) and for this reason the use of organochlorines is now restricted because of the risk of environmental contamination (Van Den Bosch 1978)

In Paraguay, as elsewhere in Latin America, rural dwellings include a range of construction materials presenting a wide variety of substrates for insecticide action. Preliminary studies of a community sprayed with lambda-cyhalothrin using bioassays with *Triatoma infestans* nymphs, indicated that wood walls seemed to retain the insecticide activity at least for three months, while similarly treated mud walls could lose activity in less than a month (Ferro et al. 1995). In laboratory conditions four different formulations were tested on four substrates and low residual effect was observed on all substrate types beyond 90 days post-spraying (Rojas de Arias et al. 2003). In consequence this study aims to look at different insecticides and formulations on different substrates typical of rural housing in Latin America and to look at the long-term effects of the insecticides on triatomine populations. The persistence of deltamethrin SC25 was also determined under field conditions, which mimicked spraying campaigns.

MATERIALS AND METHODS

Description of the communities - Two indigenous farming communities living within the Estancia Zalazar farm located in the Chaco region, 436 km west of Asuncion, and Nueva Promesa 476 km west from Asuncion were evaluated. The indigenous groups living in these communities belong to the Sanapaná and Angaité tribes, which are semi nomadic groups of the region and the tribes are mixed in these villages. The first village was settled 12 years ago and had 28 indigenous dwellings and seven farmhouses. The second, Nueva Promesa, had 74 indigenous dwellings and eight farmhouses. In Estancia Zalazar all the indigenous houses were built of wood, while farmhouses were built of mud, some of them coated with lime. In Nueva Promesa both types of dwellings were built mainly of fired bricks. The study was carried out in July 1995 during winter season when temperature ranged from 25°C to 28°C and relative humidity from 55% to 80% according to data collected during the study.

Triatomine survey - The infestation level of each house in the two communities was evaluated by manual sampling in which a team of two trained field workers equipped with forceps, torches, plastic containers, and a timer, collected triatomines for a standard period of 30 min in each house. Each container was properly identified and the level of triatomine infestation and *Trypanosoma cruzi* infection was determined in the laboratory. The level of infestation was determined one month, three months, and six months post spraying, by manual sampling as described above for the two communities described.

Evaluation of insecticide residues on filter papers - Four houses in Estancia Zalazar and five in Nueva Promesa were selected for detailed study. In each house in Estancia Zalazar, six filter papers discs (Whatman Nr 1, 90 mm diameter) were placed on aluminium foil pinned to walls, and two more discs were pinned inside the roof. Twenty-four hours after spraying the filter paper discs were removed and placed in sealed plastic bags. Two filter papers from the roof and two from the walls were sent to Roussel Uclaf (now Agrevo), UK to determine insecticide concentration by high pressure liquid chromatography (HPLC). Briefly, a reference standard solution of 0.1 g of deltamethrin was dissolved in 100 ml of extraction solvent reaching in the mix 16 µg ai/ml. Each rolled sprayed filter paper was put in a 10 ml tube and extraction solvent was added. After several shakes the liquid supernatant was used as test dilution. Using a loop injector volume, replicate volumes permitted to obtain successive duplicate chromatograms. The average area of the deltamethrin peaks was taken and mg of deltamethrin (pure/m^2) was determined.

Insecticide application - The National Service of Vector Control (Senepa) personnel sprayed all houses using deltamethrin 2.5% SC (flowable solution) supplied by Rousell Uclaf (Berkhamsted). Eight litre Hudson X-pert spray pumps were used during this operation. Target spray levels were 25 mg a.i./m^2 on the inside and outside surfaces of all walls and the inner surface of the roof. The sprayer team used standard procedures in preparing and applying the spray. Potable water was used in making the formulation. A description of each house, where the filter papers were affixed, was also made. The surface area of the house was calculated by the area of the four lateral dimensions both inside and outside, including the roof. Information about the type of building materials was also collected. After insecticide application to each house, the volume of applied solution was calculated, using the following equation: applied mg of a.i/m^2 = applied insecticide volume per house/house surface (m^2) x g a.i./litre in the spray solution x 1000

Evaluation of the insecticidal effect in sprayed houses - Activity of the insecticide was monitored by periodic bioassays on treated walls. The selected houses from Estancia Zalazar were monitored at 24 h, one month, three months and six months post-spraying by exposing 15 third instar *T. infestans* nymphs on indoor treated wall surfaces. Mud walls and lime-coated mud were chosen for these assays. Exposure was for five days and two standard exposure cones were used for each different type of surface. In Nueva Promesa insecticide effect was only evaluated at six months post-spraying in four selected houses. Cones as control groups were placed over cardboards, to avoid contact of triatomines with sprayed walls, and were then fixed on the same walls where experimental cones were placed.

Data analysis - Evaluation of factors affecting insecticide effect was made by non-parametric analysis of variance when comparison between house, substrate, mortality and post-spraying time was required. Two paired-sample t Test was applied in order to test inter-method differences between target applied and residual deltamethrin concentrations obtained under laboratory and field conditions. Mean and standard deviation were also calculated (Zar 1984).

RESULTS

In Estancia Zalazar, the filter paper residue determined by HPLC was consistently lower than the calculated applied concentration, and averaged only about half the average applied concentration (Paired samples t Test wall $p < 0.01$; roof $p < 0.01$) (Table I). In Nueva Promesa by contrast, HPLC assays were done out on duplicate filter papers from walls and roof of each tested house (Table II). No differences were found between wall and roof filter papers when duplicates were analyzed independently (Paired samples t Test wall $p > 0.05$). But when an average of the two duplicates was compared with the calculated concentration a significant difference on wall filter papers was observed ($p = 0.0017$). The averages of all HPLC assays are shown in Tables I and II. The applied doses ranged from 12.8 to 51.2 mg a.i./m^2 in walls and from 4.6 to 61.0 mg a.i./m^2 in roofs. This illustrates the unevenness and variability of applied doses during spraying campaigns, and also indicates more even coverage over roof surfaces than walls.

The evaluation of the insecticidal effect was carried out in Estancia Zalazar on the walls of the same four selected houses where filter papers were exposed and results are shown in Table III. Deposits of deltamethrin persisted in quantities sufficient to kill *T. infestans* until three months post spraying. Knock down by deltamethrin on both types of surfaces resulted in 100% final mortality. A lower insecticidal effect was observed on mud walls. Nevertheless, due to the high variability in mortality rates within replications no significant differences were observed. In Nueva Promesa, where houses built with bricks without lime and lime-coated plaster bricks were sprayed, mortality reached 20% and 100% respectively (data not shown). Mortality was not observed in any of the bioassays control groups.

In both insecticidal assays at different post-spraying times, high variability between replicates within 24 h was observed, presumably due to different residual deposi-

TABLE I

Chemical assays for deltamethrin SC25 deposits on filter papers and other substrates, at field conditions in Estancia Zalazar, Chaco region, Paraguay [a]

House number	Filter paper location [d]	Calculated applied concentration from spray deposits (mg a.i/m^2) [b]	Residual concentration of deltamethrin (mg a.i/m^2) [c]
1	Wall	24.7	12.2
	Roof		10.3
2	Wall	22.5	12.2
	Roof		12.6
3	Wall	21.6	6.7
	Roof		5.1
4	Wall	19.4	ND
	Roof		11.1
19	Wall	20.7	13.9
	Roof		ND
38	Wall	25.9	ND
	Roof		20.5
Mean ± SD	Wall	22.6 [e]	11.2 ± 3.1
	Roof		11.9 ± 5.6

a: the target dose was the commercially recommended application rate (25 mg/m^2); *b*: the initial deposits of deltamethrin were estimated from the area of the house surface treated and the amount have applied insecticide solution and the concentration of active ingredient in the spray tank; *c*: both filter papers from each location were processed together by HPLC 45 days after spraying at Roussel Uclaf Lab. Means of the two results are presented; *d*: statistical inter-methods differences were found by Paired-sample t test when residual wall ($p = 0.008$) and roof ($p = 0.009$) concentrations were separately compared with the calculated applied concentration from house spray deposits; *e*: this figure was calculated using average values of the sprayed house surfaces and the applied volume; SC: flowable solution; FP: filter paper ND: not done

TABLE II

Chemical assays of deltamethrin SC25 deposits on filter papers, at field conditions in Nueva Promesa, Chaco region, Paraguay [a]

House number	Filter paper location	Calculated applied concentration from spray deposits (mg a.i/m^2) [b]	Residual concentration of deltamethrin (mg a.i/m^2) [c,f]	
			(i) [d]	(ii) [d]
1	Wall	24.8	26.6	12.8
	Roof		61.0	7.3
2	Wall	27.0	25.5	13.8
	Roof		27.5	39.1
3	Wall	25.9	32.7	19.1
	Roof		31.0	24.2
4	Wall	37.8	51.2	25.4
	Roof		4.6	17.3
5	Wall	23.8	37.6	28.6
	Roof		50.3	56.2
Mean ± SD	Wall	26.4 [e]	34.7 ± 10.4	19.9 ± 6.9
	Roof		24.9 ± 21.8	28.8 ± 19.2

a: the applied dose was the commercially recommended application rate; *b*: the initial deposits of the deltamethrin were estimated considering the area of house surface treated, the amount of applied insecticide solution and the concentration active ingredient in the spray tank; *c*: these residual concentrations were determined by HPLC 30 days post-spraying at Roussel Uclaf Lab; *d*: duplicates; *e*: this figure was calculated using averages of the sprayed house surfaces and the applied volume of insecticide solution; *f*: no statistical differences were found by Paired-sampled t test when residual wall ($p > 0.04$) and roof ($p > 0.05$) concentration were separately compared with the calculated applied concentration from house spray deposits. Nevertheless, an average of the two wall duplicates showed significant differences when it was compared with the calculated applied concentration ($p = 0.0017$); SC: flowable solution; SD: standard deviation

tion or uneven spraying during the application process. In Estancia Zalazar, house number 4 which did not show triatomine mortality by bioassay (Table III) presented the lowest calculated applied concentration in wall sprayed deposits (Table I). In contrast, the highest mortality rate was observed in house number 1 and this showed higher calculated applied doses on walls (Tables I, III). Although high concentrations were applied on all house walls from Nueva Promesa (Table IV), lime-coated plastered brick walls showed higher mortality rates at six months post-spraying than non-plastered. In this particular case, the type of substrate may have affected the insecticide persistence (data not shown).

Re-infestation was also evaluated in both localities. In Estancia Zalazar, six months after spraying re infestation was detected by manual capture inside indigenous dwellings (Table V). In Estancia Zalazar high triatomine densities were observed before spraying. The low spray rates applied and the poor insecticide residual deposits on house substrates found after six months post-spraying, may have accelerated triatomine re-population.

DISCUSSION

This study confirms once again the initial efficacy of deltamethrin and other similar pyrethroids on triatomines when applied on different substrates at field conditions (Pinchin et al. 1980,1981, Diotaiutti & Teixeira Pinto 1991, Ferro et al. 1995, Guillen et al. 1997, Rojas de Arias et al. 1999).

However, these initial doses are not maintained on treated surfaces for more than few months (Oliveira Filho 1984, Gorla, 1991, Pinchin et al. 1994, Ferro et al. 1995, Rojas de Arias et al. 1999, Oliveira Filho et al. 2000). The persistence of residual effect on different surfaces varies according to application procedures, the nature of the structure, the formulation, the species of triatomines involved, and the environmental conditions (Oliveira Filho 1984, Diotaiutti & Teixeira Pinto 1991, Rojas de Arias 1999, Oliveira Filho et al. 2000, Rojas de Arias et al. 2003, Nakagawa et al. 2003).

In our field conditions estimated concentrations were also higher than those finally achieved. The highest variability in residue deposition was observed in roof samples

TABLE III

Effect of deltamethrin SC25 applied at 25 mg/m^2 on walls of mud and lime-coated mud, measured as percentage mortality of 3rd instar *Triatoma infestans* nymphs at different post-spraying times

		Mortality observed according to post-spraying time			
Substrates	House number	24 h	1 month	3 months	6 months
Lime-coated mud	1	100	100	100	0
		80	100	100	0
	2	80	10	0	0
		90	20	30	0
Mud	3	60	30	60	0
		20	20	40	0
	4	0	60	0	0
		70	70	0	0

Variations did not show significant differences when substrate (p = 0.5), houses and substrates (p = 0.2), time post-spraying (p = 0.06) and substrate and time post-spraying (p = 0.6) were compared. SC: flowable solution

TABLE IV

Mortality percentage average of 3rd instar *Triatoma infestans* nymphs on mud and lime-coated mud sprayed walls from selected houses in Estancia Zalazar, Chaco region, Paraguay, at different post-spraying times

	Mortality percentage according to post-spraying time			
Substrates	24 h	1 month	3 months	6 months
Lime-coated mud	87.5	57.5	57.5	0
Mud without lime	37.5	45.0	25.0	0

TABLE V

Intradomiciliary triatomine infestation at different times after spraying in Estancia Zalazar and Nueva Promesa, Chaco region Paraguay

Localities	Number of examined houses			Infestation rate at different months post-spraying			
	Baseline	Followed	Intradomiciliary infestation ^{a}	1	3	6	9
Estancia Zalazar	35	32	22.9	0%	0%	9.4%	15.6%
Nueva Promesa	82	80	6.1	0%	0%	0%	0%

a: number of infested intradomiciles/number of intradomiciles investigated per hour man

(Tables I, II), which reflects the difficulties in roof spraying. This study has demonstrated the variability in sprayed deposits, and suggests that there can be virtually untouched areas in sprayed houses or several under dosed sites. This fact could influence the survivorship of bugs causing early re-infestation. Triatomines can resist starvation for weeks and months. Nevertheless, they start getting active after 3-6 weeks of starvation (Schofield 1985, 1994). If insecticide persistence is still present on sprayed substrates and an effective initial clean-out using lowest effective dose is done, a great impact could be achieved on bug populations. Insecticidal activity for longer periods after an effective intervention should not be relevant to control operations (Guillén et al. 1997, Rojas de Arias et al. 1999, 2003).

The nature of the substrate played an important role on the residual efficacy of the insecticides. In this field study, the rapid loss in insecticide toxicity occurred on a porous surface demonstrating the strong absorption or breakdown on the insecticide (White 1982, Diotaiutti & Texeira Pinto 1991, Rojas de Arias et al. 2003).

Previous laboratory results where lime-coated mud showed effective residual effect beyond 90 days post-spraying with wettable or flowable formulations, demonstrated that this substrate acted as sealant, exposing the insecticide particles for more time on the surface (Rojas de Arias et al. 2003). In this study, lime-coated mud surfaces displayed a twofold greater capacity to kill triatomines than mud sprayed surfaces three months after treatment (Tables IV, V). Nevertheless due to the inconsistency of the results both within and between houses, no significant differences were observed. There was no insecticidal effect at six months and in coincidence with this, re-infestation began in some of the houses. The re-infestation process observed with coincidence with the poor insecticide residual effect may be attributed to re-populations of residual indoor colonies due to the low insecticide doses achieved on surfaces in the initial chemical application.

One of the most interesting findings of this work is the potential use of lime which can be applied on porous surfaces such as mud, in order to allow a more efficient performance of insecticide formulations (Tables IV, V). However, the incorporation of this strategy in a control campaign must be carefully considered in terms of its cost implications. If this strategy is used it could incur significant expense to the families taking part (Winch et al. 1994).

In conclusion, high variability of the insecticide effectiveness was observed in field conditions. Therefore, there may be sprayed areas that were virtually untouched and this fact could influence the survivorship of the bugs. In this way, persistence and variable sprays deposits could be crucial in permitting survival in sprayed houses. Further studies should be done on residual effect of interaction of insecticide on different surfaces in order to improve its persistence and operational strategies that can correct the great variabilities observed in achieved deposits, should be encouraged to find realistic and operative alternatives that can reduce the possible re-infestation in sprayed areas at a reduced cost.

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