A new aqueous suspension concentrate formulation of *cis*-permethrin and its insecticidal activity

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Abstract: Isomers of pyrethroids usually have different insecticidal activities. Permethrin, a non-cyano pyrethroid, is not an exception and *cis*-permethrin is much more active than the *trans*-isomer against *Triatoma infestans*, vector of Chagas' Disease in Argentina. The large-scale separation of *cis*- and *trans*-permethrin was performed by successive recrystallizations from ethanol-water mixtures. An aqueous suspension concentrate (flowable) formulation of pure crystalline *cis*-permethrin was prepared and assayed for its insecticidal activity on wood and ceramic surfaces against nymph V of *T infestans*. This formulation was at least three times more effective than deltamethrin, with LC_{50} values on ceramic of 0.11 $\mu g\,cm^{-2}$ and 0.33 $\mu g\,cm^{-2}$ respectively. On wood surfaces, the LC_{50} value was 0.57 $\mu g\,cm^{-2}$ compared with 3.20 $\mu g\,cm^{-2}$ for deltamethrin. Against other insect species such as *Periplaneta americana, Aedes aegypti* and *Culex quinquefasciatus*, the suspension concentrate formulation of *cis*-permethrin was, however, less effective than similar formulations of deltamethrin or β-cypermethrin.

Keywords: pyrethroids; *cis*-permethrin; suspension concentrate; geometrical isomers; *Triatoma infestans*; *Aedes aegypti*; *Culex quinquefasciatus*; *Periplaneta americana*

1 INTRODUCTION

Pyrethroid insecticides exist in different isomeric forms and the insecticidal activity depends upon the stereochemistry of the molecules. Only certain isomers are biologically active.[1]

Commercially available pyrethroids are mixtures of optical and geometric isomers. These products show high activity against insects but relatively low mammalian toxicity and have proved very effective in controlling insect pests of medical and veterinary importance, and are thus used for public health as well as agricultural purposes.[2–5]

Research into the production of new chemicals useful as insecticides has involved not only chemical modifications but also the resolution and purification of the most active isomers.

Deltamethrin, cypermethrin, β-cypermethrin, λ-cyhalothrin and β-cyfluthrin, classified as third-generation pyrethroids and comprising the more active isomers, are currently used as effective tools in National Control Programmes for the control of *Triatoma infestans* Klug, vector of Chagas' disease in Argentina.[6,7]

Permethrin, a mixture of isomers of a non-cyano pyrethroid, is not usually used in triatomid control in Latin America because of its unfavourable cost-benefit relationship[8] but is well established against other public health pests because of its low mammalian toxicity.[5]

The high insecticidal activity of the *cis*-isomer of permethrin against *T infestans* was recently described by our laboratory, as well as the antagonistic effect of the *trans*-isomer.[9,10] This difference in insecticidal activity is higher than that reported by other authors for different insect species.[11]

To complete the toxicological studies on the geometric isomers of permethrin, our laboratory established a preparative-scale separation of the *cis*- and *trans*-isomers of permethrin based on successive recrystallizations from ethanol-water mixtures, and prepared a new aqueous suspension concentrate (flowable) formulation. The insecticidal activity of this formulation on different porous surfaces against different insect species such as *T infestans*, *Aedes aegypti* L, *Culex quinquefasciatus* Say and *Periplaneta americana* (L) was assayed.

2 MATERIALS AND METHODS

2.1 Chemicals

Methanol, ethanol, propan-1-ol and propan-2-ol (Merck, Germany) were anhydrous having been dried

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over molecular sieve 4A (Aldrich, USA). All other reagents were of analytical grade.

Standard permethrin (isomeric composition *cis:trans* 45:65), *trans*-permethrin (isomeric composition *cis:trans* 1:99), *cis*-permethrin (isomeric composition *cis:trans* 99:1) were provided by Chemotécnica SA (Argentina).

Deltamethrin 25 g litre^{-1} suspension concentrate (K-othrin 2.5%) was from AgrEvo (Germany) and ß-cypermethrin 50 g litre^{-1} suspension concentrate (Sipertrin 5%) was from Chemotécnica SA (Argentina).

2.2 Biological material

Triatoma infestans:. 14-day-old nymph V, from a susceptible strain held at CIPEIN since 1974 at 28–30 °C, 50–70% RH and photo-period 12:12 h, starved 7 days before bioassays. The nymphs weighed 120–160 mg.

Aedes aegypti and Culex quinquefasciatus:. 3- to 4-day-old adult insects from a susceptible strain held at CIPEIN since 1996 at 28–30 °C, 60–80% RH and photoperiod 12:12 h.

Periplaneta americana:. 4-week-old nymphs from a susceptible strain held at CIPEIN since 1978 at 24–26 °C, 50–70% RH and photoperiod 12:12 h.

2.3 High performance liquid chromatography (HPLC)

A Jasco Familic-300 S Chromatograph (Japan) with a Quiral Pirkle column 250 × 4.6 mm (Regis, USA) and UV detector (UVIDEC 100 VI, Japan) at 220 nm was used for optical isomer separations with 2-propanol + hexane (0.1 + 99.9 by volume) as elution solvent, at a constant flow of 0.8 ml min^{-1}. A Rheodyne injector (USA) was used.[12]

A reverse-phase column 250 × 1.5 mm filled with SIL 18T (Finepak, USA) was used for geometric isomer separations.

2.4 Permethrin recrystallization

A solid fraction of commercial permethrin (Section 2.1) was filtered under vacuum and washed with ethylene glycol cooled to 0 °C. The remaining solid was recrystallized three times from ethanol + water (97.5 + 2.5 by volume) and analysed by HPLC.

2.5 Preparation of the aqueous suspension concentrate

Recrystallized *cis*-permethrin (10 g) was mixed with Supragil WP (4 g; 50 g litre^{-1}; Rhone Poulenc, France), Soprophor S/40P (4 g; 50 g litre^{-1}; Rhone Poulenc, France), and ethylene glycol (12 ml; 160 g litre^{-1}; Argentine), and a synthetic silicone (0.6 ml; 8 g litre^{-1}; Argentine) in water (50 ml) was added. The mixture was left to stand for 24 h and milled in an Alumcraft Mill (MA-70, Argentine) provided with a refrigerated jacket at 2 °C. The mixture was milled for 12 min, and xanthan gum in water (15 g litre^{-1}; 50 ml) was added. The final concentration of *cis*-permethrin was quantified by HPLC and adjusted to 50 g litre^{-1}.

2.6 Particle size determination

The particle size of the formulation was determined from the image obtained by a Scanning Electron Microscope (Philips Model 2010) and a SOLVER module from Microsoft Excel 97 using a non-lineal optimisation code (GRG2).[13]

The image was processed with Image Pro Plus (version 1.1 for Windows) software and the data processed with Microcalc Origin 4.1 (USA).

2.7 Bioassays

Triatoma infestans, A aegypti, C quinquefasciatus and *P americana* were exposed on treated wood and ceramic surfaces.

Circles of 5 cm diameter (19.6 cm^2) and 9.7 cm diameter (74 cm^2) were marked on wood and ceramic, respectively, and treated with 1.5 ml of diluted formulation. The surfaces were then allowed to dry for 24 h. Groups of 10 insects were exposed during 60 min to the treated surfaces and retained by plastic vessels 8 cm in diameter and 4 cm high. At the end of the exposure time, each group of insect was transferred to a clean jar and kept at 24–25 °C and 50% R.H. Control insects were exposed to the surfaces without formulation treatment.

Mortality was assessed up to 72 h post-treatment and LC_{50} values were calculated with software based on Probit analysis.[14]

3 RESULTS AND DISCUSSION

3.1 Permethrin recrystallization

Since permethrin is somewhat soluble in polar solvents,[15] different alcohols such as methanol, ethanol, propan-1-ol and propan-2-ol, containing various amounts of water up to 100 ml litre^{-1} were tested for suitability for recrystallization (Table 1). Although propan-1-ol + water (90 + 10 by volume) gave very good yields of *cis*-permethrin (85.8%) there were no clear correlations between the alcohol and the water content. It can be seen that ethanol + water (97.5 +

Table 1. Percentage of *cis*-permethrin recrystallized from different alcohol-water mixtures[a]

	Water (%)				
Alcohol	*0*	*1*	*2.5*	*5*	*10*
Methanol	61.0	63.7	76.3	57.0	73.0
Ethanol	58.0	60.0	72.7	55.0	64.0
n-Propanol	60.5	68.6	64.0	69.5	53.0
Isopropanol	67.7	56.0	72.2	78.3	85.8

[a] Initial permethrin *cis/trans* ratio was 44.2/55.2.

2.5) gave 72.7% *cis*-permethrin and that with three successive recrystallizations yields over 99.5% of *cis*-permethrin were obtained. The use of standard medicinal alcohol (96%) and successive recrystallizations was suggested for providing good yields of pure *cis*-permethrin on a large scale. This method proved to be very promising, and is feasible for use on a preparative or industrial scale.[15]

The spontaneous crystallization of permethrin shows that the *cis*- and *trans*-isomers crystallize with different crystalline forms. Crystallographic studies showed that both isomers crystallize separately and, although some fusion of the crystals occurred during milling, this did not affect bioactivity (Table 2).

3.2 Aqueous suspension formulation of *cis*-permethrin

Because *cis*-permethrin is a crystalline solid, it can be formulated as an aqueous suspension concentrate. It is known that the shape and size of the particles in such a formulation are important for biological activity, especially on porous surfaces. In addition, the uniformity of particle size is very important for the stability of the suspension, to facilitate spraying and ensure the bioavailability of the pyrethroid.[16]

Particle size, quantified as a function of the milling time, was obtained by digitalising the image obtained by Scanning Electronic Microscopy (SEM) at different times between 1 and 17 min. The average size was found to be about 4 μm at 12 min. After this time the particle size was constant and the distribution sharp, as can be seen in Fig 1. Longer milling times did not modify significantly the distribution of particle size. A time of 12 min was taken as standard for the milling process.

In the milling process, crystalline *cis*-permethrin was mixed with the surfactants Supragil WP and Soprophor S/40P, an alkylnaphtalene sulphonate and an ethoxylated tristyrylphenol respectively, to facilitate the wetting of the pyrethroid, and with an antifreeze agent and a silicone oil as antifoam. When the milling process was complete, xanthan gum was added as thickening agent.

The formulation thus obtained was homogenous, with adequate viscosity and suspensibility, and showing good stability over time (patent pending).

The concentration of *cis*-permethrin in the final formulation was determined by HPLC as described in Section 2.3 and adjusted to 50 g litre^{-1}.

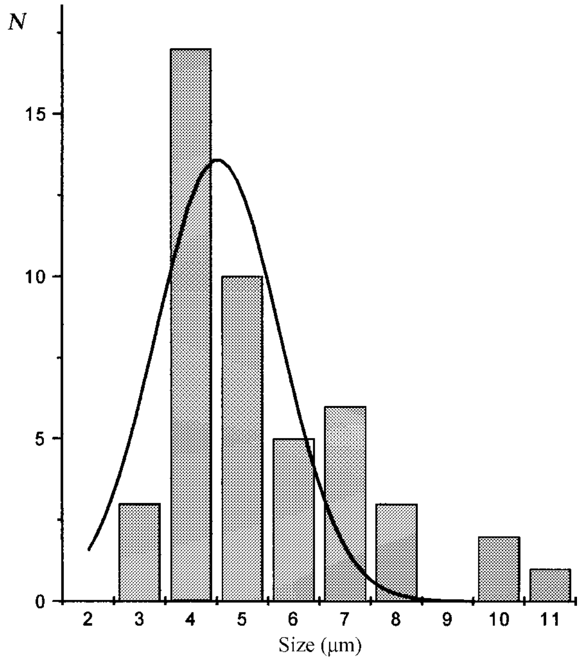

Figure 1. Particle size distribution for 12 min milling time.

3.3 Insecticidal activity

LC_{50} data for the *cis*-permethrin suspension concentrate formulation were obtained on nymph V of *T infestans* on wood and ceramic surfaces, taken as a model of porous surfaces, in comparison with a commercial suspension concentrate formulation of deltamethrin. As can be seen in Table 3, *cis*-permethrin was more effective than deltamethrin on both surfaces. The *cis*-isomer of permethrin was recently described as one of the most active pyrethroids against *T infestans*.[9,10] This high triatomicidal activity, not observed in *cis trans*-permethrin, could be explained by the less active and antagonistic *trans*-isomer masking the high toxicity of the *cis*-isomer to *T infestans*.[8,10]

However, LC_{50} values obtained for *P americana, A aegypti* and *C quinquefasciatus* on ceramic surfaces (Tables 4 and 5 respectively) show lower insecticidal activity than deltamethrin or β-cypermethrin (another cyano-pyrethroid). This result would be expected from the toxicity ratio between the *cis*-and *trans*-isomers of permethrin against different insect pests.[17] In such cases the higher toxicity of the *cis*-isomer is not enough to cause a similar difference of insecticidal effect between *cis*-permethrin and cyano-pyrethroids.

Table 2. Insecticidal activity of crystalline forms of *cis*-permethrin against *Triatoma infestans*

	LC_{50} ($\mu g\,cm^{-2}$)^a (95% CL)	
Surface	*Needles*	*Fused crystal*
Ceramic	0.11 (0.07–0.16)	0.14 (0.08–0.17)
Wood	0.57 (0.21–1.53)	0.57 (0.21–1.53)

^{a} Mean of at least two independent determinations.

Table 3. Insecticidal activity of *cis*-permethrin suspension concentrate against *Triatoma infestans* on different surfaces

	LC_{50} ($\mu g\,cm^{-2}$)^a (95% CL)	
Surface	*Deltamethrin*	*cis-Permethrin*
Ceramic	0.33 (0.15–0.71)	0.11 (0.07–0.16)
Wood	3.20 (1.40–7.57)	0.57 (0.21–1.53)

^{a} Mean of at least two independent determinations.

Table 4. Insecticidal activity of pyrethroids on ceramic surfaces against *Periplaneta americana*

Insecticide	LC_{50} ($\mu g\,cm^{-2}$)^a (95% CL)
Deltamethrin	2.06×10^{-3} (1.63–2.61)
cis-Permethrin	60.0×10^{-3} (53.6–67.3)
β-Cypermethrin	4.85×10^{-3} (4.14–5.70)

[a] Mean of at least two independent determinations.

Table 5. Insecticidal activity of pyrethroids on ceramic surfaces against *Aedes aegypti* and *Culex quinquefasciatus*

	LC_{50} ($\mu g\,cm^{-2}$)^a (95% CL)	
Insect	*β-Cypermethrin*	*cis-Permethrin*
A aegypti	2.29 (1.7–3.05)	11.5 (9.26–13.0)
C quinquefasciatus	1.00 (0.89–1.22)	14.08 (10.3–15.9)

[a] Mean of at least two independent determinations.

4 CONCLUSIONS

Until the late 1980s the most common formulations for Chagas' disease vector control campaigns were WP and EC formulations. Suspension concentrate (flowable) formulations have been used recently because of their toxicological and practical advantages.[18] Nowadays, other third-generation pyrethroids such as β-cypermethrin[7] (recently incorporated in National campaigns), β-cyfluthrin and λ-cyhalothrin, besides deltamethrin, all of them constituting the more active isomers and used as SC formulations, are safer products, less toxic and more environmentally friendly.[18,19] The high insecticidal activity of *cis*-permethrin[9,10] and its low cost relative to other pyrethroids currently used for triatomid control, besides the lower allergenic potential of the suspension concentrate formulation, gives a hope for future widespread use of the latter in Latin American campaigns against Chagas' disease vectors.

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