

Resistance to Insecticides and Effect of Synergists on Permethrin Toxicity in *Pediculus capitis* (Anoplura: Pediculidae) from Buenos Aires

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ABSTRACT Permethrin-resistant colonies of *Pediculus capitis* (De Geer) from Buenos Aires were used to establish a resistance profile and to examine resistance mechanisms. All permethrin-resistant head lice (resistance ratio from 52.8 to >88.7) were also resistant to d-phenothrin (resistance ratio from 40.86 to >48.39) and deltamethrin (resistance ratio from 16.24 to 38.06). No cross-resistance to carbaryl was found in any of the pyrethroid-resistant *P. capitis* tested. Otherwise, all resistant colonies showed low to high levels of resistance to β -cypermethrin. This pyrethroid had never been applied as a pediculicide in Argentina; however, the high level of resistance found in these permethrin-resistant colonies (resistance ratio from 9.74 to 50.97) demonstrated that pyrethroid cross-resistance occurred to this novel insecticide. Treatment with piperonyl butoxide (PBO) or triphenylphosphate (TPP) significantly decreased the toxicity of permethrin in the four colonies tested. The esterase inhibitor TPP produced lower enhancement of toxicity than the multifunction oxidase inhibitor PBO in the colonies having the highest resistance levels. Results presented here concerning the cross-resistance profile and synergism by enzyme inhibitors in permethrin-resistant head lice demonstrated that enhanced metabolism was involved in the pyrethroid resistance. However, the substantial degree of resistance that remained after synergism suggested the presence of another resistance mechanism. Cross-resistance to pyrethroid and susceptibility to the carbamate carbaryl suggested a common action mechanism.

KEY WORDS *Pediculus capitis*, insecticide resistance, cross-resistance, synergism

PERMETHRIN HAS BEEN registered for the chemical control of *Pediculus capitis* (De Geer) since the 1970s. Its use has been steadily increasing as concern has grown relating to the relatively few compounds that are effective against lice and have low mammalian toxicity (Gratz 1977). The continued use of permethrin is threatened by pyrethroid resistance.

Permethrin resistance in head lice has been reported from the Czech Republic (Rupes et al. 1995), France (Coz et al. 1993), Israel (Mumcuoglu et al. 1995), and Argentina (Picollo et al. 1998). Most of permethrin-resistant head lice showed resistance to other pyrethroid insecticides. The resistance to permethrin in head lice from Czechoslovakia was accompanied by cross-resistance to d-phenothrin and bioallethrin, and the lice from France were also resistant to d-phenothrin. In our previous study (Picollo et al. 1998), we reported that all field-collected lice that were resistant to permethrin also showed resistance to d-phenothrin and deltamethrin.

The development and spread of pyrethroid resistance in head lice occurred relatively soon after their

introduction. The lack of safe and effective alternative compounds suggests an urgent need for the development of a resistance management strategy. Basic assumptions of this management plan include the absence of cross-resistance and lack of similarity in biochemical mechanisms in head lice. In addition, the use of synergists for the inhibition of detoxifying enzymes represent not only an alternative to improve control, but a tool for elucidating resistance mechanisms.

The purpose of this study was to establish the resistance profile and evaluate resistance mechanisms in field collected head lice. Piperonyl butoxide (PBO), which inhibits mixed-function oxidases (Farnham 1998), and triphenyl phosphate (TPP), which inhibits carboxyesterases (Plapp et al. 1963), were used to determine if metabolic resistance to pyrethroid insecticides was present in head lice resistant colonies established in Buenos Aires.

Materials and Methods

Lice. Lice were collected from heads of infested children (6-12 yr old) from selected elementary schools in several areas of Buenos Aires. Head lice were collected and transported to our laboratory as

The protocol of lice collection was approved by the ad-hoc committee of the Research Centre on Pests and Pesticides and is on file in our laboratory.

Table 1. Name and location of field collections of head lice colonies in Buenos Aires

Colony	Code	Location
Marta ^a	MAR	San Fernando (province)
Luján Porteño	LP	Flores (city)
Guardia de Honor	GH	Flores (city)
Villa Maipú	VM	Villa Maipú (province)
Com. Cristiana	FER	San Fernando (province)

^a Susceptible lice colony.

previously reported by Picollo et al. (1998) and Picollo (1999). Adults and third-instar nymphs were selected for use in bioassays because no differences in susceptibility had been reported by Mumcuoglu et al. (1990).

Groups of head lice were collected from children from different neighborhoods and named accordingly (Table 1). The Marta (MAR) reference colony was collected from the heads of four nontreated children from the same family.

Chemicals. Technical grade permethrin (42.5% *cis* and 54.2% *trans*, Chemotecnica Buenos Aires, Argentina); deltamethrin (97% [AI]), AgrEvo, Buenos Aires, Argentina); d-phenothrin (94.4% [AI], Sumitomo Chemical, Osaka, Japan); carbaryl (98% [AI], Rhone Poulenc, Lyon, France); and dioctyl phthalate (98%, Aldrich, Milwaukee, WI) were used.

The synergists tested were technical grade piperonyl butoxide (PBO) (92.3% [AI], Chemotecnica Buenos Aires, Argentina) and triphenyl phosphate (TPP) (99% [AI], Janssen Chimica, Belgium).

Bioassay. Insecticide activity was evaluated by confining the lice on insecticide-impregnated filter paper, according to the method described by Maunder (1971) and Blommers and van Lennep (1978) and recommended by the World Health Organization (1981).

Stock solutions of each insecticide in dioctyl phthalate were prepared from the technical grade products. One milliliter of stock solution was diluted in 2 ml chloroform to obtain the desired concentration. Then 0.4 ml of each concentration of the final solution was spread over a Whatman No. 1 filter paper disk (7 cm diameter) and dried for 24 h. Final concentrations of insecticides on the filter paper were 0.2–90% for permethrin and d-phenothrin and 0.2–16% for deltamethrin, β -cypermethrin, and carbaryl. Final concentrations were expressed as percentage of active ingredient in stock solution. Concentrations >90% could not be tested for methodological reasons.

Synergist evaluations were done by preparing stock solutions of permethrin: synergist (1:5) in dioctyl phthalate, and the final dilution with chloroform. Control papers were prepared with the different concentrations of each synergist in dioctyl phthalate. To overcome the effect of the oily compounds in permethrin-synergist impregnated papers, another control paper was prepared using dioctyl phthalate in place of those compounds.

Batches of at least 10 lice were confined for 1 h on each impregnated paper in glass rings (4 cm diameter). At the end of the exposure period, the treated

Table 2. Susceptibility values (LC₅₀) and resistance ratios to permethrin in head lice colonies

Colony	n	Slope \pm SE	%LC ₅₀ (95% CL)	RR (95% CL) ^a
Marta ^b	240	1.13 \pm 0.13	1.02 (0.66–1.50)	—
Luján Porteño	110	—	>90.0	>88.70
Guardia de Honor	200	—	>90.0	>88.70
Villa Maipú	80	—	>90.0	>88.70
Com. Cristiana	130	1.39 \pm 0.33	53.62 (34.44–134.75)	52.80 (25.76–108.54)

Adults and large nymphs were exposed during 1 h to treated filter papers. Mortality was recorded after 18 h. Values are expressed as percentage of active ingredient in stock solution.

^a \pm 95% CI calculated by method of Robertson and Preisler (1992).

^b Susceptible lice colony.

insects were released onto a piece of untreated Whatman No. 1 filter paper (7 cm diameter) that was placed in the bottom of a plastic petri dish, and 0.1 ml water was placed on the filter paper to provide moisture for the insects. The experimental units were placed in a Lab-Line environmental chamber at 18.0 \pm 0.5°C and 70–80% RH in the dark. Untreated insects were placed on Whatman No. 1 filter paper (7 cm diameter) that contained 0.4 ml of a dioctyl phthalate chloroform solution (1:3) or 0.4 ml of synergist plus dioctyl phthalate chloroform solution. Dead and live lice were recorded after 18 h when control mortality was <5%. The criterion for mortality was inability of lice to walk over the filter paper. In all experiments, at least three replicates of each concentration were used, and data were corrected using the Abbott formula.

Statistical Analysis. Mortality data for each insecticide concentration and each insecticide and synergist concentration were used to determine the LC₅₀ using probit analysis (Litchfield and Wilcoxon 1949). The LC₅₀ was expressed as the percentage of insecticide contained in the stock solution. The resistance ratio was calculated as the ratio between LC₅₀ of the lice collected from different areas and LC₅₀ of the reference colony, and the confidence interval was calculated by the method of Robertson and Preisler (1992).

Results

Insecticide Resistance. The susceptibility to the major insecticides used in commercial products was determined for each of the head lice colonies that were from Buenos Aires. Susceptibility values (LC₅₀) to permethrin and resistance ratios compared with the reference strain are shown in Table 2. All *P. capitatus* populations, except the reference colony, showed high levels of resistance to permethrin (resistance ratio from 52.8 to >88.7). The exposition of resistant head lice to the maximum permethrin concentration (90%), generated 5% average mortality in lice from Luján Porteño, 10% from Guardia de Honor, and 13.3% from Villa Maipú.

Results of comparative tests on lice colonies against d-phenothrin and deltamethrin are shown in Table 3 and 4. All permethrin-resistant colonies also showed

Table 3. Susceptibility values (LC₅₀) and resistance ratios to d-phenothrin in head lice colonies

Colony	n	Slope ± SE	%LC ₅₀ (95% CL)	RR (95% CL) ^a
Marta ^b	120	1.57 ± 0.32	1.86 (1.27–2.75)	—
Luján Porteño	100	—	>90.0	>48.39
Guardia de Honor	148	1.54 ± 0.34	76.2 (48.98–181.12)	40.86 (21.07–79.28)
Villa Maipú	100	—	>90.0	>48.39
Com. Cristiana	90	—	>90.0	>48.39

Adults and large nymphs were exposed during 1 h to treated filter papers. Mortality was recorded after 18 h. Values are expressed as percentage of active ingredient in stock solution.

^a ± 95% CI calculated by method of Robertson and Preisler (1992).

^b Susceptible lice colony.

resistance to d-phenothrin and deltamethrin. The highest resistance to d-phenothrin was found in Luján Porteño, Villa Maipú, and Com. Cristiana. In the three colonies, mortality <50% was registered for the head lice exposed to filter paper impregnated with the maximum d-phenothrin concentration (90%). The estimated LC₅₀s for deltamethrin ranged from 0.2 to 6.25% (Table 4). These estimates were at least an order of magnitude lower than those found for permethrin and d-phenothrin. Resistance ratios for deltamethrin were also lower, ranging from 16.24 to 38.06.

Cross-resistance. No resistance to carbaryl was found in any of the *P. capitis* populations tested (Table 5). No significant difference existed in the LC₅₀ of carbaryl between reference and permethrin-resistant colonies.

Results of susceptibility tests of *P. capitis* from Buenos Aires to β-cypermethrin are shown in Table 6. All resistant populations showed low to high levels of resistance to β-cypermethrin. The LC₅₀ ranged from 0.33 to 16.9%. This pyrethroid insecticide had never been applied as a pediculicide in Argentina; thus, the resistance levels obtained for *P. capitis* from Buenos Aires (9.74–50.97), could be attributed to cross-resistance with other pyrethroid insecticides.

Table 4. Susceptibility values (LC₅₀) and resistance ratios to deltamethrin in head lice colonies

Colony	n	Slope ± SE	%LC ₅₀ (95% CL)	RR (95% CL) ^a
Marta ^b	90	1.92 ± 0.92	0.20 (0.14–0.30)	—
Luján Porteño	160	2.02 ± 0.28	5.43 (2.67–13.36)	33.07 (11.15–98.13)
Guardia de Honor	120	2.46 ± 0.42	2.67 (1.89–3.46)	16.24 (5.47–48.20)
Villa Maipú	160	1.75 ± 0.28	6.25 (4.68–8.57)	38.06 (12.83–112.96)
Com. Cristiana	100	3.31 ± 0.51	3.37 (2.69–4.21)	20.17 (6.84–59.51)

Adults and large nymphs were exposed during 1 h to treated filter papers. Mortality was recorded after 18 h. Values are expressed as percentage of active ingredient in stock solution.

^a ± 95% CI calculated by method of Robertson and Preisler (1992).

^b Susceptible lice colony.

Table 5. Cross resistance to carbaryl in pyrethroid-resistant colonies

Colony	n	Slope ± SE	%LC ₅₀ (95% CL)	RR (95% CL) ^a
Marta ^b	120	2.82 ± 0.68	3.58 (2.68–4.82)	—
Luján Porteño	200	2.23 ± 0.27	5.95 (3.75–8.92)	1.66 (1.16–2.38)
Guardia de Honor	270	2.18 ± 0.22	4.81 (3.17–7.09)	1.34 (0.96–1.88)
Villa Maipú	140	1.57 ± 0.27	6.52 (5.21–8.17)	1.68 (1.10–2.59)
Com. Cristiana	120	1.69 ± 0.28	6.11 (3.54–9.07)	1.79 (1.12–2.87)

Adults and large nymphs were exposed during 1 h to treated filter papers. Mortality was recorded after 18 h. Values are expressed as percentage of active ingredient in stock solution.

^a ± 95% CI calculated by method of Robertson and Preisler (1992).

^b Susceptible lice colony.

Effect of Synergists. The estimated LC₅₀ for *P. capitis* from Buenos Aires exposed to permethrin alone or with synergists (PBO or TPP), are shown in Fig 1. No significant differences were found in the estimated LC₅₀ for the reference colony in permethrin-PBO or permethrin-TPP treatments. The multifunction oxidase inhibitor PBO produced greater enhancement of the toxicity of permethrin in all resistant colonies than the esterase inhibitor TPP.

The combined permethrin-PBO treatment produced partial reversion in resistance populations, because the LC₅₀ decreased from >90% to lower values in Luján Porteño, Guardia de Honor, and Villa Maipú.

The combined permethrin-TPP treatment also produced lower LC₅₀ in these populations. In the Com. Cristiana colony, both synergists caused similar decreases in LC₅₀ values.

Discussion

Permethrin resistance in *P. capitis* from Buenos Aires was previously reported (Picollo et al. 1998). The four highly resistant colonies studied here showed

Table 6. Cross resistance to β-cypermethrin in pyrethroid resistant colonies

Colony	n	Slope ± SE	%LC ₅₀ (95% CL)	RR (95% CL) ^a
Marta ^b	140	2.29 ± 0.33	0.33 (0.25–0.45)	—
Luján Porteño	120	1.26 ± 0.26	16.96 (10.81–29.06)	50.97 (29.64–87.64)
Guardia de Honor	190	3.35 ± 0.43	6.31 (3.97–9.69)	18.97 (13.58–26.49)
Villa Maipú	80	1.73 ± 0.33	6.69 (3.85–10.53)	20.09 (11.61–34.76)
Com. Cristiana	110	1.43 ± 0.29	3.25 (1.89–4.94)	9.75 (5.77–16.48)

Adults and large nymphs were exposed during 1 h to treated filter papers. Mortality was recorded after 18 h. Values are expressed as percentage of active ingredient in stock solution.

^a Confidence interval (± 95% CI) calculated by method of Robertson and Preisler (1992).

^b Susceptible lice colony.

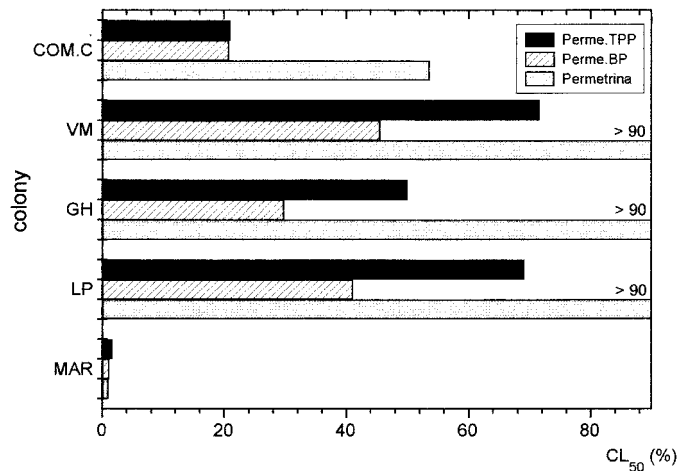


Fig. 1. Susceptibility of pyrethroid-resistant head lice exposed to permethrin alone or in the presence of synergist.

a high level of resistance to d-phenothrin, deltamethrin and β -cypermethrin. Because permethrin is present in 66.7% of pediculicide products in Argentina, in contrast with d-phenothrin (4.8%) and deltamethrin (19%), the high pyrethroid resistance found in head lice from Buenos Aires could have been caused by the intensive selection made by permethrin. Moreover, because β -cypermethrin had never been used as a pediculicide in Argentina, the high level of resistance found in the permethrin-resistance colonies demonstrates that pyrethroid cross-resistant existed.

Resistance to several pyrethroids had been reported by Rupes et al. (1995) in head lice populations from the Czech Republic. These authors reported permethrin-resistant *P. capitis* existed and that pyrethroid cross-resistance extended to include bioallethrin and d-phenothrin.

Resistance to permethrin and d-phenothrin was reported by Coz et al. (1993) for *P. capitis* from Tours, France. All the pyrethroid-resistant colonies studied here were susceptible to the insecticide carbaryl. The lack of pyrethroid cross-resistance to carbamates had been reported in other species. Priester and Georghiou (1980) found that *Culex quinquefasciatus* (Say) selected with permethrin was cross-resistant to all pyrethroids tested, but not significantly cross-resistant to the carbamate propoxur. Montagna et al. (2000) reported resistance to pyrethroids and DDT in field populations of Argentinean black flies, *Simulium bonaerense* (Coscaron & Wygodzinsky) and *S. walffhuegeli* (Enderlein), where no evidence of resistance to carbaryl had been detected.

Results of PBO and TPP applications to permethrin-resistant head lice suggested that monooxygenases (cytochrome P-450s) and esterase enzyme systems were responsible for some of the pyrethroid resistance in the colony tested. The esterase inhibitor TPP produced lower enhancement of the permethrin toxicity than the multifunction oxidase inhibitor PBO in the colonies having the highest levels of resistance (re-

sistance ratio >88.7). This was in agreement with the results of Priester and Georghiou (1980), who showed that esterases are less involved in pyrethroid degradation in insects than monooxygenases. Moreover, Hemingway et al. (1999) reported equivalent esterase activity levels in susceptible and pyrethroid-resistant Israeli *P. capitis*, but they found a weak monooxygenase-based permethrin metabolism resistance mechanism. However, the lack of synergism of d-phenothrin resistance by piperonyl butoxide suggests that a nonoxidative mechanism, such as nerve insensitivity, is also present in the resistant lice.

Pyrethroid resistance has been attributed to reduced neural sensitivity, enhanced metabolism, and reduced penetration ratio in many insects (Oppe-noorth 1985, Zerba et al. 1987). The data presented here concerning the cross-resistance profile and synergism by enzyme inhibitors, when permethrin resistance head lice are concerned, demonstrated that enhanced metabolism was involved in pyrethroid resistance. However, the substantial degree of resistance remaining after synergism suggested the presence of other resistance mechanisms. Cross-resistance to pyrethroids and the susceptibility to carbaryl suggested that a common site of pyrethroid action existed. Further investigations in the physiological mechanisms are necessary to develop a proper resistance management strategy in *P. capitis* from Buenos Aires.

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