Increased Monooxygenase Activity Associated with Resistance to Permethrin in *Pediculus humanus capitis* (Anoplura: Pediculidae) from Argentina

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ABSTRACT We studied the profile of permethrin resistance in populations of head lice infesting children 6–12 yr old in schools and their homes in and around Buenos Aires, Argentina. Five permethrin-resistant populations with different levels of resistance were collected: Hogar Loyola (HL), Republica de Turquia (RT), Hogar Mitre (HM), Guardia de Honor (GH), and Ricardo Guiraldes (RG). One susceptible population, Bandera Argentina (BA), also was collected. Their level of resistance was evaluated, and results showed resistance ratios of 13 for HL, 16 for RT, 22 for HM, 61 for GH, and 69 for RG. To elucidate the possible involvement of the cytochrome P450 monooxygenase system in conferring permethrin resistance, ethoxycoumarin-*O*-deethylase (ECOD) activity was measured in abdomens of individual third instars and adults by using a fluorometric assay. The ECOD activity was lower in the susceptible BA population (4.7 ng per louse) than in the resistant ones (13.7 ng per louse for RG, 12.3 ng per louse for GH, 8.6 ng per louse for RT, and 8.2 ng per louse for HL). ECOD activity was significantly correlated with the level of resistance in the field populations ($r = 0.97$, $P = 0.0009$), suggesting a role for cytochrome monooxygenase P450 system in permethrin resistance by head louse, *Pediculus humanus capitis* De Geer.

KEY WORDS *Pediculus humanus capitis*, monooxygenase, P450, permethrin resistance

IN ARGENTINA, FIELD POPULATIONS of the head louse, *Pediculus humanus capitis* De Geer (Anoplura: Pediculidae), have developed resistance to permethrin and other pyrethroids after intensive use of these insecticides since 1990 (Picollo et al. 1998). Recently, an extensive survey for resistance in Buenos Aires, Argentina, showed significant resistance levels in lice on children in 24 of 26 (92.3%) schools. Compared with a previously unexposed reference population, resistance ratios (RRs) to permethrin obtained by the filter paper exposure method on highly resistant populations ranged from 5.4 to >88.7 (Vassena et al. 2003). Similarly, Hemingway et al. (1999) found a high resistance level to permethrin (38.7-fold) in *P. humanus capitis* from Israel, and Pollack et al. (1999) reported that the RR for field populations from United States was 68 times higher than that obtained in Panama. Moreover, a high RR to malathion (400 times) was measured for head lice from the United Kingdom (Burgess 1995). RRs to phenothrin ranging from 20- to 160-fold were recorded for head lice from Japan (Kasai et al. 2003). Emergence of resistance to carbaryl was found in England (Downs et al. 2002).

Recent studies on permethrin-resistant head lice were focused on the possible resistance mechanisms (Bartels et al. 2001). Hemingway et al. (1999) reported high glutathione *S*-transferase and monooxygenase activities in head lice from Israel with resistance to DDT and permethrin. Lee et al. (2000) and Tomita et al. (2003) reported a molecular analysis of kdr-like resistance in permethrin-resistant populations of head lice from the United States and United Kingdom. In that work, through molecular cloning and sequencing, the authors identified two point mutations associated with permethrin resistance in the head lice. Picollo et al. (2000) found that pretreatment with piperonyl butoxide or triphenylphosphate significantly increased the toxicity of permethrin in pyrethroid-resistant head lice from Argentina and demonstrated that enhanced metabolism was involved in resistance.

The aim of this study was to examine the possible involvement of cytochrome P450 monooxygenases in conferring permethrin resistance in head lice.

Materials and Methods

Lice. Head lice were collected in 2002 from infested children at randomly selected schools from each city district in and around Buenos Aires, where permethrin based pediculicides have been intensively used since 1990. Live head lice were obtained using a fine-

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toothed antilouse comb (Nopucid, Interbelle Cosmetics, Buenos Aires, Argentina) from 6,250 children aged from 6 to 12 yr, according to a protocol approved by the ad hoc Committee of Centro de Investigaciones de Plagas e Insecticidas and archived in our laboratory. Lice were grouped and named in separate populations according to the school in which they had been collected according to our previous work (Picollo et al. 1998, 2000; Vassena et al. 2003). Five permethrin-resistant populations with different levels of resistance were collected: Hogar Loyola (HL), Republica de Turquia (RT), Hogar Mitre (HM), Guardia de Honor (GH), and Ricardo Guiraldes (RG); one susceptible population, Bandera Argentina (BA), was collected. Adults and third instars were selected at the laboratory for the bioassays (Mumcuoglu et al. 1995, Picollo et al. 2000, Vassena et al. 2003). After collection, lice were maintained without feeding in an environmental chamber (Lab-Line Instruments, Melrose Park, IL) at 18 ± 0.5°C and 70–80% RH in the dark for a maximum of 1 h before toxicological bioassays and for 15 h before biochemical assays.

Resistant populations were collected from children who had been previously exposed to insecticide treatments. These populations were named according to the district in which they were collected: GH, RT, RG, HL, and HM.

Chemicals. Technical grade permethrin (42.5% *cis* and 54.2% *trans*) was donated by from Chemotecnica (Buenos Aires, Argentina). 7-Ethoxycoumarin (7-EC) and 7-hydroxycoumarin (7-OHC) were purchased from Sigma (St. Louis, MO).

Bioassay. Serial dilutions of permethrin in acetone were prepared and applied with a 5-μl Hamilton syringe with a repeating dispenser. Each head louse was treated with 0.1 μl of the solution on the dorsal abdomen according to Vassena et al. (2003). The final dose ranged from 0.03 to 3000 μg per louse. Each treatment concentration was replicated three times by using 10 lice per replicate. Control lice were treated with acetone alone. Treated lice were placed into a petri dish over a 9-cm Whatman no. 1 filter disc moistened with 0.5 ml of water and maintained in an environmental chamber (Lab-Line Instruments) in the dark at 18 ± 0.5°C and 70–80% RH. Mortality was recorded at 18 h after treatment (Picollo et al. 1998). The criterion for mortality was inability of lice to walk from the center to the border of a 7-cm filter paper disc.

Monooxygenase Activity. Cytochrome P450 monooxygenase activity was measured using ethoxy-coumarin as substrate (ECOD activity) according to the method of Ullrich and Weber (1972) adapted for in vitro analysis by De Souza et al. (1995). Only abdomens were used, and they were dissected according to De Souza et al. (1995) and González Audino et al. (2004).

For measurements in intact tissues, the assay mixture (0.1 ml) contained one louse abdomen per well in 0.4 mM 7-EC, 0.05 M phosphate buffer (pH 7.2). Microplates were centrifuged for 30 s at 600 × *g* in a microplate centrifuge and incubated at 30°C for 4 h. The reaction was stopped with 0.1 ml of a mixture of glycine buffer (10^{-4} M, pH 10.4) and ethanol in a 50:50 ratio (vol:vol). Microplates were then centrifuged before measuring fluorescence. Fluorescence was measured using a microplate fluorescence reader (Packard Fluorocount), with 400-nm excitation and 440-nm emission filters. Enzyme activity was expressed as nanograms of ECOD produced by one abdomen after 4 h of incubation.

Statistical Analysis. Mortality data were collected using Abbott's formula (Abbott 1925). Dose–mortality data from each head louse population was subjected to probit analysis (Litchfield and Wilcoxon 1949). LD_{50} values were expressed as micrograms of permethrin per louse. After probit analysis, RRs with 95% CL were calculated for each head lice population (LD_{50}) by comparing results from resistant lice with corresponding results from the reference, nontreated population (BA) as described by Robertson and Preisler (1992). ECOD activities of louse populations were statistically analyzed using the Kruskal–Wallis/Mann–Whitney method.

Table 1. RRs to permethrin in *P. humanus capitis* (adults and third instars) from Buenos Aires

Pop	*n*	Slope ± SE	LD_{50} (μg/louse) (95% CL)	RR (95% CL)
BA[a]	240	1.45 ± 0.2	0.010 (0.003–0.023)	
HL	120	2.11 ± 0.32	0.130 (0.079–0.311)	13.36 (7.79–22.90)
RT	180	1.62 ± 0.23	0.160 (0.070–0.291)	16.13 (9.36–27.78)
HM	180	3.93 ± 0.61	0.220 (0.180–0.260)	21.93 (13.77–34.91)
GH	140	1.53 ± 0.21	0.600 (0.291–1.163)	60.95 (35.67–104.14)
RG	90	1.26 ± 0.17	0.670 (0.198–1.793)	68.60 (36.21–129.96)

[a] Reference field population.

Results

Results of toxicity tests of permethrin in field populations of head lice from Buenos Aires are summarized in Table 1. LD_{50} values are expressed as micrograms of permethrin per louse. The RR to permethrin for the five resistant populations compared with the reference population (Bandera, Argentina) ranged from 13.36 to 68.60.

The ECOD activities in the five highly resistant populations, and also in the reference population, are shown in Table 2. Mean values of deethylation of

Table 2. Levels of ECOD activity in isolated abdomens from field populations of permethrin-resistant head lice from Buenos Aires

Pop	ECOD activity (ng ECOD/louse ± SEM)	*n*
BA	4.73 ± 0.39a	74
HL	8.21 ± 0.83b	39
RT	8.57 ± 0.87b	50
HM	11.97 ± 1.53b,c	22
GH	12.3 ± 1.85b,c	62
RG	13.7 ± 1.71c	18

Values that do not share any letter are significantly different ($P < 0.05$; Kruskal–Wallis/Mann–Whitney).

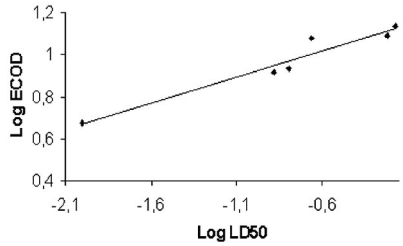

Fig. 1. Correlation between the LD_{50} to permethrin and P450 activity in field populations of *P. humanus capitis* from Argentina ($y = 0.2491x + 1.1685$, $r = 0.97$, $P < 0.001$).

7-ethoxycoumarin in louse abdomens were higher in the resistant lice. The ECOD activities were more homogeneous in the reference population than in the field populations.

The ECOD activities of louse abdomens increased with increasing resistance to permethrin in head louse populations. The ranking of enhancement of monooxygenase activity was the same as that of increasing resistance (Fig. 1). A positive linear correlation between RR and monooxygenase activity was demonstrated for the five permethrin-resistant populations ($r = 0.97$, $P = 0.0009$).

Discussion

Enhanced metabolism by oxidative enzymes is known to be a major cause of pyrethroid resistance in insects (Oppenorth 1985, Zerba et al. 1987). For head lice, we demonstrated using toxicological bioassays the importance of enhanced metabolism by monooxygenases in the pyrethroid resistance of field populations. In that work, we found that the treatment of resistant head lice with piperonyl butoxide (PBO), significantly increased the toxicity of permethrin in the four colonies tested, suggesting that this enzyme system was responsible for some of pyrethroid resistance (Picollo et al. 2000).

In this work, the activity of the monooxygenases was directly measured using ETOC as substrate on individual lice from populations exhibiting different levels of resistance to permethrin (ranging from 13.36 to 68.60). A positive correlation was established between enzyme activity and the LD_{50} to permethrin in the six field populations evaluated. Direct comparison of the ability of these populations to oxidize permethrin provides less disputable evidence in correlating pyrethroid resistance and microsomal oxidative detoxication.

Similar results were found by Hung and Sun (1989) in strains of the diamondback moth, *Plutella xylostella* (L.) exhibiting different levels of fenvalerate resistance. ECOD activity of larval homogenates showed a significant increase (from 0.62 to 2.58 nmol/min/mg protein) with increasing resistance (from 284 to >11,000) in diamondback moth strains, associating microsomal oxidation with high levels of resistance. Additionally, the role of enhanced detoxication by the cytochrome P450 monooxygenase system in a deltamethrin-resistant population of *Triatoma infestans* (Klug, 1834) from northern Argentina (RR = 7.89), was demonstrated using ECOD as substrate (González Audino et al. 2004). The activity of first instars was significantly lower in the susceptible colony (61.3 pg of ECOD per louse) than in the resistant colony (108.1 pg of ECOD per louse). Further studies on individual abdomens of field populations of *T. infestans* from northern Argentina with high resistance to pyrethroid insecticides (RR = 133.1) demonstrated a higher percentage of individuals with increased ECOD activity in the resistant population (0.56–0.64 pmol/min) compared with those of susceptible strain (0.24–0.32 pmol/min) (Picollo et al. 2005). Also, Berrada et al. (1994) demonstrated an increase in the deethylase activities by using ETOC as substrate, in a population of the psyllid *Cacopsylla pyri* (L.) with resistance to the organophosphorus insecticide monocrotophos.

In this context, the measurement of monooxygenase activity toward ETOC by using a microfluorometric technique can be useful as a diagnosis for resistance in head lice.

The high sensitivity of the technique allows the quantification of ECOD activity on individual head lice and offers the ability to identify and monitor resistant genotypes at low frequencies, an essential attribute for developing resistance management strategies and improving pesticide recommendations.

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References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Bartels, C. L., K. E. Peterson, and K. L. Taylor. 2001. Head lice resistance: itching that just won't stop. Ann. Pharmacother. 35: 109–112.

Berrada, S., D. Fournier, A. Cuany, and T. X. Nguyen. 1994. Identification of resistance mechanisms in a selected laboratory strain of *Cacopsylla pyri* (Homoptera: Psyllidae): altered acetylcholinesterase and detoxifying oxidases. Pestic. Biochem. Physiol. 48: 41–47.

Burgess, I. 1995. *Pediculus humanus capitis* in schoolchildren. Lancet 345: 730–731.

De Souza, G., A. Cuany, A. Brun, M. Amichot, R. Rahmani, and J. B. Berge. 1995. A microfluorometric method for measuring ethoxycoumarin-O-deethylase activity on individual *Drosophila melanogaster* abdomens: interest for screening resistance in insect populations. Anal. Biochem. 229: 86–91.

Downs, A. M., K. A. Stafford, L. P. Hunt, J. C. Ravenscroft, and G. C. Coles. 2002. Widespread insecticide resistance in head lice to the over-the-counter pediculicides in England, and the emergence of carbaryl resistance. Br. J. Dermatol. 146: 88–93.

González Audino, P., C. Vassena, S. Barrios, E. Zerba, and M. I. Picollo. 2004. Role of enhanced detoxication in a deltamethrin-resistant population of *Triatoma infestans* (Hemiptera, Reduviidae) from Argentina. Mem. Inst. Oswaldo Cruz 99: 335–339.

Hemingway, J., J. Miller, and K. Y. Mumcuoglu. 1999. Pyrethroid resistance mechanism in the head louse *Pediculus capitis* from Israel: implications for control. Med. Vet. Entomol. 13: 89–96.

Hung, C., and C. Sun. 1989. Microsomal monooxygenases in diamond back moth larvae resistant to fenvalerate and piperonyl butoxide. Pestic. Biochem. Physiol. 33: 168–175.

Kasai, S., M. Mihara, M. Takahashi, N. Agui, and T. Tomita. 2003. Rapid evaluation of human lice susceptibility to phenothrin. Med. Entomol. Zool. 54: 31–36.

Lee, S. H., K. Yoon, M. S. Williamson, S. J. Goodson, M. Takano-Lee, J. D. Edman, A. L. Devonshire, and J. M. Clark. 2000. Molecular analysis of *kdr*-like resistance in permethrin-resistant strain of head lice, *Pediculus capitis.* Pestic. Biochem. Physiol. 66: 130–143.

Litchfield, J. T., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Exp. Ther. 96: 99–110.

Mumcuoglu, K. Y., J. Hemingway, J. Miller, I. Ioffe-Uspensky, S. Klaus, F. Ben-Ishai, and R. Galun. 1995. Permethrin resistance in the head louse *Pediculus capitis* from Israel. Med. Vet. Entomol. 9: 427–432.

Oppenorth, J. F. 1985. Biochemistry and genetics of insecticide resistance, pp. 731–773. *In* G. A. Kerkut and G. I. Gilbert [eds.], Comprehensive insect physiology, biochemistry and pharmacology, vol. 12: Insect control. Pergamon, Oxford, United Kingdom.

Picollo, M. I., C. Vassena, A. Casadío, J. Mássimo, and E. N. Zerba. 1998. Laboratory studies of susceptibility and resistance to insecticides in *Pediculus capitis* (Anoplura: Pediculidae). J. Med. Entomol. 35: 814–817.

Picollo, M. I., C. Vassena, G. Mougabure Cueto, M. Vernetti, and E. Zerba. 2000. Resistance to insecticides and effect of synergists on permethrin toxicity in *Pediculus capitis* (Anoplura: Pediculidae) from Buenos Aires. J. Med. Entomol. 37: 721–725.

Picollo, M. I., C. Vassena, P. Santo Orihuela, S. Barrios, M. Zaidenberg, and E. Zerba. 2005. High resistance to pyrethroid insecticides associated to the ineffectiveness of field treatments in *T. infestans* (Hemiptera, Reduviidae) from the north of Argentina. J. Med. Entomol. (in press).

Pollack, R. J., A. Kiszewski, P. Armstrong, C. Hahn, N. Wolfe, H. Rahman, K. Laserson, S. Telford, and A. Spielman. 1999. Differential permethrin susceptibility of head lice sampled in the United States and Borneo. Arch. Pediatr. Adolesc. Med. 153: 969–973.

Robertson, J. L., and H. K. Preisler. 1992. Pesticide bioassays with arthropods. CRC, Boca Raton, FL.

Tomita, T., N. Yaguchi, M. Mihara, M. Takahashi, N. Agui, and S. Kasai. 2003. Molecular análisis of a para sodium channel gene from pyrethroid-resistant head lice, *Pediculus humanus capitis* (Anoplura: Pediculidae). J. Med. Entomol. 40: 468–474.

Ullrich, V., and P. Weber. 1972. The O-dealkylation of 7-ethoxycoumarin by liver microsomes. A direct fluorometric test. Hoppe-Seyler's Z. Physiol. Chem. 353: 1171–1177.

Vassena, C. V., G. Mougabure Cueto, P. González Audino, R. A. Alzogaray, E. N. Zerba, and M. I. Picollo. 2003. Prevalence and levels of permethrin resistance in *Pediculus humanus capitis* De Geer (Anoplura: Pediculidae) from Buenos Aires, Argentina. J. Med. Entomol. 40: 447–450.

Zerba, E., S. de Licastro, E. Wood, and M. I. Picollo. 1987. Insecticide: mechanism of action, pp. 103–106. *In* R. Brenner and A. Stoka [eds.], Chagas' disease vectors, vol. 3. CRC, Boca Raton, FL.

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