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## Insecticide Resistant Strains of House Flies (*Musca domestica*) Show Limited Cross-Resistance to Chlorfenapyr

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The toxicity of chlorfenapyr was evaluated against susceptible and pyrethroid resistant strains (laboratory and field collected) of house fly (*Musca domestica* L.). In one laboratory strain no cross-resistance was detected, 2.3-fold cross-resistance was detected in another strain, and a 2-fold negative cross-resistance was found in two strains. In field collected populations cross-resistance levels were also low (1.2- to 1.6-fold). These results suggest that the initial utility of chlorfenapyr for house fly control would not likely be compromised by cross-resistance, and that not all strains that have enhanced monooxygenase-mediated resistance will have negative cross-resistance to chlorfenapyr. The P450 isoform responsible for the activation of chlorfenapyr is discussed.

**Keywords:** negative cross-resistance, cytochrome P450 monooxygenases.

### INTRODUCTION

House flies (*Musca domestica* L.) are important vectors of human and animal diseases.<sup>1)</sup> Fly control is most commonly achieved with insecticides. Unfortunately, house flies have shown a remarkable ability to evolve resistance to insecticides. This trait, combined with loss of available insecticides through regulatory processes, has resulted in an urgent need for new house fly control agents. Chlorfenapyr is a relatively new and promising insecticide, with efficacy against a wide range of insects, including house flies.

The mechanism of action of chlorfenapyr appears to be unique, with a primary site of attack being the uncoupling of oxidative phosphorylation.<sup>2)</sup> This unique mechanism(s) of action suggests that resistance due to changes in the target sites of other insecticides (*i.e.* *kdr* or *Rdl*) would not result in cross-resistance to chlorfenapyr. In addition, chlorfenapyr is a protoxin requiring activation, *via* cytochrome P450 monooxygenases, to exert its toxic effects. It has been suggested that strains resistant to other insecticides (due to enhanced monooxygenase activity) might

have increased sensitivity to chlorfenapyr (due to enhanced activation of the protoxin). This seems to be the case in at least one pyrethroid resistant strain of horn fly<sup>3)</sup> and one pyrethroid resistant strain of *Heliothis virescens*.<sup>4)</sup> However, it is not clear how widespread this phenomenon would be in pyrethroid resistant insects, or what P450 might be involved.

In this study we examined the toxicity of chlorfenapyr to pyrethroid resistant strains (laboratory and field collected) of house fly. Our goal was to determine how common negative cross-resistance to chlorfenapyr is, and if the P450 involved in the activation could be potentially identified.

### MATERIALS AND METHODS

#### 1. Insects and Chemicals

Six strains of house fly were used. CS is an insecticide-susceptible strain<sup>5)</sup> and aabys is a susceptible strain with morphological markers on each autosome. LPR is a multiresistant strain having high levels of resistance to pyrethroid insecticides due to decreased cuticular penetration (*pen*), insensitivity of the target site (*kdr*) and increased oxidative metabolism<sup>6)</sup> mediated by cytochrome P450 6D1 (*i.e.* CYP6D1).<sup>7-10)</sup> The R12 and R1245 strains were isolated through a series of genetic crosses from the LPR strain<sup>11)</sup> and are pyrethroid resistant (due to CYP6D1-mediated detoxification). YPER is a multiple resistant strain with more than 18,000-fold resistance to permethrin due to *super-kdr* and monooxygenase-mediated detoxification that is not due to CYP6D1.<sup>12)</sup> Adult house flies were collected during the summer of 2003 from Florida (Alachua County) and New York (Schuyler County) with a sweep net from inside dairy barns and around calf hutches. Field collected flies were used to establish laboratory colonies. Chlorfenapyr was from American Cyanamid (now BASF, Research Triangle Park, NC) and piperonyl butoxide was from Sigma (St. Louis, MO).

#### 2. Bioassays

Insecticide was delivered in 0.5  $\mu$ l acetone to the thoracic notum of female house flies.<sup>13)</sup> Twenty 3–5 days old house flies were treated for each dose. A minimum of 3 doses giving >0% and <100% mortality after insecticide treatment was used for each experiment. Each experiment was replicated at least 3 times. The treated insects were put in 200 ml Sweetheart ice cream cups covered with cheese cloth and held at 25°C. Each cup was provided a 4 cm dental wick soaked in 15% sugar water and the dental wick was kept wet during the experiment. Mortality was assessed 24 hr after insecticide application. Insects were considered dead if they were ataxic. Bioassay data were pooled and analyzed based on standard probit analysis<sup>14)</sup> as adapted to personal computer use,<sup>15)</sup> using Abbott's correction<sup>16)</sup> for control mortality.

#### 3. Methoxyresorufin O-Demethylase (MROD) Assay

Microsomes were prepared from the abdomens of 200 female

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LPR house flies as described previously.<sup>17)</sup> Microsomal pellets were resuspended in 2 ml of resuspension buffer and stored at  $-80^{\circ}\text{C}$ . Protein was determined in triplicate for each sample using Bio-Rad protein reagent (Bio-Rad, Hercules, CA) with bovine serum albumin as the standard. Methoxyresorufin-*O*-demethylase (MROD) is a marker of CYP6D1 activity in LPR microsomes,<sup>18)</sup> and was measured using a CytoFluor<sup>®</sup> Series 4000 fluorescence multi-well plate reader (PerSeptive Biosystems, Framingham, MA) with excitation filter 530/25 and emission filter 580/40 at  $32^{\circ}\text{C}$  as previously described.<sup>19)</sup> Inhibition of MROD activity by piperonyl butoxide (positive control) and chlorfenapyr was evaluated using methods previously described.<sup>20,21)</sup>

## RESULTS AND DISCUSSION

Chlorfenapyr is highly toxic to house flies with a 24 hr  $\text{LD}_{50}$  of 99 and 70 ng/fly to the susceptible CS and aabys strains, respectively (Table 1). This toxicity is comparable with commercially used pyrethroids such as fenvalerate, bifenthrin and permethrin.<sup>6)</sup>

To investigate the potential for positive or negative cross-resistance, the toxicity of chlorfenapyr was examined in four laboratory strains of house fly that were resistant to pyrethroid (and certain other) insecticides due to monooxygenase-mediated resistance. The YPER strain was 2-fold cross-resistant to chlorfenapyr (Table 1), indicating that negative cross-resistance to chlorfenapyr is not a feature in all pyrethroid resistant house flies (even when one of the mechanisms in increased monooxygenase activity). There was no cross-resistance in the multi-resistant LPR strain of house fly. However, in the two strains that were genetically isolated from LPR, so as to have only the CYP6D1-mediated monooxygenase resistance (*i.e.* R12 and R1234), there was a 2-fold negative cross-resistance. This suggests that CYP6D1 may be involved in the metabolic activation of chlorfenapyr in house flies.

To investigate the interaction of chlorfenapyr with CYP6D1 we investigated whether chlorfenapyr would inhibit methoxyresorufin *O*-demethylase (MROD), a CYP6D1 mediated activity in LPR microsomes. The  $\text{I}_{50}$  for piperonyl butoxide inhi-

bition of MROD activity was  $3.0 \times 10^{-7}$  M, which is consistent with previous reports.<sup>20,21)</sup> However, chlorfenapyr did not inhibit MROD activity in LPR microsomes even at  $10^{-4}$  M. This suggests that the affinity of chlorfenapyr to CYP6D1 is much lower than methoxyresorufin, but does not rule out chlorfenapyr as a substrate of CYP6D1, because not all CYP6D1 substrates are inhibitors of this assay.<sup>20,21)</sup>

Toxicity of chlorfenapyr to field collected house flies was similar to the susceptible CS strain with resistance ratios of only 1.2- (Florida) or 1.6-fold (New York). The field collected populations did not show increased heterogeneity relative to the CS strain (Table 1), suggesting it is unlikely that increased levels of chlorfenapyr resistance could readily be selected for in the Florida and New York strains.

Our results suggest the initial utility of chlorfenapyr for house fly control would not likely be compromised by cross-resistance. Although negative-cross-resistance to chlorfenapyr is possible, it is not a characteristic of all pyrethroid resistant strains. Further work is needed to identify the P450 isoform(s) responsible for the metabolic activation of chlorfenapyr in house flies.

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**Table 1.** Toxicity of chlorfenapyr to eight strains of house fly

Strain	$\text{LD}_{50}$ <sup>a)</sup> (95% CI)	<i>n</i>	Slope (SE)	RR <sup>b)</sup>
CS	99 (93-105)	400	7.5 (0.8)	—
aabys	70 (67-73)	560	8.1 (0.7)	—
LPR	100 (99-110)	400	8.4 (0.9)	1.0
R12	48 (44-52)	300	6.8 (0.9)	0.5*
R1234	48 (44-52)	400	4.5 (0.5)	0.5*
YPER	230 (220-240)	845	3.4 (0.2)	2.3*
Florida	120 (110-130)	480	7.0 (0.9)	1.2*
New York	160 (110-210)	480	5.0 (1.7)	1.6*

<sup>a)</sup> In units of ng/fly.

<sup>b)</sup> Resistance ratio =  $\text{LD}_{50}$  resistant strain/ $\text{LD}_{50}$  susceptible (CS) strain.

\* Significantly different from CS based on non-overlap of confidence intervals.

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