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Rapid Report
Insecticidal activity of the enantiomers of fipronil

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Abstract: The two enantiomers of the insecticide fipronil were made by preparative HPLC. The insecticidal activities of the racemic mixture and the two enantiomers against selected agricultural or household pests (cotton stainer, *Dysdercus cingulatus* F; grain weevil, *Sitophilus granarius* L and house fly, *Musca domestica* L) were determined. There was no significant difference in acute or residual activity between the racemic mixture and the enantiomers of fipronil, indicating that there is no preferred chiral form of the compound in these key species of important insects. This observation clearly suggests that there is no major scope for marketing the insecticide in a one-enantiomer form.

Keywords: chiral; enantiomers; fipronil; insecticide; racemic

1 INTRODUCTION

Many of the molecules involved in biological processes tend to be chiral, or exhibit 'handedness'. Thus, metabolic processes often show a preference for specific chiral forms, or enantiomers. The biological significance of chirality is becoming increasingly evident. This is particularly true in the field of crop protection, where the preferred chiral form of a pesticide may be effective at significantly lower application rates, or be more specific toward a targeted pest.

The first pesticides in which differences in activity between enantiomers were acknowledged were the pyrethroids, and the commercialisation of one-enantiomer deltamethrin was a major technical achievement.[1,2] Since then several pesticides have been introduced in enantiomerically enriched form in order to enhance activity, suppress side-effects or as part of a company's post-patent strategy for the pesticide.[3]

The insecticide fipronil, discovered in 1987, has become a major insecticide with several applications ranging over agricultural to veterinary use.[4–6] It is an asymmetrical sulfoxide and thus has two enantiomers; in view of the commercial importance of fipronil, it is of interest to know the individual activities of these. The separation and characterisation of the enantiomers has been described in a patent,[7] in which a slight increase in long-term effect of the (*S*)-enantiomer on brown dog ticks *Rhipicephalus sanguineus* Latr, as well as a lower level of emesis in the treated dogs, was claimed. In the present paper, we examine the acute and residual activity of Fipronil racemic mixtures and enantiomers on Coleoptera (*Sitophilus granarius* L), Hemiptera (*Dysdercus cingulatus* F) and Diptera (*Musca domestica* L) in order to determine whether selected agricultural and household pests show a preference for specific chiral forms of fipronil.

2 EXPERIMENTAL

2.1 Separation and characterisation of the enantiomers

Pure (*R*,*S*)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile (fipronil) was extracted from a commercial formulation (Schuss®) by boiling with toluene. After one recrystallisation from toluene, the fipronil was >99% pure when assessed by GC.

Using a patented method[7] with the following modifications, separation of the enantiomers was attained. A Chiracel® OD 250 mm × 10 mm column was used as stationary phase, with isopropanol + hexane (10 + 90 by volume) as mobile phase at an elution rate of 2.4 ml min^{-1}. The enantiomers came off the column at 22 min (*R*-fipronil) and 29 min (*S*-fipronil). The solvent was removed from the collected enantiomer samples, and white crystals were obtained. The enantiomeric purity of both compounds was >99%. The enantiomers were individually tested for biological activity as described in Section 2.3. Confirmation of the absolute configurations of the

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enantiomers was achieved by recrystallisation of both compounds from toluene, by slow voluntary evaporation, and subsequent analysis by X-ray diffraction.

2.2 Insect strains

Three species of insect (cotton stainer, *D cingulatus*, grain weevil, *S granarius* and house fly, *M domestica*) were used in this study. All insects where bred under laboratory conditions at 24 °C and 70% relative humidity. For these tests, 5th-instar nymphs of *D cingulatus* were used, *M domestica* were treated at 3–6 days of age, while *S granarius* were treated as 7-day-old juveniles. Following treatment, insects where maintained on a diet of sugar or honey solution (*M domestica*), wheat seeds (*S granarius*) or cotton seeds (*D cingulatus*), unless otherwise stated.

2.3 Bioassays

Stock solutions (0.72 mg ml^{-1}) of the racemic mixture and the separate optical isomers were prepared in acetone and diluted as required for topical and surface-residue bioassays.

2.3.1 Topical bioassay

To determine the acute toxicity of the compounds, topical applications were made on all three insect species. The topical application comprised application of a single 2-µl drop (*D cingulatus* and *M domestica)* or a single 1-µl drop (*S granarius)* to the dorsal thorax using an Arnold Hand-Operated Applicator (Burkard Manufacturing Co, Herts, UK). Treated insects were transferred to observation chambers. The dose-mortality relationship for each compound was assessed (10 insects treated per dose) from five doses (0.1, 0.3, 1, 3 and 10 ng compound per insect) or with acetone alone (control). For these tests, only those *D cingulatus* individuals that had moulted to 5th-instar within the previous 24 h were selected for treatment. In addition, male and female individuals of *M domestica* were identified, pooled and treated separately.

2.3.2 Surface-residue bioassay

To determine the residual toxicity of the compounds, *M domestica* were treated by exposure to insecticide-impregnated filter paper in WHO holding tubes.[8] Filter papers were homogeneously impregnated with racemic and chiral solutions at concentrations of 0, 0.3, 1, 3, 10, 30 and 100 mg m^{-2}. After drying for 1 h to evaporate the solvent, the filter papers were placed in the holding tubes. Ten *M domestica* individuals were anaesthetised with ether and confined to each tube. After 4 h of exposure, the insects were transferred to clean holding tubes and maintained at 24 °C/70% relative humidity. Mortality was recorded after 4, 24 and 48 h.

To determine the residual toxicity on *D cingulatus* and *S granarius*, insects were treated by exposure to insecticide-impregnated filter paper placed in Petri dishes. Filter papers were homogeneously impregnated with suspensions of racemic fipronil and the separate enantiomers at concentrations of 0, 0.1, 0.3, 1, 3 and 10 mg m^{-2}. After drying for 1 h to evaporate the solvent, the filter papers were placed in the Petri dishes, and 10 insects were confined in each dish. After 72 h of exposure, insects where transferred to clean Petri dishes and maintained at 24 °C and 70% relative humidity. These insects where not provided with a diet. Mortality was recorded after 24 and 72 h.

2.4 Analysis of results

The dose-mortality relationship for each compound was plotted by fitting the data to a sigmoidal dose-response curve (GRAPHPAD PRISM, GraphPad Software, San Diego) and the LD_{50} value determined. The criterion for mortality was the lack of co-ordinated movement after being prodded. All tests where repeated at least three times. Values were considered statistically different if their 95% confidence intervals did not overlap.

3 RESULTS AND DISCUSSION

Bioefficacy comparisons where made at 24, 48 or 72 h post-treatment and confirmed the delayed action of fipronil (Fig 1) reported by Kolaczinski and Curtis.[9] Both topical and residual bioassays suggest that neither racemic fipronil nor the seperate enantiomers induce a rapid knockdown effect. As a consequence of the delayed action of fipronil, subsequent analyses of bioefficacy were performed on data collected 48 or 72 h after treatment.

Results of both residue and topical bioassays revealed that tolerance to fipronil and its enantiomers was lowest in *D cingulatus* and *S granarius*, which generally showed lower LD_{50} values than *M domestica* (Table 1). While this was at least in part because the latter were able to remove themselves from the

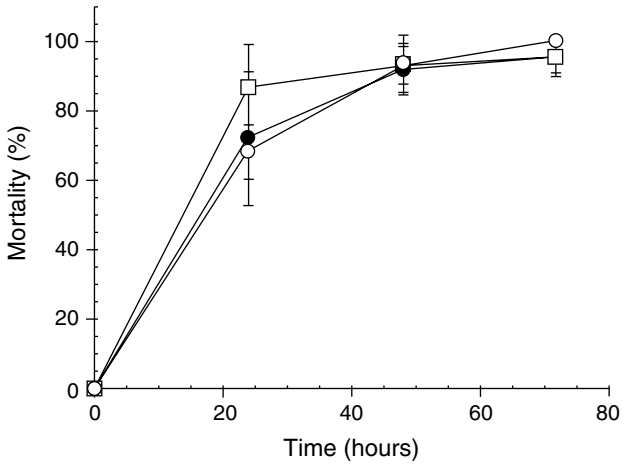

Figure 1. Mean mortality of *Dysdercus cingulatus* following topical applications of (●) racemic mixtures, (□) *R*-enantiomers or (○) *S*-enantiomers of fipronil at 3 ng per insect. Results are averages of two independent experiments ($n = 5$). Error bars indicate standard deviations from the mean.

Table 1. Surface-residue bioassay results from exposure of insects to racemic and chiral solutions of fipronil. LD_{50} values are calculated from data collected at 48 h (*Musca domestica*) or 72 h (*Dysdercus cingulatus* and *Sitophilus granarius*) after treatment

	Sigmoid regression results		
Species	Compound	LD_{50} (ng per insect)	95% confidence intervals
Dysdercus cingulatus	Racemic mixture	0.63	0.41–0.97
	R-enantiomer	0.55	0.34–0.87
	S-enantiomer	0.56	0.34–0.91
Musca domestica	Racemic mixture	15	8.50–26
	R-enantiomer	15	8.60–27
	S-enantiomer	18	10–32
Sitophilus granarius	Racemic mixture	0.80	0.57–1.10
	R-enantiomer	0.63	0.45–0.87
	S-enantiomer	0.74	0.50–1.10

Table 2. Topical bioassay results from exposure of insects to racemic and chiral solutions of fipronil. LD_{50} values are calculated from data collected at 72 h after treatment

	Sigmoid regression results		
Species	Compound	LD_{50} (ng per insect)	95% confidence intervals
Dysdercus cingulatus	Racemic mixture	1.40	0.94–2.20
	R-enantiomer	0.82	0.57–1.20
	S-enantiomer	1.10	0.68–1.70
Musca domestica	Racemic mixture	6.90	4.70–10
	R-enantiomer	7.00	5.20–9.40
	S-enantiomer	7.50	5.20–11
Sitophilus granarius	Racemic mixture	5.50	3.40–8.90
	R-enantiomer	2.20	1.30–3.70
	S-enantiomer	4.00	2.10–7.70

impregnated filter papers by clinging to the lids of the Petri dishes, *M domestica* also proved to have the highest tolerance to fipronil and its enantiomers under the topical bioassay, where the solutions were applied directly to the insects (Table 2).

The main scope of the investigation was to identify any commercially exploitable difference in the activity of the enantiomers of fipronil towards major agricultural and household pests. There was no significant difference in acute or residual activity between the racemic mixture and the individual enantiomers of fipronil (Tables 1 and 2). The principal impact of this study is the discovery that there is no preferred chiral form of fipronil against selected members of the important insect classes: Coleoptera (*S granarius*), Hemiptera (*D cingulatus*) and Diptera (*M domestica*). Hence, it seems unlikely that a costly development and marketing of a one-enantiomer version of fipronil would be justified.

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