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Disposition of fipronil in rats

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HIGHLIGHTS

- We analyzed the tissue distribution and the biotransformation of fipronil in rats.
- After a single oral dose, the highest residue levels were found in adipose tissue and to a lesser extent in adrenals.
- Residues corresponded to fipronil sulfone.
- Several other metabolites were identified in excreta, mainly glucuronide and sulfate conjugates.

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ABSTRACT

In the scientific literature, little attention has been paid to the disposition of fipronil, a phenyl pyrazole insecticide. In this study, the tissue distribution, the metabolic fate, and the elimination of fipronil was investigated in rats using radiolabeled fipronil. When a single oral dose of ^{14}C-fipronil (10 mg kg^{-1} b.w.) was given to rats, the proportion of dose eliminated in urine and feces 72 h after dosing was *ca* 4% for each route. At the end of the experiment the highest levels of radioactivity were found in adipose tissue and adrenals.

The main part of the radioactivity present in investigated tissues (adipose tissue, adrenals, liver, kidney, testes) was due to fipronil-sulfone. Five additional metabolites, isolated from urine were characterized by LC-MS/MS. Most of them are formed by the loss of the trifluoromethylsulphinyl group and subsequent hydroxylation and/or conjugation to glucuronic acid or sulfate.

In conclusion, the retention of the metabolite fipronil sulfone in tissues following fipronil administration raises the question of the potential toxicity of this insecticide.

1. Introduction

Fipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)-phenyl]-4-(trifluoromethylsulfinyl)-1-*H*-pyrazole-3-carbonitrile) is a phenyl pyrazole insecticide used to protect crops against specific soil and foliar pests (Hainzl and Casida, 1996; Hainzl et al., 1998). Fipronil is also marketed to control fleas and ticks on pets, mole crickets in turfgrass, and indoor pests such as ants and roaches (Dryden et al., 2000; Tingle et al., 2003; Gunasekara et al., 2007). The mode of action involves the blocking of the γ-aminobutyric acid (GABA) receptor, the binding to this receptor being much weaker in mammals than in insects, resulting in a substantial safety factor between these species (Gant et al., 1998; Hainzl et al., 1998). During the last decade, there has been an increasing concern about the environmental and human health effects associated with fipronil use (Chodorowski and Anand, 2004; Gunasekara et al., 2007; Lee et al., 2010). In addition to causing neurotoxicological and reproductive effects at high dosage regimen in laboratory species (Ikeda et al., 2001; Ohi et al., 2004; Szegedi et al., 2005; Galbiati Terçariol and Godinho, 2011) and mild nervous troubles in case of acute intoxication in humans (Mohamed et al., 2004; Lee et al., 2010), exposure to fipronil has been found to disrupt thyroid function in rats, producing neoplasia in the thyroid gland secondary to hormone imbalance (JMPR, 2001; Leghait et al., 2009). However, the doses at which fipronil induces thyroid cancer in this species are very high and not representative of human exposure. In addition, due to major differences in thyroid gland physiology between humans and rats, risk assessment agencies have considered rat as an inappropriate model for investigating fipronil induction of thyroid tumors (JMPR, 2001; AFSSA/AFSSE, 2005; EFSA, 2006). The determining role of metabolites and/or photodegradation products in the toxicity and ecotoxicity of fipronil has been reported by several authors (see Tingle et al., 2003 for review). Published data regarding the biotransformation of fipronil are scarce and are mainly based on microsomal incubations (Tang et al., 2004). The

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predominant pathway of fipronil metabolism is S-oxidation into the corresponding sulfone (Tang et al., 2004). This reaction, mainly catalyzed by the CYP3A4 cytochrome P450 isoform, can be considered as a bioactivation pathway since the sulfone metabolite was found to have a 6-fold greater binding affinity than the parent compound for GABA receptors in brain (Hainzl et al., 1998) and a 20-fold higher potency to block GABA-activated chloride channels in rats as compared to unchanged fipronil (Ikeda et al., 2001; Zhao et al., 2005). Fipronil-sulfone was also suspected to be more toxic than fipronil in different species (Zhao et al., 2005; Kitalugodage et al., 2011; Roques et al., 2012). Accordingly, the deleterious effect of fipronil treatment on thyroid function has been reported to be very limited in sheep, a species for which the biotransformation of fipronil into fipronil sulfone occurs at a slower rate compared with rat (Leghait et al., 2010). Furthermore, fipronil sulfone was shown to persist much longer in blood than fipronil itself (Leghait et al., 2009, 2010; Roques et al., 2012). Altogether, these data on fipronil sulfone highlight the need to better characterize fipronil metabolic pathways. More generally, a sound risk assessment of fipronil requires to better take into account its metabolites.

Some information on *in vivo* metabolic pathways was reported in different risk assessment documents but mainly based on unpublished data provided by the manufacturer for pesticide registration purposes (JMPR, 1997, 2001; AFSSA/AFSSE, 2005; EFSA, 2006).

We carried out this experiment in rat in order to investigate the disposition of ^{14}C-fipronil following a single oral administration of this compound at the dose level of 10 mg kg^{-1} b.w. The main objective of this study was to provide further insight on the distribution of residues and on the metabolic fate of fipronil in mammals by investigating the metabolic profile of this pesticide in urine and feces of treated animals.

2. Materials and methods

2.1. Chemicals

Unlabeled fipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)-phenyl]-4-(trifluoromethylsulfinyl)-1-*H*-pyrazole-3-carbonitrile, >98% pure), was purchased from Sigma–Aldrich (Saint-Quentin-Fallavier, France). [Phenyl-U-^{14}C]-fipronil with a specific activity of 1190 MBq mmol^{-1} was purchased from Scynexis Europe Ltd., UK. Before use, the purity of the radio-labeled molecule was checked by radio-HPLC and was shown to exceed 98.0%. Fipronil-sulfone (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)-phenyl]-4-(trifluoromethylsulfonyl)-1-*H*-pyrazole-3-carbonitrile (>99% pure) was obtained from Crescent Chemical Company (Islandia, NY, USA).

Dimethylsulfoxide (DMSO) and ammonium acetate were purchased from Sigma–Aldrich (Saint-Quentin-Fallavier, France). All solvents (analytical grade) were obtained from Scharlau Chemie SA (Barcelona, Spain). Ultrapure water produced with the Milli-Q system (Millipore, Saint Quentin En Yvelines, France) was used for HPLC mobile phases.

2.2. Animals

Three male Wistar rats weighing about 250 g were purchased from Iffa Credo (l'Arbresle, France). Upon arrival at the animal facility, animals were allowed at least 1 week of acclimation prior to experimentation. Animals were given free access to laboratory diet (UAR 210; Villemoisson sur Orge, France) and tap water. Rats were individually housed in stainless steel metabolic cages and were kept under a 12 h light/dark cycle. Animals were individually fed by gavage with a single nominal dose of 10 mg kg^{-1} b.w. [^{14}C]-fipronil (1.11 MBq per rat, adjusted with unlabeled fipronil) dissolved in corn oil (1 mL per rat). Urine and feces were collected once every 24 h over a 72 h period. Weights (or volumes) were measured prior to storage at −20 °C until analysis. Animals were anesthetized with ether and euthanatized by exsanguination after cervical dislocation 72 h post-dosing. Blood and tissue samples were collected for quantification of radioactivity. Adipose tissue samples were from perirenal adipose tissue. Intestinal tract was washed with NaCl 0.9% to remove contents prior sampling of duodenum portions. Each tissue sample was weighed and stored at −20 °C until analysis.

2.3. Sample processing

For total radioactivity measurement, triplicate aliquots of 150–300 μg were prepared from each sampled tissue and were precisely weighed for combustion prior to radioactivity determination. In order to characterize hepatic fipronil residues, remaining liver samples were pooled and extracted with a mixture of acetonitrile//isooctane/water (40:3.5:10, v/v/v) using a Polytron homogenizer (Kinematica, Lucerne, Switzerland) for metabolic profiling. The homogenate was centrifuged at 8000 g (4 °C, 10 min) the supernatant was removed and the pellet was extracted once more using the same procedure. The supernatants were pooled, transferred into a separatory funnel and 0.5 volume of isooctane/acetonitrile mixture (50:1, v/v) was added; then, the mixture was vigorously stirred. After decanting, radioactivity was determined in each fraction. The acetonitrile–water phase was concentrated to *ca* 4–5 mL, adjusted to pH 3 with acetic acid and extracted with a 2 g C18 cartridge (Envi-18, Supelco, Bellefonte, PA, USA). The cartridge was washed with 10 mL water, dried, and the radioactive material was eluted with 10 mL methanol. The extract was concentrated to 0.5 mL under a nitrogen stream and stored at −20 °C until analysis. Other tissues (kidney, testis, adrenals and brain) were processed similarly.

Perirenal adipose tissue sampled from each rat was pooled and extracted according to Folch's procedure (Folch et al., 1957), except that chloroform was replaced by dichloromethane. The dichloromethane fraction was evaporated to dryness and taken up by 5 mL acetonitrile/isooctane (3:2, v/v). Isooctane fraction was removed and acetonitrile fraction was evaporated to dryness under a nitrogen stream. The residual material was dissolved in 0.5 mL methanol and stored at −20 °C until analysis.

Feces (1 g) were homogenized in 50 mL ammonium acetate buffer (20 mM, adjusted to pH 3) using a Polytron homogenizer and centrifuged at 8000 g (4 °C, 10 min). The supernatant (a) was removed and the residual pellet was extracted with 50 mL acetonitrile and centrifuged identically. The organic supernatant (b) was collected, evaporated to dryness, and the residual material was dissolved in supernatant (a) and mixed with 0.1 volume methanol. This fraction was put on a 2 g C18 cartridge (Envi-18, Supelco) and the radioactive material was eluted with 10 mL methanol as previously described for hepatic residues. The methanol fraction was concentrated to 1 mL volume under a nitrogen stream. The extracts and the pellets were stored at −20 °C until analysis.

Urine samples (2 mL) were acidified with 200 μL acetic acid and diluted in 5 mL water containing 2% acetic acid. This fraction was put on a 0.5 g C18 SPE cartridge (Envi-18, Supelco), and the cartridge was washed with 10 mL water, dried, and the radioactive material was eluted with 5 mL methanol. The methanol fraction was concentrated to 0.5 mL under a nitrogen stream and stored at −20 °C until analysis.

The radioactivity was measured based on 50 μL aliquots at each step of the procedure by liquid scintillation counting.

2.4. Enzyme hydrolyses

Enzyme digestion assays with β-glucuronidase from *Escherichia coli* (type VII, Sigma Chimie, St Quentin Fallavier, France) and with