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Fipronil: uses, pharmacological and toxicological features

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Abstract

Fipronil is a phenylpyrazole pesticide widely used as insecticide in agriculture and to control ectoparasites in veterinary medicine. Taking into account that wide range of applications, its main pharmacological features and toxicity need to be known. This paper discusses how this pesticide is used, its physicochemical properties, its degradation in the environment, its biotransformation and elimination in mammals, its mechanism of action, as well as its toxicity.

Keywords: Ectoparasiticide. Fipronil. Pesticide. Toxicity.

Fipronil: usos, características farmacológicas e toxicológicas

Resumo

O fipronil é um praguicida fenilpirazol amplamente empregado com inseticida na agricultura e para o combate de ectoparasitas em medicina veterinária. Considerando esse amplo uso, é importante conhecer as principais características farmacológicas, bem como a toxicidade dessa substância. Neste artigo são abordados os seguintes tópicos: os usos desse praguicida, suas propriedades físico-químicas, sua degradação no meio ambiente, biotransformação e eliminação em mamíferos, seu mecanismo de ação e sua toxicidade.

Palavras-chave: Ectoparasiticida. Fipronil. Praguicida. Toxicidade.

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Introduction

Fipronil is a synthetic chemical widely used in agriculture to fight insects in sugarcane, rice, cotton, potato, corn and soybean crops (AGROFIT, 2007) and in veterinary medicine to control ectoparasites, such as fleas, ticks and lice (MOHAMED et al., 2004). This pesticide was developed by the French company Rhône-Poulenc Agro in the 1980s as the first phenylpyrazole pesticide and the second generation of receptor blockers of the gamma-aminobutyric acid neurotransmitter (GABA) (BOBÉ et al., 1998; IKEDA et al., 2004; MARTINS, 2009). Fipronil was first launched on the market in 1993 and was officially registered by the U.S. Environmental Protection Agency in the United States three years later (USEPA, 1996).

Taking into account the wide use of fipronil both in agriculture and in veterinary medicine, it is essential to be acquainted with the main pharmacological features and the toxicity of this substance. This paper discusses the use of this pesticide, its physicochemical properties, its degradation in the environment and in mammals, its mechanism of action and its toxicity.

To write this article, a bibliographical survey was performed that included scientific books, dissertations, theses, periodicals and traditional references found in libraries, databases and the Google Scholar browser. The databases used comprise PubMed, Scopus and ScienceDirect. The keywords to perform the searches were: fipronil, phenylpyrazole insecticide, fipronil vertebrates, fipronil invertebrates.

Development

Uses

Fipronil is used as an agricultural pesticide and as an ectoparasiticide in veterinary medicine. It is especially worth noting that Brazil is among the largest producers and exporters of agricultural products and, as a result, one of the world's largest pesticide consumers. In 2012, 477,792.44 tons of active ingredients were traded in Brazil, of which 1,068.60 tons of fipronil, São Paulo and Mato Grosso being the states that most traded pesticides in that period (IBAMA, 2012).

Fipronil, chemically derived from the phenylpyrazole or fiprole family, is a broad-spectrum agent used in agriculture as an insecticide, formicide and termiticide, especially in

sugarcane, rice, cotton, potato, maize and soybean crops (AGROFIT, 2007;). In veterinary medicine, fipronil is used against fleas, ticks, lice and other insects (MOHAMED et al., 2004; LE FAOUNDER et al., 2007). This ectoparasiticide yields highly satisfactory results in veterinary clinic, since it may applied both to puppies from eight weeks of age and to pregnant and lactating bitches to control fleas and to treat sarcoptic mange, especially in puppies (PEIXOTO et al., 2002). It may also be used as a cleaning agent in public hygiene actions against ants and cockroaches (ZHAO et al., 2005; MARTINS, 2009; ANVISA, 2016). Fipronil is also effective at low concentrations against insects resistant to other agents such as pyrethroids, organophosphates and carbamates (CONNELLY, 2001; GUNASEKARA et al., 2007a).

Regarding the use of fipronil in agriculture, Gunasekara et al. (2007a) emphasize that the application of this pesticide in rice cultivation at 12.5 g of active ingredient per hectare was more efficient than malathion at 300 g of active ingredient per hectare, providing protection against Chironomid insects for 9 to 14 days after their appearance on rice grains.

Worldwide, fipronil is a component of more than 50 products (NPIC, 2009). Fipronil's formulation is often presented as insect bait, spray and solutions aimed at pets and large animals, as well as granular products for lawns to control crickets (USEPA, 2001), which also includes liquids, powders and water-soluble granules, microgranules, negligible solids and aqueous emulsions (TINGLE et al., 2003).

In Brazil, this chemical aimed at agricultural use is marketed as Blitz®, Klap®, Regent®, Standak® and Tuit florestal® (COMPÊNDIO, 2009). For veterinary use, fipronil is sold as Frontiline® (pipette kit of 0.5 - 4.02 mL of a 10% solution, 0.25% spray), Fiprolex® (0.5-4.02 mL tube of a 10% solution) and TopLine® (1 and 5 L package at 1%; 0.32% spray).

Physical and chemical properties

According to the International Union of Pure and Applied Chemistry (IUPAC), the chemical name of fipronil is 5-amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4-[(trifluoromethyl) sulfinyl]-1H-pyrazole-3-carbonitrile and its molecular mass is 437.15 g/mol. This compound contains a CF_3-group in position 4 (Figure 1), providing this molecule with higher liposolubility, which facilitates its deposition in adipose tissue and contributes to its prolonged effect in organisms (CABONI et al., 2003).

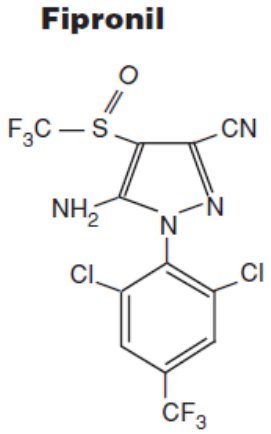

Figure 1 - Chemical structure of fipronil

Fipronil's solubility in water is 2.4 mg/L at pH 5 and 22 mg/L at pH 9. In that medium, the substance lasts approximately 14.5 days (USEPA, 1996). Its density is higher than that of water and it can be degraded by photolysis and hydrolysis under alkaline conditions (GUNASEKARA et al., 2007a).

Fipronil does not volatilize because it has relatively low vapor pressure and is only found in air if used in spray form. It features low mobility in soil, which results in low contamination potential; it also tends to dissipate facilitating its degradation by microorganisms (USEPA, 2001). Due to its wide use to control insects in crops and seeds, in addition to its application in irrigation water for rice crops (ANVISA, 2016).

Degradation in the environment, biotransformation and elimination in mammals

In the environment, this insecticide degrades rapidly by reduction, hydrolysis, oxidation and photolysis, forming five main active metabolites: 1) fipronil-sulphide (M&B 45950) - reduction product in soil; 2) fipronil-amide (RPA 200766) hydrolysis product in water and soil; 3) fipronil sulfone (M&B 46136) - oxidation product in soil; 4) fipronil-desulfinyl (M&B 46513) - the result of photolysis; and 5) fipronil-sulfonic acid (RPA 104615) (IPCS/INCHEM, 1997; BOBÉ et al., 1998; GUNASEKARA et al., 2007a; REVETON, 2007). In mammals, the main metabolite found is fipronil-sulfone (ZHAO et al., 2005).

1. Degradation in water

In aqueous solutions protected from light, fipronil remains stable on the long-term (half-life of approximately 1390 days) provided the solution is acid or neutral (pH 5.5 or 7.0) and is kept at ambient temperature (22°C ± 2°C). However, in alkaline solutions (pH 9-12), degradation occurs faster as pH increases. At pH 12, degradation is 300 times faster than at pH 9 and hydrolysis alone generates a single product only: fipronil-amide (BOBÉ et al., 1998; MARTINS, 2009; USEPA, 2001).

In aqueous solutions exposed to light, fipronil is degraded by hydrolysis and photolysis. In the presence of light, hydrolysis is faster and produces two chemicals: fipronil-desulfinil and the acid derivative of fipronil-sulfone (BOBÉ et al., 1998; GUNASEKARA et al., 2007a).

2. Degradation in soil

In soil, fipronil is usually degraded by photolysis, which is the main process of degradation in the environment, as well as by microorganisms. Therefore, its half-life varies according to soil conditions (pH, umidity, microorganisms presence, light, temperature) (GUNASEKARA et al., 2007b; MASUTTI et al., 2007).

Fipronil is not degraded when protected from light; Bobé et al. (1998) observed that more than 90% of fipronil applied to soil and unexposed to light remained unchanged for more than 96 hours. However, when exposed to light, degradation may occur at different speeds, depending on soil conditions. In all cases, a major degradation compound, fipronil desulfinil, is found.

Under normal conditions, fipronil is degraded mainly into sulfone and desufinil (ZHAO et al., 2005). This pesticide also features low dispersion capacity in soil, which highly reduces groundwater contamination. In addition, fipronil has a low to moderate adsorption rate that directly depends on the amount of organic matter contained in soil (DPR, 2011).

3. Degradation by soil microorganisms

Degradation of fipronil by microorganisms in non-sterile and loamy clay soil results in a half-life of approximately ten days at 25°C and of nine days at 35°C. Degradation takes three times longer (approximately 33 days) if the soil is sterile. The longest half-life reported lasted 342 days in clay soil. In loamy clay soil, half-life lasted 126 days (USEPA, 1996). Dissipation in anaerobic soil was faster, such as in California rice fields.

In addition to the microbiotic environment, moisture is a very important degradation factor for fipronil. Zhu et al. (2004) show that its half-life ranged from 68 to 198 days depending on soil moisture. Therefore, soils with a moisture degree of more than 50% lack aeration and thus contain less microorganisms, which results in more fipronil-sulphide as degradation product. On the other hand, soils with better aeration and a moisture degree below 50% contained more fipronil-sulfone, which is the result of a degrading process caused by aerobic microorganisms (ZHU et al., 2004).

4. Biotransformation and elimination in mammals

In mammals, the main metabolite found is sulfone, contained in liver and adipose tissue, as well as in urine. In humans, this metabolite is formed by the cytochrome P450 (ZHAO et al., 2005). In vivo studies by Durham et al. (2002) suggested that this metabolite is formed by monooxygenase activity (methoxyresorufin O-demethylase).

Mohamed et al. (2004) report that the oral administration of 4 mg/kg fipronil in rats resulted in an elimination half-life of approximately 8.5 hours while that of fipronil-sulfone was of 208 hours. This delay in eliminating the metabolite is explained by its concentration in the adipose tissue and by its high degree in the hepatic circulation.

Fipronil is mainly eliminated through feces (45-75%), but it may also be eliminated through urine (5-25%). Feces contain not only fipronil itself (M&B 46030), but also the metabolites M&B 46136, RPA 200766 and M&B 45950. Urine contains the metabolites M&B 46136, RPA 200766, M&B 45950 and M&B 45897, the latter of which features two products with an open ring (IPCS/INCHEM 1997).

Mechanism of action

Fipronil is an insecticide that interferes with the chloride channels coupled to the receptors of the *gamma*-Aminobutyric acid (GABA), acting as a non-competitive blocker of these channels. It interferes with the influx of chloride ions and thus disrupts the transmission of nerve impulses between nerve cells (RHÔNE-POULENC, 1995; USEPA, 1996; CABONI et al., 2003; IKEDA et al., 2004; GUNASEKARA et al., 2007b; NARAHASHI et al., 2007; ISLAM et al., 2012). This GABAergic antagonistic action increases the nervous impulse, leads

to excessive neural activation and the insect's death by hyperexcitation, given the fact that GABA is the main inhibitory neurotransmitter of the nervous system (OHI et al., 2004).

GABA receptors are composed of several subunits which form the ionic chlorine channel and a region that contains a recognition site for both GABA and other agonist and antagonist substances (MOHLER et al., 2004).

Three classes of GABAergic receptors are currently known: $GABA_A$ and $GABA_C$, which are ionotropic receptors, and $GABA_B$, a metabotropic G protein–coupled receptor (CHEBIB et al., 2007).

Mammalian $GABA_A$ receptors are heteroligomeric transmembrane glycoproteins composed of five subunits of seven families. At least one α, one β and one γ subunit are required for the full functioning of the receptors. It is known that *in vitro*, fipronil couples to a β3 homoligomeric receptor in a relatively weak manner and to a native receptor of insects with high affinity approximately 100 times stronger when compared to mammalian receptors (MOHAMED et al., 2004).

Ikeda et al. (2000) showed that fipronil decreases the duration of the chloride channel opening of GABAergic receptors in *Drosophila*. In $GABA_A$ receptors of mice, the insecticide decreases the mean opening time and increases the closing time, reducing the channel opening frequency and deregulating nerve cell membranes potential, leading to hyperexcitation (IKEDA et al., 2000).

Ikeda et al. (2000), in a study conducted with $GABA_A$ receptors from mice, found that fipronil slowly and reversibly blocked GABA-induced currents and that the inhibitory effect occurred in both the open and the closed receptor.

Fipronil features strong affinity for the GABAergic receptors of invertebrates, which makes it more toxic for insects than for mammals due to three factors: 1) the nervous system of insects is much simpler than that of mammals; 2) GABAergic receptors of insects and mammals are structurally different (NARAHASHI et al., 2007); and 3) insects have a glutamatergic system that is also coupled to the chloride channels, making it a critical target of fipronil (ZHAO et al., 2005; NARAHASHI et al., 2007).

Fipronil may also act on the chloride channels of the glycine receptors. $GABA_A$ receptors and glycine receptors belong to the family of Cys-loop pentameric receptors, which

are coupled to ion channels and fipronil inhibits both with the same potential (ISLAM et al. 2012). In addition, it was recently found that the degree of sentitivity of GABAergic receptors of insects towards the action of fipronil may vary according to the presence or absence of phosphorylation and dephosphorylation processes of C kinase proteins (MURILLO et al., 2011).

Toxicity

Fipronil may impact target organisms, such as insects, and non-target ones, such as aquatic organisms, birds and mammals (GUNASEKARA et al., 2007b).

1. Toxicity to insects

Fipronil is a powerful agent capable of causing dysfunction in the nervous system of insects, uncontrolled system hyperactivity and, as a result, death. In addition, Zhao et al. (2004) emphasize that fipronil may also act by inhibiting the glutamate receptors of insects, which justifies its high selectivity.

The biological activity of fipronil against *Diabrotica spp* in corn crops is very high (50% lethal dose - LD_{50} = 0.33 ng/mg). In addition to that, it is also highly efficient against the *Aedes aegypti* mosquito larvae, featuring a 50% lethal concentration (LC_{50}) of 24.8 nM in air (approximately 11.7 µg/L) within 24 hours and 15.1 nM (approximately 7.14 µg/L) within 48 hours, thus representing a possible alternative for the eradication of that mosquito, which has become a serious public health problem. Fipronil's degradation products are toxic as well: the LC_{50} of fipronil-sulfide and fipronil-sulfone in air is approximately 8.8 nM (3.79 µg/L) (TINGLE et al., 2000; GUNASEKARA et al., 2007b).

Zhao et al. (2005) observed that the LD_{50} of houseflies is 0.13 mg/kg and that GABAergic receptors of cockroaches are about 59 times more sensitive to fipronil than $GABA_A$ receptors of mice.

Fipronil's effects on the environment were surveyed by analyzing the impact caused on beetle mortality, which are important in the biological control of other pests in agriculture (GUNASEKARA et al., 2007b) and regarding changes in learning and memory, as they consequently alter the behavior of honey-producing bees (EL HASSANI et al., 2009).

2. Toxicity to aquatic organisms

Gunasekara et al. (2007b) reviewed fipronil's toxicity to aquatic organisms and noticed that this insecticide is highly toxic to many aquatic species. It also bioaccumulates in some species of shrimp, fish and water flea. Amounts in the range of ppt (ng/L) may affect microcrustaceans such as *Daphnia sp* and amounts in the range of ppb (µg/L) may affect freshwater fish, such as the *Bluegill sunfish*. Fipronil may decrease the populational growth rate of flies that use the aquatic environment for breeding, which may lead to their extinction at a concentration of 80 µg/L (STARK et al., 2005; GUNASEKARA et al., 2007b).

Beggel et al. (2010) observed that fipronil features a LD_{50} of 130 µg/L for 96 hours for saltwater fish of the species *Cyprinodon variegatus* (*Sheepshead minnow*), of 83 µg/L within 96 hours for freshwater fish of the species *Lepomis macrochirus* (*Bluegill sunfish*) and of 100 µg/L for 96 hours for the *Oncorhynchus mykiss* (*rainbow trout*) which is a freshwater fish migrating to salt water. In addition, the insecticide reduces the ability of the larvae to swim at LD_{10} for 24 hours, suggesting neurotoxicity in aquatic species at sublethal doses.

3. Toxicity in birds

Gunasekara et al. (2007b) reported that fipronil is toxic to terrestrial birds on the occasion of both acute and subacute oral exposure, presenting lower toxicity to some water birds. For some birds, this insecticide may present low LD_{50} toxicity, ranging from 2,150 to 1,120 mg/kg.

4. Toxicity to mammals

Technical fipronil (97% purity) may be classified as a category II toxic (moderately toxic) or as a category III toxic (slightly toxic), depending on the route of administration. Common signs of acute toxicity in animals include piloerection, curved posture, abnormal locomotion and diarrhea (TERÇARIOL, 2007).

Studies performed on mice show an oral LD_{50} of 92 and 103 mg/kg body weight (male and female, respectively). In addition, at oral doses of 4 and 40 mg/kg body weight, fipronil becomes bioavailable in proportion to the administered dose and features a maximum absorption time of 5.5 hours and 36 hours, respectively. These studies also show that fipronil has a mean elimination time between 183 and 245 hours (at a dose of 4 mg/kg) and between 135 and 171 hours (at a dose of 40 mg/kg), in addition to a wide distribution in tissues, predominantly in adipose tissue (IPCS/INCHEM, 1997). It was further observed that the oral

dose of 4 mg/kg body weight had a 50% absorption rate, while the oral dose of 150 mg/kg body weight resulted in a 30% absorption rate, due to saturation of the absorption process.

The dermal LD_{50} of fipronil is above 2000 mg/kg (IPCS/INCHEM, 1997). Studies in dogs showed that fipronil distribution was predominantly in the stratum corneum, the viable epidermis, the sebaceous glands and epithelial layers up to 56 days post-treatment (COCHET et al., 1997).

Acute neurotoxicity studies in rats show that the NOEL (No Observed Effect Level) and the LOEL (Lowest Observed Effect Level) were 0.5 mg/kg and 5 mg/kg, respectively, based on decreased muscle strain of hind paws seven hours after the treatment (IPCS/INCHEM, 1997).

In humans, exposure may occur through skin contact, eye contact or through accidental product ingestion. If contact is direct and brief, it may cause irritation to the skin and eyes. If small amounts of the substance are ingested, the victim may experience nausea, headache, vomiting, stomach pain, weakness, and dizziness. These symptoms are transient, since shortly after its absorption, fipronil is eliminated through feces and urine (NPIC, 2009; NCAMP, 2016). However, once absorbed by the body, this substance is distributed in tissues and releases active metabolites which accumulate mainly in adipose tissue (NCAMP, 2016).

The literature further states that fipronil is carcinogenic. In mice, for example, prolonged exposure to high doses of fipronil has caused thyroid cancer in both males and females. However, to date, there is no evidence of cancer in humans caused by exposure to this chemical (NCAP, 2005; NPIC, 2009; NCAMP, 2016).

Szegedi et al. (2005) suggest that the major causes of human intoxication by fipronil are accidental and occupational, resulting in moderate and temporary effects. Children are much more sensitive to the toxic effects of any pesticide and even if they do not present any clinical symptoms, they may show poor performance in neurobehavioral tests. Given that fact, the use of neurobehavioral tests is recommended to evaluate the neurotoxicity of chemical agents such as fipronil (TERÇARIOL, 2007).

Regarding reproductive toxicity, many chemical agents affect the reproductive system and cause changes in gestation, labor, lactation and changes in the development of the offspring (TERÇARIOL, 2007). Studies in mice have shown that fipronil causes decreased litter size, body weight of animals and mating percentage; it also reduces fertility, post-implantation and

postnatal survival of offspring, and it retarded the development of the pups. It is believed that these changes are related to fipronil's impact on the thyroid, liver and pituitary gland (TINGLE et al, 2003). Ohi et al. (2004) have shown that fipronil's adverse effects on the reproductive tract of mice are related to changes in the endocrine system, especially in the levels of progesterone and estradiol during the estrous cycle and gestation of mice.

Terçariol (2007) observed that fipronil may harm the embryofetal development and cause physiological and behavioral changes as it interferes with the steroid synthesis process (the CYP19 aromatase enzyme), which catalyzes the conversion of androgens to estrogens and is in charge of the homeostatic balance between the female and male hormones.

Recently, studies by Udo et al. (2014) have shown that oral exposure to fipronil during gestation of mice cause changes in maternal behavior and in the reflexology of the offspring. In addition, more recent studies in our laboratory have shown that treatment with oral fipronil during gestation of mice turned maternal behavior (MAGALHÃES et al., 2015), more aggressive, corroborating Udo's et al. (2014) findings. It is therefore evident that fipronil should be applied in a cautious manner during pregnancy, since it changes maternal behavior and the reflexological development of the offspring. Such disorders may be due to changes in the maternal hormonal system or in the GABAergic system.

Conclusion

Given the fact that fipronil is widely used in agriculture and as a pesticide, as well as in veterinary medicine as an ectoparasiticide, the knowledge about its pharmacological and toxicological features, especially the endocrine aspects, need to be deepened.

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