Fipronil: mechanisms of action on various organisms and future relevance for animal models studies

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Abstract. Because insects had developed resistance to several insecticides, today, neonicotinoids and fiproles are used to combat pests. The difference between this two classes of insecticides is that fipronil acts by inhibiting the receptors of nervous cells, while neonicotinoids perturbs the neuronal transmission. The suitable properties of fipronil make that its use to be more obvious on the pesticide market. Even if it is known that this insecticid e has a direct target, there are cases when its effects are negative on other organisms. In the last period, it appeared a lot of reports describing the consequences of his use not only regarding insects. Vertebrates and even humans are exposed to its bad influence. The present paper contain a description of fipronil discovery, mechanism of action and some of the main effects which occur after administrating it, as well as, relevance for animal models studies.

Keywords. Insecticide, fipronil, GABA, ecological risk, neurotoxicity, vertebrates, humans, autism spectrum disorder

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Introduction
Nowadays the use of insecticides by humans is more and more pronounced worldwide. These chemicals which were developed for many years had a great influence on people’s lifestyle. When a farmer observes that his crop is going down because the negative effect of insects, he tries to fight with them using various methods starting from removing the affected or infested leaves to apply on plants an adequate substance for certain pests. Usually, people do not think about the indirect effect of that substance and, many times, the structure of soil, the composition of the water or the organisms which are living in these habitats are affected. For example, macroinvertebrates as mosquitoes are affected due to the fact that their diet with aquatic organisms is perturbed. Aquatic organisms from microbial communities are influenced indirectly by the presence of the insecticide in the water, insecticide who arrived there through the direct use of it by humans (Muturi et al., 2017). Because of their long resistance in environment, doses of insecticides can be taken or ingerated by insects or even humans like eating fruits which contains remains of the substances spreaded against pests or contamined pollen used by bees to feed the larvae (Sánchez-Bayo et al., 2013). These are only two examples about what is happening when those substances are used and how the balance between biotope and biocenosis is disrupted. Due to the large number of chemicals which are the active substance in various pests products, insecticides have, in general, specific targets. However, their differents between provenance, chemical structures and mechanism of action leads to a certain organization of them (Gupta and Milatovic, 2014). Evidence suggests that some insecticides are inductors of neuropsychiatric features of some nervous system disease. Natural or synthetic, insecticides are widespread in different forms, extracted from plants like rotenone, an alkaloid from Derris, Lonchocarpus, Tephrosia, and Mundulea genus, known for determining Parkinson’s disease symptoms and used for fish extermination, or produced by humans through various combination of raw materials, the last are dominating the market (Gupta and Milatovic, 2014; Robea et al., 2018).

The most known synthetic insecticides are: organochlorides, organophosphates, carbamates, pyrethroids, neonicotinoids and fiproles (Ware and Whitacre, 2004; Gupta and Milatovic, 2014). In general, an insecticide acts on insect nervous system which leads to an anormal function of neurotransmitters, moment when the insecticide molecules binds in the places of a neurotrasmitter sites, more exactly dereglates the function of specific cellular channels (Bloomquist, 1999).

Fipronil (5-amino-1-[2,6-dichloro-4(trifluoromethyl)phenyl]-4-[(1R,S)-
(trifluoromethyl)sulfinyl-1H-pyrazole-3-carbonitrile) is a member of fiproles who acts on chloride channels blocking the activation of GABA (Bobé et al., 1998; Bloomquist, 1999; Mohamed et al., 2004; Ware and Whitaacre, 2004; Gwaltney-Brant, 2013; Gupta and Milatovic, 2014; Oberemok et al., 2015).

At its beginnings, fipronil was discovered and promoted by a chemical and pharmaceutical company from France named: Rhône-Poulenc (EB, 2003; Gunasekara et al., 2007; Narahashi, 2010; Hodgson, 2012). After its discovery, fipronil started to be utilised as a tool against several classes of insects (Page, 2008). Today it is produced by a german company, Badische Anilin und Soda Fabrik (BASF) as a chemical suspension packed in products with different names for plant protection, urban pest and fish control or as a veterinary instrument (Simon-Delso et al., 2014; BASF, 2018). More often, utility of fipronil is observed in dogs and cats treatments when it is administrated locally, on skin, to fight against ticks, ear mites or fleas (Sternesen, 2004; Page, 2008; NPIC, 2009; Gwaltney-Brant, 2013; Gupta and Milatovic, 2014). It was used on rabbits but the vets do not recommend its usage because of the adverse effects like: depression or a reduced appetite (Gupta and Milatovic, 2014; Varga, 2014).

Fipronil – Mechanisms of action
Fipronil is a phenylpyrazole compound which is well-known for disturbing the ligand-gated chloride channels from the cell membranes of insects (Bloomquist, 2003; Narahashi, 2010). The chloride channels are responsible with the hyperpolarization of the potential membrane, with other words, it favors the entrance in the cell of chloride ions (Bloomquist, 1999; Bloomquist, 2003; Mohamed et al., 2004). Even if the principal target is the nervous system, these channels can be affected in muscles or kidney too (Bloomquist, 2003). Binding the molecules of insecticides to the specific sites of chloride channels leads to the inhibition of GABA and glutamate neurotransmitters and, as a consequence, it determines the hyperexcitation of central nervous system (Bloomquist, 1999; Bloomquist, 2003). The entire mechanism is represented in Fig. 1.
Ecological risk

Currently, the negative effect of insecticides on indirect organisms represent a major problem for researchers considering the impossibility to manage the unpleasant issues after exposure to them. According to several reports made by the Environmental Protection Agency (2008) from United States, it were identified a series of ecological risk reflected on freshwater, estuarine, marine invertebrates and vertebrates, whose consequences depends if it is an acute or an chronic poisoning. In the Netherlands, fipronil was used illegally to eradicate the poultry red mite from the cabbage, leek and onions crops. This fact conducted to a damaging process because the aquatic crustaceans and insects populations present in the surface water became more sensible to this compound (Gunasekara et al., 2007; Tennekes, 2018). The potential role of fipronil depends also by its metabolites who are a result of various processes like: hydrolysis (fipronil-amide), reduction (fipronil-sulfide), oxidation (fipronil-sulfone) and fipronil-desulfinyl, a product of photolysis (Zhao et al., 2005; Gunasekara et al., 2007; Lin et al., 2009; Simon-Delso et al., 2014; Teerlink, 2017). The chemical formulas of fipronil and its metabolites are represented in Fig. 2.
Fipronil can be degraded by light, water or by microorganisms from soil biota (Bobé et al., 1998; Lin et al., 2009; NPIC, 2009; Simon-Delso et al., 2014). In soil it is decomposed in small molecules by the microorganisms which live there (NPIC, 2009). The principal degraded product is fipronil-sulfide, a result of reduction process (Gunasekara et al., 2007). In one report made by Lin et al. (2009), it was demonstrated that the degraded products of fipronil have a higher persistence under aerobic or anaerobic conditions than fipronil. Approximately 125 days are necessary to be removed from the soil and that because its metabolites are adherent to the soil particles (Lin et al., 2009; NPIC, 2009). Also, physiochemical properties of these compounds and the organic carbon content from soil influence the sorption of them (Ying and Kookana, 2001; Singh et al., 2014).

Due to its low solubility in the water, fipronil persists a long period in the aquatic habitats and that’s why it is considered more lethal on aquatic organisms (Ying and Kookana, 2001; Gunasekara et al., 2007; Tennekes, 2018). In water, fipronil is converted in fipronil-desulfynyl through photolysis (Gunasekara et al., 2007). There are a lot of studies all over the world which triggers an alarm semnal to take care of fipronil doses released during plant treatments (Tennekes, 2018).

Exposure of organisms to fipronil: possible neuro-relevance

Despite the fact that fipronil is used against certain pests, sometimes it affects non-target organism. Fipronil, fipronil sulfone and fipronil sulfide are
known as a toxic enhacer to aquatic organisms which are not targeted to be removed from that area and have approximately the same actions (Wu et al., 2014). Fipronil sulfone is more powerful to block the GABA$_A$ receptors from mammals than fipronil (Zhao et al., 2005).

Being an insecticide is evident that the principal target of it are insects. According to Gunasekara et al. (2007) some of the fipronil lethal doses for insects are: 1.54, 0.43 and 23.0 µg/L for several species of mosquitoes, 0.42 µg/L for midges and for one bee is 4-6.2 ng. After insects, aquatic organisms are the second target of fipronil. 16 µg/L of fipronil is the lethal dose for Daphnia pulex, 0.14 µg/L for mysid shrimp and 130 µg/L for sheephead minnow (Gunasekara et al., 2007). In one report, it was evaluate the lethal dose of fipronil on red swamp (Procambarus clarkii) and white river crayfish (Procambarus zonangulus) taking in attention the fact that fipronil can be consumed simultaneously with the organic sediment. 14.3 and 19.5 µg/L were the lethal doses of fipronil for red swamp and white river crayfish (Schlenk et al., 2001).

The great majority of the studies which were conducted on laboratory animals concluded that fipronil action is, mainly, on growth, development and reproduction of the organisms (Gibbons et al., 2014). Also, the unfavorable effect of fipronil is observed on nervous system (Hainzl and Casida, 1996; Gibbons et al., 2014; Gupta and Milatovic, 2014). For instance, in one report made by some researchers, it was found that a certain dose of this insecticide can dereglated the thyroid hormone, induce seizures or even death (NPIC, 2009; Gupta and Milatovic, 2014). Depending the mode of administration and the dose, its effects are variable (Gunasekara et al., 2007). Tremors, convulsions and hunched posture have been observed on rats when fipronil was administrated orally (Mohamed et al., 2004). The same symptoms were observed after inhalating this substance (Gupta and Milatovic, 2014). If it is a dermal exposure, for example, rabbits are more affected than rats (Gupta and Milatovic, 2014, Varga, 2014). In Table 1 are
presented some reports with species of vertebrates, doses of fipronil and the main effects which were observed by researchers.

<table>
<thead>
<tr>
<th>Taxon and species</th>
<th>Concentration</th>
<th>Mode of administration</th>
<th>Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td></td>
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<tr>
<td>Zebrafish, <em>Danio rerio</em></td>
<td>0.33 mg/L</td>
<td>Dissolved in water</td>
<td>Notochord degeneration, locomotor defects</td>
<td>Stehr et al., 2006</td>
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<td></td>
<td>0.4 mg/L</td>
<td>Dissolved in water</td>
<td>Body length reduction, spine bending</td>
<td>Yan et al., 2016</td>
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<td></td>
<td>0.4 and 0.8 mg/L</td>
<td>Dissolved in water</td>
<td>Anxiety, swimming performance perturbed, lipid peroxidation increased</td>
<td>Wang et al., 2016</td>
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<tr>
<td>Fathead minnow, <em>Pimephales promelas</em></td>
<td>0.14 mg/L</td>
<td>Dissolved in water</td>
<td>Impaired swimming</td>
<td>Beggel et al., 2010</td>
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<tr>
<td>Silver catfish, <em>Rhamdia quelen</em></td>
<td>0.03 mg/L</td>
<td>Dissolved in water</td>
<td>Changes in gene transcription</td>
<td>Beggel et al., 2012</td>
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<td></td>
<td>0.0002 mg/L</td>
<td>Dissolved in water</td>
<td>Erythrocyte damage</td>
<td>Ghisi et al., 2011</td>
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<td>Birds</td>
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<td>Bobwhite quail, <em>Colinus virginianus</em></td>
<td>0.11mg/Kg</td>
<td>Injected</td>
<td>Body mass reduction, absence of appetite, developmental abnormalities</td>
<td>Kitulagodage et al., 2011b</td>
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<tr>
<td>Chicken, <em>Gallus gallus domesticus</em></td>
<td>37.5 mg/Kg</td>
<td>Injected</td>
<td>Developmental abnormalities</td>
<td>Kitulagodage et al., 2011a</td>
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<td>Mammal</td>
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<td>Rat, <em>Rattus norvegicus</em></td>
<td>280mg/Kg</td>
<td>Injected</td>
<td>Low changes of pregnancy, weight gain reduction</td>
<td>Ohi et al., 2004</td>
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<td></td>
<td>20mg/Kg/day</td>
<td>Injected</td>
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<td>Tingle et al., 2003</td>
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</table>

People are exposed to fipronil through accidental contact, acute and chronic events or when it is about a suicide tentative (Jeyaratnam, 1990; Chodorowski and Anand, 2004). In all these cases, fipronil acts on GABA<sub>A</sub>-gated chloride channel determining hyperexcitability (Mohamed et al., 2004; Narahashi, 2010). Headache, seizures, convulsions, pneumonia or
death were some of the symptoms of fipronil ingestion (Mohamed et al., 2004; Gupta and Milatovic, 2014). Because fipronil is known to dereglate the function of GABA neurotransmitter and an abnormal function of it was found as a symptom of autism spectrum disease, this fact leads to conclude that fipronil can be used to modelate an animal model for autism (Pessah, 2008).

In some reports, it was highlighted the neurotoxic effect of fipronil. For the first time, the blocking activity of fipronil was tested on a cellular line of Drosophila (Ikeda et al., 2004). The main effect consisted in appearance of some changes in the activity of ion channels, more exactly, fipronil was responsible with shortening the period of channel opening (Grolleau and Sattelle, 2000; Ikeda et al., 2004). Furthermore, Ikeda et al. (2001) studied the activity of ion channels from dorsal root ganglion neurons of rats. 1.66 +/- 0.18 µM of fipronil was the estimated dose needed to block the GABA<sub>A</sub> receptors from dorsal root ganglion neurons of rats (Narahashi, 2010).

**Conclusions**

Fipronil is an insecticide which exerts an neurotoxic effect especially on insects by blocking the GABA-chloride channels. It and his metabolites are highly toxic to aquatic organisms according to several reports. All the information presented in this report sustains that fipronil is capable to exert direct and indirect influences on insects, aquatic and terrestrial organisms. Further studies are needed to reduce the risk of accidental exposure and to make a corelation between fipronil and autism and to validate if fipronil can be a succesfully tool for studying autism spectrum disorder in an animal model.

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