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Short Note

Testing of the fipronil's endocrine disrupting action on *Poecilia reticulata* and *Danio rerio*

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Abstract. Studies show that, an increasing number of synthesized chemicals, that are frequently used for intensive agriculture, veterinary medicine or other such fields, exhibit endocrine disrupting type of effects. These pollutants have the ability to spread through the food chain, increasing their level of concentration from producers to leading consumers. All of which require constant supervision and identification of such chemicals in every link of the food chain, especially of those which manifest endocrine disrupting properties that affect reproduction in different species of animals and fish, including humans. Pesticides are, generally, toxic for aquatic biocenosis and particularly for fish. The lack of data and further information concerning the effects of a large number of endocrine disruptors that have not yet been analyzed, impose required identification, research and supervision of their sources, propagation vectors, bioaccumulation environments, exposure risks and effects induced to animals, birds, fish and humans. Fipronil is used on a large scale both in veterinary medicine and in the treatment of agricultural crops. Applying this substance on insects and mites induces an uncontrolled activity of the central nervous system, so it is one of the insecticides on the list of suspected endocrine disruptors. In order to confirm or infirm the fipronil's function as an endocrine disruptor, testing on a model organism is advised, thus our research in conjunction with studies and results obtained in this regard, recommend using *Poecilia reticulata* and *Danio rerio* species as test subjects.

Key Words: endocrine disruptors, guppy, zebra fish, biomarkers.

Abstract. Studiile arată că, un număr tot mai mare de substanțe chimice de sinteză utilizate frecvent în agricultura intensivă, medicina veterinară sau în alte domenii de activitate, manifestă efecte de tip disruptor endocrin. Acești poluanți au capacitatea de a se propaga în lanțul trofic, concentrația lor crescând de la producători către consumatorii de vârf. Toate acestea impun o monitorizare permanentă și identificarea substanțelor chimice la nivelul fiecărei verigi a lanțului trofic și care manifestă proprietăți endocrin-disruptive cu efecte asupra reproducției la diferite specii de animale, pești și implicit la om. Pesticidele sunt, în general, toxice pentru biocenozele acvatice și în mod particular pentru pești. Lipsa datelor și a informațiilor cu privire la efectele unui număr important de disruptori endocrini ce nu au fost încă studiați, impun ca o necesitate identificarea, cercetarea și monitorizarea surselor, vectorilor de propagare, a mediilor de bioacumulare, a riscurilor expunerii și a efectelor induse la animale, păsări, pești și oameni. Fipronilul este utilizat pe scară largă atât în medicina veterinară, precum și în tratarea culturilor agricole. Aplicarea acestei substanțe împotriva insectelor și acarienilor induce o activitate necontrolată la nivelul sistemului nervos central, motiv pentru care este unul din insecticidele aflate pe lista celor suspectate că acționează ca și disruptor endocrin. Pentru atestarea sau infirmarea funcției de perturbator endocrin al fipronilului este indicat testarea pe un organism model, iar cercetările noastre coroborate cu studii și rezultate obținute în acest sens, recomandă utilizarea speciilor *Poecilia reticulata* și *Danio rerio*.

Key Words: disruptori endocrini, guppy, zebra fish, markeri.

Introduction. Pesticides are used in agriculture and public health, weed and pest control (FAO; Bretveld et al 2006). Although, pesticides can adversely affect human and animal health, as well. The consumption of foods containing such chemicals should be analyzed and tested for endocrine disruption (EDSTAC 1998; Ohi et al 2004; Țălu et al 2012; Oroian et al 2013). Exposure to these substances that can interfere with the action and biosynthesis of the endogenous hormones (endocrine disruptors = ED or EDCs) has proven to cause a number of adverse effects, with implications in the reproduction process (World Health Organization/United Nations Environment Programme 2012; Bergman et al 2012). Animal studies show: decreased fertility and presence of

malformations in the offsprings/larvae of birds, mammals and fish; a decrease in the spawning ability of fish and turtles; the effeminate behaviour of male birds and fish (European Union Prioritization List; Georgescu B. et al 2006, 2009; Georgescu C. et al 2006ab; IPCS 2002). The preliminary results of some research studies that have assessed the effects of a small concentration of ED in cell cultures and laboratory species (fish and rodents) show that the experimental procedures currently used under low level exposure conditions are presenting some deficiencies regarding the degree of precision and evaluation, aspects which could lead to the underestimation of the effects caused by endocrine disruptors (Baatrup & Junge 2001; Gourmelon & Ahtiainen 2007; Ankley et al 2009; Mnif et al 2011). In order to test the biological reaction of a chemically synthesized product, polluting the environment, that shows a deviation from the state of normality, one must resort to the use of exposure biomarkers (Walker et al 2001, 2006; Scholz & Mayer 2008).

The effect of fipronil over ecosystems. Fipronil is an insecticide/acaricide which is commonly used for the control of pests, the second generation of "phenylpyrazole insecticide" is widely used both in veterinary medicine and in the treatment of agricultural crops (Leghait et al 2009; Ohi et al 2004). It comes in the form of active substance in various commercial products. Internationally, the most widely used insecticide/acaricide with fipronil was the product called *Regent*, but which, in the last years has not been approved for commercial availability due to its high contents of active substance, 39.4 % to be exact, presenting a very high degree of risk and an extremely dangerous level of toxicity in this concentration (FAO). In small doses, fipronil has proven to be efficient in exterminating a large number of insects that affected agricultural crops (FAO 1997; Ohi et al 2004). It also shows a very good terapeutical activity and persistence against ticks and fleas that parasite on domestic animals (Leghait et al 2009; Ohi et al 2004). The way fipronil acts is inducing an uncontrolled activity in the central nervous system of insects and mites, which is then followed by their imminent death. This is also the reason why fipronil is on the list of suspected insecticides that can act as an endocrine disruptor (PAN Pesticides Database-Chemicals). The main tests performed on bioindicator species were carried out on mice (Ohi et al 2004), but there is also data about the effects of this pesticide on birds, lizards, fish, crabs, shrimps and bees (US EPA 1996; Avault 2001; Peveling et al 2003; Chandler et al 2004). Many tests performed on mice have proven the fact that fipronil delays, almost twice, the resettling period of the oestrus with female species (Ohi et al 2004) because of the hormonal, thyroid and sexual disorders it causes (US EPA 1994; Leghait et al 2009). Also, the evolution of body weight in the offsprings of the females exposed to different ratios of fipronil was significantly lower to that of the offsprings of the females from the control group (Ohi et al 2004). Studies show that the organisms which display the main risk under the action of pesticides with a potential endocrine disruption are fish (Scholz & Mayer 2008). Low concentrations of fipronil have been detected as letal to the majority of fish species tested. According to EPA, fipronil shows „a very high level of toxicity“ for the sun perch (*Lepomis gibbosus*) and „a very high level of toxicity“ for the rainbow trout (*Onchorynchus mykiss*) and according to the 2009 FAO report, LC50 in *Poecilia reticulata* is of 0.165 mg/L of fipronil. Research studies about this substance have proven with certainty that it has negative effects over the reproduction process (Chandler et al 2004), it is highly toxic for fish (Tingle et al 2003) and aquatic creatures (Gómez Manrique & Machado-Neto 2008; Gómez Manrique 2009) but there are no studies showing that it is an endocrine disruptor. Due to its proven effects over the reproduction process, it is only suspected to manifest potential endocrine disrupting effects (PAN Pesticides Database-Chemicals). To certify or to deny the fipronil's endocrine disruptor function, it is advised to experiment on a model organism, and our research (Boaru et al 2013) in conjunction with studies and results obtained in this regard, suggest using the *Poecilia reticulata* and *Danio rerio* species. The Zebrafish (*Danio rerio*) is used as a model organism because of its high percentage of fecundity and external fertilization, and the guppy fish (*Poecilia reticulata*) shows the same characteristics, except fertilization, which is internal and the

embryo develops inside the female organism (Shaddock 2009; Petrescu-Mag et al 2010; Georgescu & Georgescu 2012; Georgescu et al 2013).

***Poecilia reticulata* (the guppy fish)**, the most common species of freshwater fish have a higher tolerance concerning water quality and is a good model organism for research regarding ecotoxicology (Hedayati et al 2012). It is a viviparous species with a long life and a short reproductive cycle and the reproduction period, the sexual behavior and reproductive rates are independent of the season (Houde 1997; Baatrup & Junge 2001; Tian et al 2012). Sexual distinction is very obvious, and the male, aside the fact that it has a bright orange colour it also displays a distinctive courting behaviour (Baatrup & Junge 2001; Li & Wang 2005). The species is used to test various insecticides that proved to influence the behaviour of fish in early stages and, effects at a histological level also appeared (Polat et al 2002; Sharbridre et al 2011). It has been found that some insecticides, such as chlorpyrifos, affect the mating behaviour, with consequences regarding the survival rate and numbers of guppy fingerlings (Polat et al 2002; De Silva & Samayawardhena 2005; Sharbridre et al 2011). Because EDCs can interfere with natural hormones and cause a series of reproductive abnormalities (Tian et al 2012) the guppy fish is a model organism that can be used to assess the effects caused after exposure to xenoestrogens and to detect potential endocrine disrupting substances by tracking and analyzing some reproductive parameters (Li & Wang 2005).

***Danio rerio* (the zebrafish)** is widely used as a “biologic indicator species” due to some essential characteristics: small size and quick development (Lawrence 2007), it does not require special maintenance conditions, it’s a rustic species and it reproduces easily (Kimmel et al 1995; Spence et al 2008), it reaches sexual maturity between 3-4 months and the incubation period takes approximately 3 days (Kemadjou Njiwa et al 2004). It was selected as bioindicator species in toxicology studies on the basis of recommendations by the International Organization for Standardization and by the Organization for Economic Co-operation and Development (Ahmad & Ansari 2011). The results obtained by the exposure of this species to various pesticides showed that certainly influence the reproductive activity and dynamics, implications on embryonic development (Ansari & Ansari 2011; Kemadjou Njiwa et al 2004) and the exposure of mature *Danio rerio* fish to various fipronil concentration levels resulted in the reduction in numbers of eggs and the fecundity and hatching percentage (Boaru et al 2013).

Concluding remarks. Testing fipronil on a model organism that can confirm or infirm its endocrine disrupting function is one of the international strategic goals on identifying and monitoring chemicals with potential endocrine disruption. Tolerance in terms of water characteristics and purchasing costs of both the biologic material and maintenance costs of the *Poecilia reticulata* and *Danio rerio* species are affordable, they do not require large financial efforts and the experiments can be conducted under imposed conditions.

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