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# EVALUATION OF HISTOPHYSIOLOGICAL ALTERATIONS IN RESPIRATORY PROCESS ASSOCIATE WITH FIPRONIL ADMINISTRATION IN EUROPEAN CHUB

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## INTRODUCTION

Insecticides can reach surface waters as a result of spray drift, runoff, or intentional or accidental misapplication (EPA, 2003) and fish can suffer negative effects from insecticide exposure. Contamination of water by insecticides is mainly due to intensive agriculture combined with surface runoff and subsurface drainage, usually within a few weeks after application (Banaee et al., 2011)

Fipronil (molecular formula $C_{12}H_4Cl_2F_6N_4OS$) is a relatively new insecticide, with broad-spectrum activity against numerous insect pests, used in both agricultural and nonagricultural settings (Fent, 2014). In recent years it is used to control common household insects, in addition to being used in flea and tick treatment for pets (Fent, 2014). It is also used in seed treatments (a particular exposure to fish in Brazil where carp are co-farmed in rice fields (Clasen et al., 2012). Currently, fipronil is considered one of the most effective phenylpyrazole insecticides (Hainzl et al., 1998; Chaton et al., 2002).

The increasing use of this insecticide has raised concerns for its harmful effects on human health and the environment (Tingle et al., 2003).

The effects of insecticides on fishes are of great concern because fishes are important sources of proteins and lipids for humans and domestic animals. Fish are particularly sensitive to environmental contamination of water and, like other aquatic organisms, may be exposed to a great range of insecticides during the course of their life cycle. In fish, different insecticides can be absorbed through gills, skin or alimentary ducts (Banaee et al., 2011). The contamination of surface water by insecticides is known to have dangerous effects on the growth, survival and reproduction of fishes (Dhamgaye et al., 2020).

The phenylpyrazole insecticide, fipronil, is an N-phenylpyrazole with a trifluoromethyl-sulfinyl substituent (Fulton et al., 2013).

The mode of action of fipronil is disruption of the passage of chloride ions through the γ-aminobutyric acid (GABA)-regulated chloride channel (Cole et al., 1993; Gant et al., 1998; Ratra and Casida, 2001; Tomlin, 2006), which leads to loss of neuronal signaling, hyperexcitation, and subsequent mortality (WHO, 1997; Gant et al., 1998, Banaee, 2013). The lower binding affinity for GABA receptor in mammalian enhances selectivity for insects and increases the margin of safety for people and animals (Cole et al., 1993; Hainzl et al., 1998; Ratra and Casida, 2001).

The primary biological metabolite of fipronil - fipronil-sulfone - is reported to be twenty times more active at mammalian chloride channels than at insect chloride channels (Zhao, 2005) and have similar toxicity to the parent compound in mammals (Hainzl et al., 1998). While fipronil is easily degraded and metabolized, its metabolites are equal or even more toxic to many aquatic species than the parent compound (Wang et al., 2016; Qu et al., 2016; Júnior et al., 2017, and are also more stable in the environment (Wang et al., 2016).

Fipronil is from low to moderate persistence, and its dissipation is the result of photodegradation, hydrolysis, and volatilization (Fent, 2014). Fipronil is degraded by photolysis at desulfinyl, which is more stable to photolysis but with similar toxicity (Hainzl et al., 1998); the primary fipronil metabolite sulfone is more persistent in the environment and more toxic to fish than the parent compound (Bobe et al., 1998; Hainzl et al., 1998; Gunasekara et al., 2007).

Fipronil remains stable to breakdown in water at mildly acid to neutral pH and degrades in basic solutions with a half-life of 28 days; in studies where fipronil was exposed to light, fipronil had a half-life of 3.6 h in water (Zhao, 2005). The primary environmental metabolite (photoproduct) of fipronil - fipronil-desulfinyl - is 9-10 times more active at the mammalian chloride channel than the parent compound (Hainzl and Casida, 1996; Hainzl et al., 1998).

A series of studies have demonstrated the occurrence of fipronil and its degradation products in the aquatic environment at levels ranging between 0.001–10.004µg/l, often exceeding the acute level (0.1µg/L) of fipronil in the aquatic life benchmark of the U.S. EPA (EPA, 1996; Gan et al., 2012; Ensminger et al., 2013; Ruby et al., 2013; Budd et al., 2015; Wang et al.2016).

The toxicity of fipronil to many aquatic organisms varies from highly toxic to very highly toxic with waterborne exposure. Fipronil is highly toxic to freshwater invertebrates: in daphnids, the NOEL for fipronil was measured at 9.8 µg/l, and the LOEL was 20.0 µg/l (EPA, 1996). The fipronil-sulfone and fipronil-desulfinyl metabolites are 6.6 and 1.9 times more toxic to freshwater invertebrates, respectively, than the parent compound (EPA, 1996).

The use of fish species as bioindicators is an important environmental monitoring tool. Median lethal concentration (LC50) and dose (LD50) have been widely used to determine acute toxicity in aquatic animals. Fipronil is highly to very highly toxic to marine and freshwater: the 96 h $LC_{50}$ values of 42 mg/l for Nile tilapia, 83 mg/l for bluegill sunfish, 246 µg/l for rainbow trout, 340 µg/l for Japanese carp, and 430 µg/l for carp - Tingle et al. (2003). Fipronil 96-hour $LC_{50}$ is 0.246 mg/l for rainbow trout, 0.083 mg/l for bluegill sunfish, and 0.130 mg/l for sheepshead minnows (EPA, 1996). The metabolite fipronil sulfone have greater toxicity than the parent compound to rainbow trout (6.3 times more toxic) and bluegill (3.3 times more toxic) - Tingle et al., 2003.

The concentration of 0.142 mg/l of fipronil is considered sub lethal for *C. carpio* fry (Gupta et al. 2014); while the concentration of 0.665 mg/l is reported as the LC50 for adult of the same species (Qureshi et al. 2016).

In addition to the dose and duration of exposure, the route of administration can affect the degree of the toxicity (Driver et al., 1991; Pickford et al., 2003).

The evidence that fipronil cause toxic effects on aquatic organisms has been shown by an exposure approach in which organisms are forcedly exposed to contaminants (forced exposure) - Moreira et al., 2021. Previous studies have indicated that fish can detect and avoid contaminated environments, preventing them from suffering the toxic effects of the pollutant to which they are exposed (Moreira-Santos et al., 2019).

Fipronil accumulates in fish, the bioconcentration factor being 321 (EPA, 1996). Fish completely eliminate fipronil within 14 days of their passage into clean water (EPA, 1996). The major metabolites of fipronil in fish are fipronil sulfones and fipronil sulfides (EPA, 1996). Juvenile rainbow trout rapidly accumulated fipronil, but it was also rapidly eliminated (half-life 0.6 d) (Konwick et al., 2006). Demcheck and Skrobialowski (2003) reported fipronil concentrations as high as 5.29 mg/l in surface waters surrounded by rice agriculture.

Dallarés et al. (2020) conducted a study on the effects of dietary administration of Regent®800WG (80% fipronil) in European sea bass juveniles. Fipronil was added to the fish food (10 mg fipronil/kg feed) and the effects were studied at several time points including right before administration, 7 and 14 days after daily fipronil feed and one-week after the insecticide withdrawal from the diet (depuration period). Analyses in bile and muscle confirmed the rapid clearance of fipronil but the persistence of the metabolite fipronil-sulfone in bile even after the 7-day depuration period.

In tilapia (*Oreochromis niloticus*) exposed to fipronil in concentration of 0.8 µg/l, fipronil was transformed to fipronil sulfone in the liver and intestine under waterborne exposure rapidly transformed to fipronil sulfone in tilapia by oxidation at the sulfinyl (Li et al., 2018)

## MATERIAL AND METHOD

The study was performed with the approval of the local Committee of Bioethics according to the Romanian law 205/2004 art.7, 18, 22 and regulation number 143/400/ 2002 for care and use of animals for research purposes.

Determinations were made on European chub (*Leuciscus cephalus*), caught in the surrounding rivers of Pitesti city. The species was chosen due to the fact that it is a common species encountered in Arges River. Due to the high sensitivity of this species to industrial pollutants, enabling the chub to be used as a bio-indicator of pollution (Hajkova et al. 2007).

There were two treatment groups and a control group with three replicates and 7 fish in each group (with an average weight of 16.81±2.78 g). The fish were randomly divided into groups without determination of the male: female ratio. Fish were acclimated for 10 days prior to the test, and fed commercial fish food until the day before fipronil exposure.

After acclimation, 7 fish were randomly transferred into each tank containing 50 l of non-chlorinated well water and 0.05 and 0,1 mg fipronil/l water (without solvent). The concentrations that have been used have been established by study of the literature on fipronil levels reached in surface waters and preliminary survival test.

The immersion of fish in solution was performed after their mixing and aeration for 5 min. Waterborne administration is a common route of toxicant absorption in the aquatic environment and has advantages: simulating environmental exposure, involving no anesthesia and less handling of fish and relatively higher absorption rate constant for pesticides (Ardeshir et al., 2017). The water temperature was 18–20ºC and the immersion solution was changed every 24 h. The fish were not fed during experiments to avoid further intervention of this factor. Fish were kept in glass aquaria with gently aerated tap water, at dissolved oxygen 7.80 ± 0.28 mg/ l, pH 7,5 ± 0.45, total hardness 100 mg/l $CaCO_3$ with a natural light: dark photoperiod. The number of dead fish were recorded daily. Moreover,

to record any changes in behavior, fish were observed for about 1 h once daily.

Oxygen consumption and frequency of respiratory movements were determined at intervals of 24, 48, 72, 96, 168 and 336 h on all samples of these lots; the measurements were carried out in triplicate. The energetic metabolism, expressed by the oxygen consumption, was determined by using the closed respiratory chamber method (the oxygen dose in the water was established using the Winkler chemical method) (Picos and Nastasescu 1988; Nassar et al. 2015).

After 2 weeks of immersion in toxic substance, the fish were anesthetized with benzocaine and then sacrificed by decapitation. Pieces of gills were excised, rinsed in physiological saline and fixed in 10 % neutral formalin for 24 h. The tissue was rinsed in a graded series of ethanol to be dehydrated, cleared in xylene, embedded in paraffin, sectioned at a thickness of 5 μm (using a rotary microtome (Slee Maintz Cut 5062) and stained with hematoxylin Mayer (HE) as a general screening method. To avoid differing intensity of staining in different tissues, all sections to be stained by the same method were stained simultaneously.

Blood samples were taken from the caudal artery - three samples for each fish - (according to the method described by Picoş and Năstăsescu, 1988) two weeks after immersion in the solution of the fipronil and the average number of erythrocytes was determined (with Thoma chamber to Olympus microscope according to the method described by Picoş and Năstăsescu, 1988; Chen et al. 2015).

Values are given as arithmetic mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was performed to compare among experimental variants. Significance was accepted at $p<0.05$. Significance was accepted at $p < 0.05$.

## RESULTS AND DISCUSSIONS

Fish respond to environmental toxic changes by adapting their metabolite functions (Mishra and Shukla, 2003); they have been successfully used as a model to study the negative effects of various pesticides on the environment (Wenderlaar Bonga and Lock, 1992).

The median lethal dose (LD50) of fipronil (intraperitoneally administration) in Caspian white fish, *Rutilus frisii* kutum fingerlings was reported by 632 mg/kg suggesting it was slightly toxic to these species; the LC50 of fipronil was 572 μg/l, which was highly toxic to the fish (Ardeshir et al., 2017).

Changes in respiratory rate, oxygen consumption (during the experiment) and changes in the average erythrocyte count after two weeks of fish exposure to fipronil are shown in Table 1.

Respiratory rate in fish exposed to the effects of fipronil (0.05 and 0.1 mg/l) shows an increasing evolution in the first two days of exposure (by 9.76 and 11.96% compared to the control group), followed by a decrease progression of this physiological index until the end of the experiment (the values determined after 14 days of exposure to insecticides being 96.64, respectively 84.13% compared to the control group).

The oxygen consumption of fish intoxicated with fipronil was lower than the control values, the reductions being significant for the significance threshold $p<0.05$ after 72 hours of exposure at a concentration of 0.05 mg/l, and from the first 24 hours after exposure to 0.1 mg/l. The values determined after 14 days of toxic exposure (0.05 mg/l and 0.1 mg/l fipronil) were 12.67% and 17.66% lower compared to the control values.

Table 1. Mean ± SEM values of parameters determined on the control group (Lot I) group compared with groups treated with fipronil (Lot II – 0,05 mg/l; Lot III- 0,1 mg/l)

| Parameter | Lot I (control) | Lot II (exposed to fipronil 0,05 mg/l) | Lot III (exposed to fipronil 0,1 mg/l) |
|---|---|---|---|
| ***Breathing frequency*** | | | |
| Day 0 | 85,26±1,2513 | 87,18±0,3261 | 86,54±0,5482 |
| Day 1 | 86,12±0,8562 | 90,23±1,5632 | 96,32±0,4521* |
| Day 2 | 87,95±1,1253 | 96,54±0,6214* | 98,47±0,4156* |
| Day 3 | 85,41±0,8563 | 92,35±1,3654* | 94,63±1,3214 |
| Day 4 | 88,23±1,1156 | 90,23±0,7541 | 82,36±0,9854 |
| Day 7 | 85,21±1,4873 | 85,21±1,2365 | 78,36±1,1126* |
| Day 14 | 86,35±0,8547 | 83,45±0,2569 | 72,62±2,2142* |
| ***Oxygen consumption*** | | | |
| Day 0 | 280,42±5,2356 | 279,63±10,2358 | 274,26±11,2354 |
| Day 1 | 278,45±12,3568 | 271,56±12,3268 | 263,23±13,5624* |
| Day 2 | 276,32±14,2357 | 265,84±16,3247 | 245,36±14,2369* |
| Day 3 | 276,35±13,5681 | 261,25±11,4853* | 238,25±16,3245* |
| Day 4 | 278,23±12,3564 | 256,23±14,2356* | 235,24±11,3257* |
| Day 7 | 277,62±14,2358 | 258,32±11,8963* | 215,23±15,6247* |
| Day 14 | 281,23±12,2658 | 245,61±13,2547 * | 203,45±12,3641* |
| Red blood cells | 1,425,876 ± 111.774 | 1, 410,250 ± 86.146 | 1,286,230 ± 125.362* |
| | | | |

* Significant $p < 0.05$. Significance was calculated between control and treated groups

Also, Dhamgaye et al. (2020) reports that fipronil affected $O_2$ consumption rate of mahseer fry fish at sublethal concentration - 6.46µg/l and 12.92 µg/l): the $O_2$ consumption rate reduced progressively by 21% and 39% on 7-th day and 65% and 82% on 28th day at 6.46 and 12.92 µg/l concentrations, respectively as compared to control.

Breathing difficulty and increase in operculum movement were observed by Ghaffar et al. (2018) at common carp (*Cyprinus carpio*) exposed to fipronil at 0.08 and 0.10 mg/l concentrations. The authors also reported report convulsions, jerking, faintness and body curvature.

In a study of fipronil intoxication in European sea bass, Dallarés et al. (2020) reports an increase in the energy needs of fish, both during the period of exposure and in the period after intoxication (insecticide was administered in food).

Păunescu et al. (2016) observed a decrease in oxygen consumption, breathing frequency and an increase in the number of red blood cells on *Leuciscus cephalus* exposed to sublethal doses of herbicide Pendigan 330 EC.

The fluctuated response in respiration may be attributed to reduction in gill permeability causing a drop in oxygen consumption for which the fish compensates by increasing the ventilation volume as observed by Kalavathy et al. (2001).

The main hematological parameters in fish including red blood cell counts (RBC), hematocrit (Ht), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) may be influenced by internal and external factors.

Toxicology studies show that the disruptive action of different insecticides on the erythropoietic tissue such as kidney and spleen may decrease erythrocyte number and hemoglobin content as an anemic sign, and even lead to death of fish (Banaee, 2013).

A low red cell or hemoglobin count indicates anemia, or severe bleeding. Low hemoglobin usually means the animal has anemia. Anemia results from conditions that decrease the number or size of red cells, such as excessive bleeding, a dietary deficiency, destruction of cells because of a transfusion reaction or mechanical heart valve, or abnormality formed hemoglobin (Banaee, 2013).

Exposure of European chub to fipronil in concentration of 0.05 mg/l water does not significantly alter the average erythrocyte count after 14 days of exposure. After the same time, the fish exposed to the concentration of 0.1 mg fipronil/l water showed a significant reduction in the average number of erythrocytes (a decrease of 9.98% compared to the control group) – Table 1.

Ghaffar et al. (2018) investigate the toxic effects of fipronil on common carp (*Cyprinus carpio*). At high doses of fipronil (0,06, 0.08 and 0,1 mg/l) the authors noted severe abnormalities in clinical-hematological and biochemical parameters. The authors report decreased in erythrocyte count, hemoglobin and hematocrit and increased in mean corpuscular volume, total leukocyte count, neutrophils, monocytes, and lymphocytes in carp exposed to fipronil in various concentrations (0.04, 0.06, 0.08 and 0.010 mg/l). Erythrocyte in fipronil treated fish (0.08 and 0.10 mg/l) showed variation in size and shape: increased erythrocyte with aberrant morphological changes (pear shape, dividing erythrocyte, tear shape, vacuolated erythrocyte, erythrocytes with micronuclei, nuclear remnants and erythrocytes with eccentric nucleus).

Also, cellular abnormalities in erythrocyte of fish exposed to fipronil have also been reported (Qureshi et al. 2016).

The lower values of blood parameters could also be due to the hemolysis, rapid oxidation of hemoglobin and destruction of erythrocyte (Velmurugan et al. 2016).

Anemia following 4 days exposure to fipronil at a concentration of 0.8 mg/l was also observed by Fredianelli et al. (2019) in silver catfish, erythrocytes showing morphological alterations without increased micronucleus development.

Fish gills have many important functions: exchange of gases, transport of many mono and divalent ions, excretion of waste nitrogen, uptake and excretion of various xenobiotics (Zayed and Mohamed, 2004; Evans et al., 2005). Gills of fish are efficient biomonitoring tools due to their large surface area, which has direct and permanent contact with potential irritants (Sweidan et al., 2015) and are the first organs that respond to unfavorable environmental changes (Benli et al., 2008). Gills are a main target for toxicant entry into fish (Qureshi et al., 2016).

Histopathological investigations on different tissues of exposed fish are useful tools for toxicological studies and monitoring water pollutions (Banaee, 2013).

Any injury to the gills is followed by a chain of destructive events, which ultimately leads to the initialization of "respiratory shock". The decrease in oxygen consumption in the presence of pesticides in the environment is the result of reductions in the respiratory branchial surface. Histopathology of gill is the appropriate bioindicator to pollution monitoring.

Fipronil in water was taken up through gill along with the movement of water and blood in the gill and then transferred to blood (Clasen et al., 2012). Histopathological studies are a precise and rapid way to show the direct effect of toxicants on target organs (Ardeshir et al., 2017).

The progressive stages of tissue damage are: stage I (without alteration, normal functioning of the tissue), stage II (more severe and impair the normal functioning of the organ) and stage III, very severe

and irreversible (Poleksic and Mitrovic-Tutundzic, 1994).

Exposure of European chub to insecticide administered in water for 2 weeks determined installation of pathological changes in the gills tissues.

No histopathological changes were observed in gills of the control lot. The structural details of gills of control lot are shown in Fig. 1a. Exposure of European chub to fipronil insecticide administered in water (0.05 and 0.1 mg/l) for 2 weeks determined installation of pathological changes in the gills tissues (Fig. 1 b, c, d).

As a result of exposure to the insecticide fipronil in a concentration of 0.05 mg/l for 14 days, we found a series of histopathological changes in the gill: ruptures in the epithelial layer, a slight hypertrophy of the pillar cells, hyperemia, oedema (Fig.1, b).

In case of exposure to 0.1 mg fipronil/l water, the observed histopathological changes were: lifting of the gill lamellae, oedema, epithelial cell proliferation, secondary lamella fusion, degenerative alterations, especially in the terminal portion of the secondary lamellae.

The degenerative changes, which we observed in the gills histological structure, were presented in a moderate degree of expression.

The most frequent lesion observed in all analyzed samples of gills is the lifting of the respiratory epithelium followed by hyperplasia of the lamellar epithelium and incomplete fusion of several lamellae with greater frequency in specimens exposed to 0.1 mg fipronil/l water. These lesions correspond to stage I of gills histopathological alterations (Poleksic and Mitrovic-Tutundzic, 1994), this being related to the presence of chemical contaminants. Reducing the interlamellar space as a result of hyperplasia may cause an incomplete or complete fusion of lamellae.

Damage to gill tissue may interfere with gas exchange performance of gill and cause respiratory disorders, ion-regulation and osmoregulation dysfunction and inefficacy of the excretion of waste nitrogen metabolite in exposed fish (Cengiz and Unlu, 2006; Velmurugan et al., 2007). Some of the gill histological changes such as lifting, fusion, hyperplasia and hypertrophy generate obstacles to prevent toxicants entering into the blood (Bhagwant and Elahee, 2002).

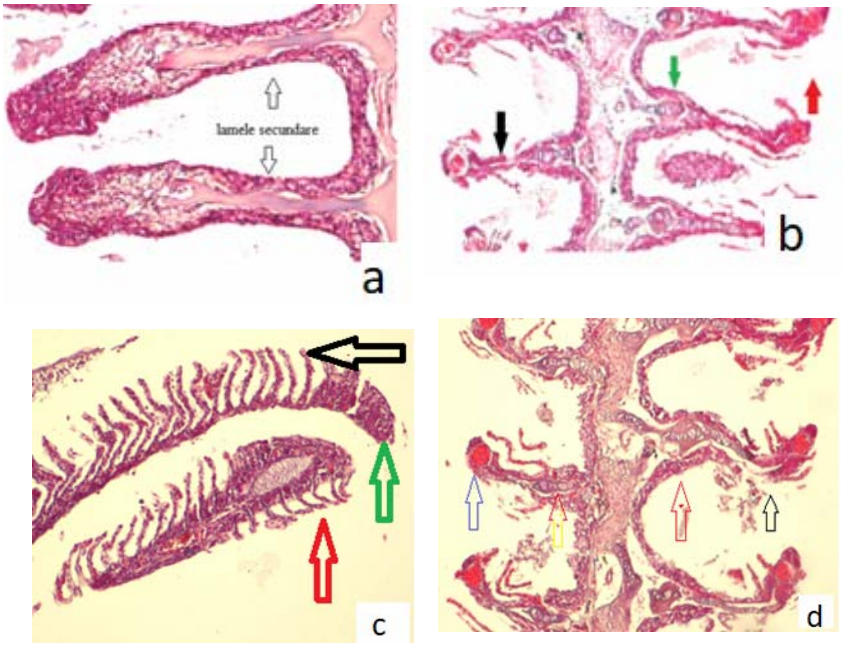


Fig. 1. Gills of European chub (*Leuciscus cephalus*) from the control and experimental group a) normal histological structure, primary (thick arrow) and secondary (narrow arrow) lamellae; b) 0.05 mg fipronil/l water - epithelial ruptures in the terminal secondary lamellae, accompanied by hemorrhage (red arrow), disorganization of the gill epithelium (black arrow), hypertrophy of the pillar cells (green arrow) c) 0.1 mg fipronil/l water - straightening of the gill slats (black arrow), fusion of the secondary slats (green arrow), edema (red arrow); d) 0.1 mg fipronil/l water - oedema with hemorrhage (blue arrow), hypertrophy of the gill epithelium and pillar cells (red arrow), disorganization of the lamellar epithelium with hemorrhage (black arrow)
HE coloring (40X)

Qureshi et al. (2016) reported disruption of primary lamellae, atrophy of secondary lamellae, lamellar degeneration and epithelial necrosis in common carp exposed to sub-acute concentrations of fipronil (400 µg/l). After 60 days of exposure to 0.23 µg/l fipronil, Ghisi et al. (2011) reported aneurisms, hyperplasia and lamellar fusion in the gills of *Rhamdia quelen*.

Gills of common carp exposed to fipronil (0.06, 0.08 and 0.10 mg/l) showed severe histopathological abnormalities, such as disorganization in the arrangements of primary and secondary lamellae, reduced size of secondary lamellae, necrosis of lamellar epithelial cells, severe congestion in cartilaginous cores, sloughing and fusion of secondary lamellae (Ghaffar et al., 2018). Telangiectasis, curling and uplifting arrangements in epithelial cells of secondary lamellae were frequently observed after day 4 of the experiment.

Fredianelli et al. (2019) also reports histopathological changes in the gills of silver catfish (*Rhamdia quelen*) exposed to fipronil insecticide in concentration of 0.8 mg/l: vascular congestion, complete fusion of secondary lamellae and epithelial cell hypertrophy.

Fipronil insecticide produces histopathological changes in the gills (aneurisms, extensive fusion and necrosis), kidneys (hemorrhage, tubular degeneration and necrosis) and liver (pyknosis and sinusoid dilation) - Ardeshir et al., 2017.

Exposure of European chub to herbicide Pendigan 330 EC administered in water for 2 weeks determined also installation of pathological gills tissues (Păunescu et al., 2016).

The route of exposure to fipronil affects its acute toxicity and also determines the main target organs of toxicity and histopathological alterations in Caspian white fish; the mean DTC values (the degree of tissue change) calculated for the exposed gill sections showed that histopathological alterations of the gills in intraperitoneal exposure was significantly less than that in waterborne exposure (Ardeshir et al., 2017).

Ardeshir et al. (2017) report to fipronil in Caspian white fish LC50 -572 µg/L which proves that the insecticide has a high toxicity (0.1–1 mg/L) according to the US Environmental Protection Agency (EPA, 2012).

Behavioral changes are the most sensitive indicators of potential toxic effects. Most insecticides affect the behavioral patterns of fish by interfering with the nervous systems and sensory receptors and consequently it can lead to disorders in the fish response to environmental stimuli (Banaee, 2013).

Exposure of European chub to fipronil at a concentration of 0.1 mg/l water induces changes in swimming movements: swimming near the surface of the water, hyperactivity and loss of balance. Experiments performed on early life stage of rainbow trout have shown that the insecticide fipronil decreased larval growth (NOEC of 6.6 mg/l; LOEC of 15 mg/l) (Tingle et al., 2003). Also, fipronil impair fathead minnow swimming ability at 142 mg/L (Beggel et al., 2010).

Gupta et al. (2014) reported that fipronil has inhibitory effects on the ATP-ase activity in the gill of *Cyprinus carpio* fry and may disturb the osmoregulatory capacity of fish and consequently, lead to fish deaths.

Exposure of common carp to fipronil showed numerous abnormalities in a dose and time dependent manner including behavioral and nervous changes with least variations (Ghaffar et al., 2018). Surface breathing, erratic swimming, extensive mucus secretion, increased operculum movement, fin tremors and swimming on one side were the characteristic changes in treated fish.

Fredianelli et al. (2019) reports that silver catfish treated at different concentrations of fipronil (0.1, 0.2, 0.3, 0.4, 0.5, and 0.8 mg/l) presented mild to severe apathy, proportionally to the concentrations used and time of exposure.

Some studies have indicated that fipronil cause several effects: seizures, hyperactivity and death in fish (Beggel et al., 2012). Moreira et al. (2021) reports reduced swimming behaviour and inhibited activity of acetylcholinesterase AChE (a biomarker of neurotoxicity) in zebrafish (*Danio rerio*) exposed to fipronil (75 and 100 µg/l). D. rerio is also capable of detecting and avoiding environmentally relevant concentrations of fipronil.

Reactions of behavioral changes in swimming have also been reported in other fish such as carp (Qureshi et al., 2016) and Nile tilapia (El-Muhr et al., 2015) exposed to fipronil. Beggel et al. (2010) reported impacts of fipronil on swimming performance and growth in larval fathead minnow at nominal concentrations ≥31 µg/l.

## CONCLUSIONS

Exposure of European chub to insecticide fipronil administered in water for 2 weeks determined installation of pathological changes in gills tissues. Also, were observed a decrease in the number of red blood cells and oxygen consumption and breathing frequency and changes in swimming movements. Our results confirm that fipronil has negative effects on European chub.

## ABSTRACT

This study was conducted to investigate the toxic effects of fipronil on *Leuciscus cephalus* (Linnaeus, 1758).

Fipronil is a phenylpyrazole insecticide discovered and developed by Rhône-Poulenc between 1985 and 1987 and released to the market in 1996. The insecticide is widely used in agricultural management to control pests and it can be leached

into aquatic ecosystems. Fipronil acts by targeting gamma-amino butyric acid (GABA) receptors and has a much higher affinity for insect than for vertebrate.

The present study evaluates the changes of some important physiological indices (energy metabolism, respiratory rate, number of red blood cells) and histopathological gills tissues in *Leuciscus cephalus* exposed to the action of Fipronil insecticide under two concentrations (0.05 and 0.1 mg fipronil/L water). Fish adaptation to laboratory conditions was conducted for two weeks in glass aquariums with a capacity of 100 l, under natural photoperiodic conditions and the oxygen dissolved in water was not below 80% of the maximum possible at the respective temperature and pressure. Feeding during this period was "*ad libitum*" once a day; during the experiments fish were not fed to avoid the additional influence of the food factor, thus allowing a better interpretation and comparison of the results. The results were interpreted statistically using SPSS 13.0 program for Windows.

The experimental samples regarded the presence of respiratory, hematological in *Leuciscus cephalus* intoxicated with Fipronil insecticide. The fipronil insecticide has changed the fish respiratory rhythm and stimulating rate of oxygen consumption. The number of erythrocytes in the fish individuals subjected for 14 days to 0.1 mg fipronil/L water was also significantly affected. Exposure of European chub to insecticide administered in water for 2 weeks determined installation of pathological changes in gills tissues.

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