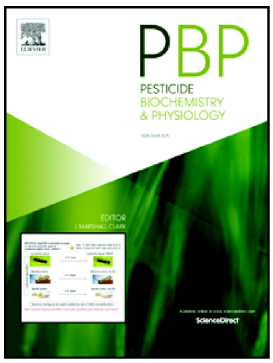


Multibiomarker responses and bioaccumulation of fipronil in Prochilodus lineatus exposed to spiked sediments: Oxidative stress and antioxidant defenses

A. Santillán Deiú, P.M. Ondarza, K.S.B. Miglioranza, F.R. de la Torre

PII: S0048-3575(21)00107-3

DOI: https://doi.org/10.1016/j.pestbp.2021.104876

Reference: YPEST 104876

To appear in: *Pesticide Biochemistry and Physiology*

Received date: 30 September 2020

Revised date: 19 April 2021

Accepted date: 17 May 2021

Please cite this article as: A. Santillán Deiú, P.M. Ondarza, K.S.B. Miglioranza, et al., Multibiomarker responses and bioaccumulation of fipronil in Prochilodus lineatus exposed to spiked sediments: Oxidative stress and antioxidant defenses, *Pesticide Biochemistry and Physiology* (2018), https://doi.org/10.1016/j.pestbp.2021.104876

Multibiomarker responses and bioaccumulation of fipronil in *Prochilodus lineatus* exposed to spiked sediments: oxidative stress and antioxidant defenses

Santillán Deiú A.[1,2], Ondarza P.M.[2,3], Miglioranza K.S.B.[2,3], de la Torre F.R.[1,2,*]

fdelatorre@unlu.edu.ar

[1]GECAP, Departamento de Ciencias Básicas e INEDES, Universidad Nacional de Luján (UNLu-CONICET). Rutas 5 y 7, Luján (6700), Argentina.

[2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

[3]Laboratorio de Ecotoxicología y Contaminación Ambiental, Instituto de Investigaciones Marinas y Costeras (IIMyC-CONICET), Universidad Nacional de Mar del Plata. D Funes 3350, Mar del Plata (7600), Argentina.

[*]Corresponding author.

Abstract

Fipronil is a current use pesticide, widely used in many crops, commonly adsorbed to sediments of aquatic environments. The purpose of this study was to evaluate the biomarker responses and fipronil distribution pattern in different matrixes (fish, sediment and water) after juveniles *P. lineatus* exposure at two environmental concentrations (5.5 and 82 μg kg^{-1}) of fipronil-spiked sediments. The levels of oxidized proteins (PO), lipid peroxidation (LPO), and enzymatic activity of superoxide dismutase (SOD), reduced glutathione content (GSH), antioxidant capacity against peroxyls (ACAP) and acetylcholinesterase (AChE) were evaluated in liver, gills and brain.

Concentrations of fipronil and its metabolites (f. desulfinyl, f sulphide and f. sulfone) were quantified by GC-ECD. F. desulfinyl was the major metabolite found in all matrixes, followed by f. sulphide in sediments, while f. sulfone was mainly accumulated in fish. Fipronil promoted oxidative stress in *P. lineatus*, as evidenced by the increases in LPO and PO levels and the decrease brain AChE activity. Fish exposed at both concentrations showed significant decrease in antioxidant capacity. Alterations in the antioxidant defenses system was evidenced in all organs. These results suggest that the occurrence of fipronil in aquatic environments can generate oxidative stress at different levels in *P. lineatus*, showing that this species is highly sensitive to the deleterious effects of fipronil and metabolites.

Keywords: Fipronil, degradation, biomarkers, fish, current use pesticides

1. INTRODUCTION

Fipronil (5-amino-1-[2, 6-dichloro-4(trifluoromethyl) phenyl]-4-[(trifluoromethyl) sulfinyl]-1H-pyrazole-3-carbonitrile)) is a phenylpyrazole pesticide used in agriculture for insects in cultures of rice, potato, corn, cotton, and, especially, sugarcane. It is also used as a component in various veterinary products, among other formulations (Wu et al., 2015). Fipronil has played an essential role in pest control because of its effectiveness at low field application rates against insects that are resistant to other insecticides such as the pyrethroids, organophosphates, and carbamates (Gunasekara et al., 2007). In insects, fipronil binds at the γ-aminobutyric acid (GABA) receptor and

blocks the GABA-gated chloride channel that regulates the passage of chloride across nerve cell membranes (Wang et al., 2016). Toxicity in mammals is believed to be the result of blockade of $GABA_A$ receptor chloride channels the nervous system, although with a lower potency than the GABA blockade in insect. However, this pesticide could be highly toxic to many non-target organisms, such as fish, aquatic invertebrates and terrestrial birds (Schaaf, 2015; Wu et al., 2015).

In Argentina, the use of agrochemicals has strengthened along with agricultural expansion. The continuous use of these substances raises the concern about their behavior, environmental fate and potential adverse effects (Solis et al., 2018). The use of fipronil has also been reported in soybeans and corn agricultural practices, being the main crops developed in Argentina (Souza Casadinho, 2009). Moreover, fipronil is also applied in cotton, yerba mate, tobacco, citric and livestock as insecticide, and as acaricide for lice and tick in domestic treatments (Villamil Lepori et al., 2013; Schaaf, 2015). Considering the widely use of fipronil on different crops and non-agricultural outdoor uses, information regarding its environmental fate and bioavailability is of great concern (Brennan et al., 2009).

Fipronil can often reach aquatic environments via surface runoff or drift from aerial or ground based spraying applications. Some inputs to the environments have reported concentrations up to 5.5 $\mu g.kg^{-1}$ in sediments of rivers and lakes receiving runoff (Schlenk et al., 2001). Besides, from non-agricultural sources, fipronil can enter in the aquatic environments as treated effluents and also as biosolids applied on land that came from sludges reaching a mean concentration of 91 $ug.kg^{-1}$ total fiproles (Sadarias et al 2019). In the aquatic environment, fipronil is rapidly partitioned between water

and sediments (Maul et al., 2008; Tingle et al., 2003). Among physicochemical properties, fipronil is moderately hydrophobic (log K*ow*= 4.0) and it could be degraded to its major metabolites: f. desulfinyl, f. sulfone, f. sulphide and f. amide by the processes of photolysis, oxidation, reduction and hydrolysis, respectively (Bobé et al., 1998). Once adsorbed to particles, the fipronil and its metabolites are relatively persistent, being the degradation products more persistent than the parental compound (Lin et al., 2008; 2009; Brennan et al., 2009). Contaminated sediments may be toxic to aquatic life through direct contact or as a food resource (Hirsch, 1998). Studies with sediment-associated fipronil have mainly been focused on acute toxicity for aquatic organisms, while sublethal responses have received limited research attention and mainly are focused on benthic invertebrates (Moran et al., 2012). In this context representative data using a sensitive test organism was reported by Maul et al. (2008) showing effects on growth of larval *Chironomus tentans* exposed to spiked sediments with fipronil, f. sulphide and f. sulfone from 83 to 111 µg kg^{-1}. Moreover, effects of fipronil and its metabolites on oxidative stress also have been reported in *Eupemphix nattereri* tadpoles after spiked sediments exposure at concentrations of 35, 120 and 180 µg kg^{-1} (Gripp et al., 2017).

Biomarkers are a useful tool for monitoring the aquatic environment and evaluate possible toxic effects on organisms caused by xenobiotics, such as pesticides. In this context it has been reported that prolonged exposure to those pesticides could induce the production of reactive oxygen species (ROS), leading to an oxidative stress condition in fish (Stara et al., 2013; Hatami et al., 2019; Jiao et al., 2019). Previous studies have shown that fipronil exposure in fish promote oxidative stress leading to

lipid peroxidation and protein carbonization (Clasen et al., 2012; Wang et al., 2016; Gripp et al., 2017).

Prochilodus lineatus (Order Characiformes) is a strict illiophagus (mud and detritus feeder) leading to be in direct contact with pollutants in both water and sediment. This species is currently considered as a suitable bioindicator of pesticide pollution due to be highly sensitive, under laboratory and field conditions (Cazenave et al., 2009; Pereira et al., 2013; Moreno et al., 2014). Since *P. lineatus* constitutes one of the most important species of the Parano-Platense ichthyofauna (Bonetto et al., 1970), and due to the scarce information about the impacts of fipronil on fish and particularly on this species, it was developed this work. Thus, the aim of the present study was to evaluate the biomarkers responses in *P. lineatus* juveniles after exposure to fipronil-spiked sediments at two environmental relevant concentrations, as well as to know the distribution of fipronil and its metabolites in the aquatic environment (water, sediment, suspended particulate matter (SPM) and fish organs).

2. MATERIALS AND METHODS

2.1 Test organisms

Juveniles of *P. lineatus* (body weight: 12.8 ± 0.2 g; n=24) were supplied by a fish farming facility of 25 de Mayo, Misiones province, Argentina. The fish were acclimated for two weeks in 50 L glass aquaria with running well water under temperature 21 °C ± 1, pH 8.5, dissolved oxygen (DO) 8.5 ± 0.2 mg O_2 L^{-1} and conductivity 1100 ± 50 µS cm^{-1}. During this period, they were fed everyday with commercial fish food (Truchas Crumbles, Gepsa) containing 36% protein.

2.2 Sediment preparation

Surface sediment was collected from a site located in Las Flores creek (Luján, Buenos Aires province, Argentina) with scarce anthropic impact. Sediment of this site has been previously characterized and assigned as a reference by other authors (Ronco et al., 2008, Peluso et al., 2013; Scarcia et al., 2014). Once in the laboratory, sediment was processed, debris and macrofauna was manually extracted, then sediment was dried at room temperature. Subsequently, dry sediment was ground and sieved with a mesh size of 1190 microns allowing to pass up to sand-sized particles. Sieved sediment was then placed into amber glass containers and assigned to control and fipronil-spiked sediments with 5.5 µg kg^{-1} (C1) and 82 µg kg^{-1} (C2). Spiked sediments were prepared in a glass mortar by mixing fipronil Analytical standard (Pestanal Sigma-Aldrich 98.6% pure) dissolved in ethanol with an initial inoculum of sediment and after homogenized, transferred to amber glass containers. This procedure was repeated until all the sediment was fortified. Then, the sediments were continuously mixed in an end-over-end system for 15 days. Organic carbon content of sediment was about 5% and sediment composition was previously characterized by Scarcia (2014) with the following grain size distribution: sand 61%, clay 12% and silt 27%. Spiking concentrations C1 and C2 were selected considering maximum reported levels in the environment (Schlenk et al, 2001) and a toxicity threshold level arbitrarily defined as the 25% value of LC50 of a *Hyalella Azteca* 10 day sediment test normalized to 5% organic carbon content (Hintzen et al. 2009).

2.3 Experimental design

The study was conducted according to the recommendation of the local and National Institutes of Health Guidelines (Resolution 672-15, National University of Lujan). Maintenances and experiments with fish were followed with national and institutional guidelines (CONICET, 2005) for the protection of animal welfare and National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Whole sediment static bioassays were conducted in glass aquaria containing 44 L of unchlorinated tap water and 6 kg of control (C) or fipronil-spiked sediment (C1 and C2). After the addition of the sediment, the system was allowed to stabilize for 24 h before adding the organisms. Fish were randomly distributed to each experimental treatment (n=8) and remained exposed for 15 days under constant aeration and photoperiod (12L/12D); no food was supplied during the assay. In order to evaluate the dispersion and availability of fipronil and its metabolites in the different matrixes (water, sediment and SPM), a similar test was conducted simultaneously in aquaria without fish for both assayed concentrations (C1 and C2). Water quality parameters including temperature, dissolved oxygen, pH and conductivity were monitored periodically. Ammonium levels in the water were measured during the experiment following standard procedure (APHA, 2005). Water (150 mL) and sediment (10 g) samples from all treatments and aquaria were collected at 24 hours (after system stabilization) and 15 days (end of experiment), and then stored at -20 °C for fipronil quantification. After the 15 days of exposure, *P. lineatus* were anesthetized with 10 mg L^{-1} MS-222 (3-aminobenzoic acid ethyl ester methane sulfonate salt, Sigma-Aldrich) (Topic Popovic et

al., 2012) solution and body weight and total length were recorded and euthanized by an incision behind the operculum. Then liver, gills and brain were removed, weighed and kept at -80 °C until biomarker and chemical analyses. Condition factor (CF = (total weight / total length^3) x 100) and hepatosomatic index (HSI = (liver weight / total weight) x 100) were calculated according to Bagenal and Tesch (1978) and Sloof et al. (1983), respectively. Bioconcentration factor (BCF) was calculated for each organ (liver and gills) as the ratio between total fipronil concentration in the organ (µg kg^{-1}) to the respective total concentration in the surrounding media: sediment (µg kg^{-1}) and water + SPM (µg L^{-1}) for both concentration groups.

2.4 Chemical analysis

2.4.1 Standard materials and reagents

Identification and quantification of fipronil and its metabolites f. desulfinyl, f. sulphide and f. sulfone, were performed using external standard solutions from AccuStandard (USA), whereas PCB #103 (Ultra Scientific, USA) was used as internal standard. High purity n-hexane, acetone and dichloromethane (residue analysis grade) were used as solvents for the analytical procedure. Anhydrous sodium sulfate and silica gel were purchased from Merck Inc. (Germany).

2.4.2 Extraction and clean-up procedure

Water, SPM, sediment samples and fish organs were analyzed.

Previous to the extraction, water samples (150 mL) were filtered with micro-glass fiber filters (1.2 µm pore size, 47 mm ID) in order to obtain SPM. Then, water samples

(n=16) (20 mL) were extracted using 12 mL acetone:dichloromethane (DCM) mixture (2:1 v/v,) by shaking during to 2 h. Then, the organic layer was removed and concentrated under nitrogen flow to 1 mL for additional clean-up with silica gel chromatography (previously activated to 200 °C, 24 h). The column was preconditioned with 5 mL of DCM and eluted with 30 ml of acetone:DCM mixture (2:1 v/v). The purified fraction was then concentrated in hexane to 1 mL in amber glass vials, and kept at -20 °C until gas chromatographic analyses

After filtration, SPM filters (n=12) and sediment (n=16) samples (5 g) were ground in a mortar with anhydrous sodium sulfate (2.5 g) and then extracted with 25 mL acetone:DCM mixture (1:1 v/v) via sonication for three times (15 minutes, 20 °C, 400 W, 40 Khz). Extracts were centrifugated (10 minutes, 960 g, 20 °C) to separate the organic layer, and then solvent was transferred to vials and concentrated under nitrogen flow to 1 mL.

Liver and gills (n=12) from 6 fish randomly sampled (2 fish from each aquaria) were weighed and dried with anhydrous sodium sulfate until a homogeneous powder was obtained. Then, these samples were extracted with acetone:DCM mixture (1:1) (twice the volume necessary to cover the sample) via sonication in the same conditions previously described for SPM and sediment. After centrifugation, the organic layer was removed and concentrated in vials to 1 mL. Extracts of SPM filters, sediment and fish organs were further purified with silica gel chromatography, as was previously described for water samples.

2.4.3 Analytical procedure

Fipronil and the three metabolites f. sulfone (oxidation), f. sulphide (reduction) and f. desulfinyl (photolysis) were analysed. These compounds were identified and quantified using a gas chromatograph Shimadzu 17-A equipped with a ^{63}Ni electron capture detector (GC-ECD). The capillary column used was SPB-5 (30 m, 0.25 mm ID, 0.25 μm film thickness, Supelco, USA). One microliter was injected on a splitless mode (275 °C) and detector was kept at 310 °C. The oven temperature was held at 100 °C for 1 min, followed by an increase of 20 °C min^{-1} up to 180 °C (held for 1 min), 1 °C min^{-1} up to 215 °C (held 1 min). Helium was used as carrier (1.5 mL min^{-1}) and nitrogen (1.5 mL min$^{-1)}$ as make-up gas. Identification and quantification of compounds were performed by injection of fipronil and its metabolites standard solutions and PCB #103 as internal standard.

2.4.4 Quality control and assurance

Procedural and instrumental blanks were analyzed throughout the procedure to check for possible laboratory contamination. The laboratory-spiked sediment was a successfully procedure with recoveries between 78 to 87%. Fipronil or metabolites levels in the blanks were below the detection limit. Surrogate recovery (PCB #103) was greater than 90%. Method detection limits were calculated according to Keith et al. (1983), and ranged between 0.01-0.03 ng mL^{-1}.

2.5 Biochemical analyses

2.5.1 Sample preparation

Aliquots of -80 °C frozen tissues were weighed and homogenized in ice with buffer pH 7.4 (0.1 M NaH_2PO_4; 0.15 M KCl; 1 mM EDTA; 1 mM DTT; 10% v/v glycerol) according to Nilsen et al. (1998). Aliquots of initial homogenate was used for lipid peroxidation determination and the remaining homogenate was centrifuged (20 min, 10,000 g, 4 °C) and the supernatant fraction was reserved for the evaluation of the other biochemical parameters.

2.5.2 Oxidative damage

Lipid peroxidation (LPO) was measured by the thiobarbituric acid reaction (TBARS) according to Oakes and van der Kraak (2003). The initial homogenate was added to a mixture composed of 8.1% sodium dodecyl sulfate (SDS), 20% acetic acid (pH 3.5), 0.8% thiobarbituric acid (TBA) and butylated hydroxytoluene (BHT) 1,407 mM solutions and incubated at 95 °C for 1 h. After cooling, Milli-Q water and n-butanol (99.4%) were added, and the mixture was centrifuged (10 min, 200 g, 15 °C) to separate the organic layer. The chromogen formed was measured by fluorometry at an excitation wavelength of 516 nm and an emission wavelength of 560 nm using a microplate reader (BioTek Synergy HT). The malondialdehyde concentration was expressed as nmol TBARS g^{-1} of wet tissue; tetramethoxypropane was used as an external standard.

The protein oxidation (PO) level was evaluated according to Reznick and Packer (1994) with modifications (Ansaldo et al., 2007), by detecting the formation of protein hydrazones as a result of the reaction of dinitrophenyl hydrazine (DNPH) with protein carbonyls. After the protein hydrazone formation, they were precipitated using TCA 30% and then washed 3 times with ethanol:ethyl acetate (1:1 v/v). After the final

wash, the protein was solubilized in 1 mL of urea (6 M in 20 mM potassium phosphate, pH 2.5) and then incubated at 37 °C for 45 min. The final solution was centrifuged (10,000 g, 5 min) to remove any insoluble material. The carbonyl content was calculated from the absorbance measurement at 375 nm (UV-VIS 1800 Shimadzu), using an absorption coefficient ε= 22,000 M^{-1} cm^{-1}. The results were expressed at nmol carbonyl mg $protein^{-1}$.

2.5.3 Antioxidant defenses

Superoxide dismutase (SOD) activity was evaluated following an indirect method involving the inhibition of cytochrome c reduction by the competition with SOD for the superoxide anion radical ($O_2^{\cdot-}$) formed by the xanthine/xanthine oxidase system (McCord and Fridovich, 1969). Changes in the absorbance were recorded by spectrophotometry at 550 nm in a UV-VIS 1800 Shimadzu. The activity was expressed as units of SOD mg $protein^{-1}$, where 1 SOD unit (U) is defined as the enzyme quantity that causes 50% of inhibition of reduction of cytochrome c per minute.

The content of reduced glutathione (GSH) was determined according to the method of Beutler (1963), by the reaction of glutathione with the color reagent 5,5-dithiobis-2-nitrobenzoic acid (DTNB), forming a thiolate anion (TNB), which was measured at 412 nm (UV-VIS 1800 Shimadzu). The GSH levels were expressed in nmol GSH mg $protein^{-1}$.

Total antioxidant capacity against peroxyl radical (ACAP) was determined according to Amado et al. (2009) by fluorometry (ex/em 485/525 nm) (BioTek Synergy HT) in samples treated or not with a peroxyl radical generator. Peroxyl radicals were produced by thermal (37 °C) decomposition of 2,2'-azobis(2-methylpropionamidine)

dihydrochloride (ABAP; 4 mM; Aldrich) and measured with the fluorogenic compound 2',7'-dichlorofluorescein diacetate (H_2DCF-DA) at a final concentration of 40 µM in according to the methodology and further modifications adopted by Monserrat et al. (2014). The results were expressed as difference of fluorescence units (FU) at 30 min in the same sample with and without ABAP and standardized to FU at 30 min without ABAP (background area). For interpretative purposes, a small bar in the graph means a higher antioxidant capacity and viceversa.

2.5.4 Cholinesterase activity and protein estimation

Acetylcholinesterase activity (AChE) was measured only in brain supernatant fractions, according to Ellman et al. (1961) using acetylthiocholine iodide as substrate and dithiobisnitrobenzoic acid (DTNB). The change in absorbance was recorded at 412 nm (UV-VIS 1800 Shimadzu) at 10 sec intervals for 2 min. AChE activity was calculated as nmol min^{-1} mg $protein^{-1}$.

The total protein quantity of the supernatant fractions was measured by the method of Lowry et al. (1951) using bovine serum albumin as standard.

2.5.5 Statistical analysis

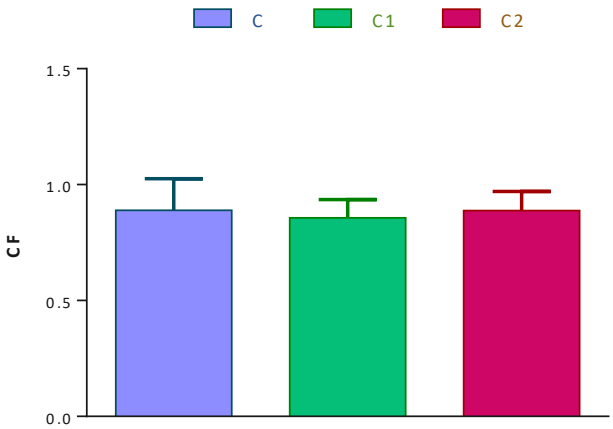
Biological parameters are reported as mean ± standard error and were first tested for normality and homoscedasticity using Kolmogorov and Levene tests, respectively. Variables that had not a normal distribution and/or homogeny of variance were transformed using Log and tested again, prior to parametric analysis. Differences between fipronil concentrations (C1 and C2) and control group (C) were evaluated

using One-way analysis of variance, followed by a posteriori Tukey test. Differences were considered statically significant when $p<0.05$. All statistical analysis was performed by the InfoStat software (Di Rienzo et al., 2012).

3. RESULTS AND DISCUSSION

No mortality was registered along the bioassays indicating that the concentrations used were sublethal for juveniles of *P. lineatus*.

The health condition of *P. lineatus* was evaluated by the CF and HSI physiological parameters, (Fig. 1). CF is a useful parameter that reflects general condition of the fish and HSI could provide information about possible liver abnormalities (van der Oost et al., 2003). Both in combination are used as a general indicator of potential pollution impacts. In this context, our results indicated that under the assayed conditions, exposure to spiked sediments with fipronil did not promote any difference in these parameters when compared to control fish.



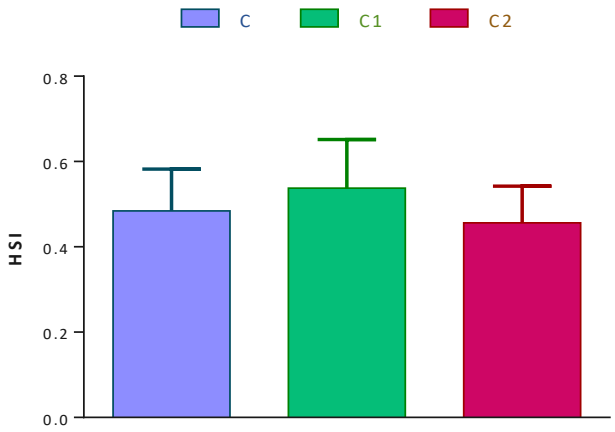


Figure 1. Physiological indices of *P. lineatus* after exposed for 15 days to control conditions (C) or fipronil (C1 = 5.5 µg kg^{-1}, C2 = 82 µg kg^{-1}) (mean ± SD). * indicates significant differences between control and exposed group ($p \leq 0.05$).

3.1 Water quality parameters in aquaria

The physico-chemical parameters of the water such as temperature, dissolved oxygen, pH and conductivity were similar as in the acclimated period detailed previously and remained constant throughout the experimental period (Table 1). No differences were observed between control conditions (C) and fipronil concentrations (C1 and C2) and also between aquaria with and without fish. *P. lineatus* is a fish species adaptable to a broad range of environmental conditions were optimal fingerling survival was recorded at temperature between 19 – 23 °C and pH range 4.0 - 9.8 (Vidal, 1967; Zaniboni-Filho et al., 2002). Maximum ammonium concentrations were found in aquaria with fish at C1 and C2 treatments (0.58 and 0.84 mg $N\text{-}NH_4^+$ L^{-1}). Moreover, all values measured were lower than the maximum permitted quantity (1.13 mg $N\text{-}NH_4^+$ L^{-1}) according the National Law N° 24051 as well as the recommended aquatic life chronic criteria by USEPA, 1999 (1.2 mg $N\text{-}NH_4^+$ L^{-1}).

Table 1. Physico-chemical parameters of the water measured on aquaria with/without fish under control conditions (C) or exposed to fipronil concentrations, 5.5 µg kg^{-1} (C1) and 82 µg kg^{-1} (C2). Values are expressed as mean ± standard deviation.

	Aquaria with fishes			**Aquaria without fishes**		
	C	**C1**	**C2**	**C**	**C1**	**C2**
Temperature (°C)	21.7 ± 0.5	23.3 ± 1.7	23.2 ± 1.6	20.4 ± 1.2	20.3 ± 1.2	20.6 ± 1.2
Dissolved Oxygen (mg O_2 L^{-1})	8.4 ± 0.2	8.0 ± 0.3	8.08 ± 0.4	8.8 ± 0.2	8.7 ± 0.2	8.5 ± 0.1
pH (pH units)	9.2 ± 0.1	9,2	9,2	9.5 ± 0.1	9.4 ± 0.1	9.3 ± 0.1
Conductivity (µS cm^{-1})	1115 ± 48	1099 ± 52	1099 ± 47	1116 ± 17	1150 ± 47	1132 ± 45
Ammonium (mg N-NH_4^+ L^{-1})	0.16 ± 0.19	0.58 ± 0.29	0.84 ± 0.49	0.21 ± 0.35	0.38 ± 0.31	0.33 ± 0.21

3.2 Chemical analysis

The nominal and measured concentrations of fipronil and its metabolites in the water, sediments and SPM in each exposure aquaria without and with fish are presented in Tables 2 and Table 3, respectively. The levels of fipronil or metabolites were low of detection limits in control group.

Table 2. Concentrations of fipronil and its metabolites f. desulfinyl, f. sulphide and f. sulfone in water, sediments and suspended particulate matter (SPM) (mean ± standard deviation) in aquaria without fish.

	24 h		*15 d*	
	Sediment (µg kg^{-1} d wt)	**SPM (µg kg^{-1} d wt)**	**Sediment (µg kg^{-1} d wt)**	**SPM (µg kg^{-1} d wt)**

Control				
Fipronil	<LD	<LD	<LD	<LD
F. Desulfinyl	<LD	<LD	<LD	<LD
F. Sulfide	<LD	<LD	<LD	<LD
F. Sulfone	<LD	<LD	<LD	<LD
C1 (5.5 µg kg-1)				
Fipronil	4.24 ± 0.18	68.66	1.30 ± 0.35	29.95
F. Desulfinyl	0.36 ± 0.17	34.64	0.3 ± 0.08	36.26
F. Sulfide	0.16 ± 0.07	14.22	0.33 ± 0.05	21.80
F. Sulfone	<LD	35.77	<LD	24.52
C2 (82 µg kg-1)				
Fipronil	74.45 ± 4.13		40.25 ± 4.69	22.83
F. Desulfinyl	0.55 ± 0.06		0.76 ± 0.08	41.12
F. Sulfide	<LD		16.34 ± 2.53	26.85
F. Sulfone	<LD		0.94 ± 0.18	8.07

<LD: below limit of detection.

Table 3. Concentrations of fipronil and its metabolites f. desulfinyl, f. sulphide and f. sulfone in sediments and suspended particulate matter (SPM) (mean ± standard deviation) in aquaria with fish.

	24 h			*15 d*		
	Water ($\mu g\ L^{-1}$)	**Sediment ($\mu g\ kg^{-1}$ d wt)**	**SPM ($\mu g\ kg^{-1}$ d wt)**	**Water ($\mu g\ L^{-1}$)**	**Sediment ($\mu g\ kg^{-1}$ d wt)**	**SPM ($\mu g\ kg^{-1}$ d wt)**

Control						
Fipronil	<LD	<LD	<LD	<LD	<LD	<LD
F. Desulfinyl	<LD	<LD	<LD	<LD	<LD	<LD
F. Sulfide	<LD	<LD	<LD	<LD	<LD	<LD
F. Sulfone	<LD	<LD	<LD	<LD	<LD	<LD
C1 (5.5 µg kg^{-1})						
Fipronil	0.41 ± 0.29	5.24 ± 0.45	42.03	0.19 ± 0.01	2.16 ± 0.35	39.7
F. Desulfinyl	0.09 ± 0.02	0.78 ± 0.04	39.01	0.14 ± 0.03	0.72 ± 0.02	25.74
F. Sulfide	<LD	0.59 ± 0.22	23.97	<LD	0.51	22.74
F. Sulfone	0.06 ± 0.05	<LD	<LD	0.14 ± 0.03	<LD	39.76
C2 (82 µg kg^{-1})						
Fipronil	0.32 ± 0.19	64.12 ± 5.70	33.37	0.25 ± 0.07	23.21	19.75
F. Desulfinyl	0.05 ± 0.01	0.66 ± 0.06	52.98	0.12	0.5	24.14
F. Sulfide	<LD	0.74 ± 0.13	34.75	<LD	10.53	34.18
F. Sulfone	0.08 ± 0.03	0.83 ± 0.06	34.71	0.18 ± 0.03	2.06	36.03

<LD: below limit of detection.

In aquaria without fish, fipronil sediment concentrations measured after 24 h of exposure were between 80-90% from the nominal concentrations (Table 2). The metabolite f. desulfinyl was present at both concentrations at levels lower than 10% of nominal fipronil. Moreover at C1, f. sulphide showed concentrations close to 3% of nominal fipronil. After 15 days of exposure, levels of parental were found to be 30 - 50% of the fipronil nominal concentrations (C1 and C2). At the lowest concentration (C1), f. sulfone was below the detection limit; however at C2, all metabolites were present. In all cases, at 24 h and 15 days the levels of parental fipronil were higher than metabolites. Previous work carried out in the laboratory lead to check the constant controlled conditions, with high prevalence of an aerobic environment. This fact supports the

occurrence of Fp. desulfinil as the main metabolite found, as product of photolysis. Moreover, the concentrations of Fp. sulphide markedly lower than the others metabolites also supports this statement.

In the case of SPM, after 15 days, parental and metabolites were present at both concentrations, being f. desulfinyl the major compound in this matrix.

A similar trend was observed in aquaria containing fish (Table 3). Thus, at 24 h of exposure, the measured concentrations in sediments of parental fipronil were almost 100 % for C1 and 80 % for C2. All metabolites were present at C2, at concentrations lower than 1 % of the parental compound, while f. desulfinyl and f. sulphide were found also at C1 with levels close to 10% of the nominal fipronil concentration. Shorter $t_{1/2}$ values (about 10-14.5 days) have been reported for fipronil in pond water and aerobic sediment (Zhu et al., 2004), and the major degradation products were f. desulfinyl and f. sulphide (Gunasekara et al., 2007). In the present work, at 15 days of exposure there was a drop of parental fipronil to 30-40% of the nominal concentration. In the case of metabolites, the levels were similar to those found at 24 h, with the exception of f. sulphide at C2 with concentrations 10-fold higher.

For both, C1 and C2 groups, fipronil and its metabolites f. desulfinyl and f. sulphide were detected in SPM at 24 h. F. desulfinyl was the main metabolite found representing 37 % (C1) and 34 % (C2) for total fipronil level (Table 3). In the present work, at 24 h in C2 group, f. sulfone was detected being 20 % of total fipronil. After 15 days, SPM showed a reduction of parental compound concentrations (5 % C1 and 40 % C2 from the initial levels). In the case of metabolites, the concentrations were slightly lower in almost all samples with the exception of f. sulfone at both concentrations.

Levels of parental fipronil and metabolites, when present, were below 0.5 ug L^{-1} in all water samples (Table 3).

At C1 group, f. desulfinyl was the main metabolite found in all matrix, mainly in sediment and SPM, along the time exposure. This result could be associated to the resuspension of spiked sediment with the swimming of fish, increasing the photodegradation of fipronil. Meanwhile, at C2 group f. sulphide was the major metabolite detected in all matrixes with a significant increase in concentration along time. It is known that under anoxic environment fipronil is reduced to the f. sulphide form (Ying and Kookana, 2002).

It is important to note that the metabolite f. sulphide showed in sediment a significant increase of its concentrations at 15 days of exposure under both treatments. This fact could be related to the compositions of the sediment in the assay conditions which produce a more reducing environment at 15 days leading to fipronil reduction to the f. sulphide metabolite. On the other hand, a prevalence of f. desulfinyl form in SPM, mainly at 24 h of exposure with fish and in both time conditions without fish, could be related to the high exposure of UV light which produce the photodegradation of the parental fipronil.

The levels of fipronil and its metabolites in gills and liver of *P. lineatus* are presented in Table 4. Contaminant concentrations in gills and liver followed the distribution pattern: f. sulfone > fipronil > f. desulfinyl at both treatments, while the f. sulphide metabolite levels were <LD.

Table 4. Concentrations of fipronil and its metabolites in fish and bioconcentration factors (BCF) after 15 days of exposition.

Fish organs	*Liver*	*Gills*
Control		
Fipronil	<LD	<LD
F. Desulfinyl	<LD	<LD
F. Sulphide	<LD	<LD
F. Sulfone	<LD	<LD
C1 (5.5 µg kg^{-1})		
Fipronil	172.4 ± 40.2	38.1 ± 4.8
F. Desulfinyl	95.5 ± 2.5	28.6 ± 0.7
F. Sulphide	<LD	<LD
F. Sulfone	380.2 ± 14.5	57.1 ± 2.0
BCF	191.0	6.3
C2 (82 µg kg^{-1})		
Fipronil	915.8 ± 406.6	67.9 ± 16.6
F. Desulfinyl	390.2 ± 86.3	37.8 ± 0.5
F. Sulphide	<LD	<LD
F. Sulfone	1,332.8 ± 571.8	168.2 ± 2.0
BCF	72.7	15.5

<LD: below limit of detection. Fish organ concentrations are expressed as µg kg w wt^{-1}

Liver showed higher concentrations of fipronil and its metabolites than gills in both exposed groups. The bioconcentration factors (BCF) were calculated for liver (liver/sediment) and gills (gill/ (water + SPM) (Table 4). As expected, *P. lineatus* liver exposed to fipronil concentrations showed higher BCFs values (191.0 and 72.7 for C1 and C2, respectively) than gills (6.3 and 15.5 for C1 and C2), indicating that a greater bioaccumulation occurs in the liver.

The higher concentrations of fipronil and metabolites found in liver in relation to gills at both experimental groups could be related to liver is the most important organ for storage and transformation of pollutants, in addition to the fact that spiked sediment was the main food source for the fish.

Xenobiotics substances may be metabolized in liver by non-synthetic alteration (oxidation, reduction or hydrolysis) of the parental compound of the substances and conjugation by phase I and phase II enzymes (Commandeur et al., 1995). Fipronil is transformed during phase I to f. sulfone by the cytochrome 450 (Wu et al., 2014). In this sense, f. sulfone constituted the main metabolite found in both organs at both experimental concentrations followed by the metabolite f. desulfinyl. It is important to note, that the metabolite f. sulfone was not detected in sediment, indicating that the metabolism of fipronil to f. sulfone has occurred in both organs. Moreover, there were not differences between the ratios f. sulfone/fipronil in both organs at both concentrations, probably due to the fipronil levels does not affect the rate of metabolism in liver and gills. A similar behavior was observed for the ratios f. desulfinyl/fipronil in both organs at both experimental concentrations.

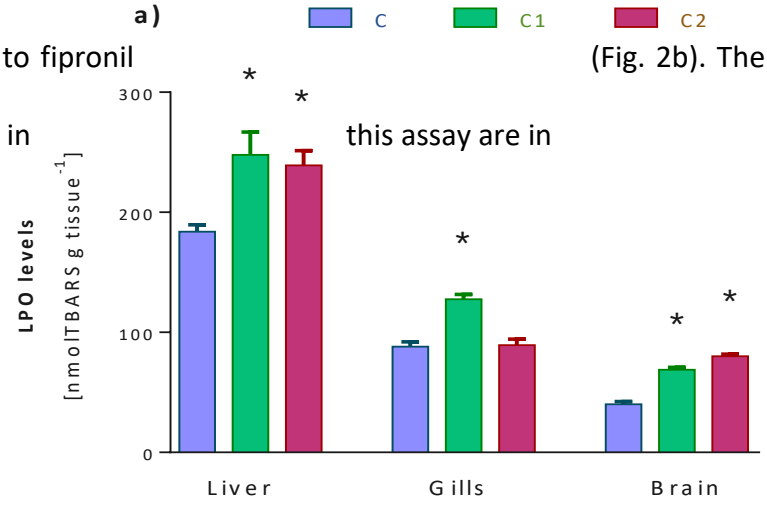
Other reports have documented similar values of fipronil bioaccumulation in fish (Baird et al., 2013). Thus, this study conducted a subchronic test with fathead minnows (*Pimephales promelas*) exposed to fipronil in water and sediment and reported f. sulphide as a major metabolite formed in sediment, while exposed fish rapidly accumulated fipronil and f. sulfide and transformed the majority to f. sulfone. Tissue-specific accumulation, biotransformation and elimination of fipronil in tilapia (*Oreochromis niloticus*) were reported by Li et al. (2018), where higher levels of fipronil

and f. sulfone were detected in liver and intestine. By other hand, Qu et al. (2018) showed that fipronil was rapidly transformed to f. sulfone and f. sulphide in loach (*Misgurnus anguillicaudatus*) liver in a 72-h acute toxicity test.

3.3 Biomarkers responses

Fipronil exposure induced significant lipid damage on fish in all the studied organs. In this context, LPO was verified in liver (35% C1 and 30% C2), gills (45% C1 only) and brain (72% C1 and 99% C2) (Fig. 2a). Similarly, a previous study with *Cyprinus carpio* juveniles showed an LPO increase in liver, gills and brain when were exposed to fipronil (0.65 µg L^{-1}) (Menezes et al., 2016). Furthermore, Gripp et al. (2017) also demonstrated that fipronil promoted an increase in LPO levels in *Eupemphix nattereri* tadpoles after sediment fortified exposure at three concentrations (35 µg kg^{-1}, 120 µg kg^{-1} and 180 µg kg^{-1}). Previous studies have been reported that fipronil could be responsible of formation of reactive oxygen species (ROS) and promote damage in macromolecules such as lipids, proteins and DNA (Wang et al., 2016; Gripp et al., 2017). Damage to proteins due to the direct action of ROS may even cause a loss of enzyme activity (Wong et al., 2010), while lipid peroxidation (LPO) cause alteration of cell membrane permeability and integrity (Valavanidis et al., 2006).

When oxidative damage in proteins was considered, PO levels showed a significant increase in liver (34% C1 and 108% C2) and in brain (65% C1 and 85% C2) after fish exposure to fipronil (Fig. 2b). The increments of PO observed in this assay are in



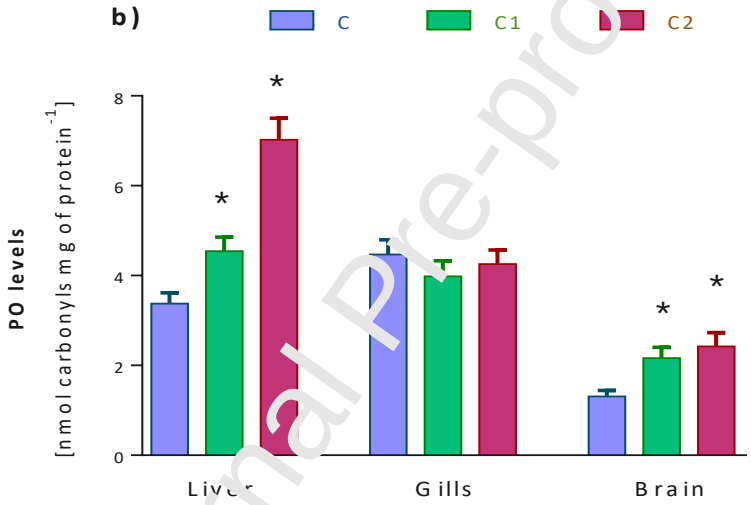


Figure 2. Lipid peroxidation (LPO) (a) and protein oxidation (PO) (b) levels in liver, gills and brain of *P. lineatus* exposed to control conditions (C) or fipronil (C1 = 5.5 µg kg^{-1}, C2 = 82 µg kg^{-1}) (mean ± SD). * Indicates significant difference between control and exposed group ($p \leq 0.05$).

agreement with PO levels reported in liver by Clasen et al. (2012) when juveniles of *C. carpio* were exposed for 30 and 90 days to 0.65 mg L^{-1} fipronil.

Organisms are able to protect themselves from these damages by means an antioxidant defense system consisting in antioxidant enzymes (e.g. SOD, CAT) and non-enzymatic defenses (e.g. GSH) that acts by reducing the concentration of ROS and maintaining the redox state in cells and organisms (da Silva Barreto et al., 2018).

Changes in SOD activity and GSH content in *P. lineatus* promoted by fipronil exposition are shown in Figure 3.

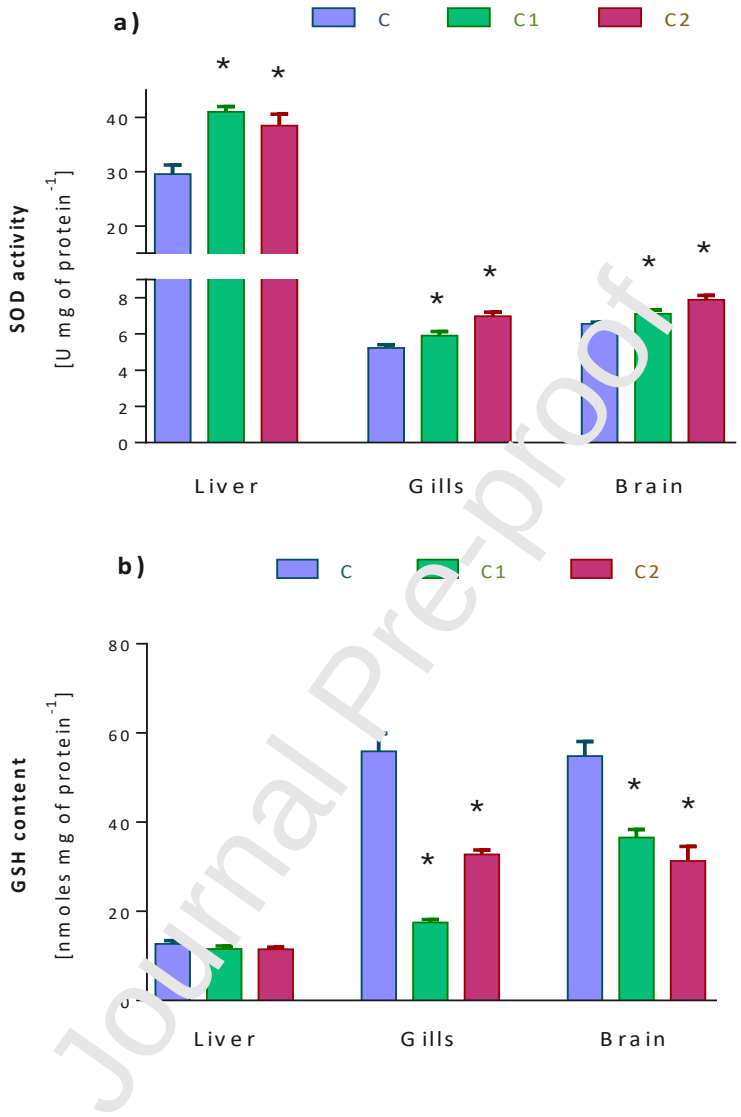


Figure 3. Antioxidant defenses in liver, gills and brain of *P. lineatus* exposed to control conditions (C) or fipronil (C1 = 5.5 µg kg^{-1}, C2 = 82 µg kg^{-1}). (a) SOD activity; (b) GSH content (mean ± SD). * Indicates significant differences between control and exposed group ($p \leq 0.05$).

In this study, a significant increase in SOD activity was observed after exposure to both concentrations in liver (39% C1 and 30% C2), gills (13% C1 and 34% C2) and brain (8%

C1 and 20% C2) (Fig. 3a). Alterations in SOD activity in the three organs are probably a response to the LPO and PO high levels detected for protecting fish against oxidative stress. Gupta et al. (2013), observed an increase in SOD activity in liver when *C. carpio* were exposed for 15 days to sublethal concentration (0.142 mg L^{-1}) of fipronil. Liver has an important role in the detoxification of pesticides. Thus, the significant increase on hepatic SOD activity could contribute to scavenge ROS generated by fipronil exposure. In gills and brain, a lower increment of SOD activity was observed; therefore, our results may indicate that SOD was not the principal antioxidant defense in these two organs against fipronil exposure.

The GSH, as a non-enzymatic antioxidant defense, is considered the first line of defense against ROS, participating in many cellular reactions by directly neutralizing pro-oxidants or acting as a substrate for catalyzing enzymatic reactions (Palermo et al., 2015). As the most abundant intracellular antioxidant, GSH is a major antioxidant which scavenges hydroxyl radicals in cells (Wang et al., 2016). The cell's ability to reduce glutathione is the key to how effectively the cell can manage the oxidative stress (Matés, 2000). In this study, GSH content showed a significant decrease in gills (29% C1 and 13% C2) and brain (33% C1 and 43% C2) after fish exposure to fipronil-spiked sediment (Fig. 3b). These results suggest that GSH behave as the main cellular defense in the detoxification of these organs. On the contrary, no significant alteration was observed in GSH levels in liver for all experimental treatments (Fig. 3b). The lack of response in the hepatic GSH content of *P. lineatus* exposed to fipronil could be explained that the principal detoxification in liver was promoted by SOD activity. Our

results are in concordance with those reported in GSH content of liver, gills and brain of *C. carpio*, after acute exposure to fipronil and buprofezin (Ghazanfar et al., 2018). Determination of ACAP can provide a better understanding of how antioxidants interact with ROS (Amado et al., 2009). The advantage of this rapid technique is the capacity to establish an integrated antioxidant response of an organism against ROS. The alteration in the antioxidant capacity could be a consequence of an oxidative damage state. A few studies evaluated the influence of toxicant in alterations of total antioxidant capacity of aquatic organism (Monserrat et al., 2014; Mardirosian et al., 2015). The exposure to fipronil promoted a significant increase in ACAP values in liver (25% C1 and 36% C2), showing a decrease in total antioxidant capacity (Fig. 4).

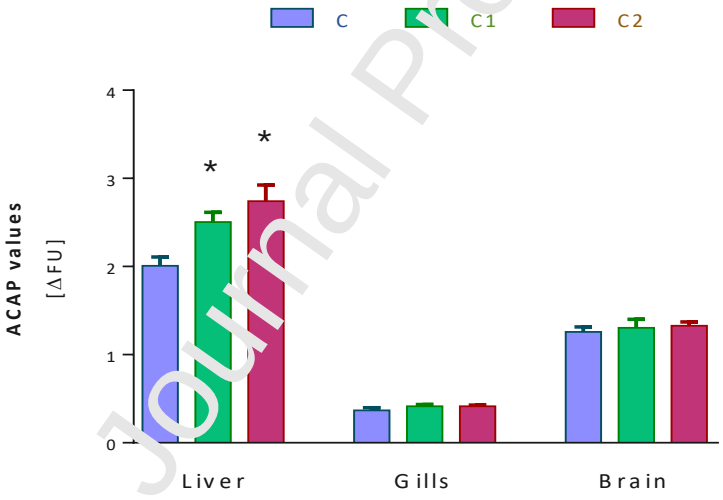


Figure 4. Total antioxidant capacity against peroxyl radical (ACAP) in liver, gills and brain of *P. lineatus* exposed to control conditions (C) or fipronil (C1 = 5.5 µg kg^{-1}, C2 = 82 µg kg^{-1}) (mean ± SD). * Indicates significant differences between control and exposed group ($p \leq 0.05$).

Besides, gills and brain ACAP showed no differences in fish exposed compared to the control group. In agreement, da Silva Barreto et al. (2018) showed that acute exposure of the pesticide chlorothalonil (100 µg L^{-1}) promoted a reduction in ACAP values in the

estuarine polychaete *Laeonereis acuta*. In our study, fipronil exposure promoted a decrease in hepatic antioxidant capacity meaning in a loss of functionality and irreversible damage evidenced by the highest levels of LPO and PO. The lack of variation in the antioxidant capacity in gills and brain could be consequence of the alteration in GSH content. Amado et al. (2009), demonstrated that liver samples spiked with GSH showed a reduction in ACAP values, which means higher antioxidant capacity. Although we didn't observe a decrease in ACAP in gills and brain, we neither observed an increase. So, the alterations in GSH content promoted by fipronil prevent the reduction of antioxidant capacity in gills and brain.

Alterations of brain acetylcholinesterase (AChE) activity in fish are frequently used and reported as a rapid method for ecotoxicological evaluations. In this context, inhibition of AChE activity in fish is recognized as an effect of several pesticides, such as carbamates, organophosphates and even heavy metals (Bocquené et al., 1998; Monserrat et al., 2002; Modesto and Martinez, 2010). Our results are in concordance with these previous studies. AChE activity was significant lower in brain after exposure to C2 (18%) treatment compared to control group (Fig. 5).

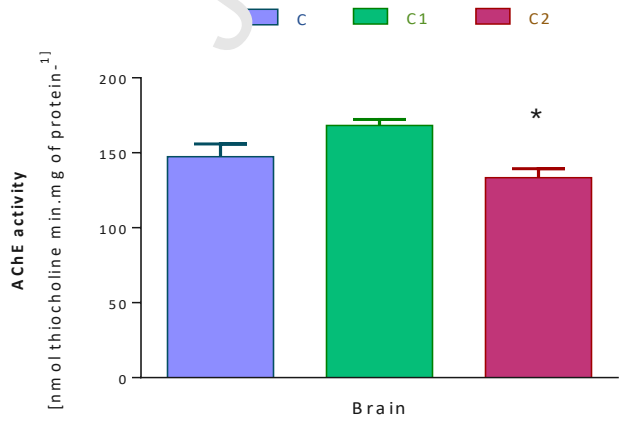


Figure 5. AChE activity in brain of *P. lineatus* exposed to control conditions (C) or fipronil (C1 = 5.5 µg kg^{-1}, C2 = 82 µg kg^{-1}) (mean ± SD). * Indicates significant differences between control and exposed group ($p \leq 0.05$).

Menezes et al. (2016) also found that fipronil can cause an inhibition of AChE activity in brain when *C. carpio* was exposed to a sublethal concentration. Among CUPs widely used for agricultural pests, chlorpyrifos is another than cause alterations in AChE. In a study with *Danio rerio* exposed to chlorpyrifos (400 µg L^{-1}), an inhibition of AChE was detected (Rodríguez-Fuentes et al., 2015).

According to the distribution and metabolism of total fipronil, no differences were observed between aquaria with or without fish. The percentages of recovery of parental fipronil and metabolite formation throughout the time of exposure were similar between both aquaria. Total fipronil concentrations were found to be distributed mainly in the sediment and to a lesser extent in water, which justifies the lipophilic character of fipronil and its reduced mobility towards the water matrix. Among the metabolites found, the presence of f. desulfinil in all matrices stands out, as a result of the photodegradation of fipronil because the aquaria were exposed to artificial light. In sediment and SPM, f. sulfide is other detected metabolite, product of a reduction reaction generated in the sediment. In fish, the main compound detected at high levels was f. sulfone, due to oxidation inside the fish.

Tested concentrations of fipronil and time exposure were sufficient to promote effects on the evaluated biomarkers in the selected fish species without affecting survival. In this context our results show that, even though physiological indexes were not responsive, fipronil promote oxidative damage to lipids and proteins and alter the antioxidant defense system in the three organs evaluated. It should be pointed out

that liver was the target organ of fipronil exposure, mainly as a result of a significant increase in lipid peroxidation and oxidized proteins and a decrease in antioxidant capacity. Antioxidant defenses (mainly mediated by SOD) were not sufficient to counteract this damage. In this organ the high levels of total fipronil and high BCF values detected, resulted in significant bioaccumulation particularly of f. sulfone and parental fipronil. Since f. sulfone is more toxic to aquatic organisms than parental fipronil, the main cause of oxidative damage in the liver could be attributed to the f. sulfone metabolite and secondly to the parental compound.

4. CONCLUSIONS

The present study is to our knowledge, the first report to evaluate the accumulation of fipronil and its potentially harmful effects on *Prochilodus lineatus* juveniles under an experimental fortified sediments assay. The levels of parental fipronil and its metabolites, mainly f. desulfinyl and f. sulphide, were higher in sediment and SPM than in water, which is an important factor to be considered for possible toxic effects in benthic organisms. Furthermore, fipronil, f. desulfinyl and f. sulfone were accumulated by fish, both in liver and gills, with the higher concentrations found in liver. Antioxidant defenses were differentially triggered mainly by SOD and GSH, moreover, lipid and protein oxidative damage were elicited in all three organs. The brain damage by the highest fipronil concentration exposure was denoted by an AChE inhibition response. The important decrease in total antioxidant capacity observed in the liver highlights that this organ was the most affected by fipronil exposure. The

multibiomarker responses elicited in the native fish proved to be a valuable endpoint of the toxicity exerted by bioavailable forms of fipronil in aquatic environments.

ACKNOWLEDGEMENTS: All sampling was performed under permission and complies with current national legislation. We thank to Julián Maggio for they support on the experiments. This study was funded by the Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET (PIP N° 0596/ 2014-2016, F. de la Torre), by Agencia Nacional de Promoción Científica y Tecnológica, ANPCyT (PICT 2015-2016, K. Miglioranza) and the Departamento de Ciencias Básicas e INEDES, Universidad Nacional de Luján. This work is part of the PhD thesis of A. Santillán Deiú.

REFERENCES

Amado, L.L., Longaray Garcia, M., Ramos, P.B., Freitas, R.F., Zafalon, B., Ribas Ferreira, J.L., Yunes, J.S., Monserrat, J.M., 2009. A method to measure total antioxidant capacity against peroxyl radicals in aquatic organisms: Application to evaluate microcystins toxicity. *Science of The Total Environment* 407: 2115–2123.

Ansaldo, M., Sacristan, H., Wider, E. (2007). Does starvation influence the antioxidant status of the digestive gland of *Nacella concinna* in experimental conditions? *Comparative Biochemistry and Physiology, Part C* 146: 118–123.

APHA, American Public Health Association. (2005). Standard Methods for the Examination of Water and Wastewater. Washington, DC, USA.

Bagenal, T.B., Tesch, F.W. (1978). Methods for assessment of fish production in fresh waters. In: Bagenal, T.B. (Ed), Age and growth, Blackwell Scientific Publications, Oxoford 101-136.

Baird, S., Garrison, A., Jones, J., Avants, J., Bringolf, R., Black, M. (2013). Enantioselective toxicity and bioaccumulation of fipronil in fathead minnows (*Pimephales promelas*) following water and sediment exposures. *Environmental Toxicology and Chemistry* 32: 222–227.

Beutler, E., Duron, 0., Kefly, B.M. (1963). Improved method for the determination of blood glutathione. *The Journal of Laboratory and Clinical Medicine* 61: 882-888.

Brennan, A. A., Harwood, A. D., You, J., Landrum, P. F., Lydy, M. J. (2009). Degradation of fipronil in anaerobic sediments and the effect on porewater concentrations. *Chemosphere* 77: 22–28.

Bobé, A., Meallier, P., Cooper, J. F., Coste, C. M. (1998). Kinetics and mechanisms of a biotic degradation of fipronil (hydrolysis and photolysis). *Journal of Agricultural and Food Chemistry*, 46: 2834–2839.

Bocquené, G., Galgani, F. (1998). Biological effects of contaminants: Cholinesterase inhibition by organophosphate and carbamate compounds. *ICES Techniques in Marine Environmental Sciences* 22: 1-12.

Bonetto, A.A., Cordiviola de Yuan, E., Pignalberi, C. (1970). Nuevos datos sobre poblaciones de peces en ambientes lénticos permanentes del Paraná Medio. *Physis* 30: 141–154.

Cazenave, J., Bacchetta, C., Parma, M. J., Scarabotti, P. A., Wunderlin, D. A. (2009). Multiple biomarkers responses in *Prochilodus lineatus* allowed assessing changes in the

water quality of Salado River basin (Santa Fe, Argentina). *Environmental Pollution* 157: 3025-3033.

Clasen, B., Loro, V.L., Cattaneo, R., Moraes, B., Lópes, T., de Avila, L.A., Zanella, R., Boschmann Reimche, G., Baldisserotto, B. (2012). Effects of the commercial formulation containing fipronil on the non-target organism *Cyprinus carpio*: implications for rice-fish cultivation. *Ecotoxicology and Environmental Safety* 77: 45-51.

Commandeur, J.N., Stijntjes, G.J., Vermeulen, N.P. (1995). Enzymes and transport systems involved in the formation and disposition of glutathione S-conjugates. Role in bioactivation and detoxication mechanisms of xenobiotics. *Pharmacological Reviews* 47: 271-330.

CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), (2005). Marco Ético de Referencia para las Investigaciones Biomédicas en Animales de laboratorio, de granja y obtenidos de la naturaleza. Buenos Aires, Argentina. [en línea] https://www.conicet.gov.ar/wp-content/uploads/OCR-RD-20050701-1047.pdf

da Silva Barreto, J., de Melo Tarouco, F., de Godoi, F., Geihs, M. A., Abreu, F., Fillmann, G., Sandrini, J. Z. da Rosa, C. E. (2018). Induction of oxidative stress by chlorothalonil in the estuarine polychaete *Laeonereis acuta*. *Aquatic Toxicology* 196: 1–8.

Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M., Robledo C.W. InfoStat versión 2020. Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL http://www.infostat.com.ar

Ellman, G.L., Courtney, K.D., Valentino, A.J., Featherstone, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology* 7: 88–95.

Ghazanfar, M., Shahid, S., Qureshi, I.Z. (2018). Vitamin C attenuates biochemical and genotoxic damage in common carp (*Cyprinus carpio*) upon joint exposure to combined toxic doses of fipronil and buprofezin insecticides. *Aquatic Toxicology* 196: 43–52.

Gripp, H.S., Freitas, J.S., Almeida, E.A., Bisinoti, M.C. Moreira, A.B. (2017) Biochemical effects of fipronil and its metabolites on lipid peroxidation and enzymatic antioxidant defense in tadpoles (*Eupemphix nattereri* Leiuperidae). *Ecotoxicology and Environmental Safety* 136: 173-179.

Gunasekara, A.S., Troung, T., Goh, K.S., Spurlock, F., Tjeerdema, R.S. (2007). Environmental fate and toxicology of fipronil. *Journal of Pesticide Science* 32: 189-199.

Gupta, S.K., Pal, A.K., Sahu, N.P., Saharan, N., Mandal, S.C., Prakash, C., Akhtar, M.S., Prusty, A.K. (2012). Dietary microbial levan ameliorates stress and augments immunity in *Cyprinus carpio* fry (Linnaeus, 1758) exposed to sublethal toxicity of fipronil. *Aquaculture Research* 43: 893-906.

Hatami, M., Banaee, M., Nematdoost Haghi, B. (2019) Sub-lethal toxicity of chlorpyrifos alone and in combination with polyethylene glycol to common carp (*Cyprinus carpio*). *Chemosphere* 219: 981-988.

Hintzen, E.P., Lydy, M.J., Belden, J.B. (2009) Occurrence and potential toxicity of pyrethroids and other insecticides in bed sediments of urban streams in central Texas. Environ. Pollut. 157, 110–116.

Hirsch, M.P. (1998). Toxicity of silver sulfide-spiked sediments to the freshwater amphipod (*Hyalella azteca*). *Environmental Toxicology and Chemistry* 17: 601-604.

Jiao, W., Han, Q., Xu, Y., Jiang, H., Xing, H., Teng, X. (2019). Impaired immune function and structural integrity in the gills of common carp (*Cyprinus carpio* L.) caused by chlorpyrifos exposure: Through oxidative stress and apoptosis. *Fish and Shellfish Immunology* 86: 239-245.

Keith, L.H., Crumett, W., Wentler, G. (1983). Principles of environmental analysis. *Analytical Chemistry* 55: 2210–2218.

Li, H., You, J., Wang, W. (2018). Multi-compartmental toxicokinetic modeling of fipronil in tilapia: Accumulation, biotransformation and elimination. *Journal of hazardous materials* 360*:* 420-427.

Lin, K., Haver, D., Oki, L., Gan, J. (2008). Transformation and sorption of fipronil in urban stream sediments. *Journal of Agricultural and Food Chemistry* 56: 8594–8600.

Lin, K., Haver, D., Oki, L., Gan, J. (2009). Persistence and sorption of fipronil degradates in urban stream sediments. *Environmental Toxicology and Chemistry* 28: 1462-1468.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951). Protein measurement with Folin phenol reagent. *The Journal of Biological Chemistry* 193:265–275.

Mardirosian, M.N., Lascano, C.I., Ferrari, A., Bongiovanni, G.A., Venturino, A. (2015). Acute toxicity of arsenic and oxidative stress responses in the embryonic development of the common South American toad *Rhinella arenarum*. *Environmental Toxicology and Chemistry* 34: 1009–1014.

Matés, J.M. (2000). Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology* 153: 83-104.

Maul, J.D., Brennan, A.A., Harwood, A.D., Lydy, M.J. (2008). Effect of sediment-associated pyrethroids, fipronil, and metabolites on *Chironomus tentans* growth rate, body mass, condition index, immobilization, and survival. *Environmental Toxicology and Chemistry* 27: 2582–2590.

McCord, J.M., Fridovich, I. (1969). Superoxide dismutase. An enzymatic function for erythrocuprein (hemocuprein). *The Journal of Biological Chemistry* 244: 6049–6055.

Menezes, C., Leitemperger, J., Murussi, C., de Souza Viera, M., Adaime, M.B., Zanella, R., Loro, V.L. (2016). Effect of diphenyl diselenide diet supplementation on oxidative stress biomarkers in two species of freshwater fish exposed to the insecticide fipronil. *Fish Physiology and Biochemistry* 42: 1357-1368.

Modesto, K.A., Martinez, C.B.R. (2010). Roundup causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish *Prochilodus lineatus*. *Chemosphere* 78: 294-299.

Moran, P.W., Calhoun, D.L., Nowell, L.H., Kemble, N.E., Ingersoll, C.G., Hladik, M.L., Kuivila, K.M., Falcone, J.A., and Gilliom, R.J. (2012). Contaminants in stream sediments from seven U.S. metropolitan areas—Data summary of a National Pilot Study: U.S. Geological Survey Scientific Investigations Report 2011–5092, 66 p.

Monserrat, J.M., Bianchini, A., Bainy, A.C.D., 2002. Kinetic and toxicological characteristics of acetylcholinesterase from the gills of oysters (*Crassostrea rhizophorae*) and other aquatic species. *Marine Environmental Research* 54: 781–785.

Monserrat, J.M., Garcia, M.L., Ventura-Lima, J., González, M., Ballesteros, M.L., Miglioranza, K.S.B., Amé, M.V., Wunderlin, D.A. (2014). Antioxidant, phase II and III responses induced by lipoic acid in the fish *Jenynsia multidentata* (Anablapidae) and its influence on endolsulfan accumulation and toxicity. *Pesticide Biochemistry and Physiology* 108: 8-15.

Moraes, B.S., Loro, V.L., Pretto, A., da Fonseca, M.B., Menezes, C., Marchesan, E., Reimche, G.B., de Avila, L.A. (2009). Toxicological and metabolic parameters of the teleost fish (*Leporinus obtusidens*) in response to commercial herbicides containing clomazone and propanil. *Pesticide Biochemistry and Physiology* 95: 57-62.

Moreno, N.C., Sofia, S.H., Martinez, C.B.R. (2011). Genotoxic effects of the herbicide Roundup Transorb® and its active ingredient glyphosate on the fish *Prochilodus lineatus*. *Environmental Toxicology and Pharmacology* 37: 448-454.

National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th ed. Washington (DC): National Academies Press (US); 2011.

Nilsen, B.M., Berg, K., Goksøyr, A. (1998). Induction of Cytochrome P450 1A (CYP1A) in fish. A biomarker for environmental pollution. From: *Methods in Molecular Biology, Cytochrome p450 Protocols* 107: 423-438. Edited by Phillips I.R. and Shephard E.A. © Humana Press Inc., Totowa, NJ.

Oakes, K.D., van der Kraak, G.J. (2003). Utility of the TBARS assay in detecting oxidative stress in white sucker (*Catostomus commersoni*) populations exposed to pulp mill effluent. *Aquatic Toxicology* 63: 447–463.

Palermo, F.F., Risso, W.E., Simonato, J.D., Martinez, C.B. (2015). Bioaccumulation of nickel and its biochemical and genotoxic effects on juveniles of the neotropical fish *Prochilodus lineatus*. *Ecotoxicology and Environmental Safety* 116: 19–28.

Peluso, L., Rossini, G. B., Salibián, A. and Ronco, A. (2013). Physicochemical and ecotoxicological based assessment of bottom sediments from the Luján River basin, Buenos Aires, Argentina. Environ Monit Asses. 185(7), 5993-6002.

Pereira, L., Fernandes, M.N., Martinez, C.B.R. (2013). Hematological and biochemical alterations in the fish *Prochilodus lineatus* caused by the herbicide clomazone. *Environmental Toxicology and Pharmacology*, 35: 1-8.

Qu, H., Ma, R., Wang, F., Gao, J., Wang, P., Zhou, Z., Liu, D. (2018). The effect of biochar on the mitigation of the chiral insecticide fipronil and its metabolites burden on loach (*Misgurnus.anguillicaudatus*). *Journal of Hazardous Materials* 360: 214-222.

Reznick, A.Z., Packer, L. (1994). Oxidative damage to proteins: spectrophotometric method for carbonyl assay. *Methods in Enzymology* 233: 357–363.

Rodríguez-Fuentes, G., Rubio Escalante, F.J., Noreña-Barroso, E., Escalante-Herrera, K.S., Schlenk, D. (2015). Impacts of oxidative stress on acetylcholinesterase transcription, and activity in embryos of zebrafish (*Danio rerio*) following Chlorpyrifos exposure. *Comparative biochemistry and physiology. Toxicology & Pharmacology: CBP* 172-173: 19–25.

Ronco, A., Peluso, L., Jurado, M., Rossini, G.B., Salibián, A. (2008). Screening of sediment pollution in tributaries from the southwestern coast of the Rio de la Plata estuary. *Latin American Journal of Sedimentology and Basin Analysis* 15: 67–75.

Sadaria, A.M., Labban, C.W., Steele, J.C., Maurer, M.M., Halden, R.U. (2019) Retrospective nationwide occurrence of fipronil and its degradates IN U.S. wastewater and sewage sludge from 2001-2016, Water Research 155: 465-473.

Schaaf, A. A. (2015). Valoración de impacto ambiental por pesticidas agrícolas. *Observatorio Medioambiental* 18: 87-96.

Schlenk, D., Huggett, D.B., Allgood, J., Bennett, E., Rimoldi, J., Beeler, A.B., Block, D., Holder, A.W., Hovinga, R., Bedient, P. (2001). Toxicity of fipronil and its degradation products to *Procambarus* sp.: field and laboratory studies. *Archives of Environmental Contamination and Toxicology* 41: 325– 332.

Sloof, W., Van Kreijl, C.F., Baars, A.J. (1983). Relative liver weights and xenobiotic metabolizing enzymes of fish from polluted surface in the Netherlands. *Aquatic Toxicology* 4: 1-14.

Solis, M., Bonetto, C.A., Marrochi, M.N., Paracampo, A.H., Mugni, H.D. (2018). Aquatic macroinvertebrate assemblages are affected by insecticide applications on the Argentine Pampas. *Ecotoxicology and Environmental Safety* 148: 11-16.

Souza Casadinho, J. (2009). La problemática del uso de plaguicidas en argentina. Modelos productivos e impacto en el ambiente. Red de Acción en Plaguicidas y sus Alternativas de América latina RAPAL. [en línea] http://www.rapal.org/articulos_files/Plaguicidas_Argentina.pdf 15/4/2013

Stara, A., Kristan, J., Zuskova, E., Velisek, J. (2013). Effect of chronic exposure to prometryne on oxidative stress and antioxidant response in common carp (*Cyprinus carpio* L.). *Pesticide Biochemistry and Physiology* 105: 18-23.

Tingle, C.C.D., Rother, J.A., Dewhurst, C.F., Lauer, S., King, W.J. (2003) Fipronil: environmental fate, ecotoxicology and human health concerns. *Reviews of Environmental Contamination and Toxicology* 176: 1–66.

Topic Popovic, N., Strunjak-Perovic, I., Coz-Rakovac, R., Barisic, J., Jadan, M., Persin Berakovic, A., Sauerborn Klobucar, R. (2012). Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. *Journal of Applied Ichthyology* 28: 553-564.

USEPA, United States Environmental Protection Agency. (1999). Update of ambient water quality criteria for ammonia. Washington, DC: Office of Water, Office of Science and Technology.

Valavanidis, A., Vlahogianni, T., Dassenakis, M., Scoullos, M. (2006). Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicology and Environmental Safety* 64: 178–189.

van der Oost, R., Beyer, J., Vermeulen, N.P.E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology* 13: 57–149.

Vidal, J.C. (1967). Contribución al estudio biológico del sábalo de los ríos Paraná y Uruguay. Secr. Est. Agric. y Ganadería: 1-51.

Villamil Lepori, E., Bovi Mitre, G.B., Nassetta, M. (2013). Situación actual de la contaminación por plaguicidas en Argentina. *Revista Internacional de Contaminación Ambiental* 29: 25-43.

Wang, X., Martínez, M.A., Wu, Q., Ares, I., Martínez-Larrañaga, M.R., Anadón, A., Yuan, Z. (2016). Fipronil insecticide toxicology: oxidative stress and metabolism. *Critical Reviews in Toxicology* 46: 876-899.

Wong, C.M., Marcocci, L., Liu, L., Suzuki, Y.J. (2010). Cell signaling by protein carbonylation and decarbonylation. *Antioxidants and Redox Signaling* 12: 393-404.

Wu, H., Gao, C., Guo, Y., Zhang, Y., Zhang, J., Ma, E. (2014). Acute toxicity and sublethal effects of fipronil on detoxification enzymes in juvenile zebrafish (*Danio rerio*). *Pesticide Biochemistry and Physiology* 115: 9-14.

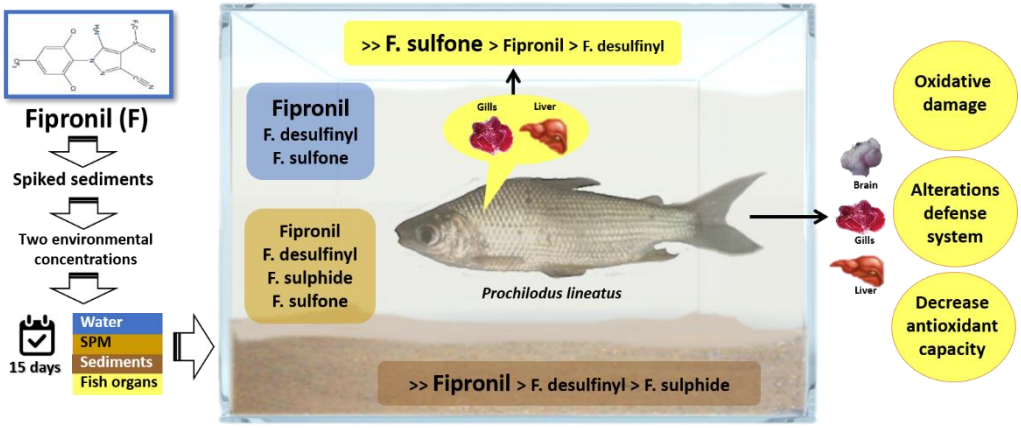
Wu, J., Lu, J., Lu, H., Lin, Y., Wilson, P.C. (2015). Occurrence and ecological risks from fipronil in aquatic environments located within residential landscapes. *The Science of The Total Environment* 518-519: 139-147.

Ying, G., Kookana, R. (2002). Laboratory and field studies on the degradation of fipronil in soil. *Australian Journal of Soil Research* 40: 1095-1102.

Zaniboni-Filho, E., Meurer, S., Golombieski, J.I., Silva, L.V.F., Baldisserotto, B. (2002). Survival of *Prochilodus lineatus* (Valenciennes) fingerlings exposed to acute pH changes. *Acta Scientiarum* 24: 917-920.

Zhu, G., Wu, H., Guo, J., Kimaro, F.M.E. (2004). Microbial degradation of fipronil in clay loam soil. *Water Air and Soil Pollution* 153: 35–44.

Graphical abstract



Highlights

- Exposure to fipronil (5.5 ug.kg^{-1}) was enough to promote adverse effects in *P. lineatus.*
- The metabolite fipronil sulfone was highly accumulated in liver and gills.
- Fipronil promoted oxidative damage in liver and gills.
- Liver was the most affected organ by fipronil exposure.