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DNA damage and oxidative stress induced by imidacloprid exposure in different tissues of the Neotropical fish *Prochilodus lineatus*

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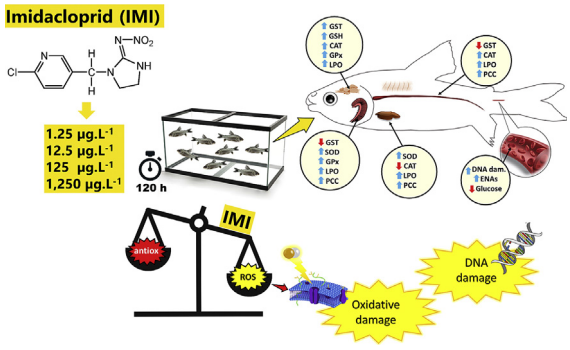
HIGHLIGHTS

- We evaluated acute effects of Imidacloprid (IMI) on the fish *Prochilodus lineatus*.
- IMI promoted oxidative damage in different fish tissues.
- IMI promoted hypoglycemia in the exposed fish.
- DNA damage was detected in erythrocytes of fish exposed to IMI.
- Liver, kidney and gill were the most affected organs by IMI exposures.

GRAPHICAL ABSTRACT



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ABSTRACT

Imidacloprid (IMI), a systemic neonicotinoid insecticide widely used in worldwide scale, is reported in freshwater bodies. Nevertheless, there is a lack of information about IMI sublethal effects on freshwater fish. Thus, the aim of this study was to identify the potential hazard of this insecticide to the South American fish *Prochilodus lineatus* exposed for 120 h to four IMI concentrations (1.25, 12.5, 125, and 1250 µg L^{-1}). A set of biochemical, genotoxic and physiological biomarkers were evaluated in different organs of the fish. IMI exposure induced significant changes in the enzymatic profiles of *P. lineatus*, with alterations in the activity of biotransformation and antioxidant enzymes in different tissues. Redox balance of the tissues was affected, since oxidative damage such as lipoperoxidation (LPO) and protein carbonylation (PCC) were evidenced in the liver, gills, kidney and brain of fish exposed to different IMI concentrations. Fish exposed to all IMI concentrations showed decreased blood glucose indicating an increase of energetic demand. DNA damage was evidenced by the comet test, in the erythrocytes of fish all the concentrations evaluated. We integrated these results in the Integrated Biomarker Response (IBR) index, which evidenced that the organs most affected by IMI exposure were the liver and kidney, followed by the gills. Our results highlight the importance of investigating different target tissues after IMI exposure and show the sublethal effects of IMI in some of them; they also warn to the possible consequences that fish living in freshwater ecosystems can suffer due to IMI exposure.

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1. Introduction

Neonicotinoids are one of the most important chemical classes of insecticides due to their high efficacy against a broad spectrum of insects and the versatility of their use, together with the fact that they are potent at low doses, relatively non-volatile, and highly water-soluble (EXTOXNET, 1996; Tomlin, 2000). Today this class of insecticides comprises at least seven major compounds with a market share of more than 25% of total global insecticide sales (Bass et al., 2015) and is replacing older classes such as organophosphate and carbamate insecticides worldwide (Jeschke et al., 2011). Neonicotinoids act selectively on insect nicotinic acetylcholine receptors (nAChRs), as agonists (Jeschke et al., 2011), competing for theses receptors in the postsynaptic membrane (Tomizawa and Casida, 2005; Anderson et al., 2015). Nicotinoids are much more toxic to insects than vertebrates due in part to differences in their interactions with the binding site of receptors (Tomizawa and Casida, 2009). However, Tennekes (2010) and Mason et al. (2012) suggested that neonicotinoid insecticides might be contributing to the decline of insectivorous birds in Europe, and fish, amphibians, bats, and birds around the world.

Imidacloprid (IMI) was the first neonicotinoid launched in 1991 and has become one of the major products in many pest control programs (Jeschke et al., 2011; Goulson, 2013). In Brazil, IMI was the tenth best-selling active ingredient during 2013, with approximately 8 thousand tons of compound sold (IBAMA, 2013). The use of IMI to control terrestrial pests could potentially result in unintended transport to aquatic habitats and indirect contamination through spray drift, atmospheric deposition, soil erosion, and runoff (CCME, 2007). A number of governmental agencies have developed guidelines on the acceptable IMI concentrations in surface water for the protection of aquatic organisms, ranging between 0.0083 (RIVM, 2014), 0.23 (CCME, 2007), and 1.05 $\mu g\,L^{-1}$ (USEPA, 2014). However, several studies have detected IMI concentrations in surface water greater than the guidelines values, in different aquatic environments around the world. Concentrations up to 49 $\mu g\,L^{-1}$ were already measured (Starner and Goh, 2012; Main et al., 2014; Gibbons et al., 2015; Struger et al., 2017), although the highest concentrations mentioned above were detected in water bodies close to farming areas (Sánchez-Bayo and Goka, 2005; Van Kijk et al., 2013; Morrissey, 2015). In Brazil, IMI concentrations of 2.18 (Bortoluzzi et al., 2006) and 3.65 $\mu g\,L^{-1}$ (Becker et al., 2009) have already been detected in aquatic environments influenced by agricultural activities. In a study of the risk classification of different neonicotinoids in Brazilian surface waters, Miranda et al. (2011) reported that IMI presents a greater risk of promoting serious effects on non-target organisms compared to other neonicotinoids. Additionally, according to Albuquerque et al. (2016), neonicotinoids together with other currently used insecticides such as fipronil, represent the greatest potential risk to local aquatic environments when compared to other classes of pesticides.

Despite IMI is being reported in surface waters at concentrations below those which will cause mortality to freshwater fish (SERA, 2005; Tišler et al., 2009), sublethal effects may occur, such as physiological stress and DNA damage (Gibbons et al., 2015). However, there is a lack of studies concerning IMI sublethal effects on fish. In this context, the aim of the present study was to evaluate the potentially harmful effects of IMI on biochemical, physiological, and genetic parameters of the Neotropical freshwater fish *Prochilodus lineatus*. This fish is an ecologically and economically important benthic species, widely found in rivers of the South and Southeast of Brazil where it comprises a large part of the ichthyomassa (Taylor et al., 2006). Besides, *P. lineatus* is commonly used in ecotoxicological studies given its sensitivity to pesticides (Langiano and Martinez, 2008; Modesto and Martinez, 2010; Pereira et al., 2013; Moreno et al., 2014). Additionally, this species meets the criteria of the OECD (1992) for selection of species for acute toxicity tests.

2. Material and methods

2.1. Fish handling, experimental design, and sampling

Juveniles of *P. lineatus* (n = 40; 23.3 ± 4.7 g; 12.6 ± 0.9 cm; mean ± SD) were supplied by the Fish Hatchery Station of the State University of Londrina. Fish were acclimated for five days in a 300 L tank containing dechlorinated tap water under constant aeration. The room photoperiod was fixed at a 12:12 h light/dark cycle. On the second and fourth days of acclimation, fish were fed with a commercial fish diet containing 36% protein (Guabi, Brazil). Feeding was suspended 24 h prior to the beginning of the experiments and the animals were not fed during the experiments.

After acclimation, the fish were randomly divided into five groups (n = 8 fish per group) and maintained in glass aquaria containing 80 L of dechlorinated tap water. One group was kept under control conditions only in clean water (IMI0) and the other four groups were exposed to different nominal concentrations of active ingredient from the commercial formulation of Imidacloprid® (48% a. i.- Nortox S.A. Brazil): 1.25 (IMI1.25), 12.5 (IMI12.5), 125 (IMI125), and 1250 $\mu g\,L^{-1}$ (IMI1250) for five days, under static conditions and daily water renewal. Imidacloprid is acutely toxic to adult fishes at relatively high concentrations, over 80 $mg\,L^{-1}$; however, juvenile fishes are considerably more susceptible (Cox, 2001). Thus, the lower IMI concentration tested was defined as an environmentally relevant concentration (1.25 $\mu g\,L^{-1}$) and the others were defined from this concentration in geometric progression of ratio 10 to a maximum concentration of 1.25 $mg\,L^{-1}$, which would be safe for juveniles of *P. lineatus*.

Water temperature, pH, dissolved oxygen, and conductivity were monitored throughout the experiment using a multiparameter water quality meter (HORIBA U-52, Japan). Every day, water samples (500 mL) were collected from all aquaria for analysis of IMI concentration, before water renewal. Aquaria were partially covered to avoid photo degradation of IMI.

After the exposure period, fish were anesthetized with benzocaine (0.1 $g\,L^{-1}$) and blood samples were collected from the caudal vein and processed for comet assay and hematological parameters. After blood sampling fish were quickly sacrifice by medullar sectioning for the removal of liver, gills, posterior kidney, brain, and axial muscle. Organs were immediately stored in liquid nitrogen for biochemical analysis. In addition, subsamples of the liver were processed for comet assay.

Individual tissue samples were homogenized (1:10 w/v) in a phosphate buffer solution (0.1 M; pH 7.0 or 7.5 for AChE analysis). Samples were centrifuged (15,000xg, 20 min, 4 °C) and the supernatants were stored at −80 °C for subsequent biochemical analysis. For all biochemical biomarkers, the protein content o was determined according to Bradford (1976).

2.2. Chromatographic analysis of imidacloprid in water samples

Water samples were collected daily during the fish exposure period in clean amber glass bottles and were analyzed without the solid phase extraction (SPE) step. The concentrations of IMI in water samples were determined according to the method validated by Montagner et al. (2014). Quantification of the IMI was performed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The analysis was performed using an Agilent 1200 Series LC system equipped with binary pump, automatic injector, and thermostatized column compartment. Chromatographic separation was performed with a Zorbax SB-C18 column (2.1 × 30 mm,