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The pyrethroid λ-cyhalothrin induces biochemical, genotoxic, and physiological alterations in the teleost *Prochilodus lineatus*

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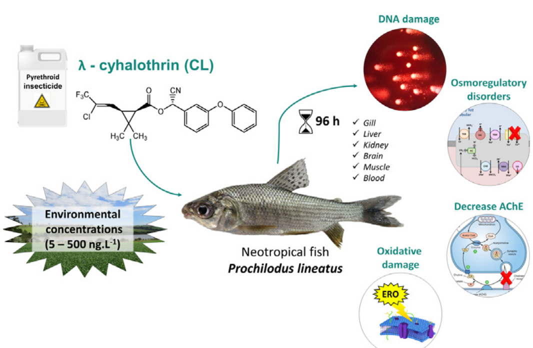
HIGHLIGHTS

- We evaluated acute effects of a λ-cyhalothrin (CL) formulation on the fish *Prochilodus lineatus*.
- CL promoted oxidative damage in different fish tissues.
- CL promoted a decreased in the muscle acetylcholinesterase activity.
- CL also promoted osmoregulatory disorders.
- DNA damage was detected in erythrocytes of fish exposed to CL.

GRAPHICAL ABSTRACT



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ABSTRACT

The λ-cyhalothrin (CL) is a globally used pyrethroid insecticide that has been detected in different water bodies worldwide. However, studies on the effects of CL on freshwater fishes are still incipient. In this context, we evaluated the acute effects of a commercial formulation containing CL (Karate Zeon® CS 50) in juveniles of the teleost *Prochilodus lineatus* exposed for 96 h to four concentrations of the active ingredient (5, 50, 250 and 500 ng.L^{-1}). Biochemical, physiological, and genotoxic biomarkers were evaluated in different organs of the fish. Exposure to CL induced significant changes in the enzymatic profiles of *P. lineatus*, with specific alterations in biotransformation enzymes and antioxidant defence in different tissues. Lipid peroxidation was observed in fish gills and kidney. Increases in esterases were observed in the liver of fish exposed to all CL concentrations evaluated, whereas acetylcholinesterase activity decreased in the muscles of fish at all concentrations. CL also promoted osmoregulatory disorders, with decreases in calcium and magnesium gill ATPases, with consequent hypocalcaemia, in addition an increase in sodium-potassium ATPase activity was observed in the gills of fish exposed to the highest CL concentration, probably in order to compensate a reduction in plasma sodium. Besides, increases in DNA damage were observed in the erythrocytes of fish exposed to all CL concentrations. Thus, despite the low CL concentrations and the short exposure time, this pyrethroid caused hematological adjustments, oxidative stress, osmoregulatory disorders, and DNA damage in *P. lineatus*, showing that the species is highly sensitive to the deleterious effects of CL.

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1. Introduction

Over the past three decades, the worldwide pattern of insecticide use has been changing, and a decline in the production of high-toxic compounds to mammals, such as organophosphates and carbamates, has been the most notable modification (Kumar et al., 2014). Meanwhile, the use of pyrethroid insecticides is on the rise on a worldwide scale since they are potent and efficient insect control agents and have a rapid action and selective toxicity that is relatively harmless to mammals and birds (Soderlund and Bloomquist, 1989; Goulding et al., 2013; Haverinen and Vornanen, 2014). However, several studies have shown that pyrethroids are among the most toxic pesticides for aquatic organisms, including fish (US EPA, 2001; Saxena and Seth, 2002; Pimpão et al., 2007; Gu et al., 2007; Carriquiriborde et al., 2009; Ansari et al., 2011; Muranli and Güner, 2011; Werner and Young, 2018). Due to their lipophilic character, pyrethroids have a high rate of absorption through fish gills, which may partly explain the high sensitivity of this group to pyrethroid exposure (Polat et al., 2002), and can bioaccumulate in fish (Corcellas et al., 2015; Brander et al., 2016; Clasen et al., 2018). In addition, fish seem to have a deficient enzyme system to hydrolyse these compounds (Viran et al., 2003), leading to slower rates in their metabolization and elimination compared with birds and mammals (Bradbury and Coats, 1989; Fishel, 2005; Brander et al., 2016).

As a result of agricultural runoff, forest spraying, and direct spray procedures in water bodies, pyrethroids can enter aquatic environments and accumulate in sediments due to their high adsorption coefficient (Werner and Young, 2018). This characteristic can cumulatively increase the concentrations of these compounds in sediments of water bodies, thus increasing the risk for freshwater fish, especially the benthic species (Marino and Ronco, 2005; Velmurugan et al., 2007; Kutluyer et al., 2015).

Although pyrethroids are widely used around the world, there is limited information concerning environmental concentrations of these insecticides in surface waters (Weston et al., 2009; Domagalski et al., 2010; Weston and Lydy, 2012; Jabeen et al., 2015; Stehle and Schulz, 2015). Concentrations of λ-cyhalothrin (CL) in surface waters ranging from 346 ng.L^{-1} in rivers of Greece (Tsaboula et al., 2016) to 797 ng.L^{-1} in agricultural regions of the southern United States (Anderson et al., 2013) were already reported in the literature. Additionally, in tropical regions CL has been detected in sediments at concentration of 983 ng.L^{-1} in agroecosystems of Costa Rica (Carazo-Rojas et al., 2018), and in Brazilian rivers at concentrations of 1.32 ng g^{-1} (Hunt et al., 2016), 1 ng g^{-1} to 5 ng g^{-1} (Miranda et al., 2008), and 19.7 ng g^{-1} to 60.0 ng g^{-1} (Possavatz et al., 2014).

The insecticidal activity and toxicity of the pyrethroids are dependent on the stereochemistry of the compound. Cis isomers are usually more toxic than trans isomers, and the introduction of the α-cyano group increases the toxicity of the molecule for both insects and mammals (Casida et al., 1983; Brander et al., 2016). There are two groups of pyrethroids with distinctive poisoning symptoms, distinguished by an alpha-cyano group in their structure. While those without an alpha-cyano group (type I pyrethroids, e.g., cismethrin, permethrin, bifenthrin) exert their neurotoxicity primarily through interference with sodium channel function in the nervous system, type II pyrethroids (with alpha-cyano group, e.g., cyhalothrin, deltamethrin, cypermethrin) can affect additional ion-channel targets such as chloride and calcium channels (Werner and Young, 2018).

The CL is a type II α-cyano pyrethroid current use as an insecticide for a wide range of target insects, it has also been adopted for the management of pests or in public health campaigns in the control of disease vectors (Muranli and Güner, 2011). The main action mode of pyrethroids is the change in the permeability of voltage-dependent sodium channels of nerve cells. Pyrethroids modify the activation and inactivation kinetics of the sodium channels, resulting in the prolonged opening of individual channels, causing membrane depolarization, repetitive discharges, and synaptic disturbances and leading to the symptoms of intoxication by hyperexcitability (Vijverberg and vanden Bercken, 1990; Palmquist et al., 2012). In Brazil, 253 pyrethroid compounds are registered to commercial formulations, with CL being the pyrethroid with the highest number of registrations (n = 72) (BRASIL, 2018).

The freshwater fish *Prochilodus lineatus* (Valenciennes, 1836) has been used as a suitable biological model to study the effects of pesticides because it is sensitive to herbicides (Langiano and Martinez, 2008; Modesto and Martinez, 2010a, 2010b; Santos and Martinez, 2012; Pereira et al., 2013; Moreno et al., 2014) and insecticides (Maduenho and Martinez, 2008; Bacchetta et al., 2011; Poletta et al., 2013; Loteste et al., 2013; Vieira et al., 2018). In this context, our objective is to evaluate the potential toxic effects of an λ-cyhalothrin formulation in biochemical, physiological, and genotoxic biomarkers of the fish *P. lineatus* after acute exposure to environmentally relevant CL concentrations.

2. Material and methods

2.1. Experimental design

Juveniles of *P. lineatus* (n = 40; weight, 21.57 ± 3.86 g; length, 11.15 ± 0.70 cm) were obtained from the Fish Hatchery Station of the State University of Londrina. They were acclimated for seven days in 300-L tanks containing clean, dechlorinated water and with constant aeration, under a 12:12-h light/dark photoperiod. During this period, commercial feed (Guabi®, 36% protein content) was given to the fish every 48 h, and the feed was interrupted 24 h before the tests and during the exposure. The physical and chemical parameters of the water were monitored throughout the acclimation and exposure using a multiparameter water quality meter (HORIBA U-52, Japan) (temperature = 22.86 ± 0.071 °C; pH = 7.88 ± 0.023; dissolved oxygen = 7.26 ± 0.028 mg O_2.L^{-1}; conductivity = 0.102 ± 0.004 mS cm^{-1}).

After the acclimation period, the fish were randomly divided into five groups (n = 8 fish/group) and kept in glass aquaria containing 80 L of dechlorinated water. One group was kept under controlled conditions only in clean water (CL_0), and the other four groups were exposed to different nominal CL concentrations from the commercial formulation Karate Zeon© CS (Syngenta S.A., Brazil), as follows: 5 (CL_5), 50 (CL_{50}), 250 (CL_{250}), and 500 (CL_{500}) ng.L^{-1}. The nominal concentrations of CL were calculated considering the content of the active ingredient (CL) in the commercial formulation (5% a.i.). Fish were exposed to the different treatments over 96 h, under semi-static conditions, with a complete renewal of the test solution every 24 h, once pyrethroids can be degraded by chemical processes, mainly hydrolysis and photolysis, and via aerobic and anaerobic biodegradation (Laskowski, 2002).

The letal concentration (LC_{50}) values over the 96 h for different fish species varied between 0.21 and 2.3 μg.L^{-1} (Maund et al., 1998), presenting high toxicity to these organisms. Thus, we sought to use safe CL concentrations for *P. lineatus* that would not cause mortality, starting from a minimum concentration of 5 ng.L^{-1} and increasing in a geometric progression of 10 to the maximum concentration of 5000 ng.L^{-1}. However, as there was 100% mortality at the highest concentration after 24 h, an intermediate concentration was added to the experiment (250 ng.L^{-1}).

After the exposure period, the fish were anaesthetized with