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Acute toxicity of pyrethroid-based insecticides in the Neotropical freshwater fish *Brycon amazonicus*

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Abstract

Pyrethroids are insecticides widely used in agriculture to control ectoparasites and biological vectors. They can reach the water bodies by leaching and or runoff. Fishes are highly sensitive to pyrethroids and the nervous system sensibility and the deficient drug metabolism are the clues but the toxicity mechanisms are yet unclear. The acute toxicity assays allow evaluating the potential, environmental risks of specific pesticides. Type II pyrethroids are becoming widely used and there is no law concerning the limits of use to this kind of pesticide in Brazil. The LC_{50};96h was evaluated for three pyrethroid based-insecticides (PBI): cypermethrin, deltamethrin and λ-cyhalothrin in fish *Brycon amazonicus.* The LC_{50};96h for the cypermethrin based-insecticide (CBI) was 36 µg L^{-1}; for deltamethrin based-insecticide (DBI) was 2.6 µg L^{-1}; and for λ-cyhalothrin (LBI) was 6.5 µg L^{-1}. During the tests some behavioral alterations were registered just after the exposure; they were more evident at the highest xenobiotics concentrations. These alterations were indicative of asphyxia and nervous system damages. The three insecticides are highly toxics to *B. amazonicus* and the degree of toxicity is: deltamethrin> λ-cyhalothrin> cypermethrin. The behavioral alterations observed are worrying since long-term exposure to sublethal concentrations can affect survival and reproductive ratios.

Keywords: cypermethrin, deltamethrin, teleost, lethal concentration, pesticide, λ-cyhalothrin

INTRODUCTION

Pyrethroids are synthetic compounds analogous to pyrethrins, which are derived from *Chrysanthemum cinerariaefolium*. These insecticides are widely used in agriculture for the low toxicity to mammals and birds (Elliott, 1976; Soderlund *et al*., 2002). In fish farms they are used to control ectoparasites and biological vectors (Hart *et al*., 1997; EMEA, 2003; ANVISA, 2007; USEPA, 2008). In addition, they reach the water bodies by leaching and or runoff. Pyrethroids are chemically classified in two families: those carrying the α-cyano group such as deltamethrin, cypermethrin, λ-cyhalothrin and cyfluthrin (type II); and those without the α-cyano group such as bifenthrin, permethrin, resmethrin (type I). Substitution of hydroxyl group of the molecule by the α-cyano group increases the insecticide potency (Soderlund *et al*., 2002).

Pyrethroids affect the permeability of the Na^+ voltage-dependent channels in the nervous cells. This effect leads to membrane depolarization and synaptic disturbances responsible for the hyper excitability observed in cases of intoxication (Narahashi, 1996; Soderlund *et al*., 2002). Other mechanisms of action should be inhibition of calcium channels, inhibition of Ca^+ enzymes and Ca^+/Mg^+ ATPases (Narahashi, 1991; Coats, 2008). Pyrethroids exhibit low toxicity to mammals and birds, however, fishes are very susceptible to them (Coats, 2008). The rate of toxicity to fishes is in a range of micrograms per liter (Jones, 1995; Maund *et al*., 1998; USEPA, 2008; Güner, 2009; Saravanan *et al*., 2009) and this occurs because the fish nervous system is deficient

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to metabolize such chemicals (Demounte, 1989; Haya, 1989). The biotransformation of *cis* and *trans*-cypermethrin in trout (*Oncorhynchus mykiss*) is very low when compared to that observed in frog (*Rana temporaria*), rats (*Mus musculus*) and quail (*Coturnix coturnix japonica*) (Edwards *et al*., 1987).

Brazilian laws establish the maximum concentration of permethrin at 20 μg L^{-1} to drinking water (BRASIL, 2005) but type II pyrethroids are becoming widely used and there are no legal limits for the use of this class of pesticide. The efficiency of type II pyrethroids is greater than that of type I, and they are currently registered by the National Agency of Sanitary Vigilance (ANVISA) and the Agriculture Ministry (MAPA). Nowadays there are nearly ten pyrethroids-based insecticides allowed by law in Brazil, and cypermethrin, deltamethrin and λ-cyhalothrin are the most employed (MAPA, 2013). Identification and assessment of environmental risks caused by xenobiotics require previous experimental evaluation through pilot toxicity tests. This strategy allows the proper management of environments to prevent future adverse events and it is essential to establish official regulations of use. Determination of dose-response in proper aquatic organisms is an important step in that process (USEPA, 2002). This kind of biological assay evaluates drastic and harmful effects in the organism exposed to a chemical over a short span, usually four days. Immobility for invertebrates and mortality for fish are the main criteria to evaluate the lethality, which are expressed in Lethal Concentration (LC_{50}) of the chemical over 50% of the animal sampling and extended to the animal population (Zagatto & Bertoletti, 2006).

Despite the increased studies on LC_{50} of pyrethroids in fish species (Vijayavel & Balasubramanian, 2007; Güner, 2009; Kumar *et al.*, 2009; Kumar *et al*., 2011), no data are available about the LC_{50} of pyrethroids in matrinxa *Brycon amazonicus* (Spix & Agassiz 1829), a freshwater fish of warm waters. For this purpose, the endpoints considered above the acute toxicity of the type II pyrethroid-based insecticides (PBI) cypermethrin, deltamethrin and λ-cyhalothrin were gauged in the freshwater teleost matrinxa *Brycon amazonicus* which will provide fundamental data to appropriate regulation of these PBIs.

MATERIAL AND METHODS

Animal acquisition

Juveniles *Brycon amazonicus* (10.5 ± 3 g and 7.5 ± 1 cm) were kindly provided by the fish farm Pollettini (Mogi Mirim, SP, Brazil). The fish were held into 2000L tanks to acclimate for four weeks under controlled temperature 25.5 ± 0.5 °C, pH 7.4 ± 0.3, dissolved oxygen 5.7 ± 0.4 mg L^{-1}, alkalinity 26.5 ± 0.3 mg L^{-1} of HCO_3^+ and hardness 18.0 ± 0.1 mg of $CaCO_3$ (APHA, 1980). Over that period, the fish were fed to satiety twice a day with pellets containing 32% of crude protein.

The experimental protocol was previously approved by the Institutional Committee of Ethics to Animal Research under PIN – CEEA 04/2008 and CEUA 056/2011.

Chemicals

Cypermethrin commercial formulation Galgotrin® (250g i.a. L^{-1}) and deltamethrin commercial formulation Keshet® (25 g i.a. L^{-1}) were supplied by Milenia Agrosciencias S.A (Londrina, PR, Brazil). Lambda-cyhalothrin commercial formulation Trinca Caps® (250 g i.a. L^{-1}) was provided by DVA (Campinas, SP, Brazil).

Acute Toxicity: Lethal Concentration (LC_{50};96h)

The acute toxicity tests for the PBIs were performed in a static system daily monitored with constant aeration and kept the same water quality of the acclimation tanks (APHA, 1980), averting any disturbances (OECD, 1992). The fish density was 1.0 g per liter (IBAMA, 1987, OECD, 1992) and the experimental aquaria were 250 L. The fish feeding was discontinued 24 hours before the tests. All insecticides were directly dissolved in the water of the tanks in appropriate concentrations. The volume of pure insecticide dispensed in the tank water was calculated according to the active ingredient (a.i.) concentration of the commercial formulation per volume of tank. The information concerned the solvent is not provided by manufactures. The LC_{50};96h were calculated with the trimmed Spearman-Karber method by the LC_{50} JSPEAR Program (Hamilton *et al.*, 1977) with a confidence interval of 95%. Some behavioral patterns were observed and reported along experiment.

Cypermethrin based-insecticide (CBI)

This LC_{50};96h was performed with 72 fish (30.5 ± 5 g and 12.5 ± 1 cm) equally distributed into eight aquaria with 0, 5, 10, 20, 30, 40, 70 e 100 μg a.i. L^{-1} for 96h.

Deltamethrin based-insecticide (DBI)

This LC_{50};96h was performed with 70 fish (16 ± 3 g e 11 ± 1 cm) equally distributed into seven aquaria with 0, 1.4, 2.4, 3.4, 4.4, 5.4, 6.4 μg a.i. L^{-1} exposed for 96h.

Lambda-cyhalothrin based-insecticide (LBI)

This LC_{50};96h was performed with 63 fish (31.2 ± 6 g e 13.6 ± 1 cm) equally distributed into seven aquaria with 0, 5, 6, 7, 8, 9 e 10 μg a.i. L^{-1} exposed for 96h. Mortality records and removal of dead fish were done at each 24 hours in all experimental tests.

RESULTS

The LC_{50};96h of CBI to *B. amazonicus* was 36 μg L^{-1}, with lower and upper limits of 30 μg L^{-1} and 40 μg L^{-1}, respectively (Table 1). The LC_{50};96h of DBI was 2.6 μg L^{-1}, with lower and upper limits of 2.2 μg L^{-1} and 3.1 μg L^{-1}, respectively (Table 2). The LC_{50};96h of LBI was 6.5 μg L^{-1}, with lower and upper limits of 6 μg L^{-1} and 7 μg

L^{-1}, respectively (Table 3). Mortality was ascertained only after the 24 initial hours for all PBIs. During the tests, some behavioral alterations were observed and reported just after the exposure and were more evident at the highest xenobiotics concentrations (Table 4). The increased opercular movement, loss of equilibrium and erratic swimming were observed for all exposures of PBIs. However, just fish acutely exposed to CBI presented loss of color (light color), while fish exposed to deltamethrin presented circular swimming.

Table 1 - Mortality of *B. amazonicus* exposed to cypermethrin based-insecticide (Galgotrin®) for 96 hours.

*a.i. (µg L^{-1})	Final n	Mortality (%)
0	9	0
5	9	0
10	9	0
20	9	0
30	9	0
40	1	88
70	0	100
100	0	100

The initial number of fish was n=9; *a.i.- active ingredient concentration of the commercial formulation.

Table 2 - Mortality of *B. amazonicus* exposed to deltamethrin based-insecticide (Keshet®) for 96 hours.

a.i.* (µg L^{-1})	Final n	Mortality (%)
0	10	0
1.4	10	0
2.4	5	50
3.4	4	60
4.4	1	90
5.4	0	100
6.4	0	100

The initial number of fish was n=10; *a.i.- active ingredient concentration of the commercial formulation.

Table 3 - Mortality of *B. amazonicus* exposed to lambda-cyhalothrin based-insecticide (Trinca-caps®) for 96 hours.

a.i.* (µg L^{-1})	Final n	Mortality (%)
0	9	0
5	9	0
6	4	55.5
7	4	55.5
8	2	77.7
9	1	88.8
10	0	100

The initial number of fish was n=9; *a.i.- active ingredient concentration of the commercial formulation.

Table 4 Behavioral patterns of *Brycon amazonicus* exposed to lethal concentrations of three pyrethroids-based insecticides for 96 hours.

Parameters	CBI	DBI	LBI
Opercular movement increased	+	+	+
Loss of equilibrium	+	+	+
Loss of color	+		
Erratic swimming	+	+	+
Sudden swimming followed by interruption	+	+	
Circular swimming		+	

CBI = cypermethrin based-insecticide, DBI = deltamethrin based-insecticide, LBI = λ-cyhalothrin based-insecticide.

DISCUSSION

The toxicity of the PBIs in the present study can be considered very high to *B. amazonicus*, since the LC_{50};96h for all insecticides were lower than 0.1 mg L^{-1} (Zucker, 1985). In fish, the reasons for the high toxicity of pyrethroids is not yet clear but the nervous system sensibility and the deficient drug detoxification metabolism are the clues (Coats, 2008). Carboxylesterases catalyze the hydrolysis of pyrethroids and fish seems to be deficient of these enzymes retarding the drug detoxification and clearance (Bradbury and Coats, 1989; Demoute, 1989; Haya, 1989). Pyrethroids are lipophilic compounds, what increases the absorption rate through the gills and the toxicant sensibility (Viran *et al.*, 2003; Kumar *et al.*, 2011). In addition, the pyrethroids can inhibit the Ca^{2+}channels and the Ca^{2+}/Mg^{2+} ATPases. Because certain ATPases are involved in ion regulation, this alteration has also been considered a secondary toxic mechanism of pyrethroids related to the osmoregulation disorders (Coats, 2008).

The LC_{50};96h to the PBI assayed allows us to establish their toxicity degree as: deltamethrin > λ-cyhalothrin > cypermethrin for the commercial formulations of this study. This toxicity is different of that observed for fingerlings of *Oreochromis* sp which is λ-cyhalothrin> deltamethrin> cypermethrin (Bajet *et al.*, 2012). That difference might be attributed to some factors such as developmental stage, commercial formulation, or even to a species-specific trait. In fish, the toxicity of cypermethrin varies between 0.4 µg L^{-1} (Bradbury e Coast, 1989) and 400 µg L^{-1} (Kumar *et al.*, 2009) as depicted in Table 5. Likewise, the LC_{50} of deltamethrin and λ-cyhalothrin may vary among fishes (Table 5). Solubility is another important factor, which determines the toxicity of pesticides in water (Saha & Kaviraj, 2008). In addition, the toxicity of pyrethroids is dependent of the molecule stereochemistry.

Every isomeric form of the pyrethroid displays its typical toxicity and the most commercial formulations have a fixed isomeric ratio (Kumar *et al.* 2011). This is well observed to the four isomeric forms of fenvalerate in *Danio rerio* (Ma *et al.*, 2009); to γ-cyhalothrin and λ-cyhalothrin in *Macrobrachium nippoensis* (Wang *et al*. 2007); and to *cis*-cypermethrin e *trans*-cypermethrin in *Salmo gairdneri* (Edwards *et al.*, 1987). All those differences among the LC_{50};96h values for

Table 5 Acute toxicity (Lethal Concentration LC_{50}) of fish species exposed to type II pyrethroids for 96 hours.

Cypermethrin		
Species	**LC_{50} (μg L^{-1})**	**References**
Brycon amazonicus	36	Present study
S. erythropthalmus	0.4	Bradbury & Coats (1989)
Tilapia nilotica	2.2	Bradbury & Coats (1989)
Poecilia reticulata	9.43	Yılmaz *et al*. (2004)
Rhamdia quelen	193	Borges *et al*. (2007)
Channa punctatus	400	Kumar *et al*. (2009)
Deltamethrin		
Brycon amazonicus	2.6	Present study
Cyprinus carpio	1.45	Svobodova *et al*. (2003)
Cyprinus carpio	3.5	Lakota *et al*. (1989)
Oreochromis niloticus	15.4	Golow & Godzi (1994)
Oreochromis mossambicus	250	Vijayavel & Balasubramanian (2007)
Lambda-cyhalotrhin		
Brycon amazonicus	6.5	Present study
Gambusia affinis	1.10	Güner (2009)
Brachydanio rerio	1.94	Wang *et al*. (2007)
Clarias batrachus	5.0	Kumar *et al*. (2011)
Channa punctatus	7.92	Kumar *et al*. (2007)

the species emphasize the importance of assessing the toxic characteristics of the current commercial formulation of pyrethroids to several native species. These assessments could be used to estimate the potential environmental risks of these specific pyrethroids.

The behavioral alterations observed in *B. amazonicus* over the acute toxicity tests were similar for all PBIs. Those alterations are usually reported for pyrethroids and comprise tremors, fast and erratic swimming, loss of equilibrium, water surface breathing and lethargy (Werner & Moran, 2008). Moreover, asphyxia, nervous system damages and possible metabolic alterations are evidences of acute exposure. Lambda-cyhalothrin produces hyperactivity, loss of equilibrium, accelerated swimming, increase of opercular beating frequency and convulsions in *Clarias batrachus* (Kumaret *et al.*, 2011); the same signals observed in *B. amazonicus*. On the other hand, *C. batrachus* displays skin darkness (Kumar *et al.*, 2011) differently of that observed in *B. amazonicus* exposed to cypermethrin. Alterations in the opercular beating are also observed in the common carp *Cyprinus* carpio, exposed to fenvalerate (Reddy *et al*., 1992). The Neotropical silver catfish *Rhamdia quelen* exposed to sublethal concentrations of cypermethrin exhibits some alterations (Borges *et al*., 2007) similar to those observed in *B. amazonicus*. In addition to the behavioral and physiological damages from acute exposure to pyrethroids, the swimming performance can be reduced leading to increased susceptibility to predators and consequent death, as observed in rainbow trout *Oncorhynchus mykiis* exposed to deltamethrin (Goulding *et al*., 2013). Other damages can also be observed in consequence of long-term exposure to pyrethroids such as decrease of survival and reproduction rates, and alterations of cohort behavior as observed in brown trout *Salmo truta* exposed to cypermethrin (Jaensson *et al*., 2007). Moreover, the reduction in the fish growth can be a consequence of the exposure of contaminants. The feeding rate, absorption rate, metabolic rate and absorption efficiency are reduced in *O. mossambicus* exposed to deltamethrin (Vijayavel & Balasubramanian, 2007).

Pyrethroids insecticides present low water solubility, low residence time in water and high absorbance into particulate matter (Rasmussen *et al.,* 2008; Bajet *et al*., 2012), which may decrease its toxicity in field conditions. However, these pesticides are very toxic even at low concentrations, posing risks to non-target aquatic populations. The present data could be useful in the assessment of risks of pyrethroids insecticides. Low concentrations of pyrethroids, as reported in this study, are usual in water environments (Marino & Roco, 2005; Belluta *et al*., 2010) and they are close to that reported to fish farms in some countries (EMEA, 2003; Haya, 2005). The presented susceptibility of the Neotropical fish species to these xenobiotics is pivotal to establish secure levels of these compounds in freshwater. Given that, acute toxicity tests can contribute to evaluate potential hazards caused by pesticides that reach aquatic environments. It is important to consider that injuries caused by sublethal concentrations of xenobiotics have high ecological importance, and could affect an entire population.

Conclusion

The evaluation of the LC_{50};96h to the three PBIs type II tested in *B. amazonicus* showed that they are highly toxic and the degree of toxicity is: deltamethrin> λ-cyhalothrin> cypermethrin. The behavioral alterations observed in *B. amazonicus* are very concerning, since long term exposure to sublethal concentrations can affect survival and reproductive ratios.

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