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Hepatic Enzymes Activity in the Fish *Prochilodus Lineatus* (Valenciennes, 1836) After Sublethal Cypermethrin Exposure

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Abstract *Prochilodus lineatus,* a fish, was exposed to sublethal concentrations of cypermethrin: 0.075, 0.150, and 0.300 μg L^{-1} and a control group (without cypermethrin) for 96 h. Five specimens were exposed in each concentration for triplicate (n = 60). Hepatic biochemical values and behavioral changes were studied. The results revealed a significantly higher level of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase in hepatic tissue at different concentrations of cypermethrin tested compared to control ($p < 0.05$). A decrease in total protein was observed in exposed groups but not significantly ($p > 0.05$). This study provides information to know toxic mechanisms of cypermethrin on hepatic enzymes of *P. lineatus* that are poorly understood.

Keywords *Prochilodus lineatus* · Hepatic enzymes · Cypermethrin

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In the last years, loss of biodiversity, population reduction, and destruction of aquatic habitats have been registered as a result of industrial and farming activities that use riverine systems as receiver or vehicle of their wastes. Biocides are extensively used in the agricultural zone of the Parana River basin in Argentina (Simoniello et al. 2009). Synthetic pyrethroid pesticides account for over 30 % of the global pesticide use. Among them, cypermethrin is a type II pyrethroid compound classified as a toxicity class II chemical (moderately toxic, WHO (2004) hazard classification). It is effective against a wide range of insect pests in cereals, fruits (including citrus), cotton, forestry, ornamentals, rape, soybeans, tobacco, tomatoes, vegetables, vines, coffee, cocoa, and other crops.

Freshwater fish are highly susceptible to the toxic effects of cypermethrin (Das and Mukherjee 2003). Nevertheless, there is very scarce information about the injurious actions of this compound in Neotropical fish (Parma et al. 2007; Borges et al. 2007; Simoniello et al. 2009). The estimation of early signs of danger using biomarkers in an ecosystem provides useful information for environmental biomonitoring and allows the development of control strategies and prevention measures (Markert et al. 2003). *Prochilodus lineatus* (Pisces, Prochilodontidae) is the most abundant fish species (60 % of the total ichthyomass of the Parana River) and the main resource in commercial fisheries in the Middle Paraná River (Rossi et al. 2007). Besides, *P. lineatus* is a bottom feeder that comes in contact with sediments and the sediment–water interface, where pyrethroid insecticides are expected to accumulate (Cazenave et al. 2009).

The tests of hepatic function constitute a form to study the liver and its possible functional alterations. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) are enzymes involved in the metabolism of amino acids, and their alterations allow

the identification of tissue damage in organs such as the liver and kidney (Begun 2005; Borges et al. 2007). Two general hepatic enzyme categories are used to evaluate liver function: On the one hand, AST and ALT, which increased activity, indicate damage of the hepatic tissue, and ALP increase is indicative of an obstruction in intra- and extrahepatic biliary system.

The objective of this work was to quantify the levels of hepatic enzymes: AST, ALT, and ALP together with total protein (TP) in liver of *Prochilodus lineatus*, exposed to sublethal concentrations of cypermethrin to get a better understanding of the effects of these chemical and the adaptive metabolic responses of this species.

Materials and Methods

Fishes were obtained from a pristine environment next to Santa Fe city (Argentina) at 31°39′36″S and 60° 35′ 26″W; they were transported to the INALI bioassay laboratory in oxygenated recipients. A total of 60 juvenile specimens (mean weight: 41.7 ± 21.7 g, and length: 113.31 ± 19.49 mm) were used to carry out the experiment. For acclimation purpose, fish were held in 150 L tanks in well-aerated dechlorinated water for 2 weeks and were fed daily.

Experiment was conducted in 25 L glass aquaria, with 12:12-h light/dark cycles, pH 6.7 ± 0.1, total hardness 315 ± 7.1 ppm $CaCO_3$, temperature 24 ± 1°C, and dissolved oxygen 5.9 ± 0.5 mg/L. Fish feeding was suspended 24 h before the beginning of the experiment.

For the experiment, 45 fishes (5 fishes per tank, and three replicates per concentration) were exposed to the following sublethal concentrations (Parma et al. 2007) during 96 h: 0.075, 0.150, and 0.300 μg L^{-1} of cypermethrin. Test solutions were prepared from a commercial formulation containing 25 % active ingredient (SHERPA O®). Another 15 specimens were used as intra-assay control without chemical. Concentration of the formulation in each tank was determined by HPLC obtaining 90 % of active ingredient with respect to the value informed in the label, in all cases analyzed. Considering breakdown in water, under normal environmental temperatures and pH, cypermethrin is stable to hydrolysis with a half-life greater than 50 days and to photodegradation with a half-life greater than 100 days (EXTOXNET 2010). The static test method of subacute toxicity test was used.

Mortality, immobility, and behavioral alterations (frequency and speed of swimming and frequency of erratic movements) in fish were registered every 24 h.

At the end of experiment, fish were removed from aquaria, anesthetized with benzocaine according to Parma et al. (2007), and then were killed and dissected. The livers were immediately frozen in liquid nitrogen and stored at −80°C until biochemical determinations were carried out.

Fish livers were homogenized in 10 volumes (w/v) of 0.25 M sucrose buffer, pH 7.4, and then centrifuged at 3,000*g* for 20 min in a refrigerated centrifuge at 4°C to remove cell debris, and clear cell-free extracts were used as enzyme source, according to David et al. (2004).

The activity of AST, ALT, ALP, and TP levels in liver homogenate was measured using commercial kits (Wiener Lab®). All samples were analyzed in triplicate, at 37°C, using a spectrophotometer. Enzymatic activities data of homogenate livers were expressed as U L^{-1}, and liver TP data expressed as g dL^{-1}.

Statistical analysis was performed using the software SPSS 14.0 for Windows. Variables were tested for normality with Kolmogorov–Smirnov test, and homogeneity of variances between groups was verified by Levene test. Data from enzymatic and metabolic parameters were analyzed by one-way ANOVA followed by the Tukey's test or Kruskal–Wallis followed by the Mann–Whitney U test, depending on data homogeneity and normality assumption. Linear regressions were applied to analyze the concentration–effect relationship and the relation between enzyme activities and size of the animals. A difference of $p < 0.05$ was considered statistically significant.

Results and Discussion

No mortality or behavioral alterations were observed in the fish exposed to sublethal concentration of cypermethrin. No difference was found between replicates in any treatments ($p < 0.05$) for any of the parameters evaluated, so all results are informed as mean values ±SE per experimental group.

TPs at different concentrations of cypermethrin were as follows: 0.462 ± 0.10, 0.445 ± 0.06, 0.363 ± 0.02, and 0.347 ± 0.09 at control, 0.075, 0.150, and 0.300 μg L^{-1}, respectively. Liver protein concentration showed non-significant changes ($p > 0.05$) at the end of cypermethrin exposure to different concentration assayed; similar results were found in *Clarias batrachus* exposed to 0.07 mg L^{-1} cypermethrin for 10 days (Begun 2005).

The sublethal toxicity of cypermethrin on enzymatic parameters of *P. lineatus* was presented in Fig. 1. The fishes showed significantly higher activities of AST, ALT, and ALP in the exposed groups at all concentrations compared to controls (ANOVA, $p < 0.01$ in all cases). Results showed that AST, ALT, and ALP activities presented a significant increase in 0.300 μg L^{-1} when compared with controls ($p < 0.001$ in all cases, *U* test), while only AST and ALP showed significant increase in 0.150 μg L^{-1} cypermethrin concentration ($p < 0.001$ in both cases, *U* test). Linear regression analysis showed a concentration–effect relationship only in the case of AST

($p < 0.05$). No relationships were observed between the values of the different enzymes and weight or length of the animals when was used as a factor ($p > 0.05$).

Activities of AST and ALT are considered sensitive indicators of stress (Gould et al. 1976). The enzymatic increase observed in this investigation is a clear indication of shunting of amino acids into TCA cycle through oxidative deamination and active transamination. Such a phenomenon was necessary to cope up with the energy crisis during pyrethroid exposure (David et al. 2004). About this, in Begun (2005), AST and ALT activities in hepatic and gill tissues were elevated throughout the exposure to cypermethrin during 10 days. While in Prashanth and Neelagund (2008), a progressive increase was observed in the activities of ALT and AST in all the organs (gill, liver, and muscle) of *Cirrhinus mrigala* exposed to cypermethrin. Similar results were obtained by Tiwari et al. (2012) in *Labeo rohita* exposed to sublethal doses of cypermethrin for 24 h and 96 h, causing significant ($p < 0.05$) time- and dose-dependent alterations in the activity of enzymes ALT and AST, in liver and muscle tissues of fish. Increases in AST and ALT levels indicate that fish are under toxic stress. The amino acids appear to be mobilized to get transamination to 2-keto acids, for use in the production of energy-rich compounds (David 2004).

In the present study, ALP increased but only significantly at the highest concentration. In jundiá (*Rhamdia quelen*), the activity of ALP significantly increased at almost all periods of exposure and concentrations of cypermethrin used (Borges et al. 2007). On the other hand, Dobsikova et al. (2006) found a decrease in ALP levels in blood plasma of common carp (*Cyprinus carpio*) exposed to cypermethrin (96-h LC50). Das and Mukherjee (2003) found that ALP activity in the brain of *Labeo rohita* was inhibited only after 45 days of exposure to doses of 1/10 and 1/50 of 96-h LC50.

ALP shows minimal activities in normal hepatic tissue; this enzyme is located mostly within the biliary canaliculi and epithelial cells comprising the bile ducts. The elevated serum ALP activities are regularly attributed to hepatobiliary origin. Also, increase in ALP activities is frequently associated with the administration of drugs (Ramaiah 2007).

Toxic effects of cypermethrin were assessed in *P. lineatus,* an important fish species in neotropical region, to estimate its influence on hepatic biochemical parameters. In a previous report, the influence of cypermethrin in erythrocytes of the same species was evaluated using the comet assay and results showed significant differences between exposed groups and controls at all tested concentrations ($p < 0.05$) (Simoniello et al. 2009). In summary, *P. lineatus* is a promising sentinel organism for the evaluation of substances potentially harmful in aquatic environments.

This study provides important information to know toxic mechanisms of cypermethrin on hepatic enzymes of *P. lineatus,* poorly understood up to now.

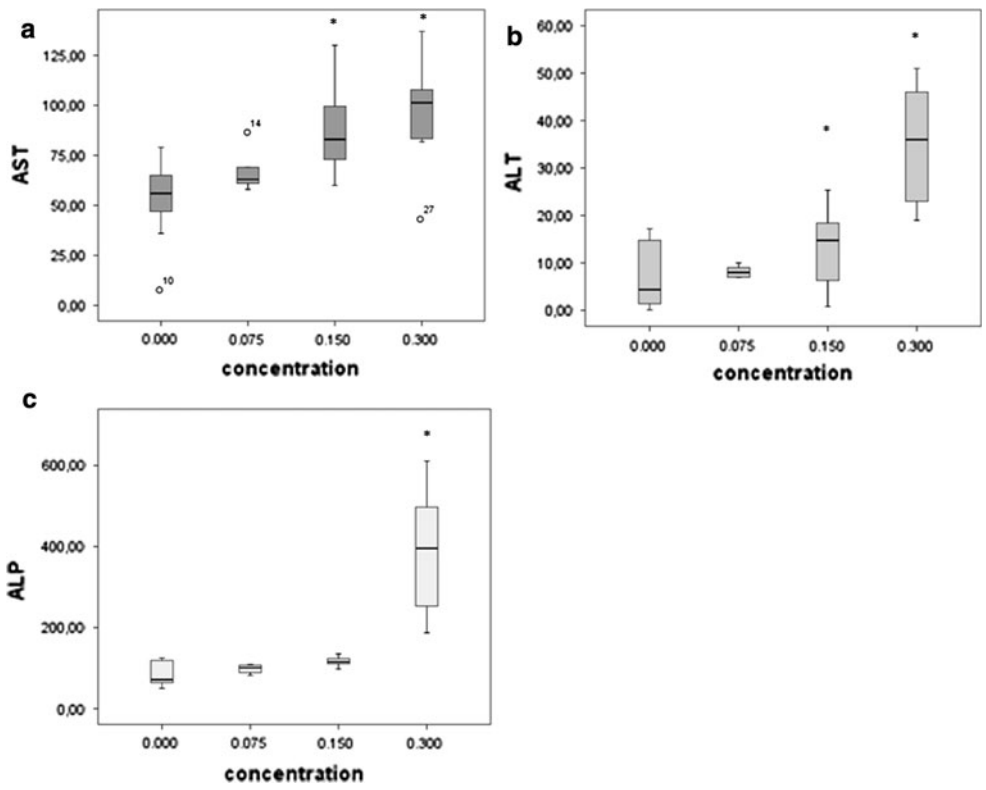

Fig. 1 Enzymatic parameters: **a** AST (U L^{-1}), **b** ALT (U L^{-1}), **c** ALP (U L^{-1}) observed in *P. lineatus* liver for different treatment of cypermethrin. *Boxplots* showing enzymatic activities in control and cypermethrin-exposed fishes. *Boxes* are limited by first and third quartiles divided by median; thin vertical lines represent minimum and maximum values except when outliers are present. *$p < 0.05$ Mann–Whitney *U* test

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