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Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus*

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Abstract

The toxicity of Roundup, a glyphosate-based herbicide widely used in agriculture, was determined for the Neotropical fish *Prochilodus lineatus*. The 96 h-LC_{50} of Roundup was 13.69 mg L^{-1}, indicating that this fish is more sensitive to Roundup than rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). These differences should be considered when establishing criteria for water quality and animal well-being in the Neotropical region. Short-term (6, 24 and 96 h) toxicity tests were then performed to evaluate the effects of sub-lethal concentrations of the herbicide (7.5 and 10 mg L^{-1}) to *P. lineatus*. Roundup did not interfere with the maintenance of the ionic balance and there was no significant alteration in plasma cortisol levels in Roundup-exposed fish. However an increase in plasma glucose was noted in fish exposed to 10 mg L^{-1} of the herbicide, indicating a typical stress response. Catalase liver activity also showed an increase in fish exposed to 10 mg L^{-1} of the herbicide, suggesting the activation of antioxidant defenses after Roundup exposure. In addition, Roundup induced several liver histological alterations that might impair normal organ functioning. Therefore, short-term exposure to Roundup at subletal concentrations induced biochemical, physiological and histological alterations in *P. lineatus*.

Keywords: Acute toxicity; Biotransformation; LC_{50}; Liver histopathology; Osmoregulation; Oxidative stress; Roundup; Stress response

1. Introduction

Glyphosate is a broad-spectrum non-selective herbicide used for inhibition of unwanted weeds and grasses in agricultural, industrial, urban, forestry and aquatic landscapes (Çavas and Könen, 2007). It is perhaps the most important herbicide ever developed and its use continues to expand particularly in applications involving plant varieties that are genetically modified to tolerate glyphosate treatments (Williams et al., 2000). In Brazil, glyphosate is the most widely used herbicide and its consumption has increased 95% in the period from 2000 to 2004. In the state of Paraná (southern Brazil) 4562 tons of glyphosate were used in corn and soybean culture between 2000 and 2001 (Inoue et al., 2003). High concentrations of glyphosate were detected in water near to intense cultivation areas in southern Brazil (da Silva et al., 2003). The major formulation is Roundup, in which glyphosate is formulated as isopropylamine salt and a surfactant, polyethoxylene amine (POEA), is added to enhance the efficacy of the herbicide (Tsui and Chu, 2004; Releya, 2005). Due to its high water solubility and extensive usage (especially in shallow water systems), the exposure of non-target aquatic organisms to this herbicide is a concern (Tsui and Chu, 2003).

The acute toxicity of glyphosate is considered to be low by the World Health Organization (WHO, 1994). However, commercial glyphosate formulations are more acutely toxic than glyphosate (Amarante et al., 2002; Peixoto, 2005). Surfactants such as POEA in Roundup are the principal toxic component in the formulated products based on glyphosate to aquatic organism (Tsui and Chu, 2003). In a review of toxicological data, Giesy et al. (2000) found POEA to be more toxic to fish than glyphosate. Neskovic et al. (1996) carried out acute toxicity tests with carp (*Cyprinus carpio*) and found the median lethal concentration in 96 h (96 h-LC_{50}) of glyphosate to be fairly high, 620 mg L^{-1}. However, considering the formulated product Roundup, 96 h-LC_{50} varied from 2 to 55 mg L^{-1}, depending on the fish species, life stage and test conditions (Jiraungkoorskul et al., 2002).

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Nevertheless, glyphosate alone or with its formulation products was previously considered to be harmless in normal usage and at chronic exposure in previous testing approaches (Williams et al., 2000). However, toxic effects of Roundup at sub-lethal concentrations have now been demonstrated in fish (Marc et al., 2004). Sub-lethal concentrations of glyphosate, corresponding to less than 2% of the LC_{50}, caused ultrastructural damage in the liver of *C. carpio* (Szarek et al., 2000). Histological alterations were also observed in liver, gills and kidneys of Nile tilapia (*Oreochromis niloticus*) after acute and chronic exposure to sub-lethal concentrations of Roundup (Jiraungkoorskul et al., 2002, 2003).

Despite the fact that Roundup is widely used in Brazil, only a limited amount of information is available on its toxic effects to native freshwater fishes. Recently, acute effects of Roundup on metabolic and enzymatic parameters of *Leporinus obtusidens* and *Rhamdia quelen* were investigated by Glusczak et al. (2006, 2007). Apart from these studies, toxicological responses of Neotropical fishes to glyphosate-based herbicides remain poorly understood.

Prochilodus lineatus (Order Characiformes, Family Prochilodontidae) is native to the south and southeast regions of Brazil. This fish represents a well suited species to environmental monitoring as it is a bottom feeder animal which is in contact with xenobiotics in water and in sediment, and is sensitive to variations in water quality (Camargo and Martinez, 2006; Simonato et al., in press).

Thus, to obtain more information about the threat imposed by the use of glyphosate-based pesticides to Neotropical fish species this work was designed to determine the toxicity of Roundup to *P. lineatus* and to evaluate the responses of this fish at biochemical, physiological and histological levels, after acute exposure to sub-lethal concentrations of the herbicide.

2. Materials and methods

2.1. Toxicity tests

Juveniles of *P. lineatus*, weighing 16.32 g±8.35 (mean±S.E., $n=144$), were supplied by the Hatchery Station of Universidade Estadual de Londrina. Prior to the toxicity tests, fish were acclimated to laboratory conditions for a minimum of seven days in a 600-L tank with aerated dechlorinated water ($T \cong 22$ °C; pH ≅ 7.5; OD ≅ 8 mgO_2 L^{-1}; conductivity ≅ 90 µS cm^{-1}; $Na^+ \cong 0.086$ mM; $K^+ \cong 0.030$ mM; $Cl^- \cong 0.103$ mM; hardness ≅ 80 mg L^{-1} $CaCO_3$). During this period, fish were fed with commercial pellet food each 48 h. Animals were not fed during the toxicity tests.

Short-term (6, 24 and 96 h) static toxicity tests were performed to evaluate the toxicity of Roundup to *P. lineatus*. Preliminary tests were carried out to determine the appropriate concentration range for testing the chemical. The commercial formulation of glyphosate, Roundup (360 g glyphosate L^{-1} or 41% of glyphosate, Monsanto do Brasil LTDA), was used. Experiments were performed in 100 L glass aquaria containing 8 fish each, with continuously aerated dechlorinated water, with the same characteristics described for the acclimation period. The tests consisted of five groups of fish exposed to one of five Roundup concentrations (7.5, 10, 15, 20 and 30 mg L^{-1}) and a control group exposed only to water, without the herbicide. The number of dead fish was recorded every 6, 24 and 96 h, and the values of the median lethal concentration (LC_{50}) were estimated by the trimmed Sperman–Karber method (Hamilton et al., 1977).

To evaluate Roundup effects, fish were exposed to two sub-lethal concentrations of the herbicide, corresponding to 55 and 75% of the 96 h-LC_{50}, or only to clean water (control groups), in 100 L glass aquaria containing 8 fish each. One experimental group for each Roundup concentration plus one control group were terminally sampled at: 6, 24 and 96 h. Replicates were carried out for each experimental time. The physical–chemical characteristics of the water during sub-lethal tests for both Roundup concentrations, in all the exposure periods, remained stable. The mean values (±SE) for control and experimental groups were, respectively, temperature: 21.7±0.3 °C and 21.7±0.3; pH: 7.5±0.1 and 7.4±0.1; dissolved oxygen: 7.2±0.1 and 7.2±0.1 mg O_2. L^{-1}; conductivity: 91.7±3.0 and 94.3±2.9 µS cm^{-1}.

Immediately after removal from the aquaria, the fish were anesthetized with benzocaine (0.1 g L^{-1}), and blood samples were taken from the caudal vein into heparinized plastic syringes. Subsequently animals were killed by cervical section and the livers were immediately removed and divided in two parts. One part was stored at −80 °C for enzymatic assays and the other was placed in Bouin's fixative for histopathological analysis.

2.2. Physiological and biochemical analysis

Blood samples were centrifuged (5 min, 5000 ×*g*) and plasma samples were stored frozen (−20 °C). Plasma sodium was measured by flame photometry. Plasma chloride concentration was determined by the thiocyanate method using a commercial kit (Analisa, Brazil). Plasma osmolarity was determined with a freezing point osmometer. Plasma glucose was analyzed using a colorimetric commercial kit (Glucox 500-Doles Reagentes, Brazil) based on the glucose–oxidase reaction. Cortisol was analyzed with a commercial immunoenzymatic kit (Diagnostic Systems, USA) and the reading carried out in a microplate reader at 450 nm.

Fish livers were homogenized in 10 volumes (w/v) of ice-cold 0.1 M K-phosphate buffer (pH 7.0) and centrifuged (14000 ×*g*) for 20 min at 4 °C, to obtain the supernatant for glutathione-*S*-transferase (GST) and catalase analyses. GST activity was determined as described by Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The change in absorbance was recorded at 340 nm and the enzyme activity was expressed in nmol min^{-1}.mg liver protein. Catalase activity was estimated from the rate of consumption of hydrogen peroxide (Beutler, 1975). Change in absorbance was recorded at 240 nm and enzyme activity was expressed in µmol min^{-1}.mg liver protein. Concentration of protein in the supernatant was measured by the method of Lowry et al. (1951). All samples were analyzed in duplicate.

2.3. Histopathological analysis

Liver samples were fixed in Bouin's fixative, embedded in paraffin and sectioned (5 µm). The slides were stained with