Environmental Toxicology and Pharmacology 56 (2017) 241–248

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Research Paper

Effects of glyphosate-based herbicide on pintado da Amazônia: Hematology, histological aspects, metabolic parameters and genotoxic potential

Fernando Rafael de Moura[a], Ritane Rose da Silva Lima[b], Ana Paula Simões da Cunha[b], Patrícia da Costa Marisco[b], Danilo Henrique Aguiar[c], Marina Mariko Sugui[a], Adilson Paulo Sinhorin[a], Valéria Dornelles Gindri Sinhorin[a,*]

[a] Programa de Pós-Graduação em Ciências Ambientais, Laboratórios Integrados de Pesquisas em Ciências Químicas (LIPEQ), Instituto de Ciências Naturais, Humanas e Sociais, Universidade Federal de Mato Grosso, Campus Universitário de Sinop, Sinop, Mato Grosso, Brazil
[b] Instituto de Ciências da Saúde, Universidade Federal de Mato Grosso, Campus Universitário de Sinop, Sinop, Mato Grosso, Brazil
[c] Instituto de Ciências Naturais, Humanas e Sociais, Universidade Federal de Mato Grosso, Campus Universitário de Sinop, Sinop, Mato Grosso, Brazil

ARTICLE INFO

Keywords:
Hybrid fish
Histology
Metabolism
Roundup Original®
Water contamination

ABSTRACT

Roundup Original® is an herbicide widely used in Mato Grosso's agriculture and it may contamine water bodies, being an unforeseen xenobiotic to aquatic organisms, particularly fish. This study investigated the effects on the hybrid fish jundiara (*Leiarius marmoratus* × *Pseudoplatystoma reticulatum*) of an environmentally relevant exposure to this herbicide. Glucose levels in liver, muscle and plasma decreased after exposure to 1.357 mg L^{-1} of Roundup Original® (glyphosate nominal concentration), while glycogen levels reduced in liver and muscle for different times. Elevated cholesterol and triglycerides revealed an adaptive response. Protein and lactate levels also increased during the experiment, however no changes were observed for muscle lactate. Increment of the transaminases suggests damage to the liver cells. After 96 hours of exposure, reductions in all hematological parameters were observed, whereas the micronucleus test findings showed genotoxic scenery. Histological analysis did not display pathological alterations of the hepatic tissue. The results obtained provide valuable data for noticing the effects of pollutants on non-target organisms.

1. Introduction

Agriculture is dependent on the use of chemical substances for crop protection and also to increase production. Herbicides are widely used in field crops to control the presence of weeds, however they potentially threaten non-target organisms (Çavas and Könen, 2007; Samanta et al., 2014). Parvez and Raisuddin (2005) pointed out that the response of aquatic organisms when subjected to a pollutant results in modification of the organism's cellular and biochemical biology, leading to major alterations in tissues, physiology and even behavior. Glyphosate-based herbicides are extremely common throughout the world due to their cost-effectiveness and because they are not considered to bioaccumulate in organisms (Giesy et al., 2000). However, aquatic organisms, in particular fish, seem to be more sensitive to glyphosate than mammals (Lushchak et al., 2009). Most glyphosate formulas contain the presence of a surfactant. Modesto and Martinez (2010) state that the critical part of the herbicide formula is the surfactant due to its higher toxicity than the primary agent. The Roundup Original® formula contains polyoxyethylene amine (POEA), already demonstrated to be more toxic than glyphosate to aquatic organisms (Santos et al., 2005).

Grisolia (2002) used the micronucleus test to demonstrate how three different dosages of Roundup (42, 85 and 170 mg Kg^{-1}) were able to induce genotoxicity in *Tilapia rendalii* even though in the same experiment the higher dosages of 50, 100 and 200 mg Kg^{-1} did not cause the same effect in mice. Metabolical disorders were also observed in fish exposed to glyphosate-based herbicides such as the reduction of glycogen and glucose in muscle of *L.obtusidens* exposed to 3, 6, 10, and 20 mg L^{-1} of Roundup (Glusczak et al., 2006), increase in liver protein levels and reduction of plasma cholesterol in surubim exposed to 2.25, 4.5, 7.5, and 15 mg L^{-1} of Roundup Original® (Sinhorin et al., 2014), and also a variety of histological, biochemical and physiological modifications in *P. lineatus* exposed to 7.5 and 10 mg L^{-1} of Roundup (Langiano and Martinez, 2008).

The state of Mato Grosso is considered the largest producer of transgenic soybean in Brazil, and as a consequence is paralleled by the intense use of pesticides in these monocultures, increasing the likelihood of environmental contamination by pollutants (Shiogiri et al., 2012). Sinhorin et al. (2014) state that Mato Grosso is also part of the

* Corresponding author.
E-mail addresses: valeriadgindri@gmail.com, valeriadgindri@hotmail.com (V.D.G. Sinhorin).

http://dx.doi.org/10.1016/j.etap.2017.09.019
Received 17 August 2017; Received in revised form 25 September 2017; Accepted 27 September 2017
Available online 29 September 2017

Legal Amazon and as a result is influenced by several rivers where fishing is a widespread practice. The aquaculture sector within this region has been increasing due to a reduction in availability of native fish as a result of over-fishing.

Based on a similar situation observed by Samanta et al. (2014), fish in Mato Grosso may be exposed to cumulative doses of glyphosate-based herbicides due to the contamination of rivers, streams and ponds from crop residue runoff as mentioned by Armas et al. (2007). These authors indicated that although glyphosate was below the limit of detection in the water samples analyzed in their study, it was in counterpart the most detected molecule among the tested substances, with an elevated solubility in water, suggesting its potencial as a water contaminant.

This study aims to assess how the above cited situation is being reproduced in this region by evaluating the hybrid fish "pintado da Amazônia" or "jundiara" (*Leiarius marmoratus* × *Pseudoplatystoma reticulatum*) and its feedback to an acute laboratorial exposure to a sublethal concentration of Roundup Original® through the evaluation of metabolic, histologic, hematologic and genotoxic parameters. This species was chosen as there is limited information on how this hybrid fish reacts to herbicide exposure, and also because this species is an important component in the region's commercial fisheries.

2. Material and methods

2.1. Chemicals

Roundup Original® (480 g L^{-1} containing isopropylamine salt of glyphosate, 360 g L^{-1} acid equivalente, *N*-(phosphonomethyl) glycine (glyphosate) and 684 g L^{-1} of inert ingredients), Monsanto, St. Louis, MO, USA was used in this experiment. Paraphenylphenol and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The kits for determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), plasma glucose, plasma protein levels, plasma cholesterol and plasma lactate were purchased from Labtest®, Diagnostics SA, Minas Gerais, Brazil. Other reagents used in the experiment were of the highest analytical grade.

2.2. Fish

Juvenile jundiara (85.0 ± 10.0 g and 18.0 ± 2.0 cm) were obtained from a fish farm in Sinop, MT, Brazil. Prior to experimentation, fish were acclimatized to laboratory conditions for 7 days in 300 L fiberglass tanks containing aerated and dechlorinated water under natural photoperiod conditions (12 h light/12 h dark). During this period fish were fed once per day with commercial fish pellets containing 42% crude protein. The physico-chemical parameters of the test water were measured every day (mean ± SD): temperature 27 ± 1·0 °C, pH 6.84 ± 0·4, dissolved oxygen 6.31 ± 0.5 mg L^{-1}, hardness 18 ± 2.0 mg L^{-1} $CaCO_3$, nonionized ammonia 0.05 ± 0.02 mg L^{-1} and nitrite 0.06 ± 0.01 mg L^{-1}. Pellet residue and feces were removed by suction and filtration every other day.

2.3. Experimental design

After the acclimatization period, fish were randomly distributed among eight different groups: four non-exposure groups (6, 24, 48 and 96 h), the control groups, and four exposure groups (6, 24, 48 and 96 h) exposed to 1.357 mg Gly a.e L^{-1}, from Roundup Original® diluted in a stock solution (100 mg L^{-1}). For this experiment we chose the concentration equivalent to 10% of LC_{50}, 1.357 mg L^{-1}, based on previous tests (data not shown) and according to the values of glyphosate (nominal concentration) used in agricultural areas in Brazil, which range between 0.36 to 2.16 mg L^{-1} (Rodrigues and Almeida, 2005; Moreno et al., 2014; Sánchez et al., 2017) and in view of the repeated applications of the herbicide in most developing countries, the concentration in the aquatic ecosystem may be higher, thus, suggesting the relevance of our test concentration (Nwani et al., 2013). Fish were placed into 50 L tanks, with continuously aerated dechlorinated water, with 3 fish per tank. Water quality parameters were monitored and maintained at similar levels to the acclimatization period throughout the experiment, since fish were kept in a static system. All tests were performed in triplicate (total of 24 tanks). The Roundup Original® solution was added to the experimental tanks. At the end of exposure for each time period, exposed and corresponding control fish were removed from the tanks, anesthetized with eugenol (50 mg L^{-1}) (Kreutz et al., 2011), and caudal vein blood was drawn using a heparinized syringe. Fish were killed by medular section. Liver and muscle samples were removed by dissection. Samples were stored at −80 °C for further analysis. This study was approved by the Committee Guidelines (Comitê de Ética no Uso de Animais of the Universidade Federal de Mato Grosso). Reference number: 23108.780290/11-5.

2.4. Determination of metabolic variables

Glycogen was determined in liver and muscle tissue following the method of Bidinotto et al. (1997). Glucose and lactate were measured according to Duboie et al. (1956) and Harrower and Brown (1972), respectively. Protein content was determined through the method described by Lowry et al. (1951).

Blood samples were centrifuged and the blood plasma collected and stored at −80 °C for analysis. Plasma glucose and total protein levels were analyzed using colorimetric commercial kits based on the glucose oxidase method and biuret reactions, respectively. Plasma lactate and cholesterol were determined using enzymatic commercial kits based on lactate oxidase-peroxidase and esterase-oxidase reactions, respectively. ALT and AST activity was determined using kits based on Ultraviolet-International Federation of Clinical Chemistry and Laboratory Medicine (UV-IFCC) kinetic reactions.

2.5. Histological analysis

For the histological examination liver portions were removed and immediately fixed in 10% formaldehyde and dehydrated through a graded series of ethanol (70 to 95%). Following this the portions were embedded in historesin and sectioned at 3 μm thickness with a microtome. These sections were rehydrated in distilled water and then stained using hematoxylin and eosin (HE). To examine the presence of abnormalities such as necrosis, vacuolation or inflammation of the tissue, the Miotic 2.0 data analysis and processing software was used.

2.6. Hematologic parameters

An aliquot of blood was used to determine hemoglobin (Hb), hematocrit (Ht), total erythrocytes (RBC) and total leukocytes (WBC). Samples were analyzed using biochemical analyzer (XT-18000 Sysmex, Roche, Hitachi Ltd, Tokyo, Japan) at a Clinical Analysis Laboratory. This equipment uses the electric resistance detecting method (impedance technology) with hydro dynamic focusing to measure RBC, Ht and other parameters. Fluorescence flow cytometry is used to measure WBC, reticulocyte count and platelets count.

2.7. Micronucleus test

A blood aliquot of the sample fish was smeared onto clean glass slides and air-dried overnight. Slides were fixed using ethanol for 15 minutes, then washed with distilled water and stained with Giemsa 10% for 20 minutes. Slides were then permanently mounted using Permount® resin between the slide and coverslip and stored for analysis. Two slides were prepared for each fish and the number of micronuclei present in 1000 erythrocytes were scored for each slide under × 100 magnification (Grisolia, 2002).