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Effects of Roundup Transorb on fish: Hematology, antioxidant defenses and acetylcholinesterase activity

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ABSTRACT

Roundup Transorb® (RDT) is a glyphosate-based herbicide containing a mixture of surfactants. The objective of this work was to evaluate the effects of this herbicide on the Neotropical fish *Prochilodus lineatus*. Juvenile fish were acutely exposed (6, 24 and 96 h) to 1 mg L^{-1} of RDT (RDT 1), 5 mg L^{-1} of RDT (RDT 5) or only water (control) and blood samples for hematological analysis, liver for antioxidants analysis, and brain and muscle for acetylcholinesterase (AChE) determination, were collected. RDT effects were more evident in fish exposed to the higher concentration of the herbicide. Hematologic alterations appeared only after 96 h exposure, when fish showed an increase in the hematocrit and in the number of both red and white blood cells. After 6 h exposure fish showed a transient reduction in superoxide dismutase and catalase activity. RDT also inhibited glutathione-S-transferase, after 6 and 24 h of exposure. The reduction in these enzymes is probably related to the occurrence of lipid peroxidation (LPO) in fish exposed to the herbicide for 6 h. LPO returned to control levels after 24 and 96 h exposure to RDT, when fish showed an increased activity of glutathione peroxidase. The content of reduced glutathione also increased after 96 h exposure. Thus, after 24 and 96 h the antioxidant defenses were apparently enough to combat ROS, preventing the occurrence of oxidative damage. The exposure to RDT for 96 h led to an inhibition of AChE in brain and muscle at rates which may not be considered a life-threatening situation.

1. Introduction

The glyphosate N-phosphomethyl-glycine is a post-emergent herbicide widely used in several types of cultures. Numerous commercial formulations containing glyphosate as the active ingredient have become popular around the world due to their effective action and low toxicity to mammals (Corbera et al., 2005). Roundup Transorb® (RDT), an example of an herbicide that uses glyphosate as the active principle, is classified as harmful to the environment (Class III). This product was launched into the American market in 1998 for weed control in the culture of sugarcane, coffee and plants of the genus *Citrus*. The main feature of this product in relation to other formulations is its rapid translocation of about 60 min. The so-called Transorb® technology enables the faster delivery of the product and in larger quantities to the root of the invader plant, offering less risk of loss in adverse conditions such as rain. What allows for the rapid translocation of the active principle is the surfactant used in this formulation.

The surfactant is a critical part of an herbicide formulation as it assists adhesion to, and wetting and spreading on the leaf, and uptake through the leaf. Several types of surfactants are used and these substances, which are usually highly toxic, are responsible for the toxicity of the products used. The surfactant used in the formulation of RDT is a mixture containing 15% of polyoxyethylene amine (POEA) and other unspecified surfactants (Howe et al., 2004). Several studies have demonstrated that this product is more toxic than the original Roundup® formulation (Howe et al., 2004; Santos et al., 2005); however, the literature about the toxicity of RDT is still scarce, especially for animals.

Teleost fish have proved to be good models to evaluate the toxicity and effects of contaminants on animals, since their biochemical responses are similar to those of mammals and of other vertebrates (Sancho et al., 2000). *Prochilodus lineatus* is an economically important neotropical fish species, commonly found in rivers of the south and southeast regions of Brazil and considered as a potential bioindicator species (Martinez and Souza, 2002; Martinez et al., 2004; Camargo and Martinez, 2006). In this fish acute exposures to sublethal concentrations of Roundup® original induced DNA impairment (Cavalcante et al., 2008), hematological changes and liver histological alterations (Langiano and Martinez, 2008), and altered antioxidant defenses (Modesto and Martinez, 2010).

The exposure of fish to several types of chemical agents may induce changes in several hematological variables (Heath, 1995), which are frequently used to evaluate fish health (Martinez and Souza, 2002). The study of blood parameters in fishes has been

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widely used for the detection of physiopathological alterations in different conditions of stress (Nussey et al., 1995). Hematological parameters such as hematocrit, hemoglobin, number of erythrocytes and white blood cells are indicators of toxicity with a wide potential for application in environmental monitoring and toxicity studies in aquatic animals (Sancho et al., 2000; Barcellos et al., 2003).

Some biochemical parameters also represent fine tools for evaluating the effects of contaminants and for environmental monitoring (Ahmad et al., 2004). These biomarkers include acetylcholinesterase (AChE). Changes in AChE activity might be related to various contaminants (Payne et al., 1996; Miron et al., 2005; Ferrari et al., 2007). Organophosphate and carbamate insecticides are AChE inhibitors (Monserrat et al., 2002), but several studies have also shown that formulations containing glyphosate can also inhibit AChE activity in fishes (Glusczak et al., 2006, 2007; Modesto and Martinez, 2010). The determination of AChE activity is normally done in brain and muscle tissue because the neuromuscular system of fish is principally cholinergic and its activity is essential for normal muscle behavior and function (Payne et al., 1996). Inhibition of AChE in brain produces alterations in behavior, and in muscle it leads to hyperstimulation of muscle fibers, which may cause tetania, paralysis and death (Kirby et al., 2000). In fishes, brain AChE has been studied in greater depth than muscle AChE (Ferrari et al., 2007).

Many pollutants can induce the formation of reactive oxygen species (ROS) (Ahmad et al., 2000), such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot-}$), and hydroxyl radical ($^{\cdot}OH$). Due to their high reactivity, these species may damage lipids, proteins, carbohydrates and nucleic acids (Hermes Lima, 2004). To neutralize ROS, animals have an antioxidant defense pathway constituted of antioxidant enzymes such as superoxide dismutase (Cu–Zn SOD), catalase (CAT) and glutathione peroxidase (Se-GPx), as well as non-enzymatic antioxidants such as glutathione tripeptide (GSH).

When the animal's defenses are insufficient to neutralize ROS, oxidative damage may occur, and one of the most serious damages is membrane lipid peroxidation (Scandalios, 2005). Herbicide-induced lipid peroxidation has already been described for various fish species (Sevgiler et al., 2004; Glusczak et al., 2006, 2007; Modesto and Martinez, 2010). Therefore, both the activity of antioxidant enzymes and the occurrence of oxidative damage have been proposed as indicators of pollutant-mediated oxidative stress (Ahmad et al., 2000; Li et al., 2003).

In view of the above, and considering the lack of knowledge about the toxic potential of the herbicide Roundup Transorb® to aquatic animals and the growing use of this herbicide, the objective of this work was to evaluate its effects on hematological and biochemical parameters of *P. lineatus*.

2. Materials and methods

2.1. Animals

Juveniles of *P. lineatus* with mean weigh of 9.97 ± 2.5 g (mean ± SD, n = 108), supplied by the University Hatchery Station, were acclimated for one week on 300 L tanks containing dechlorinated and aerated water. The physical and chemical parameters were kept nearly constant (mean ± SE): temperature 23.0 ± 1.8 °C; pH 6.9 ± 1.2; dissolved oxygen 6.9 ± 0.7 mg O_2 L^{-1}; conductivity 69.9 ± 1.6 μS cm^{-1} and hardness 44 mg $CaCO_3$ L^{-1}. During this period, the fish were fed at 48 h intervals with a commercial fish feed containing 36% of protein. Feeding was suspended 48 h prior to testing and the animals were not fed during the experiments.

2.2. Experimental design

The commercial formulation of Roundup Transorb® (480 g glyphosate L^{-1}, Monsanto do Brasil LTDA) at two nominal concentrations 1 and 5 mg L^{-1} was used. These concentrations were defined taking into account: (i) the current application rates of glyphosate-based herbicides, according to which a water body with no interception vegetation can have a maximum concentration of 3.7 mg glyphosate L^{-1} (Giesy et al., 2000), which corresponds to 7.7 mg of Roundup Transorb L^{-1}; (ii) the half-life of glyphosate, which is 7 d; (iii) the results of preliminary tests where *P. lineatus* exposed to 1 and 5 mg L^{-1} of RDT survived throughout the 96 h of exposure while fish exposed to 10 mg L^{-1} of RDT did not.

For the acute toxicity tests, the animals were placed in 100 L aquaria filled with dechlorinated and continually aerated water, with a density of six fish per aquarium. For each experimental period (6, 24 and 96 h) a toxicity test was run wherein one group of fish was exposed only to water (control), another to water containing 1 mg L^{-1} of RDT (RDT 1), and a third to water containing 5 mg L^{-1} of RDT (RDT 5). All toxicity tests were carried out in duplicate in accordance with OECD guideline (OECD, 1992). Water parameters were monitored during the experiments (mean ± SE): temperature 25.3 ± 0.4 °C; pH 7.4 ± 0.1; dissolved oxygen 6.0 ± 0.8 mg O_2 L^{-1}; conductivity 59.5 ± 5.8 μS cm^{-1} and hardness 44 mg $CaCO_3$ L^{-1}.

After exposure, the fish were removed from the aquaria, immediately anesthetized with benzocaine (0.1 g L^{-1}), and caudal vein blood drawn with a heparinized syringe. The animals were then killed by medullar section, measured and weighed, and samples of liver, brain and white muscle were removed by dissection. The samples were frozen at −80 °C until use.

2.3. Hematologic parameters

An aliquot of the blood was used for the determination of hematocrit, by microcentrifugation in capillary tubes and the whole blood hemoglobin by the cyanmethemoglobin method in a spectrophotometer at 540 nm. Total erythrocytes (RBC) were counted on improved Neubauer hemocytometer using blood samples fixed in formol citrate. Total leukocytes (WBC) were counted in blood smears stained with routine panoptics stains (Laborclin Ltda, BR) Two thousand WBC were analyzed on each slide for the total count and at least 200 leukocytes on each slide for the differential count.

2.4. Liver enzymes

The livers were weighed, homogenized (10× volume) in potassium phosphate buffer 0.1 M (pH 7.0), centrifuged (20 min, 15,000g, 4 °C), and the supernatant removed for analysis of the biochemical parameters. Glutathione-S-transferase (GST) activity was determined according to the methodology proposed by Keen et al. (1976), following the complexation of the reduced glutathione (GSH) with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm, and expressed as nmol of conjugated CDNB min^{-1} mg of $protein^{-1}$. Copper–zinc superoxide dismutase (CuZn–SOD) activity was determined by the method of Flohé and Otting (1984). This method is based on the measurement of the inhibition of the reduction rate of cytochrome c by the superoxide radical, at 550 nm. SOD activity was expressed in U SOD mg of $protein^{-1}$, with one U of SOD corresponding to the quantity of enzyme that promoted the inhibition of 50% of the reduction rate of cytochrome c. Catalase (CAT) activity was determined according to the technique described by Beutler (1975), by monitoring the H_2O_2 decomposition from the decrease of absorbance at 240 nm. CAT activity was expressed in μmol H_2O_2 min^{-1} mg of $protein^{-1}$. Selenium-dependent glutathi-

one peroxidase (Se-GPx) activity was determined by the method of Hopkins and Tudhope (1973), based on NADPH oxidation in the presence of GSH (0.95 mM) and H_2O_2 at 340 nm. GPx activity was expressed in μmol oxidized NADPH min^{-1} mg of $protein^{-1}$.

2.5. Non-enzymatic antioxidant

Reduced glutathione (GSH) levels were estimated according to Beutler et al. (1963), using 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) following Monteiro et al. (2009) and Thomaz et al. (2009). Supernatants of the acid extracts (1:1 v/v with 12% TCA) were added to 0.25 mM DTNB in 0.1 M potassium phosphate buffer, pH 8.0, and thiolate anion formation was determined at 412 nm against a GSH standard curve. GSH content was expressed in μg of GSH mg of $protein^{-1}$.

2.6. Lipid peroxidation (LPO)

Lipid peroxidation (LPO) was determined by measuring the lipid hydroperoxide (HP) levels using the FOX method (Ferrous Oxidation–Xylenol orange) as described by Jiang et al. (1991). Samples previously deproteinized (with 10% TCA) were incubated for 30 min at room temperature with a reaction mixture containing 0.25 mM $FeSO_4$, 25 mM H_2SO_4, 0.1 mM xylenol orange and 4 mM butylated hydroxytoluene in 90% (v/v) methanol. HP levels were measured spectrophotometrically at 560 nm, using a standard curve of cumene hydroperoxide (CHP) ranging from 0.0004 to 0.1 mM. HP was expressed as μmol CHP mg $protein^{-1}$.

2.7. Brain and muscle acetylcholinesterase (AChE)

Brain and muscle samples were homogenized (10× volume) in potassium phosphate buffer (0.1 M, pH 7.5), centrifuged (20 min, 15,000*g*, 4 °C), and the supernatant was removed for analysis of AChE. Enzyme activity was determined based on the colorimetric method of Ellman et al. (1961) adapted for reading on microplates, according to Alves Costa et al. (2007). The final concentration of the acetylthiocholine iodide substrate employed was 9 mM, while that of the DTNB color reagent was 0.5 mM for both tissues. Absorbance was determined in a microplate reader at 415 nm and the enzyme activity was expressed in nmol DTNB min^{-1} mg of $protein^{-1}$.

The protein concentration in all the samples of all the assays was determined by the method of Lowry et al. (1951), using bovine albumin as standard.

2.8. Statistical analysis

For each treatment group (control, RDT 1 and RDT 5), at each period of exposure (6, 24 and 96 h), 12 animals were sampled. However, the amount of tissue sampled from one fish was not enough for the determination of all the parameters and thus samples were distributed in order to get at least five or six fish samples for each analysis. For all the parameters analyzed, differences among control and experimental groups, at each experimental time, were analyzed by parametric (one-way ANOVA) or non-parametric (Kruskall-Wallis) analysis of variance, depending on the distribution of the data and the homogeneity of the variances. Where appropriate, multiple-range test (Tukey test) was used to identify the differences. Values of $P \leqslant 0.05$ were considered significant.

3. Results

During the tests, there was no fish mortality in any of the experimental groups and the water parameters were monitored and kept constant.

The results of the hematological parameters analyzed are shown in Table 1. A significant increase in hematocrit and number of erythrocytes in the fish of group RDT 5 was found after 24 and 96 h of exposure. There was also a significant increase in the total number of leukocytes (WBC) and lymphocytes and a reduction in the number of neutrophils in the same group after 96 h of exposure.

Considering the primary antioxidants enzymes, SOD and CAT activity showed a significant reduction in the fish exposed for 6 h to the highest concentration of herbicide, and after 24 h exposure CAT activity decreased significantly in fish exposed to both concentrations of the herbicide (Fig. 1a and b). Fish exposed to RDT for 96 h did not show any significant alteration in SOD and CAT activ-

Table 1
Blood parameters of *Prochilodus lineatus* exposed only to water (control), to 1 mg L^{-1} of Roundup Transorb® (RDT 1) or to 5 mg L^{-1} of Roundup Transorb® (RDT 5), for different experimental periods (6, 24 and 96 h).

		Hb (g 100 mL^{-1})	Ht (%)	RBC (×10^6 mm^{-3})	WBC (×10^3 mm^{-3})	
6 h	Control	7.45 ± 0.47	32.2 ± 1.2	2.89 ± 1.40	201.3 ± 8.7	
	RDT 1	8.05 ± 0.89	33.9 ± 0.9	2.88 ± 1.60	201.5 ± 6.4	
	RDT 5	7.56 ± 0.74	32.5 ± 0.5	2.87 ± 1.04	202.7 ± 4.8	
24 h	Control	7.85 ± 0.53	31.3 ± 0.7	2.29 ± 0.13	202.9 ± 5.1	
	RDT 1	8.58 ± 0.62	34.6 ± 1.6	2.27 ± 0.10	203.4 ± 4.1	
	RDT 5	6.96 ± 0.47	35.2 ± 1.0*	2.38 ± 0.11*	202.8 ± 6.8	
96 h	Control	8.04 ± 0.78	33.5 ± 1.0	2.54 ± 0.85	201.7 ± 7.4	
	RDT 1	8.21 ± 1.01	34.6 ± 1.9	2.53 ± 1.00	201.9 ± 6.7	
	RDT 5	7.89 ± 1.04	36.9 ± 0.5*	2.65 ± 0.79*	220.1 ± 4.7 *	
		Differential leukocyte counts (%)				
		lym	neut	mon	eos	bas
6 h	Control	54.9 ± 0.9	38.4 ± 1.7	5.1 ± 0.9	0.8 ± 0.4	0.8 ± 0.3
	RDT 1	55.1 ± 0.9	38.5 ± 1.4	5.0 ± 0.8	0.9 ± 0.8	0.6 ± 0.4
	RDT 5	55.2 ± 0.8	37.9 ± 1.8	5.1 ± 0.5	0.9 ± 0.7	0.9 ± 0.5
24 h	Control	54.5 ± 1.1	39.0 ± 1.1	4.9 ± 1.1	0.7 ± 0.5	0.9 ± 0.6
	RDT 1	54.8 ± 0.7	38.8 ± 0.7	5.0 ± 0.6	0.9 ± 0.7	0.5 ± 0.4
	RDT 5	55.0 ± 0.9	38.8 ± 1.0	4.8 ± 1.2	0.8 ± 0.6	0.6 ± 0.3
96 h	Control	54.7 ± 0.8	39.2 ± 0.9	4.8 ± 0.9	0.8 ± 0.4	0.5 ± 0.4
	RDT 1	54.1 ± 0.4	39.8 ± 0.8	4.8 ± 0.7	0.9 ± 0.6	0.4 ± 0.2
	RDT 5	60.1 ± 0.5*	34.9 ± 0.4 *	4.0 ± 0.5	0.4 ± 0.3	0.6 ± 0.3

The values represent means ± SE (*n*: 5–6).
* Significantly different from respective control ($p \leqslant 0.05$). Hb = hemoglobin, Ht = hematocrit, RBC = erythrocytes count, WBC = leukocytes count, lym = lymphocytes, neut = neutrophils, mon = monocytes, eos = eosinophils and bas = basophils.

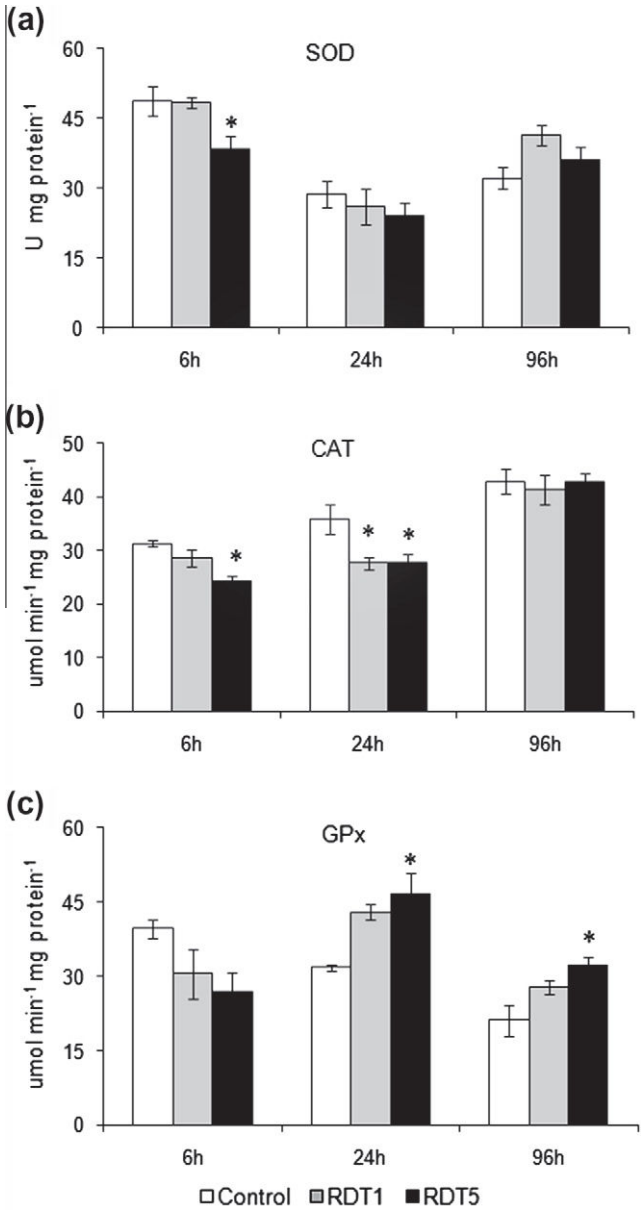

Fig. 1. Hepatic activity of superoxide dismutase (a), catalase (b) and glutathione peroxidase (c) of *Prochilodus lineatus* exposed to 1 mg L^{-1} (RDT 1) or 5 mg L^{-1} (RDT 5) of Roundup Transorb® or only water (control), for different experimental periods (6, 24 and 96 h). Data are means ± SE (n = 5 or 6). *Different from respective control, $P \leqslant 0.05$.

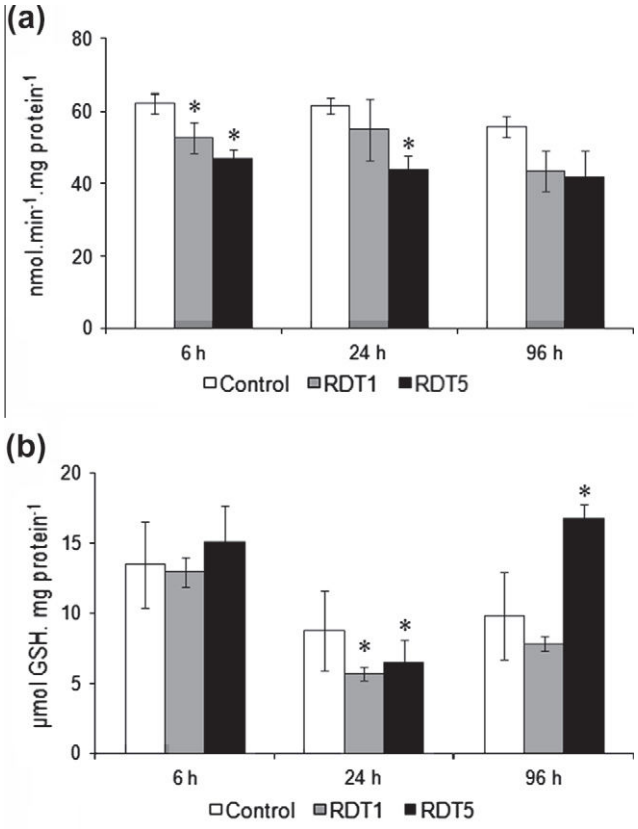

Fig. 2. Activity of glutathione-S-transferase (a) and GSH content (b) in the liver of *Prochilodus lineatus* exposed to 1 mg L^{-1} (RDT 1) or 5 mg L^{-1} (RDT 5) of Roundup Transorb® or only water (control), for different experimental periods (6, 24 and 96 h). Data are means ± SE (n = 6). *Different from respective control, $P \leqslant 0.05$.

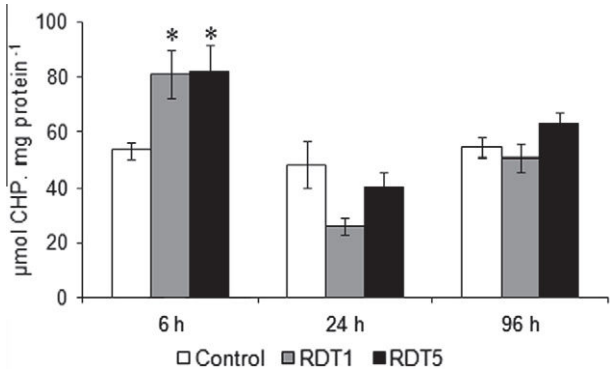

Fig. 3. Lipid hyperoxide concentrations, expressed as the concentration of cumene hydroperoxide (CHP), in the liver of *Prochilodus lineatus* exposed to 1 mg L^{-1} (RDT 1) or 5 mg L^{-1} (RDT 5) of Roundup Transorb® or only water (control), for different experimental periods (6, 24 and 96 h). Data are means ± SE (n = 6). *Different from respective control, $P \leqslant 0.05$.

ity. On the other hand, GPx activity increased significantly after 24 and 96 h of exposure to RDT 5 (Fig. 1c).

GST activity was significantly reduced in fish exposed for 6 h to both RDT concentrations and in fish exposed for 24 h to RDT 5. After 96 h exposure to both RDT concentrations GST activity remained reduced in comparison with respective control, but no significant difference was detected (Fig. 2a). GSH levels decreased after 24 h exposure to both concentrations of the herbicide, but fish of group RDT 5 presented a significant increase in GSH levels after 96 h exposure (Fig. 2b).

The LPO levels significantly increased in the liver of fish exposed to both concentrations of RDT for 6 h (Fig. 3). Significant alterations in hepatic LPO levels were not registered in fish exposed to RDT for longer periods.

AChE activity in the brain of *P. lineatus* showed significant reductions after 96 h exposure to RDT 1 (decrease 11.75%) and RDT 5 (decrease 17.88%) in comparison with respective controls (Fig. 4a). In muscle tissue, the activity of the enzyme was significantly reduced by 21.25% in fish exposed to RDT 5 for 96 h (Fig. 4b).

4. Discussion

Some recent studies have addressed the effects of Roundup original on fish (Çavas and Könen, 2007; Glusczak et al., 2007; Lushchak et al., 2009; Modesto and Martinez, 2010) however, to our knowledge, this is the first study that focus on the effects of an-

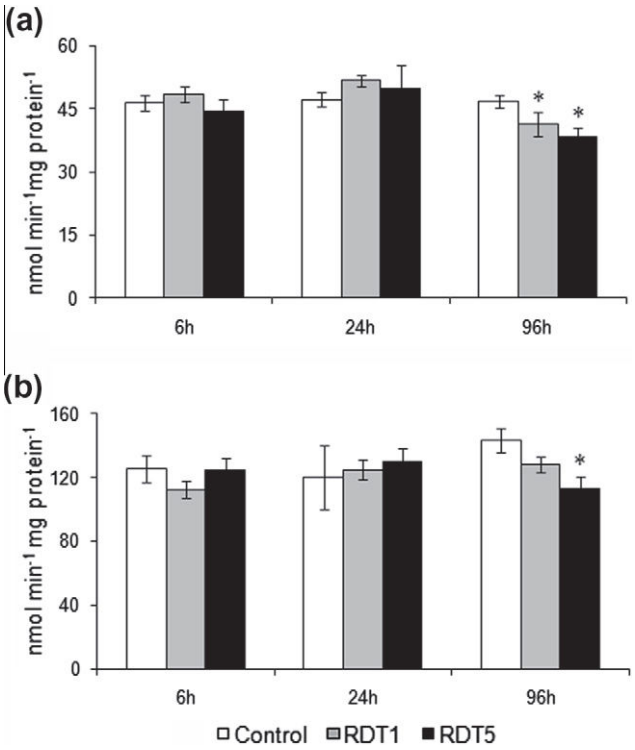

Fig. 4. Acetylcholinesterase activity in brain (a) and muscle (b) tissue of *Prochilodus lineatus* exposed to 1 mg L^{-1} (RDT 1) or 5 mg L^{-1} (RDT 5) of Roundup Transorb® or only water (control), for different experimental periods (6, 24 and 96 h). Data are means ± SE ($n = 6$). *Different from respective control, $P \leqslant 0.05$.

other glyphosate-based herbicide, the Roundup Transorb, on fish. This work showed that this herbicide is an inhibitor of brain and muscle acetylcholinesterase, and promotes important changes in the hematology and antioxidant defenses of the Neotropical fish species *P. lineatus*.

Hematological parameters in fish can significantly change in response towards chemical stressors; however, these alterations are non-specific to a wide range of substances. Some of these changes may be the result of the activation of protective mechanisms (Cazenave et al., 2005) such as the results of the blood parameters observe in the present work. Fish exposed to the higher concentration of RDT for 24 and 96 h showed an increase in hematocrit and in the number of erythrocytes, indicating the release of erythrocytes from blood deposits and/or from hemopoetic tissues into the blood stream (Svodova et al., 1994). This finding disagrees with the results reported by Glusczak et al. (2006), who observed a decrease in hematocrit, number of erythrocytes and hemoglobin of *Leporinus obtusidens* exposed to different concentrations of Roundup® original (3, 6, 10 and 20 mg L^{-1}) for 96 h. This difference in response may be explained by the fact that Roundup Transorb and Roundup original are herbicides with different formulations, although they use the same active principle, glyphosate. Besides the xenobiotic type, it is also important to point out that the effects of environmental toxicants on hematological characteristics of fish vary according to the target species (Elahee and Bhagwant, 2007). This is another explanation for the variation on the hematological responses observed in this work and in the work by Glusczak et al. (2006), using another fish species *L. obtusidens*.

White blood cell numbers can also be affected by a variety of physiological and environmental factors and the responses normally found when fish are subjected to an array of toxicants are lower percent lymphocytes and higher percent neutrophils (Witeska, 2005). However, fish exposed to RDT showed a different pattern of response, with an increase in the total number of leukocytes and lymphocytes followed by a decrease in neutrophils. These alterations can be the result of the activation of the immune system in the presence of contaminant, which in turn may be an adaptive response of the organism resulting in a more effective immune defense (Barreto-Medeiros et al., 2005).

As for the antioxidant enzymes, the reduction in SOD activity after 6 h of exposure to the higher concentration of the herbicide may be related to the production of oxidants. It is known that there is a complex pathway of interaction among the enzymes involved in the animal's antioxidant system and that the activity of one enzyme influences the activity of other enzymes. It is also known that the substrate of product of some of the antioxidant enzymes can also influence the activity of others. An excess of hydrogen peroxide may reduce SOD activity, while the superoxide anion may be responsible for decreased CAT activity (Bagnyukova et al., 2006). Thus, it is reasonable to assume that hydrogen peroxide is responsible for the reduction observed in SOD activity. This hypothesis is confirmed by the fact that the CAT activity was also reduced in the fish after 6 and 24 h of exposure to the herbicide, favoring the accumulation of hydrogen peroxide in the cell. Similarly, this reduction in CAT activity may be due to superoxide ions, which are probably not being neutralized efficiently by SOD. The SOD–CAT system is the first line of defense against oxygen toxicity, due to the inhibitory effects on the formation of oxyradicals (Pandey et al., 2003), and these enzymes are frequently used as biomarkers, indicating the production of reactive oxygen species (ROS) (Monteiro et al., 2006). In the present work, inhibition of these two enzymes is a sign of interference in the antioxidant defenses of the fish occurring in the first 24 h of exposure to the herbicide.

The decrease in the hepatic activity of GST in fish exposed to the herbicide for 6 and 24 h reinforces the idea of the presence of oxidants that would lead to the inactivation of the enzymatic activity (Bagnyukova et al., 2006) considering that GST is sensitive to products of the Haber–Weiss reaction (Hermes-Lima and Storey, 1993). The inhibition of GST was also observed in the liver of goldfish after 96 h exposure to Roundup original (Lushchak et al., 2009). Conversely, when *P. lineatus* was exposed to Roundup original Modesto and Martinez (2010) found a significant increase in liver GST after 24 and 96 h exposure. These results show that both types of glyphosate-based herbicides can have different effects on the same fish species and that the effect of the same product may vary among different fish species.

The significant increase of GPx activity in the fish from RDT 5 group, after 24 and 96 h of exposure, indicates that the antioxidant pathway was stimulated, probably due to the increased production of peroxides. Although this enzyme acts principally in the removal of organic peroxides, it is also involved in the metabolization of hydrogen peroxide (Zhang et al., 2004; Maran et al., 2009). Thus, the activation of GPx in 24 h may indicate a response to compensate the inhibition of CAT in this period of exposure.

Glutathione, the major non-protein thiol of cells, is involved in the cellular defense against the toxic action of oxyradicals (Schuliga et al., 2002). This low molecular mass thiol can be easily oxidized and serve as a sink for free radicals and other reactive species (Hermes Lima, 2004). Variations in cellular glutathione content are considered indicators of the degree and duration of exposure to oxidant pollutants in fish (Dautremepuits et al., 2009). In the present study, a transitory decrease in liver GSH after 24 h exposure to both RDT concentrations was followed by a raise in GSH content in fish exposed to the highest concentration of the herbicide. This increase after 96 h exposure to RDT 5 probably represents an adaptation following herbicide exposure. Increases in GSH level have been observed in response to nonlethal concentrations in acute toxicity studies (Schuliga et al., 2002). Augmented GSH content

was previously reported in fish after acute exposure to the herbicide simazine (Oropesa et al., 2009). Zhang et al. (2004) have reported that during a moderate oxidative stress, the GSH levels in fish liver can increase as an adaptive mechanism by means of an increased synthesis. Protective and adaptive roles of GSH against oxidative stress-induced toxicity are well established in aquatic animals (Regoli and Principato, 1995; Otto and Moon, 1995).

When not neutralized, reactive oxygen species can react with membrane lipids (Ahmad et al., 2000), producing lipid peroxidation, which is considered one of the main consequences of oxidative stress (Hermes Lima, 2004). In this work, the occurrence of lipid peroxidation was indicated by a transient increase in the levels of lipid hydroperoxide (LH) in fish exposed to both concentration of the herbicide for 6 h. However, LH levels returned to control levels after 24 and 96 h exposure to RDT, indicating that longer exposures to RDT did not affect lipid peroxidation in the liver of *P. lineatus*. Thus, it can be inferred that the antioxidant defense in 6 h of exposure was insufficient, leading to increased lipid peroxidation as a function of the presence of RDT. In fact, significant decreases in SOD, CAT and GST activities were observed after 6 h exposure to RDT. In longer experimental times, these defenses returned to basal levels and then they were apparently enough to combat the ROS, preventing the occurrence of this oxidative damage. Lushchak et al. (2009) using a similar method to quantify lipid peroxidation found that the herbicide Roundup original also does not affect lipid peroxidation in the liver of the goldfish after 96 h of exposure.

The exposure to RDT for 96 h lead to a inhibition of AChE in the brain by 11.7–17.9% and in the muscle by 21.3%. This rate of AChE inhibition may not be considered a life-threatening situation since available investigations show that fish are capable of tolerating over 90% AChE inhibition (Day and Scott, 1990; Oruc and Usta, 2007). However it can cause muscle hyperactivity which leads to intracellular ATP depletion and enhances the generation of reactive oxygen free radicals which may play an important role as mediators of skeletal muscle damage and inflammation (Yang et al., 1996). According to these authors, accumulation of oxygen free radicals might be a consequence of AChE inhibitor-induced muscle hyperactivity. Following this idea, it may be inferred that the increased GSH content and GPx activity in the liver after 96 h exposure to RDT might be a protection against ROS generated as a consequence of the muscle AChE inhibition.

This inhibition of the activity of acetylcholinesterase in the brain and muscle of fish exposed to RDT is consistent with other studies that have also reported the inhibition of this enzyme in *P. lineatus* (Modesto and Martinez, 2010) and other species of fish exposed to Roundup original (Glusczak et al., 2006, 2007). This inhibition is much more like to be due to a common surfactant used in both formulated product than to glyphosate itself (Giesy et al., 2000).

The results obtained in this work allow us to conclude that the formulation of Roundup Transorb® promotes alterations in hematologic and biochemical parameters of *P. lineatus* which were more evident in fish exposed to the higher concentration of the herbicide. Hematologic alterations appeared only after 96 h exposure, when fish showed an increase in the hematocrit and in the number of both red and white blood cells, which probably represent an adaptive response to help the organism to counteract the herbicide effects. After 6 h exposure fish showed a transient reduction in superoxide dismutase and catalase activity. RDT also inhibited glutathione-S-transferase, after 6 and 24 h of exposure. The reduction in these enzymes is probably related to the occurrence of lipid peroxidation (LPO) in fish exposed to the herbicide for 6 h. However, LPO returned to control levels after 24 and 96 h exposure to RDT, when fish showed an increased activity of glutathione peroxidase. The content of reduced glutathione also increased after 96 h exposure. Thus, after 24 and 96 h the antioxidant defenses were apparently enough to combat ROS, preventing the occurrence of oxidative damage. The exposure to RDT for 96 h led to an inhibition of AChE in brain and muscle but at rates which may not be considered a life-threatening situation.

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