



Effects of the surfactant polyoxyethylene amine (POEA) on genotoxic, biochemical and physiological parameters of the freshwater teleost *Prochilodus lineatus*

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ARTICLE INFO

Article history:

Received 17 April 2014

Received in revised form 11 June 2014

Accepted 15 June 2014

Available online 20 June 2014

Keywords:

Antioxidant
DNA damage
Endocrine disruptor
Glyphosate
Oxidative stress
Roundup®

ABSTRACT

The surfactant polyoxyethylene amine (POEA) is added to several formulations of glyphosate herbicides that are widely used in agriculture and can contaminate aquatic ecosystems. In the present study, an integrated approach examining genotoxic, biochemical and physiological parameters was employed to evaluate acute effects of POEA on the Neotropical fish *Prochilodus lineatus*. Juvenile fish were exposed to 0.15 mg·L⁻¹ (POEA 1), 0.75 mg·L⁻¹ (POEA 2) and 1.5 mg·L⁻¹ (POEA 3) of POEA or only water (CTR), and after 24 h exposure samples of blood and liver were taken. Compared with CTR, liver of fish exposed to POEA 2 and POEA 3 showed increased activity of 7 ethoxyresorufin-O-deethylase and increased content of glutathione, whereas the activity of glutathione-S-transferase was diminished. On the other hand, fish of the group POEA 1 showed an increase in the activity of superoxide dismutase and in the occurrence of lipid peroxidation. Fish exposed to POEA 3 presented increased hepatic activity of glutathione peroxidase and reduced plasma cortisol. The exposure to POEA at all concentrations tested caused an increase in plasma lactate and a decrease in the hepatic activity of catalase, in the number of red blood cells and in hemoglobin content. The comet assay used for analyzing DNA damage in blood cells indicated the genotoxicity of the surfactant at all concentrations tested. Taken together these results show that POEA can cause effects at various levels, such as hemolysis, DNA damage and lipid peroxidation, which are directly related to an imbalance in the redox state of the fish.

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1. Introduction

Herbicides are a group of chemicals of high environmental risk due to their growing use, presence in aquatic environments (Tsui and Chu, 2003), and their capacity to affect non-target organisms such as fish (Copatti et al., 2009). Besides the active ingredient, adjuvant compounds are added to herbicide formulations in order to improve the efficacy of the commercial product. Among the large and heterogeneous group of adjuvant substances, nonionic surfactants stand out due to their widespread use (Foy, 1996). However, there are few data on the risks and environmental toxicity of these compounds (Krogh et al., 2003). The assessment of the toxicity of the herbicide formulations is made almost exclusively based on the active ingredient, while the possible effects of adjuvant compounds are overlooked by environmental toxicologists and environmental protection agencies (Guilherme et al., 2012a).

Glyphosate-based herbicides are widely used around the world and rank first in the market of herbicides in Brazil (IBAMA, 2010). Governmental regulatory agencies and international organizations indicate low toxicity and environmental risk from direct exposure to glyphosate (Health Canada, 1991; EPA, 1993; WHO, 1994). However, several authors have found that commercial glyphosate formulations pose higher risk than glyphosate alone (Tsui and Chu, 2003; Howe et al., 2004).

Roundup® is a broad spectrum herbicide that represents one of the most commonly applied formulations in the world (Fan et al., 2013) and is used in agriculture, ornamental gardens and – some formulations are approved for application to – aquatic habitats (Giesy et al., 2000). In addition to glyphosate as the active ingredient, Roundup® has the nonionic surfactant polyoxyethylene amine (POEA) in its formulation, which seems to be directly related to the toxicity of the product (Howe et al., 2004). Several studies have already demonstrated that POEA is more toxic than the active ingredient and the formulated product itself (Tsui and Chu, 2003). Besides its high toxicity, the surfactant persistence in aquatic environments, ranging from 21 to 42 days, is longer than the half-life of the active ingredient which ranges from 7 to 14 days (Giesy et al., 2000). Despite the indication of the high toxicity of POEA there are few studies related to its effects on non-target organisms. The sublethal effects of this surfactant were investigated on growth and energetic

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reserves in the freshwater crayfish *Cherax quadricarinatus* (Frontera et al., 2011), on genotoxic parameters of the fish *Anguilla anguilla* (Guilherme et al., 2012a) and on oxidative stress parameters and acetylcholinesterase activity of the fish *Carasius auratus* (Fan et al., 2013).

Toxicity tests with aquatic organisms represent an effective tool for assessing the effects of pollutants on organisms. The Neotropical freshwater fish *Prochilodus lineatus* is commonly found in the south and southeast of Brazil and has been widely used in ecotoxicological studies due to their sensitivity to a wide variety of environmental pollutants such as herbicides (Modesto and Martinez, 2010a,b; Pereira et al., 2013).

The biotransformation of xenobiotic compounds includes different enzyme systems and several types of substrates in order to produce compounds more water-soluble, thus facilitating their excretion (Simonato et al., 2011). In fish, the isoenzyme class of the cytochrome P450 family responsible for the biotransformation of a broad range of xenobiotics is the CYP1A subfamily (Benedetti et al., 2007). The induction of this enzyme by contaminants is commonly measured by the activity of ethoxyresorufin-O-deethylase (EROD) (Whyte et al., 2000). Some studies have already shown changes in EROD activity after exposure of fish to herbicides (Santos and Martinez, 2012). Along the biotransformation process, the enzyme glutathione-S-transferase (GST) participates in the detoxification of lipophilic contaminants by catalyzing conjugation reactions with endogenous substrates which enhance the water solubility of contaminants, facilitating their elimination (Stegeman et al., 1992). The toxicity of many contaminants can be modulated by the induction of GST activity (Carletti et al., 2008), however, fish exposed to the herbicide Roundup® may exhibit a reduction in the activity of this enzyme (Lushchak et al., 2009; Modesto and Martinez, 2010a; Menezes et al., 2011).

Pesticides, among other types of xenobiotics, can induce the generation of reactive oxygen species (ROS) (Modesto and Martinez, 2010a). Due to the high reactivity, these ROS can interact with lipids, proteins, carbohydrates and nucleic acids and cause oxidative damage (Hermes Lima, 2004). Herbicides are able to induce carbonylation and thiol oxidation as post-translational modifications of proteins (Braconi et al., 2008) and these processes are suggested to play a key role in herbicide-induced toxicity (Braconi et al., 2011). In order to promote the neutralization of ROS, aerobic organisms have antioxidant defenses and oxidative damage occurs when these defenses are insufficient to neutralize ROS, promoting oxidative stress, which is related to several pathological processes, including lipid peroxidation (LPO) and DNA damage. Both the induction of LPO (Guilherme et al., 2010; Modesto and Martinez, 2010a,b; Menezes et al., 2011) and genotoxic effects (Cavalcante et al., 2008; Guilherme et al., 2012b) of Roundup® have already been described in different tissues for a variety of fish, but it is not yet clear the contribution of the surfactant for these effects of formulations.

Studies point the ability of Roundup® to interfere with blood parameters of fish (Glusczak et al., 2006; Modesto and Martinez, 2010b), and although they are not specific responses, hematological parameters should be considered good indicators of toxicity for their sensitivity to several contaminants (Van der Oost et al., 2003). Rise of blood cortisol and glucose has been reported in many fish species as the most used responses to quantify stress in these organisms (Barton and Iwana, 1991). Studies point to the ability of certain pesticides to mimic hormones or exert endocrine disrupting activity (Cericato et al., 2008), resulting in impaired stress response which may adversely affect the health of fish.

In this context, in the present study, an integrated approach examining biochemical, genotoxic and physiological parameters was employed in order to evaluate acute effects of the surfactant POEA on the fish *P. lineatus*.

2. Material and methods

2.1. Animals

Juveniles of *P. lineatus* (Valenciennes, 1836) with 12.48 ± 0.32 g body mass and 12.28 ± 0.11 cm total length (mean \pm SEM) were

provided by the Fish Hatchery Station of the State University of Londrina. Fish were acclimated for 7 days in 300 L-tanks with constant aeration and dechlorinated water. Physical and chemical parameters of the water were monitored daily using a multiparameter probe and the values were (mean \pm SEM): temperature 21.4 ± 0.57 °C; pH 6.9 ± 0.4 ; dissolved oxygen 7.1 ± 0.2 mg·L⁻¹; conductivity 91.5 ± 0.97 µS cm⁻¹ and 12 h:12 h photoperiod. Animals were fed on the second, fourth and sixth day of acclimation, and the feeding was suspended 24 h before and during the experiments.

2.2. Experimental design and sampling

After acclimation, animals were submitted to static acute toxicity tests for 24 h in 100 L-glass aquaria containing 80 L of water. For testing, animals were divided into four groups (n = 8 in each): a control group (CTR), with fish exposed only to dechlorinated water and three experimental groups exposed to different concentrations of POEA: 0.15 mg·L⁻¹ (POEA 1), 0.75 mg·L⁻¹ (POEA 2) and 1.5 mg·L⁻¹ POEA (POEA 3). All tests were carried out in duplicate in accordance with OECD guideline (OECD, 1992). Water parameters remained stable over the experiment (mean \pm SEM): temperature 21.4 ± 0.9 °C; dissolved oxygen 8.43 ± 0.6 mg·L⁻¹; pH 7.2 ± 0.5 and conductivity 112 ± 2.8 µS cm⁻¹. The POEA concentrations were defined considering previous tests already performed in our laboratory with *P. lineatus* exposed to 1 and 5 mg·L⁻¹ Roundup Transorb® (Modesto and Martinez, 2010b) and 10 mg·L⁻¹ Roundup® (Modesto and Martinez, 2010a), which corresponds to 0.15 mg·L⁻¹, 0.75 mg·L⁻¹ and 1.5 mg·L⁻¹ POEA, respectively, as the surfactant is typically 15% of the glyphosate formulations (Giesy et al., 2000).

After exposure fish were anesthetized with benzocaine (0.12 g·L⁻¹) and after the blood was withdrawn from the caudal vein, the animals were killed by medullar section for removal of liver. These procedures are in accordance with protocols approved by the Ethics Committee on Animal Experimentation of the State University of Londrina (Process 35004.2011.18). The liver samples were stored in ultrafreezer (−80 °C).

2.3. Physiological assays

Aliquots of blood were immediately used for the determination of hematocrit (Hct), by microcentrifugation (1200 g; 5 min) in capillary tubes. The hemoglobin (Hb) content was determined by the cyanmethemoglobin method in a spectrophotometer at 540 nm, using a commercial kit (Analisa, Brazil). The number of red cells per mm³ of blood (RBC) was determined using an improved Neubauer hemocytometer.

Blood samples were centrifuged (1870 g 10 min) and plasma samples were stored in the freezer (−20 °C). The plasma cortisol was measured by immunoassay using a commercial kit (Monobind Inc., USA) in a microplate reader at 450 nm. The plasma glucose was analyzed by the glucose oxidase method using a commercial kit (Labtest Diagnóstica, Brazil) in a microplate spectrofluorometer at 505 nm. Plasma lactate was determined using a commercial enzymatic kit (Labtest Diagnóstica, Brazil) at 550 nm.

2.4. Biochemical assays

Liver samples were weighed and homogenized (1:10 mass/vol) in phosphate buffer (0.1 M; pH 7.0), centrifuged (20 min; 13,000 g; 4 °C) and the supernatant was separated for analysis of biochemical parameters. The protein concentration in the liver homogenate was measured by the method of Lowry et al. (1951), using bovine serum albumin as standard.

CYP1A was determined by the activity of ethoxyresorufin-O-deethylase (EROD) according to Eggens et al. (1992), following Simonato et al. (2011). The activity of glutathione-S-transferase (GST)

was determined according to Keen et al. (1976) following the complexation of glutathione (GSH) with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm. Superoxide dismutase (SOD) activity was determined according to McCord and Fridovich (1969), by measuring the inhibition of the reduction rate of cytochrome c by the superoxide radical, at 550 nm and 25 °C. Catalase (CAT) activity was determined according to Beutler (1975) by the rate of decomposition of H₂O₂ assessed by absorbance decrease at 240 nm. Glutathione peroxidase (GPx) activity was estimated according to Hopkins and Tudhope (1973) by NADPH oxidation in the presence of H₂O₂ at 340 nm. Glutathione reductase (GR) activity was indirectly determined by the reduction of NADPH in the presence of glutathione disulfide (Carlberg and Mannervik, 1975), at 340 nm. The concentration of glutathione (GSH) was determined according to Beutler et al. (1963), following Simonato et al. (2011).

Lipid peroxidation (LPO) was determined by two assays: i) First is from the oxidation of Fe²⁺ in the presence of xylenol orange (FOX assay) according to Jiang et al. (1991). LPO was expressed in μmol cumene hydroperoxide $\cdot \text{mg protein}^{-1}$, using a cumene hydroperoxide (CHP) standard curve. ii) Second is from the production of malondialdehyde (MDA), which is one of the end products of lipid peroxidation, following Satoh (1978). LPO was expressed in μmol MDA $\cdot \text{mg protein}^{-1}$, using a MDA standard curve.

2.5. DNA damage

The alkaline comet assay with erythrocytes was performed according to Singh et al. (1988), with some modifications described by Ramsdorf et al. (2009). Immediately after sampling, an aliquot of blood of each animal was mixed with fetal bovine serum (1:1000). Up to 24 h later an aliquot of this mixture was then added to the low melting point agarose (0.5%), placed on a glass slide previously coated with normal melting point agarose and remained in the refrigerator for 30 min. Then the slides were subjected to: a) lysis: 1 h at 4 °C, protected from light, in lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 10% DMSO, 1 mL Triton X-100, pH 10.0); b) DNA denaturation: 30 min in the dark in an electrophoresis buffer (0.3 N NaOH, 1 mM EDTA, pH > 13); c) electrophoresis: 20 min, 300 mA, 25 V, 1 V cm^{-1} ; and d) neutralization: three rinses for 5 min each with buffer (0.4 M Tris, pH 7.5). Slides were fixed with absolute ethanol for 10 min and kept under refrigeration until cytological analyses.

The slides, stained with gelRed were analyzed under a Leica microscope (DM 2500) adapted for fluorescence/epifluorescence at 1000 \times magnification. All slides were blind-reviewed. The extent of DNA damage was quantified by the length of DNA migration, which was visually determined in 100 randomly selected and non-overlapping nucleoids per fish. DNA damage was classified in four classes 0: no visible damage; 1: a short tail smaller than the diameter of the nucleus; 2: a tail length 1 to 2 times the diameter of the nucleus; 3: a tail length > two times the diameter of the nucleus. The score of DNA damage for 100 comets was obtained by multiplying the number of cells in each class by the damage class, and ranged from 0 (all undamaged) to 300 (all maximally damaged).

2.6. Statistical analysis

After checking for normality and homoskedasticity, the results of the different parameters for fish of the control group (CTR) and of groups POEA 1, POEA 2 and POEA 3 were compared to each other by a parametric analysis of variance (ANOVA) and differences among treatment groups were identified by the Student–Newman–Keuls (SNK) multiple comparison test. Results are presented as mean \pm 1 SEM. Values of $p < 0.05$ were considered significant.

3. Results

During the tests, there was no mortality in any experimental group. Water parameters remained stable over the experiment (mean \pm SEM): temperature 21.4 ± 0.9 °C; dissolved oxygen 8.43 ± 0.6 $\text{mg} \cdot \text{L}^{-1}$; pH 7.2 ± 0.5 and conductivity 112 ± 2.8 $\mu\text{S cm}^{-1}$.

3.1. Biochemical parameters performed in liver

Fish of the groups POEA 2 and POEA 3 showed higher EROD ($p = 0.044$) compared to animals of the control group (Fig. 1A). On the other hand, GST activity was significantly lower ($p = 0.021$) in fish of the groups POEA 2 and POEA 3 in relation to control animals (Fig. 1B).

Animals of the group POEA 1 presented hepatic activity of SOD significantly higher ($p = 0.031$) in relation to their control group (Fig. 2A). The antioxidant enzyme catalase (CAT) was significantly lower ($p = 0.030$) in all groups exposed to the surfactant, compared to control animals (Fig. 2B). The hepatic activity of the GPx was significantly higher ($p = 0.002$) in fish of the group POEA 3 (Fig. 2C) in comparison to CTR, POEA 1 and POEA 2 groups. The GR showed no variation ($p = 0.062$) in its activity after the exposure of animals to different POEA concentrations (Fig. 3A). GSH levels in the liver were significantly higher ($p = 0.005$) in fish of the groups POEA 2 and POEA 3 compared to the control and POEA 1 groups (Fig. 3B).

Lipid peroxidation (Fig. 4) was significantly higher in the liver of animals of the group POEA 1, in relation to CTR, POEA 2 and POEA 3 groups, both by the FOX method ($p \leq 0.001$) and the TBARS method ($p = 0.007$).

3.2. DNA damage in blood cells – comet assay

The scores of DNA damage in blood cells of fish of POEA 1, POEA 2 and POEA 3 groups were significantly higher ($p \leq 0.001$) when

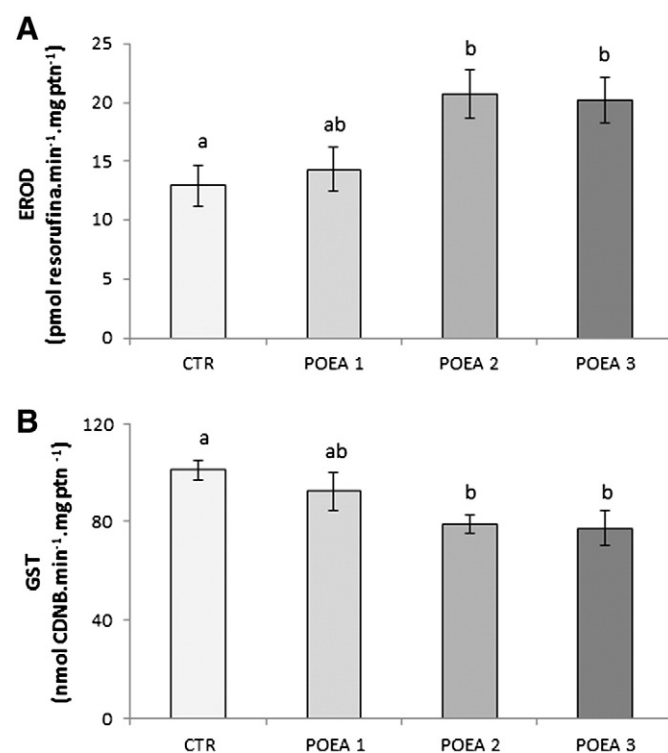


Fig. 1. Hepatic activity of EROD (A) and GST (B) of *Prochilodus lineatus* exposed for 24 h only to water (CTR) or to $0.15 \text{ mg} \cdot \text{L}^{-1}$ (POEA 1), $0.75 \text{ mg} \cdot \text{L}^{-1}$ (POEA 2) and $1.5 \text{ mg} \cdot \text{L}^{-1}$ (POEA 3) of POEA. Values are mean \pm SE ($N = 7-10$). Different letters indicate statistical difference between groups ($p < 0.05$).

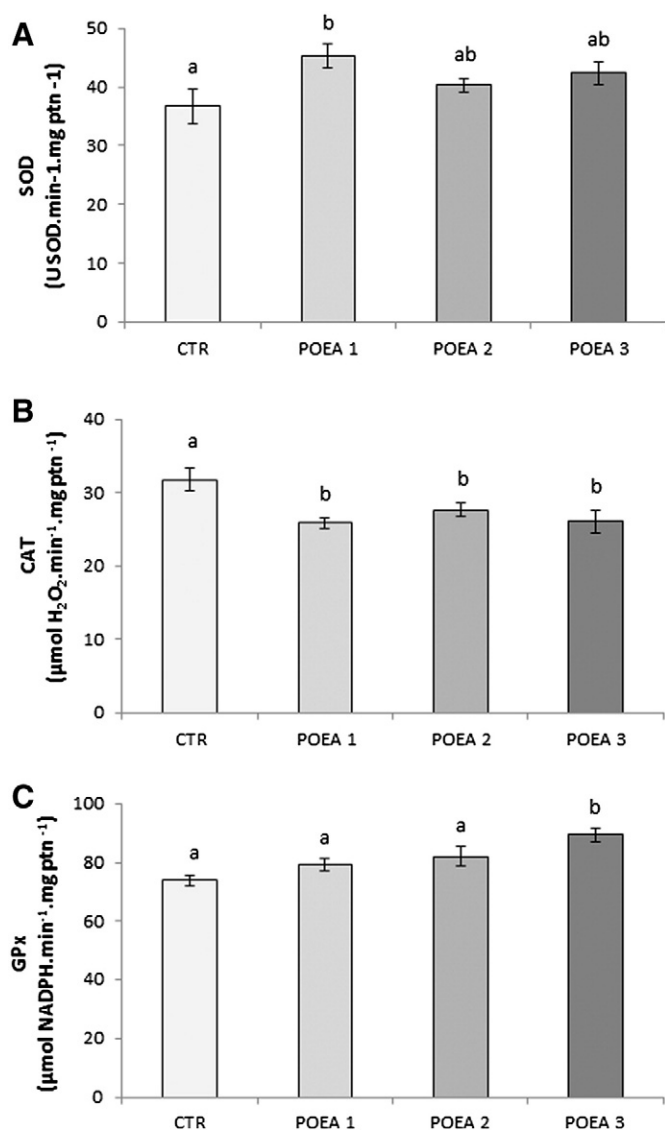


Fig. 2. Hepatic activity of SOD (A), CAT (B) and GPx (C) of *Prochilodus lineatus* exposed for 24 h only to water (CTR) or to 0.15 mg·L⁻¹ (POEA 1), 0.75 mg·L⁻¹ (POEA 2) and 1.5 mg·L⁻¹ (POEA 3) of POEA. Values are mean \pm SE (N = 7–12). Different letters indicate statistical difference between groups ($p < 0.05$).

compared with CTR (Fig. 5). The most frequent type of damage in these fish was the class 2 (POEA 1: 49%; POEA 2: 40% and POEA 3: 42.1%).

3.3. Physiological parameters

The plasma cortisol of fish from group POEA 3 was significantly lower ($p = 0.020$) when compared with the fish in the control, POEA 1 and POEA 2 groups (Fig. 6A). However plasma glucose did not change significantly ($p = 0.182$) in any of POEA concentrations compared with CTR (Fig. 6B). On the other hand, fish of POEA 1, POEA 2 and POEA 3 groups showed significantly higher concentrations of plasma lactate in relation to control ($p = 0.001$).

The exposure of fish to POEA did not change the hematocrit value ($p = 0.659$), compared with the CTR (Fig. 7A). However, hemoglobin content (Fig. 7B) and RBC (Fig. 7C) of fish of POEA 1, POEA 2 and POEA 3 groups were significantly lower in relation to control ($p = 0.001$ for both).

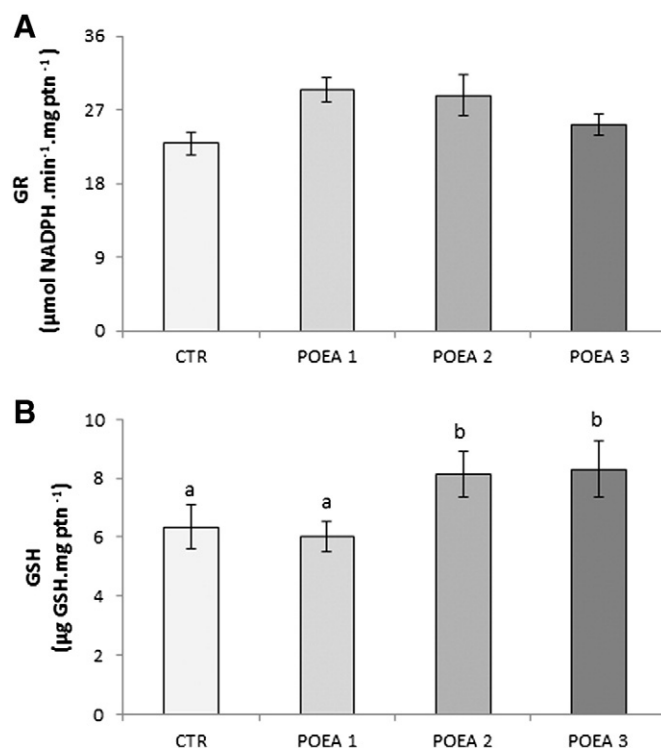


Fig. 3. GR activity (A) and concentration of GSH (B) in the liver of *Prochilodus lineatus* exposed for 24 h only to water (CTR) or to 0.15 mg·L⁻¹ (POEA 1), 0.75 mg·L⁻¹ (POEA 2); 1.5 mg·L⁻¹ (POEA 3) of surfactant POEA. Values are mean \pm SE (N = 6–12). Different letters indicate statistical difference between groups ($p < 0.05$).

4. Discussion

Toxicity tests with glyphosate and Roundup® have been frequently employed for assessing the effects of these compounds on physiological (Gluszczak et al., 2006; Langiano and Martinez, 2008; Modesto and Martinez, 2010a,b), biochemical (Gluszczak et al., 2007) and genetic parameters (Cavalcante et al., 2008; Guilherme et al., 2012b; Moreno et al., 2014) in different fish species. Data in the literature describe the greater toxicity of the formulated product when compared with the active compound. Some authors associate this increase with the addition of surfactants to the commercial product (Tsui and Chu, 2003). Within this context this study evaluated the toxic effects of the surfactant POEA on the native fish species *P. lineatus*.

The induction of the catalytic activity of CYP1A in fish exposed to organic compounds is well established (Simonato et al., 2011). As evidenced in this study, the exposure of *P. lineatus* to the two higher concentrations of the surfactant caused a significant increase in the catalytic activity of CYP1A, but there are no data on metabolic pathways of the surfactant. A reduction in the hepatic activity of GST in fish exposed to POEA 2 and POEA 3 suggests the presence of oxidizing agents capable of inhibiting of its activity, considering GST sensitivity to pro-oxidants (Bagnyukova et al., 2006). The exposure of *P. lineatus* to Roundup Transorb® for 6 and 24 h also caused an inhibition in the activity of hepatic GST (Modesto and Martinez, 2010b). The same result was observed in goldfish exposed for 96 h to Roundup® (Lushchak et al., 2009). Chronic exposure of the fish *Rhamdia quelen* to lower concentrations of Roundup® (0.45 and 0.95 mg·L⁻¹) also promoted a reduced the activity of this enzyme (Menezes et al., 2011). The results observed in this study corroborate the data presented in the literature and suggest that inhibition of GST, consistently checked for formulated products, may be related to the addition of the surfactant to the herbicide and to the pro-oxidant effects exerted by POEA.

Regarding antioxidant enzymes, it was observed higher SOD activity at the lowest concentration of the surfactant and lower CAT activity at

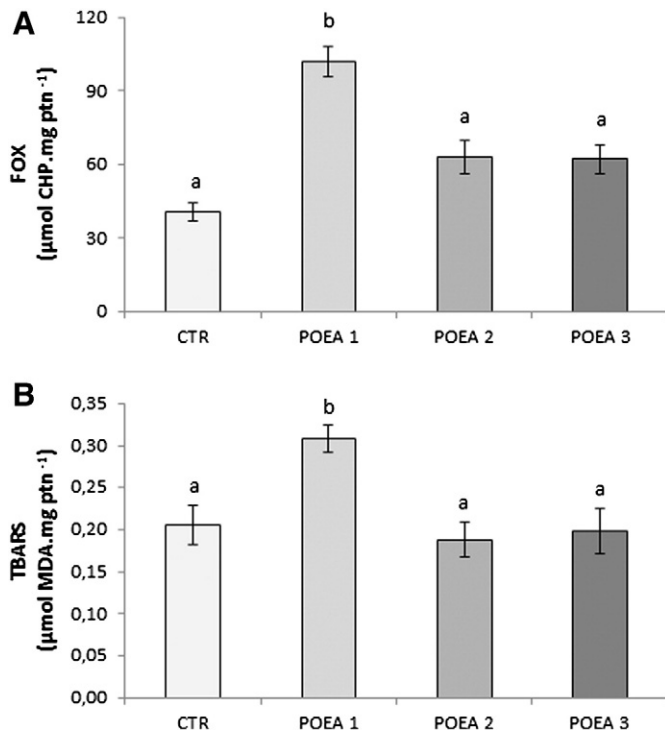


Fig. 4. Hepatic concentration of cumene hydroperoxide (A) and MDA (B) in *Prochilodus lineatus* exposed for 24 h only to water (CTR) or to 0.15 mg·L⁻¹ (POEA 1), 0.75 mg·L⁻¹ (POEA 2) and 1.5 mg·L⁻¹ (POEA 3) of POEA. Values are mean ± SE (N = 7–11). Different letters indicate statistical difference between groups ($p < 0.05$).

all concentrations. The relationship between the production of reactive oxygen species and induction in the activity of antioxidant enzymes is quite complex (Halliwell and Gutteridge, 2005). The adaptive responses to combat ROS may be influenced by the products generated by ROS itself. An excess of hydrogen peroxide can reduce the activity of SOD, while the accumulation of superoxide anion may be responsible for inhibiting the activity of catalase (Bagnyukova et al., 2006). These data reinforce the idea that SOD activity increases until the superoxide anion radical increases to a threshold (Oruc, 2012), after that the excess of this radical promotes the inhibition of catalase. Studies of acute exposure of *P. lineatus* to Roundup® also found the inhibition of liver catalase activity (Modesto and Martinez, 2010a). In this way, inhibition of liver catalase showed in both the formulated products and the surfactant suggests that the change in the activity of this enzyme may be a specific interference of POEA to the antioxidant defenses of the fish.

The increase in GPx activity in fish exposed to POEA 3 indicates stimulation of the antioxidant pathway, probably due to increasing

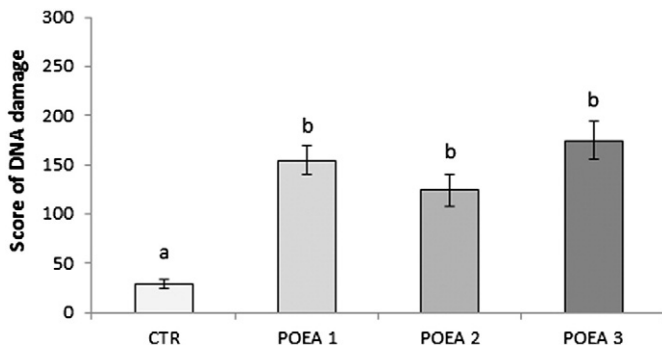


Fig. 5. Score of DNA damage in blood cells of *Prochilodus lineatus* exposed for 24 h only to water (CTR) or to 0.15 mg·L⁻¹ (POEA 1), 0.75 mg·L⁻¹ (POEA 2) and 1.5 mg·L⁻¹ (POEA 3) of POEA. Values are mean ± SE (N = 7–14). Different letters indicate statistical difference between groups ($p < 0.05$).

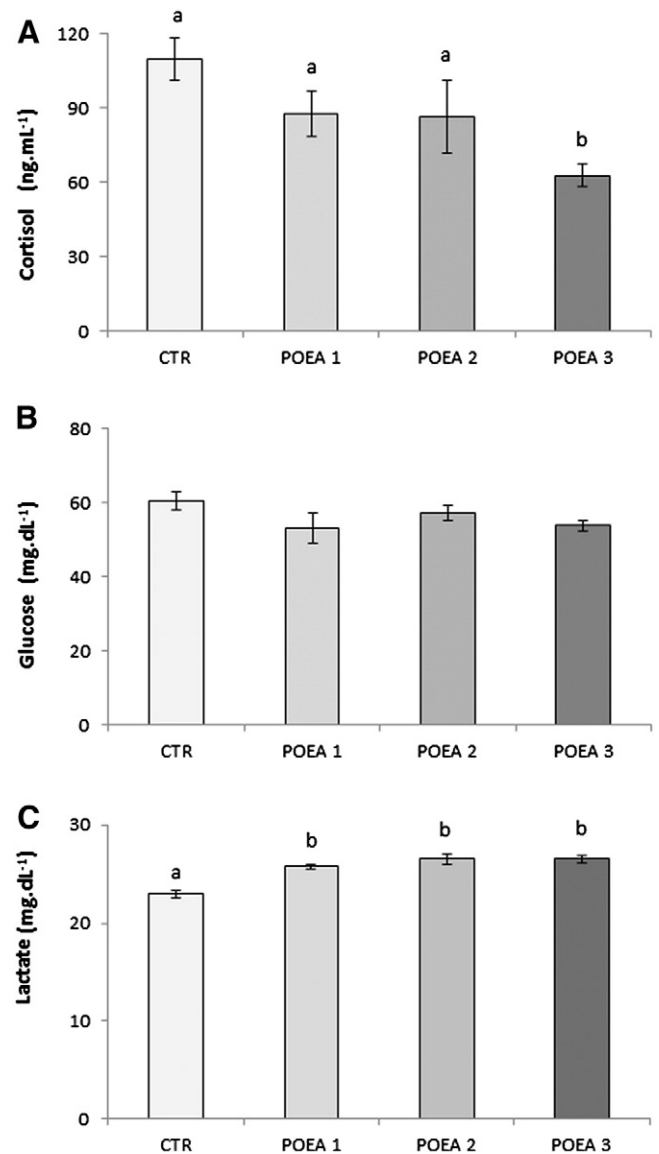


Fig. 6. Plasma concentrations of cortisol (A), glucose (B) and lactate (C) of *Prochilodus lineatus* exposed for 24 h only to water (CTR) or to 0.15 mg·L⁻¹ (POEA 1), 0.75 mg·L⁻¹ (POEA 2) and 1.5 mg·L⁻¹ (POEA 3) of POEA. Values are mean ± SE (N = 6–10). Different letters indicate statistical difference between groups ($p < 0.05$).

peroxide concentrations. Modesto and Martinez (2010b) showed the same result after exposure of *P. lineatus* to Roundup Transorb®. These authors reported an increase in GPx activity as a compensatory response to inhibition of catalase.

In the present study, an increased concentration of hepatic GSH was observed for POEA 2 and POEA 3. This shows the important antioxidant role that glutathione plays as first line of defense (Ahmad et al., 2000) as it may directly act on reactive oxygen species in order to promote their neutralization and prevent oxidative damage (Hermes Lima, 2004). These data combined with results found for lipid peroxidation indicate that the increase in hepatic concentrations of GSH may be related to the protection against oxidative damage. Other studies also observed increase of GSH in response to sublethal concentrations of Roundup® (Modesto and Martinez, 2010a). Zhang et al. (2004) reported that hepatic GSH content of fish may rise as a result of an adaptive mechanism to oxidative status, and that this change comes from increased synthesis of GSH, which probably occurred in the present work since there was no increase in the activity of the GR. In addition to the antioxidant role, GSH is also related to the modulation of aryl hydrocarbon

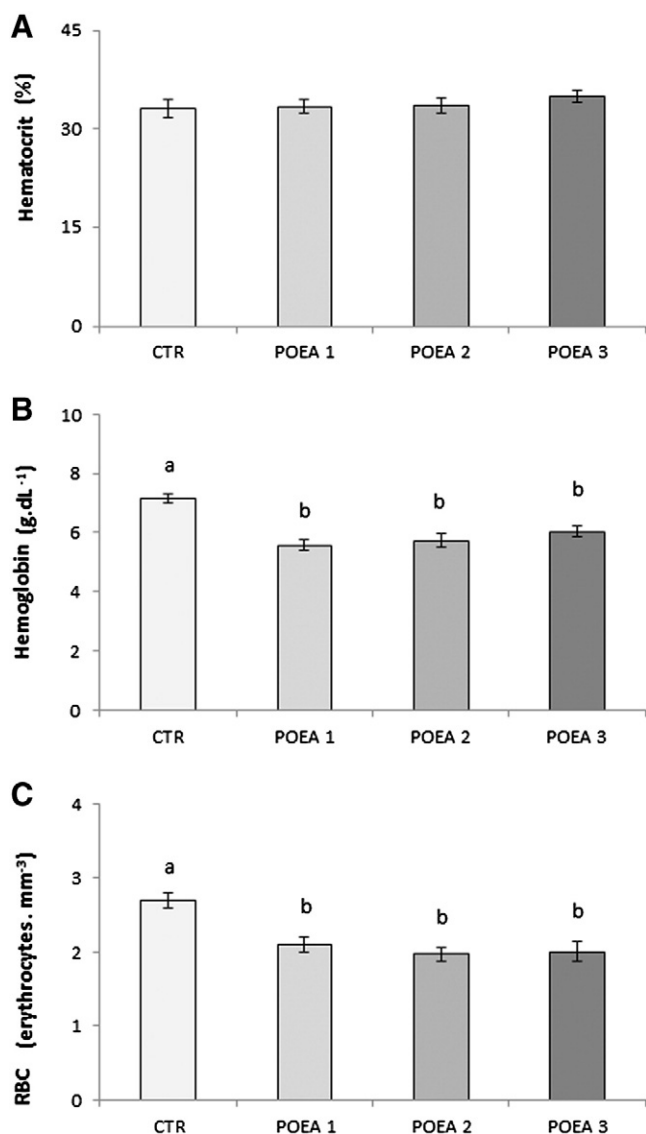


Fig. 7. Hematocrit (A), hemoglobin content (B) and number of erythrocytes per mm³ of blood (C) of *Prochilodus lineatus* exposed for 24 h only to water (CTR) or to 0.15 mg·L⁻¹ (POEA 1), 0.75 mg·L⁻¹ (POEA 2) and 1.5 mg·L⁻¹ (POEA 3) of POEA. Values are mean ± SE (N = 12–16). Different letters indicate statistical difference between groups ($p < 0.05$).

receptor (Ah), and the increase in the GSH content may promote an increase in the expression and catalytic activity of CYP1A, demonstrating the interaction between GSH and cytochrome P450 (Van der Oost et al., 2003). This relationship can be observed herein, as there was an increase in both the contents of GSH, as in EROD activity, in fish exposed to POEA 2 and POEA 3.

If not neutralized, reactive oxygen species can react with lipid membranes (Ahmad et al., 2000), producing lipid peroxidation, which is considered one of the consequences of oxidative stress (Hermes Lima, 2004). In the present study, lipid peroxidation was shown by an increase in both lipid hydroperoxide levels (FOX), which is derived from the early stage of lipid peroxidation as well as by increased levels of malondialdehyde (TBARS), which is the final result of lipid peroxidation (Hermes Lima, 2004), in fish exposed to POEA 1. Thus, it can be inferred that the antioxidant defenses were inadequate to this concentration, leading to increased lipid peroxidation. It is suggested that for fish exposed to POEA 2 and POEA 3, the LPO was prevented by the increase in hepatic content of GSH. Several studies have shown lipid peroxidation in different fish species exposed to Roundup®

(Guilherme et al., 2010; Modesto and Martinez, 2010a,b; Menezes et al., 2011), and the present study corroborates data of the literature, pointing a potential contribution of POEA to the LPO observed after exposure to Roundup® formulations.

The level of DNA integrity has been proposed as a sensitive indicator of genotoxicity for various compounds (Zhu et al., 2005). In this study, the results obtained with blood cells highlight that POEA is genotoxic for the fish *P. lineatus* as it was observed significantly higher DNA damage scores in of fish exposed to all POEA concentrations. These data confirm the study of Guilherme et al. (2012a), where the exposure of *A. anguilla* to POEA was also capable of causing DNA damage. Other studies have already shown the genotoxic effects of two glyphosate formulations for *P. lineatus*, Roundup® (Cavalcante et al., 2008) and Roundup Transorb® (Moreno et al., 2014), as well as that glyphosate itself is genotoxic to gill cells and erythrocytes of *P. lineatus* (Moreno et al., 2014). Actually, recent studies rule out synergistic interaction of glyphosate and surfactant to promote non-specific DNA damage (Guilherme et al., 2012a) and show that both the active ingredient (glyphosate), and the surfactant POEA has genotoxic activity.

Concerning stress response in fish, some studies have already shown that Roundup® interferes with cortisol release in *R. quelen* (Cericato et al., 2008), and promotes an increase in plasma glucose levels in *P. lineatus* (Langiano and Martinez, 2008). In fact, in the present study, plasma cortisol concentration after exposure of fish to POEA 3 was decreased, indicating that higher concentration of the surfactant interferes with stress response. Moreover, plasma glucose concentration after 24 h of exposure to POEA was not altered. The lack of a hyperglycemic response may be related to the interference that the surfactant exerted on cortisol release. It is well described in the literature that the hyperglycemic response in many teleost species is maintained by the action of adrenaline and cortisol (Wendelaar Bonga, 1997). Thus, the reduced response of cortisol after POEA exposure can be suggested to be responsible for the maintenance of basal levels of blood glucose levels. Accordingly, it can be suggested that POEA alone is not capable of invoking a hyperglycemic response.

The exposure of *P. lineatus* to POEA reduced the number of blood cells (RBC) indicating cell lysis. This idea is supported by the large amount of DNA damage found in blood cells of fish exposed to POEA and also by oxidative effects found in this study. Other studies also point to the decrease in RBC of fish exposed to Roundup® (Gluszczak et al., 2006; Salbego et al., 2010). Given the result presented by this study and literature data, it is possible to infer the contribution of the POEA in decreased RBC of fish exposed to the herbicide. The exposure of *P. lineatus* to the surfactant also caused a decrease in hemoglobin content, probably directly related to the decrease in RBC. The decrease in the hemoglobin content leads to functional hypoxia. Some studies have already reported hypoxia after exposure of fish (Gluszczak et al., 2006) to Roundup®. This hypoxia in *P. lineatus* exposed to the surfactant is further sustained by increased plasma lactate observed in fish exposed to the three concentrations of POEA evaluated in this study. In the literature, some studies relate directly the situation of hypoxia with increased plasma lactate (Panepucci et al., 2001; Herbert and Steffensen, 2005) and suggest that the increase in plasma lactate levels under hypoxic conditions denotes an increased anaerobic metabolism. Thus, the effects of POEA on hematological parameters suggest that the surfactant contributes to the destruction of erythrocytes and can therefore lead to functional hypoxia.

5. Conclusions

Based on the present results, it can be concluded that some of the effects observed after fish exposure to glyphosate-based herbicides may be related to the addition of the surfactant to the commercial product. The results showed damage at various levels, and these damages such as hemolysis, DNA damage and lipid peroxidation may be directly

related to an imbalance in the redox state of the fish. Therefore, the exposure to POEA generates a condition of oxidative stress.

Acknowledgments

The authors thank the Hatchery Station of State University of Londrina for the supply of fish. This work is part of the Master Dissertation of C.D.C. Navarro (Programa Multicêntrico de Pós Graduação em Ciências Fisiológicas) who received a scholarship from the Brazilian Council for Scientific and Technological Development (CNPq). This study was supported by grants from the Brazilian Institute of Aquatic Toxicology (INCT-TA, CNPq: 573949/2008-5). C.B.R. Martinez is research fellow from CNPq.

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