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Genotoxic effects of the herbicide Roundup Transorb® and its active ingredient glyphosate on the fish *Prochilodus lineatus*

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ABSTRACT

Roundup Transorb® (RT) is a glyphosate-based herbicide and despite its wide use around the world there are few studies comparing the effects of the active ingredient with the formulated product. In this context the purpose of this study was to compare the genotoxicity of the active ingredient glyphosate with the formulated product RT in order to clarify whether the active ingredient and the surfactant of the RT formula may exert toxic effects on the DNA molecule in juveniles of fish *Prochilodus lineatus*. Erythrocytes and gill cells of fish exposed to glyphosate and to RT showed DNA damage scores significantly higher than control animals. These results revealed that both glyphosate itself and RT were genotoxic to gill cells and erythrocytes of *P. lineatus*, suggesting that their use should be carefully monitored considering their potential impact on tropical aquatic biota.

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1. Introduction

The use of herbicides in agriculture can result in their presence in several ambient matrices, including aquatic environments, since agrochemicals can easily reach surface waters from agricultural fields by runoff and leaching (Konstantinou et al., 2006; Borggaard and Gimsing, 2008). Since the 1970s, glyphosate [N-(phosphonomethyl) glycine] is one of the herbicide with largest participation in the world market, due to its efficiency to control invasive plants (Williams et al., 2000; Solomon and Thompson, 2003; Rodrigues and Almeida, 2005; Duke and Powles, 2008). It inhibits plant growth through

interference with the production of essential aromatic amino acids by inhibition of the enzyme enolpyruvylshikimate phosphate synthase (EPSP) (Amarante et al., 2002). The large use of glyphosate is partly explained by its application to genetically modified plant varieties (Williams et al., 2000) that have a gene which confers resistance to the herbicide molecule, preventing the blockage of the EPSP action, thus the metabolic pathway is not interrupted and the plants develop normally (Coutinho et al., 2005). In addition, different formulations of glyphosate are used in silvicultural practices and urban environments (Borggaard and Gimsing, 2008). The intensive use of this herbicide represents a concern regarding to contamination of aquatic ecosystems, since it has been shown that

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glyphosate and its products are more mobile and persistent in aquatic environments (Silva et al., 2003; Kolpin et al., 2006) than earlier research has indicated (Giesy et al., 2000).

Today, varieties of glyphosate-based formulations such as Roundup® are registered in more than 100 countries and are available under different brand names. Among these formulations we can highlight Roundup Transorb® (RT), which was developed for use during rainy periods due to the fact that its absorption by the plant takes only 1 h, while the other formulations of Roundup® need at least 4 h to be absorbed (Rodrigues and Almeida, 2005). RT contains glyphosate (480 g L^{-1}) and inert ingredients which are reported as a mixture of surfactants containing 15% of polyethoxylene amine (POEA) (Howe et al., 2004). This agrochemical is classified as moderately toxic (Class III), according to the toxicological classification, and dangerous to the environment, according to the Hazard Potential Classification (Monsanto, 2010).

According to the World Health Organization, the toxicity of glyphosate is low (WHO, 1994). However, the glyphosate-based products are generally more toxic for fish (Amarante et al., 2002; Peixoto, 2005), mainly due to the addition of surfactants (Tsui and Chu, 2003). In a review by Giesy et al. (2000) it is indicated that the glyphosate LC₅₀ for the rainbow trout (*Oncorhynchus mykiss*) is 86 mg L^{-1} , whereas the LC₅₀ (96 h) of Roundup® for *Prochilodus lineatus* is 13.69 mg L^{-1} (Langiano and Martinez, 2008) and the LC₅₀ (96 h) of RT for *O. mykiss* is 18 mg L^{-1} (FISPQ, 2008). Although these three works deal with different species, it can be observed that the volume of Roundup® and RT necessary to cause the death of 50% of the fish was about 5–6 times smaller than that of glyphosate.

Considering the importance of approaches involving the genotoxic effects of pollutants on aquatic organisms, as well as the scarcity of information regarding these genetic damage produced by RT and glyphosate on fish, this study was designed to assess DNA damage in fish exposed to glyphosate and RT, using the comet assay. This assay, also known as SCGE (single cell gel electrophoresis assay), is one of the most widely used test for the evaluation of DNA strand breaks in aquatic animals, including studies *in vivo* and *in vitro* (Ohe et al., 2004; Monteiro et al., 2011); this technique allows the detection of direct (Paz-y-Minó et al., 2002) or indirect damage (Vanzella et al., 2007) as well as the repair of damage in the DNA molecule (Jha, 2008). The comet assay detects damage to the DNA induced by alkylating, intercalating and oxidizing agents and various tissues can be used to perform the assay, such as gill and blood (Dhawan et al., 2009). Some studies have indicated the comet assay as a sensitive and useful method for the detection of DNA damage in laboratory studies using the neotropical fish *P. lineatus* (Vanzella et al., 2007; Cavalcante et al., 2008; Monteiro et al., 2011; Santos and Martinez, 2012).

In this context the purpose of this study was to compare the genotoxicity of the active ingredient glyphosate with the formulated product RT, in order to clarify whether the surfactant of the RT formula may exert toxic effects on the DNA molecule, since its composition has not been revealed yet. It is believed that the higher toxicity of RT is most likely due to the addition of surfactants to increase the effectiveness of the product (Solomon and Thompson, 2003), however, there are few studies comparing the effects of the active ingredient with the formulated product to evaluate the toxicity of each of

them and also verify whether the active ingredient contributes to this possible toxicity. In order to meet our goal we used the comet assay to investigate whether the glyphosate and the formulated product, the herbicide Roundup Transorb® (RT), exert genotoxic effects on erythrocytes and gill cells of the fish *P. lineatus*.

2. Materials and methods

2.1. Acclimation

Juveniles of *P. lineatus* weighing $7.9 \pm 2.1 \text{ g}$ (mean \pm SD, N = 144) were provided by the Hatchery Station of Londrina State University. Fish were acclimated for seven days in 300-L tanks filled with dechlorinated water (temperature: 23°C ; pH: 7.3), continuously aerated and kept under the 12/12 h photoperiod. Animals were fed every 48 h, except the day before the start and during the toxicity tests.

2.2. Experimental design

After acclimation, animals were exposed to the formulate product Roundup Transorb® (RT) or only to the active ingredient glyphosate (GLY) in static acute toxicity tests performed in 100L glass aquaria containing 6 fish each. In one set of experiments fish were exposed to the commercial formulation of Roundup Transorb® ($480 \text{ g glyphosate L}^{-1}$, Monsanto do Brazil Ltda) at two nominal concentrations: 1 mg L^{-1} (RT1) and 5 mg L^{-1} (RT5) or only to clean water (negative control or NC) for 6, 24 and 96 h. These concentrations of RT were defined taking into account: (a) the predicted concentrations of Roundup following direct application to water that could result in a maximum concentration of 9.0 mg L^{-1} (Giesy et al., 2000); (b) the results of a previous work (Modesto and Martinez, 2010) that showed that *P. lineatus* exposed to both concentration of RT survived throughout the 96 h of exposure but exhibited alterations in hematologic and biochemical parameters. In another set of experiments fish were exposed to glyphosate (Sigma-Aldrich; CAS no. 1071-83-6) at two nominal concentrations 0.48 mg L^{-1} (GLY 0.48) and 2.40 mg L^{-1} (GLY 2.4), or only to clean water (negative control or NC), for 6, 24 and 96 h. These glyphosate concentrations correspond to the amount of glyphosate salt in both concentrations of Roundup Transorb® tested in this work. Positive control (PC) groups, consisting of fish injected with the clastogenic agent cyclophosphamide (0.04 mg g^{-1} , Sigma – CAS no. 64-86-8) and transferred to glass aquaria containing water under the same conditions as the NC were sampled 6, 24 and 96 h after injection. During the tests water was continuously monitored for temperature, dissolved oxygen, pH and conductivity.

2.3. Fish sampling and cellular dissociation

After each exposure period, the fish were removed from the aquaria and anesthetized with benzocaine (0.1 g L^{-1}), for collection of blood from the caudal vein. This procedure followed the standard protocols approved by the Committee for Animal Experimentation of Londrina State University. An aliquot of blood was stored in microtubes containing Phosphate Buffered Saline (PBS: NaCl 126.6 mM, KCl 4.8 mM, CaCl 1.5 mM, NaHCO₃

3.7 mM, Na₂HPO₄ 8.9 mM, NaH₂PO₄ 2.9 mM) and kept on ice for the comet assay. Then, the animals were killed by cervical section for the removal of the gills.

After removing and cleaning the gills with PBS, the gill filaments were excised and transferred to microtubes containing PBS and kept on ice until cell dissociation. The method for cellular dissociation was based on Cavalcante et al. (2008). Briefly, gill filaments were sectioned and pieces were incubated for 15 min at 30 °C in 0.05% trypsin (diluted in PBS Ca²⁺ and Mg²⁺ free) and homogenized by periodic manual inversion at room temperature for tissue dissociation. After that, the solution was filtered (30 µm mesh size) into a tube containing a fetal calf serum 10% to halt the enzymatic digestion. The resultant solution was centrifuged (10 min, 1000 g) and the pellet was resuspended in PBS to be used in the comet assay.

2.4. Comet assay

Before performing the comet assay the cell viability of the erythrocytes and gill cells obtained after each exposure was determined by the Trypan blue exclusion method. For each sample, 100 cells were analyzed using a Neubauer chamber and the viability was expressed as the percentage of viable cells (white cells) in the total number of cells. At least 80% of cells should be viable to run the comet assay (Tice et al., 2000).

The Comet assay was performed using the alkaline (pH > 13) version of the assay developed by Singh et al. (1988), with the modifications detailed by Cavalcante et al. (2008). Basic steps of the assay for the two cell types were executed as follows: (a) lysis: 1 h, at 4 °C, protected from light, in a lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 10% DMSO, 1 mL Triton X-100, pH 10.0); (b) DNA unwinding: 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; (d) neutralization: three washes for 5 min each in 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 (15 µL of GelRed diluted in 45 mL of distilled water and 5 mL of NaCl 1 M), were analyzed with a Leica microscopy (DM 2500) equipped with a blue excitation filter (450–490 nm) and a barrier filter of 515 nm at 1000× 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 select and non-overlapping cells per fish. DNA damage was classified in four classes: class 0 – no visible damage; class 1 – a short tail smaller than the diameter of the nucleus; class 2 – a tail length 1–2 times the diameter of the nucleus; class 3 – a tail length >2 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). Results for DNA damage in each cell type were expressed as the mean score of DNA damage for each treatment group, for each exposure period.

2.5. Statistical analyses

The results of the comet assay were compared among different treatments NC × RT1 × RT5 × PC and NC × GLY 0.48 × GLY

2.4 × PC, for each experimental time (6, 24 or 96 h), using one-way analysis of variance (ANOVA). When indicated (significant value of F) differences between treatments were analyzed by the post hoc Student–Newman–Keuls test (SNK). The decision to use parametric tests was based on analysis of normality and homogeneity of variance. Statistical significance was designated as $p < 0.05$.

3. Results

Water characteristics remained stable along the experiments and the mean values (\pm SD) considering both RT and GLY groups, and respective control groups, were for temperature: 23.3 ± 2.2 °C, dissolved oxygen: 7.1 ± 0.3 mg O₂ L⁻¹, conductivity 72.0 ± 2.0 µS cm⁻¹ and pH: 7.4 ± 0.1. No mortality was registered along toxicity tests neither in the control groups (NC and PC) nor in the RT and GLY groups.

The mean score of DNA damage in erythrocytes of fish exposed to both concentrations RT (Fig. 1A) was significantly higher in comparison to respective NC after 24 h ($p < 0.001$) and 96 h ($p < 0.001$) exposure, while no significant increase was observed after 6 h of exposure ($p = 0.683$). For the gill cells the mean score of DNA damage in fish exposed to both concentrations of glyphosate (Fig. 1B) were significantly

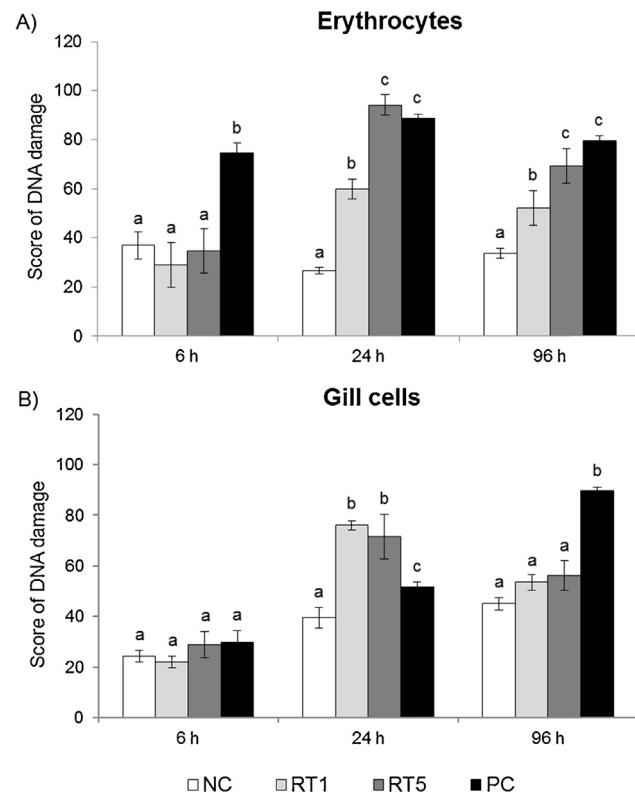


Fig. 1 – Comet scores in erythrocytes (A) and gill cells (B) of *Prochilodus lineatus* exposed to Roundup Transorb® at two concentrations 1 mg L⁻¹ (RT1) and 5 mg L⁻¹ (RT5) and the respective negative control (NC) and positive control (PC), at each period of exposure (6, 24 and 96 h). Values are presented as mean \pm SE (n: 5–6). For each experimental period, the different letters indicate significant differences between the groups ($p < 0.05$).

Table 1 – Mean frequency of nucleoids observed in each comet class (0, 1, 2 and 3) and the number of damaged nucleoids per fish (mean \pm SE) in erythrocytes and gill cells of *Prochilodus lineatus* exposed to Roundup Transorb® at two concentrations 1 mg L^{-1} (RT1) and 5 mg L^{-1} (RT5), and the respective negative control (NC) and positive control (PC), at each period of exposure (6, 24 and 96 h), taking into account the total number of fish (N).

Time	Group	N	Damage classes (%)				Damaged nucleoids (mean \pm SE)
			0	1	2	3	
Erythrocytes							
6 h	NC	6	63.0	37.0	0.0	0.0	37.0 ± 5.5 A
	RT1	6	71.0	29.0	0.0	0.0	29.0 ± 4.4 A
	RT5	6	65.3	34.7	0.0	0.0	43.7 ± 8.9 A
	PC	6	26.2	72.7	0.0	0.0	73.7 ± 3.8 B
24 h	NC	6	73.3	26.7	0.0	0.0	26.7 ± 1.5 A
	RT1	6	41.3	57.2	1.3	0.0	58.5 ± 7.2 AB
	RT5	6	12.5	80.8	6.6	0.0	87.5 ± 1.3 B
	PC	6	13.5	87.8	0.3	0.0	88.2 ± 1.7 B
96 h	NC	6	66.3	33.7	0.0	0.0	33.7 ± 2.0 A
	RT1	6	47.8	52.2	1.6	0.3	52.2 ± 3.3 B
	RT5	6	30.7	67.2	1.0	0.0	68.2 ± 6.2 C
	PC	6	21.5	76.7	0.0	0.0	78.0 ± 1.8 C
Gill cells							
6 h	NC	6	75.7	24.3	0.0	0.0	24.3 ± 2.3 A
	RT1	6	78.0	22.0	0.0	0.0	22.0 ± 2.4 A
	RT5	6	74.7	25.2	0.0	0.0	25.2 ± 5.2 A
	PC	6	70.2	29.8	0.0	0.0	29.8 ± 4.7 A
24 h	NC	6	60.7	39.2	0.2	0.0	39.3 ± 4.3 A
	RT1	6	21.9	75.0	0.5	0.0	75.5 ± 1.9 B
	RT5	6	42.0	57.5	0.5	0.0	70.8 ± 7.6 B
	PC	6	49.2	50.2	0.7	0.0	50.8 ± 2.1 C
96 h	NC	6	55.0	45.0	0.0	0.0	45.0 ± 2.4 A
	RT1	6	45.0	53.2	0.2	0.0	53.3 ± 3.1 A
	RT5	6	43.8	55.3	0.3	0.0	55.7 ± 5.9 A
	PC	5	10.4	89.4	0.2	0.0	89.6 ± 1.0 B

Different letters indicate significant differences between the number of damaged nucleoid, for the same tissue at the same exposure time ($p < 0.05$).

higher in comparison to respective NC only after 24 h exposure ($p = 0.007$), and no significant increase was observed after 6 h ($p = 0.33$) and 96 h ($p = 0.158$) of exposure. In both tissues analyzed from fish exposed to RT the frequency of class 1 damage was predominant (varying from 80 to 22%) and the occurrence of damage of class 2 and class 3 was very low and varied from 6.6% to 0% (Table 1).

Fish exposed to both concentrations of glyphosate showed mean score of DNA damage in erythrocytes (Fig. 2A) significantly higher in comparison to respective NC after 6 h ($p = 0.01$) and 96 h ($p = 0.014$) exposure and no significant increase was observed after 24 h of exposure ($p = 0.248$). For the gill cells the mean score of DNA damage was significantly higher than respective NC only after 6 h exposure to 2.4 mg L^{-1} of glyphosate ($p = 0.02$), and no significant increase was observed after 24 h ($p = 0.529$) and 96 h ($p = 0.315$) of exposure to any glyphosate concentration (Fig. 2B). Fish exposed to glyphosate also showed, in both tissues, that the frequency of class 1 damage was predominant (varying from 62.8 to 17.2%) and the occurrence of damage of class 2 and class 3 was low and varied from 10.2% to 0% (Table 2).

The scores of damage as well as the number of damaged nucleoids for the positive control groups were significantly

higher in comparison to respective NC for erythrocytes and gill cells over the three experimental periods (Figs. 1 and 2 and Tables 1 and 2). The only exception was the gill cells of fish from PC group that was run together with RT after 6 h exposure that showed both score of damage and number of damaged nucleoids not statistically different from respective NC (Fig. 1B and Table 1).

4. Discussion

Our findings regarding RT and glyphosate effects on DNA of *P. lineatus*, revealed by the comet assay, confirm the potential of both the formulated herbicide as its active ingredient to induce DNA damage in erythrocytes and gill cells of this neotropical fish. Our results also showed that blood and gill cells of *P. lineatus* can respond differently to DNA damage, reinforcing the importance of using different tissues as complementary tools for detecting genotoxicity in fish. In our study we found that erythrocytes of fish exposed to both concentrations of RT (1 and 5 mg L^{-1}) and glyphosate (0.48 and 2.4 mg L^{-1}) showed DNA damage after 96 h, while gill cells did not. On the other hand, both types of cells showed damage in their DNA after

Table 2 – Mean frequency of nucleoids observed in each comet class (0, 1, 2 and 3) and the number of damaged nucleoids per fish (mean \pm SE) in erythrocytes and gill cells of *Prochilodus lineatus* exposed to glyphosate at two concentrations 0.48 mg L^{-1} (GLY 0.48) and 2.4 mg L^{-1} (GLY 2.4), and the respective negative control (NC) and positive control (PC), at each period of exposure (6, 24 and 96 h), taking into account the total number of fish (N).

Time	Group	N	Damage classes (%)				Damaged nucleoids (mean \pm SE)
			0	1	2	3	
Erythrocytes							
6 h	NC	6	83.0	15.2	0.0	0.0	$17.0 \pm 4.6 \text{ A}$
	GLY 0.48	6	72.0	22.3	5.5	0.2	$28.0 \pm 3.2 \text{ A}$
	GLY 2.4	6	69.5	19.3	10.2	1.0	$30.5 \pm 2.4 \text{ A}$
	PC	6	26.2	72.7	1.0	0.0	$73.7 \pm 3.8 \text{ B}$
24 h	NC	6	82.3	15.8	1.8	0.0	$17.7 \pm 3.8 \text{ A}$
	GLY 0.48	6	66.7	33.3	0.0	0.0	$33.3 \pm 6.7 \text{ A}$
	GLY 2.4	6	76.7	19.5	3.7	0.0	$23.3 \pm 4.1 \text{ A}$
	PC	6	13.5	87.8	0.3	0.0	$88.2 \pm 1.7 \text{ B}$
96 h	NC	6	55.0	41.6	3.4	0.0	$35.0 \pm 2.1 \text{ A}$
	GLY 0.48	6	39.7	59.8	0.5	0.0	$60.3 \pm 7.2 \text{ B}$
	GLY 2.4	6	36.0	62.8	1.2	0.0	$64.0 \pm 5.3 \text{ B}$
	PC	6	21.5	76.7	1.3	0.0	$78.0 \pm 1.8 \text{ C}$
Gill cells							
6 h	NC	6	84.0	14.2	1.8	0.0	$16.0 \pm 4.4 \text{ A}$
	GLY 0.48	5	71.2	22.0	6.6	0.2	$28.8 \pm 3.4 \text{ B}$
	GLY 2.4	6	69.5	19.3	10.2	1.0	$30.5 \pm 2.4 \text{ B}$
	PC	6	70.2	29.8	0.0	0.0	$29.8 \pm 4.7 \text{ B}$
24 h	NC	6	80.2	16.7	2.5	0.0	$19.2 \pm 7.1 \text{ A}$
	GLY 0.48	6	79.8	18.0	2.2	0.0	$20.0 \pm 1.5 \text{ A}$
	GLY 2.4	6	78.3	19.8	1.8	0.0	$21.7 \pm 3.4 \text{ A}$
	PC	6	49.2	50.2	0.7	0.0	$50.8 \pm 2.1 \text{ B}$
96 h	NC	5	71.2	28.8	0.0	0.0	$28.8 \pm 8.4 \text{ A}$
	GLY 0.48	6	82.8	17.2	0.0	0.0	$17.2 \pm 6.6 \text{ A}$
	GLY 2.4	5	72.0	23.8	3.4	0.6	$27.8 \pm 4.9 \text{ A}$
	PC	5	10.4	89.4	0.2	0.0	$89.6 \pm 1.0 \text{ B}$

Different letters indicate significant differences between the number of damaged nucleoid, for the same tissue at the same exposure time ($p < 0.05$).

24 h of exposure to RT and after 6 h of exposure to glyphosate. Similarly, Cavalcante et al. (2008) evaluating the genotoxic effects of Roundup® on erythrocytes and gill cells of *P. lineatus* also observed that the DNA lesions in erythrocytes cells persisted after 96 h, whereas in gill cells they did not persist. Significant decrease in DNA damage was also observed in gill cells of *Anguilla anguilla* exposed to $58 \mu\text{g L}^{-1}$ Roundup® for 3 days in comparison to one day exposure (Guilherme et al., 2012). Among other things, these latter authors attributed their findings to DNA-repair system and/or cell turnover in gill cells epithelium, which apparently, under certain circumstances, can show an intense cell-division rate (Pacheco et al., 1993). In the current study, the incidence of DNA damage in erythrocytes and absence of such damage in gill cells after 96 h of exposure to both concentrations of RT and GLY could be attributed to intrinsic differences in the repair enzyme system and/or turnover cell in both cell types. However, further studies are still necessary to confirm this hypothesis.

As shown, the erythrocytes of fish exposed to RT presented an increased damage score to the DNA molecule after 24 and 96 h. A possible explanation for the genomic lesions in erythrocytes lasted up to 24 and 96 h could be that the amount of DNA damage within these cells might have been greater than the capacity of the repair enzyme system to fix such damage.

Gold fish (*Carassius auratus*) exposed to 5 mg L^{-1} of Roundup® for 48 and 96 h also showed DNA damage that persisted over time (Çavas and Konen, 2007). Our results show that both RT and Roundup®, two glysophate-based products, are genotoxic to these fish under these conditions.

The results of the comet assay with erythrocytes of *P. lineatus* exposed to glyphosate showed increase DNA damage after 6 and 96 h of exposure with both concentrations tested; however, after 24 h, the score of damage returned to the basal level. The fact that there was no damage after 24 h of exposure may be ascribed to the activity of the DNA repair system working to fix the ruptures caused by the exposure to glyphosate, which was no longer possible after 96 h, when the damage to the DNA molecule reappeared.

Guilherme et al. (2012), assessing the genotoxic effects of glysophate on *Anguilla anguilla* exposed to 0.018 and 0.036 mg L^{-1} of this herbicide for 24 and 72 h, found a significant increase in the DNA damage of erythrocytes after exposure to the two above cited concentrations. The presence of damage after 24 h of exposure differs from the response obtained in this work. Although these authors used larger individuals than the ones used here, this difference may be due to the greater sensitivity of *A. anguilla*, or else because the repair system in this species could fix the damage caused

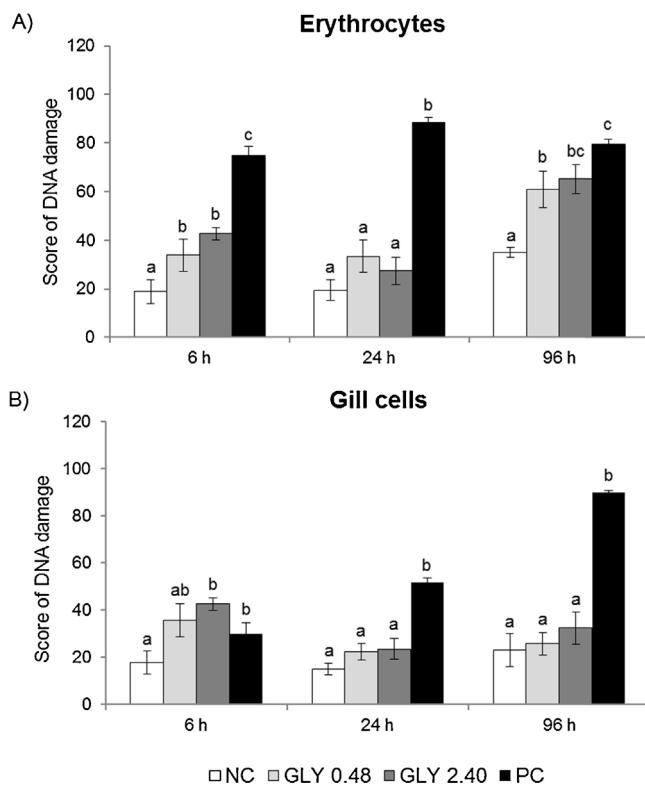


Fig. 2 – Comet scores in erythrocytes (A) and gill cells (B) of *Prochilodus lineatus* exposed to glyphosate at two concentrations 0.48 mg L^{-1} (GLY 0.48) and 2.4 mg L^{-1} (GLY 2.4) and the respective negative control (NC) and positive control (PC), at each period of exposure (6, 24 and 96 h). Values are presented as mean \pm SE ($n: 5-6$). For each experimental period, the different letters indicate significant differences between the groups ($p < 0.05$).

by the exposure to glyphosate. On the other hand, Cavalcante et al. (2008) tested the concentration of 10 mg L^{-1} of Roundup®, which also has the active ingredient glyphosate, in *P. lineatus* and obtained results similar to those found in the current work. The results obtained by these authors compared to the results of the present study indicate that *P. lineatus* responded to glyphosate in the same way they do to the formulated product, despite having been exposed to lower concentrations.

Comparing the analysis of DNA damage in erythrocytes of fish exposed to glyphosate and RT, we noted that both had genotoxic effects. These results contradict some information found in the literature reporting that the active ingredient is less toxic than the formulated product (Solomon and Thompson, 2003). Then, we concluded that the active ingredient may be contributing to the genotoxicity of the formulated product.

In addition to erythrocytes, other types of cells, including gill cells, can be used to investigate damage to the DNA molecule through the comet assay (Dhawan et al., 2009). Gill cells have been widely used because this organ is in direct contact with the contaminants present in the water. The assessment of DNA damage in these cells showed that glyphosate was genotoxic only after 6 h of exposure to the

highest concentration tested, and RT was genotoxic after 24 h of exposure for both concentrations tested. These results show either that both glyphosate and RT have a less strong effect on this tissue, or that the repair enzyme system is operating efficiently, not allowing damage during the other experimental times. Cavalcante et al. (2008) found DNA lesions in gill cells of *P. lineatus* at both 6 and 24 h of exposure, showing that Roundup® exerted a genotoxic effect for a longer time when compared to glyphosate.

Grisolia (2002) states that studies using agrochemicals are showing the existence of differences between the active ingredients and their formulations with respect to mutagenicity and that the increased toxicity of the formulated products is due to surfactants or inert compounds that are more toxic than the mutagenic compounds.

In this work, cyclophosphamide was used as positive control, since it is a compound that, when metabolized in the cells, can produce mutations, chromosomal aberrations, and micronuclei in a variety of cells (Anderson et al., 1995). As we observed from the results of the comet assay with both erythrocytes and gill cells, cyclophosphamide caused higher damage to the DNA than those found in NC, thus confirming its genotoxic potential and attesting the sensitivity of both our model and our biological test. Cyclophosphamide has also been used in other studies, whereby its potential toxicity was observed (Cavalcante et al., 2008; Çavas and Konen, 2007).

5. Conclusions

Our results indicate that both glyphosate and RT cause damage to the DNA molecule of *P. lineatus*, corroborating that the active ingredient can contribute to the genotoxicity of the formulated product, and that both act with more intensity in erythrocytes than in gill cells. Gills cells have probably more defenses than other tissues because they are in direct and permanent contact with the contaminants in the water. Taking together these results revealed that both glyphosate itself as well as the formulated product RT were genotoxic to gill cells and erythrocytes of *P. lineatus*, suggesting that their use should be carefully monitored considering their potential impact on tropical aquatic biota.

Conflict of interest

Nothing declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.etap.2013.12.012>.

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