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Oxidative stress caused by a commercial presentation of glyphosate in two indigenous fish species of importance in Colombia

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Abstract

The use of glyphosate to eradicate poppy (*Papaver somniferum*) and coca (*Erythroxylon coca*) plants is of great concern in Colombia due to potential effects on the environment. Juveniles of white cachama (n=36) (*Piaractus brachypomus*) (22.7 ± 2.9 g) and yamú (*Brycon amazonicus*) (n=36) (11.7 ± 1.1 g) were exposed for 96 h to three glyphosate concentrations (0, 5 and 15 ppm, v/v, as Roundup®). Enzymes linked to oxidative stress in liver and gills were measured after the exposure. The main oxidative stress changes happened in the gills of glyphosate-exposed cachama and liver of both species. There was a significant decrease in reduced glutathione (GSH) and glutathione-s-transferase (GST) activity of both species. Catalase activity was significantly reduced in livers of exposed cachama. Lipid peroxidation had a significant increase in glyphosate-exposed cachama (liver and gills) and yamú (liver). The biochemical changes found in both species and both organs indicate the oxidative effects of glyphosate as Roundup® despite the asymptomatic and non-lethal exposures.

Keywords: Glyphosate; Fish; Oxidative Stress; Toxicology; Colombia

1. Introduction

Glyphosate (N-phosphono-methyl-glycine) is a wide-spectrum, systemic herbicide massively used in Colombia to control illegal crops such as poppy (*Papaver somniferum*) and coca (*Erythroxylon coca*) [1, 2]. Different reports suggest toxic effects of glyphosate and the surfactants of the commercial preparations, among them: changes in hormonal profiles and reproductive variables of humans [3] and fish [4], genotoxic effects on peripheral blood cells [5], and disruption of cell cycle in sea urchin embryos [6] among others. Conversely, other reports suggest minimal toxic effects on humans [7] and frogs [8, 9].

Primary toxic effects on fish after exposure to glyphosate, the surfactants used as vehicles of the commercial preparations and the final mixtures have been reported in Colombia. Various fish species of importance in the Colombian aquaculture showed different degrees of susceptibility to the herbicide [10-13]. Amongst the species that showed high susceptibility to acute waterborne exposures are bocachico (*Prochilodus magdalenae*) and yamú (*Brycon amazonicus*). On the other hand, species such as red and Nile tilapia (*Oreochromis sp*, and *Oreochromis niloticus*, respectively) showed effects at higher exposure concentrations in different experiments. The most remarkable effects reported in the fish species are respiratory signs (gasping at the surface accompanied by methemoglobinemia; microcirculatory changes and lamellar fusion in the branchial tissue) [10], central nervous system changes (frantic swimming, concentration-dependent alterations in cholinesterase activity and changes in some clinical biochemistry parameters such as AST (aspartate amino-transferase) and ALT (alanine amino-transferase) activities [11, 12].

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Despite massive aerial use of glyphosate-based herbicides on coca and poppy plants during the last 35 years, eradication of these illegal crops has not been fully successful in Colombia. Nowadays, more than 200,000 hectares are cultivated with such crops that are harvested to produce coca paste and heroine in several geographical regions of Colombia.

In the present work, acute-96h, waterborne exposures to a commercial presentation of glyphosate (0, 5 and 15 ppm, v/v) were carried out in juveniles of white cachama (*Piaractus brachypomus*) and yamú (*Brycon amazonicus*), two indigenous fish species of importance in Colombia. Biochemical changes linked to oxidative stress in gills and liver, were used as biomarkers to investigate toxic effects.

2. Material and methods

2.1 Fish and waterborne exposures

Juveniles of both species were acclimated for four weeks prior to the experimental phase in the Laboratory of Aquatic Toxicology, School of Veterinary Medicine, Universidad Nacional de Colombia. Roundup® (N-phosphono-methyl-glycine isopropilamine salt, 48% E.C.) was added to 10-gallon-glass tanks, where fish were randomly distributed (n=12 / treatment, 3 replicates). Water temperature was kept at 26.0 ± 1.0 °C. Fish were not fed during the 96 h experimental time. Experimental waterborne concentrations of the herbicide (5 and 15 ppm, v/v as Roundup®) were selected based on preliminary investigations [11].

All the protocols for acclimation, experimental phase, sampling and humane euthanasia of fish were previously approved by the Bioethics Committee of the School of Veterinary Medicine and Animal Science, Universidad Nacional de Colombia, Bogotá (Project A/3517-1, IFS).

2.2 Oxidative stress tests

After the 96h-exposure, fish were transferred to an ice-filled container, weighed and measured. The fish were sacrificed by severing the spinal cord. Gill and liver tissues were dissected and rinsed with ice-cold saline to remove debris. Afterwards, tissue was minced and homogenized (phosphate buffer 0.1 M, pH 7.4) and centrifuged (12,500 r.p.m. for 30 minutes at 4°C, Labofuge 400R ™). Supernatants (S-9, post-mitochondrial fraction) were stored at −70 °C for further biochemical analysis [14].

Activities of glutathion-s-transferase (GST), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), as well as reduced glutathion concentration (GSH) and lipid peroxidation (LPO) were measured spectrophotometrically in both species and organs. Samples were analyzed in either duplicates or triplicates. Protein quantification of S-9 fraction was performed by the bicinchoninic acid method (562 nm, Pierce 23227 ™). GST was measured by mixing 2.25 ml buffer sodium phosphate (0.1M, pH 7.4), 0.05 ml 1-chloro2,4-dinitrobenzene (CDNB) (1mM), 0.1 ml GSH (1mM) and 0.1 ml S-9 fraction. Changes in absorbance at 340 nm determined activity as nmols CDNB/min/mg protein [15]. SOD was carried out based on the ferricytochrome C method. The reaction was performed at 550 nm and reaction mixture consisted of buffer phosphate (pH 7,4, 50 mM), EDTA 0.1mM, xantine, cytochrome C and xantine oxidase. Activity was expressed as SOD units/mg protein (1 unit = amount of enzyme to produce 50% inhibition of ferricytochrome C reduction) [16, 17]. CAT activity was based on the transformation of hydrogen peroxide (H_2O_2) into water and oxygen. Mixture reaction consisted of 1.95 ml buffer phosphate (0.1 M, pH = 7.4), 1 ml H_2O_2 (0.019 M) and 0.05 ml S-9 fraction. The lowering in absorbance (240 nm) due to the H_2O_2 depletion determined activity as nmols H_2O_2/min/mg protein. GPx was measured mixing 1.44 ml buffer phosphate (0.5 M, pH = 7.0), 0.1 ml EDTA (1 mM), 0,1 ml sodium azide (1 mM), 0.05 ml glutathion reductase (15 IU/ml), 0.1 ml reduced GSH, 0.1 ml NADPH (0.2 mM), 0.1 ml H_2O_2 (0.25 mM) and 0.1 ml S-9 fraction. NADPH oxidation was recorded at 340 nm [18]. GPx activity was reported as nmols oxidized NADPH/min/mg protein (molar extinction coefficient = 6.22 x 103 M^{-1} cm^{-1}) [19]. GSH was measured by precipitating S-9 fraction with sulfosalicilic acid (4%) 1:1, keeping at 4°C during 1 h before centrifugation (3000 r.p.m., 4°C). Supernatant (0.5 ml) was mixed with 2.3 ml buffer phosphate (0.1 M, pH=7.4) and 0.2 ml DTNB (5,5-dithiobis-2-nitrobenzoic acid). GSH concentration (nmols GSH/mg protein) was determined based on a calibration curve at 412 nm [19, 20]. LPO was determined by the TBARS method (thiobarbituric acid reactive substances). One ml of S-9 was incubated (37°C/ 1 h), then 1 ml trichloroacetic acid (5%) and 1 ml tiobarbituric acid (0.67%) were pipetted to the mixture and centrifuged (3000 r.p.m./ 10 min). Supernatant was heated (90°C/10 min). After cooling at room temperature, absorbance measured at 535 nm served to calculate LPO as nmols MDA/h/mg protein (extinction coefficient, 1.56 x 105 M^{-1} cm^{-1})[19].

2.3 Experimental design and statistical analysis of results

Experimental design was a factorial (2x3), where 2 represented the fish species (white cachama and yamú) and 3, the experimental herbicide concentrations (0, 5 and 15 ppm). Each tank was selected as the experimental unit and each fish as the sampling unit. Results were compared by means of ANOVA (95% confidence) followed by Tukey test using the SAS® software.

3. Results and discussion

Neither of the experimental fish in both species showed symptoms or died during the 96 h exposure. The results of the oxidative stress tests are presented in Table 1 and Figure 1. In 10 out of the 24 oxidative stress tests (two organs, two species, six parameters), at least one of the herbicide concentrations induced a significant change as compared to the others. For most of the cases, enzymatic activities were lower in glyphosate-exposed fish than in controls. GSH concentration was significantly reduced in the gills and liver of cachama and yamú as compared to controls (Figure 1A). On the other hand, LPO in glyphosate-exposed fish increased significantly as compared to the controls, particularly in liver and gills of cachama and liver of yamú (Figure 1B). Gills of cachama showed the major changes in GST, CAT, GSH and LPO as well as GST in yamú. Liver in both species showed significant changes in GST, GSH and LPO due to the glyphosate exposure.

A report published in 2015 by the IARC (International Agency for Research on Cancer), the specialized cancer agency of the World Health Organization (WHO), classified glyphosate as *probably carcinogenic to humans,* category 2A [21]. This results in serious concerns around the world given the massive use of this herbicide and thus the potential implications in public health. The main mechanisms through which glyphosate may cause cancer are linked to oxidative stress and genotoxic-related pathways according to the IARC review on many glyphosate-related research papers [22, 23, 25]. Oxidative stress is reported in fish as a common pathway induced by glyphosate with consequences at different biochemical levels [24]. In the present investigation, waterborne exposures to a commercial preparation of glyphosate induced various oxidative-stress responses in white cachama and yamú, two indigenous finfish species found mainly in bodies of water in the east of Colombia.

SOD decreased in both gills and liver of cachama after glyphosate exposure whereas no changes happened in yamú. The same effect was found by Guilherme et al. (2012) [26] in *Anguilla Anguilla* at 58 and 115 ppb Roundup® for 1 to 3 days; Lushchak et al. (2009) in goldfish (*Carassius auratus*) at 2.5, 5.0, 10 and 20 ppm Roundup® [27]; Modesto and Martínez (2010) in *Prochilodus lineatus* at 10 ppm for 6, 24 and 96-h [28] and Topal et al. (2015) in rainbow trout at 5 ppm commercial glyphosate for 48 h [29].

CAT showed the same reduced activity as SOD in the gills and liver of cachama in the present investigation. Reduced CAT activity was reported in rainbow trout exposed to commercial glyphosate for 24 h (2.5 ppm) and 48h to 96h (10 ppm) [30]. On the contrary, in other fish-based studies, glyphosate caused increased CAT activity in *Anguilla anguilla* gills [26] as well as in goldfish liver (1.5-fold) and kidney (1.3-fold) [27].

Reduced glutathione (GSH) and glutathione-s-transferase (GST) had remarkable changes in the present work. One of the most significant effects caused by glyphosate was the lowering of GSH concentration in white cachama gills and yamú liver at 15 ppm Roundup®. The report by Lushchak et al. (2009) [27] indicates low glutathion reductase (GR) activity in liver, kidney and brain of goldfish after 96 h exposures to 0 to 20 ppm Roundup®. GR catalyzes the production of GSH from the oxidized form. On the other hand, *Prochilodus lineatus* exposed to 10 ppm Roundup® had increased [GSH] after 24 h [28]. GST is a phase II biotransformation enzyme that catalyzes the conjugation of electrophilic compounds with the tripeptide glutathione playing a more important role than GPx in fish [30]. GST had significant lower activities in the two fish species of the present study when exposed to 15 ppm. Likewise, goldfish exposed to Roundup® showed a 29-34% reduction in GST as compared to controls (2.5-20 ppm for 96 h) [27]. However, the patterns were different in *Prochilodus lineatus* when this neotropical species was exposed to 10 ppm for 96 h [28]. GPx was the oxidative stress enzyme that showed the least changes after the glyphosate exposure in the present investigation.

Table 1 Oxidative stress parameters in controls and glyphosate-exposed fish (5 and 15 ppm). Values expressed as means ± standard error (n=12/treatment).

Oxidative stress parameter	Experimental treatments	White cachama		Yamú	
		Gills	Liver	Gills	Liver
Glutathion transferase (GST)	Control	154.2 ± 12.4 [a]	200.1 ± 11.8 [a]	274.9 ± 27.6 [a]	191.1 ± 16.5 [a]
	5 ppm	119.4 ± 15.6 [a,b]	211.3 ± 15.6 [a]	313.6 ± 21.3 [a]	193.1 ± 21.5 [a]
	15 ppm	88.7 ± 5.2 [b]	208.6 ± 25.7 [a]	196.0 ± 10.4 [b]	209.1 ± 22.8 [a]
Superoxide dismutase (SOD)	Control	4.2 ± 0.5 [a]	1.3 ± 0.2 [a]	9.7 ± 1.0 [a]	7.5 ± 0.7 [a]
	5 ppm	3.9 ± 0.4 [a]	1.2 ± 0.1 [a]	7.9 ± 0.7 [a]	8.9 ± 0.9 [a]
	15 ppm	2.8 ± 0.3 [a]	0.8 ± 0.1 [a]	9.0 ± 0.9 [a]	6.4 ± 1.0 [a]
Catalase (CAT)	Control	6.2 ± 1.3 [a]	90.7 ± 6.8 [a]	26.9 ± 2.2 [a]	124.3 ± 11.1 [a]
	5 ppm	3.2 ± 0.4 [a,b]	69.1 ± 5.2 [b]	27.0 ± 1.5 [a]	142.1 ± 15.7 [a]
	15 ppm	3.5 ± 0.5 [b]	30.2 ± 2.9 [c]	26.7 ± 1.7 [a]	140.1 ± 15.5 [a]
Glutathion peroxidase (GPx)	Control	50.3 ± 7.3 [a]	37.8 ± 5.1 [a]	25.3 ± 4.4 [a]	21.9 ± 3.5 [a]
	5 ppm	46.0 ± 6.7 [a]	40.3 ± 4.5 [a]	25.3 ± 3.0 [a]	33.6 ± 5.5 [a]
	15 ppm	34.5 ± 5.8 [a]	31.2 ± 8.2 [a]	34.9 ± 3.2 [a]	21.8 ± 4.5 [a]

Different letters within the same column indicate statistically significant differences among treatments for each species, organ and oxidative stress parameter ($p < 0.05$, ANOVA). GST expressed as nmols CDNB/minute/mg protein, SOD as units/minute/mg protein, CAT and GPx as nmols/minute/mg protein.

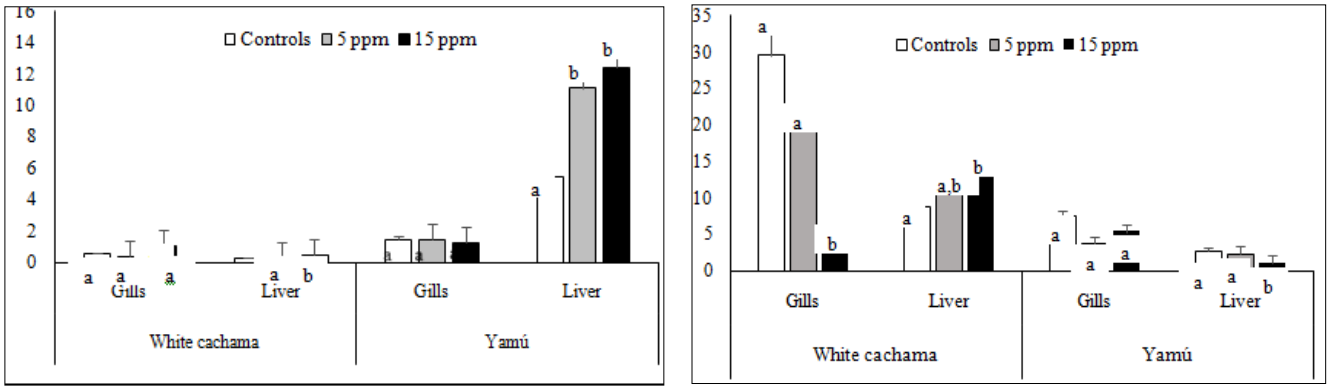

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Figure 1 a) LPO and b) GSH concentration in liver and gills of glyphosate exposed fish and controls (means ± S.E. Different letters indicate statistically significant differences among treatments for each organ and species, $p < 0.05$)

LPO was the endpoint that displayed the most relevant changes after glyphosate exposure in both species. In both organs of cachama, LPO was significantly higher in 15 ppm glyphosate as compared to controls. In yamú liver, LPO was higher at both concentrations (5 and 15 ppm) than in controls. The silver catfish (*Rhamdia quelen*) exposed to 0.2 and 0.4 ppm Roundup® for 96 h showed significant LPO in muscle [31]. In the same pattern, *Prochilodus lineatus* exposed to 10 ppm had increased concentration of TBARS in the liver after 24 and 96 h [28]. Another study in the fish species *Rhamdia quelen* indicated that glyphosate (1.2 ppm) was not a cause of LPO in the liver [32]. This latter study tested lower experimental concentrations than the present work. Species-specific response of fishes has to be considered when comparing susceptibilities among them to oxidative stress due to glyphosate. In fact, the present investigation demonstrated that cachama was more susceptible to LPO in liver and gills at 15 ppm than yamú.

A key factor for understanding the changes in oxidative stress after glyphosate exposure might be the time point at which reactions are measured. Rainbow trout exposed to glyphosate during 96 h had a significant increase in SOD, CAT and GPx that took place during the 6-24 h exposure time. On the contrary, by the 96 h time point, most of these reactions were at the lowest catalytic level [29], as it happened in this investigation. Interestingly, in the present work, low catalytic activity in oxidative stress reactions was accompanied by a significant increase in LPO which indicates that the ROS scavenge capacity diminished significantly by the 96h experimental time, leading to damage in lipid membranes of gills and liver. An important research question resides in determining if the oxidative stress caused by glyphosate is due to the active principle, the surfactants/vehicles present in the commercial products or both. Surfactants, such as the nonionic polyoxyethylene amine (POEA), enhance the efficacy of glyphosate by increasing the kinetics of intracellular passage [33, 34]. In order to confirm the effects of surfactants, Navarro & Martinez (2014) [35] tested different concentrations of POEA in the freshwater teleost *Prochilodus lineatus*. The surfactant by itself caused liver LPO at 0.15 ppm after 24 h of exposure. The use of commercial formulations are more common in experimental designs considering that the actual application in the field proceeds with the whole mixture (active principle + surfactant). Glyphosate, as the active principle, does not exert its herbicide action without the presence of surfactants in the mixture.

4. Conclusion

This study demonstrated oxidative stress effects after non-lethal, asymptomatic exposures to 5 and 15 ppm of a commercial presentation of glyphosate for 96 hours. In 10 of the 24 tests (6 biochemical endpoints x 2 species x 2 organs), there was a significant difference in at least one of the herbicide-tested concentrations as compared to the controls.

Differences in oxidative stress responses between the two species (yamú and cachama) and the two tested organs (gills and liver) were found in the present work. White cachama had a higher degree of LPO in both organs than yamú, whereas the gills were more affected than the liver in both species.

The present investigation and others where oxidative stress is linked to glyphosate exposure are important to understand the toxicology of this herbicide and the new developments with regard to the cancer risks. The link between oxidative stress and carcinogenic potential of glyphosate and its commercial presentations makes relevant the present results in these two native fish species of Colombia considering the massive use of this herbicide and likely implications in wildlife and public health.

Compliance with ethical standards

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Disclosure of conflict of interest

No competing financial interests or of any other nature exist for the present investigation.

Statement of ethical approval

The present investigation was performed based on the approval and guidelines of the *Bioethics Committee of the School of Veterinary Medicine and Animal Science, Universidad Nacional de Colombia* [Ethics Approval Protocol Project A-3517/1 - 2008].

References

[1] Morris K. Plan Colombia: parallels drawn with Vietnam war. Lancet 2007; 357:52.

[2] Marshall EJP, Solomon KR, Carrasquilla G. Coca (*Erythroxylum coca*) control is affected by glyphosate formulations and adjuvants. J Toxicol Environ HealthA. 2009;72: 930–936.

[3] Richard S, Moslemi S, Sipahutar H, et al. Differential effects of glyphosate and Roundup® on human placental cells and aromatase. Environ Health Persp. 2005; 113:716-720.

[4] Soso AB, Gil LJ, Ranzani-Paiva MJ, et al. Chronic exposure to sub-lethal concentration of a glyphosate-based herbicide alters hormone profiles and affects reproduction of female Jundiá (*Rhamdia quelen*). Environ Toxicol Pharmacol. 2007; 23:308-313.

[5] Paz-y-Miño C, Sánchez ME, Arévalo M, et al. Evaluation of DNA damage in an Ecuadorian population exposed to glyphosate. Gen Mol Biol. 2007; 30:456-460.

[6] Marc J, Mulner-Lorillon O, Bellé R. Glyphosate-based pesticides affect cell cycle regulation. Biol Cell. 2004; 96:245-249.

[7] Solomon KR, Anadon A, Carrasquilla G, et al. Coca and poppy eradication in Colombia: Environmental and human health assessment of aerially applied glyphosate. Rev Environ Contam Toxicol. 2007; 190:43–125.

[8] Bernal MH, Solomon KR, Carrasquilla G. Toxicity of glyphosate and Cosmo-Flux to larval Colombian frogs: laboratory acute toxicity. J Toxicol Environ Health A. 2009; 72:961–965.

[9] Triana TM, Montes CM, Bernal MH.Efectos letales y subletales delglifosato (Roundup Activo®) en embriones de anuros colombianos. Act Biol Col. 2013; 18(2):271-278.

[10] Eslava-Mocha PR, Ramírez WF, Rondón IS. Sobre los efectos tóxicos del glifosato y sus mezclas: impacto en peces nativos. Universidad de Los Llanos, Villavicencio (Colombia). 2007. 150 p.

[11] González JF, Ochoa DM, Figueredo D, et al. Efectos tóxicos del Roundup® (glifosato) en tilapia roja (*Oreochromis sp.*), yamú (*Brycon amazonicus*) y bocachico (*Prochilodus magdalenae*). Rev Med Vet Zoot, 2007; 54:113-119.

[12] González JF, Ochoa DM, González CA, et al. Contaminación de las aguas con glifosato y sus efectos tóxicos en ictiofauna nativa de Colombia. 2012. Universidad Nacional de Colombia. Bogotá, D.C. 71 p.

[13] Eslava-Mocha PR, Vargas-Pulido A, León-Pinzón AL, et al. Pathological effects and lethal concentration of two nonionic, tallowamine-polyethoxylate surfactants in white cachama (*Piaractus brachypomus*). Water Air Soil Pollut. 2019; 230: 286.

[14] Vodicnik MJ, Elcombe CR, Lech JJ. The effect of various types of inducing agents on hepatic microsomal monooxygenase activity in rainbow trout. Toxicol Appl Pharmacol. 1981; 59:364-374.

[15] Habig WH, Pabst MJ, Jakoby WB. Glutathione-s-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem. 1974; 249: 7130-7139.

[16] McCords JM, Fridovich I. Superoxide dismutase an enzymic function for erythrocuprein (hemocuprein). J Biol Chem. 1969; 244 (22): 49-55.

[17] Morales AE, Pérez-Jiménez A, Hidalgo MC, et al. Oxidative stress and antioxidant defenses after prolonged starvation in *Dentex dentex* liver. Comp Biochem Physiol. 2004; 139C: 153–161.

[18] Dorval J, Hontela A. Role of glutathione redox cycle and catalase in defense against oxidative stress induced by endosulfan in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*). Toxicol Appl Pharmacol. 2003; 192: 191–200.

[19] Fatima M, Ahmad I, Sayeed I, et al. Pollutant-induced over-activation of phagocytes is concomitantly associated with peroxidative damage in fish tissues. Aquat Toxicol. 2000; 49: 243–250.

[20] Ahmad I, Hamid T, Fatima M, et al. Induction of hepatic antioxidants in freshwater catfish (*Channa punctatus* Bloch) is a biomarker of paper mill effluent exposure. Biochim Biophys Acta. 2000; 1523: 37-48.

[21] IARC (International Agency for Research on Cancer). Some organophosphate insecticides and herbicides. Monographs on the evaluation of carcinogenic risks to humans. 2015; vol. 112. Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, France. 464 p.

[22] Muñoz JP, Silva-Pavez E, Carrillo-Beltrán D, et al. Ocurrence and exposure assessment of glyphosate in the environment and its impact on human beings. Environ Res. 2023; 231(116201).

[23] Lei Q, Yan-Mei D, Hong C, et al. Glyphosate based-herbicide disrupts energy metabolism and activates inflammatory response through oxidative stress in mice liver. Chemosphere 2023; 315(137751).

[24] Faria M, Bedrossiantz J, Rosas Ramírez JR, et al. Glyphosate targets fish monoaminergic systems leading to oxidative stress and anxiety. Environ Inter. 2021; 146 (106253).

[25] Guyton KZ, Loomis D, Grosse Y, et al. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. Lancet Oncol. 2015; 16:490-491.

[26] Guilherme S, Gaivão I, Santos MA, et al. DNA damage in fish (*Anguilla anguilla*) exposed to a glyphosate-based herbicide:elucidation of organ-specificity and the role of oxidative stress. Mutation Res. 2012; 743:1-9.

[27] Lushchak OV, Kubrak OI, Storey JM, et al. Low toxic herbicide Roundup® induces mild oxidative stress in goldfish tissues. Chemosphere 2009; 76:932-937.

[28] Modesto KA, Martínez C. Roundup® causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish *Prochilodus lineatus*. Chemosphere 2010; 78:294-299.

[29] Topal A, Atamanalp M, Uçar A, et al. Effects of glyphosate on juvenile rainbow trout (*Oncorhynchus mykiss*): transcriptional and enzymatic analyses of antioxidant defence system, histopathological liver damage and swimming performance. Ecotoxicol Environ Saf. 2015; 111:206–214.

[30] Stephensen E, Sturve J, Förlin L, et al. Effects of redox cycling compounds on glutathione content and activity of glutathione-related enzymes in rainbow trout liver. Comp Biochem Physiol C. 2002; 133:435-442.

[31] Glusczak L, dos SantosD, Moraes BS, et al. Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (*Rhamdia quelen*). Comp Biochem Physiol C. 2007; 146:519-524.

[32] Ferreira D, Costa de Motta A, Kreutz LC, et al. Assessment of oxidative stress in *Rhamdia quelen* exposed to agrichemicals. Chemosphere 2010; 79:914-921.

[33] Gehin A, Guillaume YC, Millet J, et al. Vitamins C and E reverse effect of herbicide-induced toxicity on human epidermal cells HaCaT: a biochemometric approach. Int J Pharm. 2005; 288:219-226.

[34] El-Shenawy NS. Oxidative stress responses of rats exposed to Roundup® and its active ingredient glyphosate. Environ Toxicol Pharmacol. 2009; 28:379-385.

[35] Navarro CD, Martinez CBR. Effects of the surfactant polyoxyethylene amine (POEA) on genotoxic, biochemical and physiological parameters of the freshwater teleost *Prochilodus lineatus*. Comp Biochem Physiol C. 2014; 165: 83–90.

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