academicJournals

Vol. 8(3), pp. 25-33, March, 2016
DOI:10.5897/JECE2015.0362
Article Number: 29BD0C957807
ISSN 2141-226X
Copyright ©2016
Author(s) retain the copyright of this article
http://www.academicjournals.org/JECE

Journal of Environmental Chemistry and Ecotoxicology

Full Length Research Paper

Sensitivity, ecotoxicity and histopathological effects on neotropical fish exposed to glyphosate alone and associated to surfactant

Claudinei da Cruz[1]*, Silvia Patrícia Carraschi[2], Natália Sayuri Shiogiri[2], Adilson Ferreira da Silva[2], Robinson Antonio Pitelli[2] and Marcia Rita Fernandes Machado[3]

[1]University Center of Barretos Educational Fundation. Av. Prof. Roberto Frade Monte no 389, 14.783-226, Barretos (SP), Brazil.
[2]Weed Science Environmental Research Studies of the College of Agricultural and Veterinary Science at Unesp, Via de Acesso Prof. Dr. Paulo Donato Castellane, Zona Rural, s/ n°, Jaboticabal, SP, Brazil.
[3]Department of Morphology and Animal Physiology of the College of Agricultural and Veterinary Sciences at Unesp from Jaboticabal, SP, Brazil.

Received 13 August, 2015; Accepted 22 December, 2015

The aim of this research was to evaluate neotropical fish sensitivity (*Piaractus mesopotamicus, Phallocerus caudimaculatus, Hyphessobrycon eques*, and *Brachydanio rerio*) to a reference substance (potassium chloride); to estimate the lethal concentration (LC_{50}; 96 h) for glyphosate, formulated as Rodeo® alone and in association with 0.5 and 1.0% Aterbane® BR surfactant and to evaluate the histopathology of the gills, liver, and kidney from the fish after acute exposure. *P. caudimaculatus* and *H. eques* are good bioindicators like *B. rerio* because they have similar sensitivity. The LC_{50};96 h for glyphosate alone and in association with 0.5% Aterbane® BR was similar (>975.0 mg L^{-1}) for all the fish. Aterbane® BR alone was the most toxic substance to *P. caudimaculatus* (5.81 mg L^{-1} LC_{50};96 h) and glyphosate associated to 1.0% Aterbane® BR was more toxic to *H. eques* (411.91 mg L^{-1} LC_{50};96 h). The glyphosate alone and in association with Aterbane® BR was classified as practically non-toxic, whereas Aterbane® BR alone was considered moderately toxic for the tested organisms. The histopathological effects caused by glyphosate exposure on gills, liver, and kidneys are reversible, except for the liver necrosis on *P. caudimaculatus. H. eques, P. caudimaculatus*, and *P. mesopotamicus* present great potential to be used as standard organisms for herbicides monitoring and the use of glyphosate without surfactant addition is enough to cause histological alterations on *H. eques* and *P. caudimaculatus*, which makes them possible to be applied on environmental monitoring studies as biomarkers.

Key words: Bioindicator, pesticide, histology, biomarker, gill, herbicide.

INTRODUCTION

Glyphosate (GFT) is the most used herbicide for weed control on several crops, due to its wide action spectrum,

*Corresponding author. E-mail: cruzcl@yahoo.com.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

Table 1. Concentrations of the substances used on the toxicity assays.

Substances	Fish			
	P. mesopotamicus	***P. caudimaculatus***	***H. eques***	***B. rerio***
KCL (g L^{-1})	1.0, 1.5, 2.0, 2.5	0.5, 1.0, 1.5, 2.0, 2.5	1.0, 1.5, 2.0, 2.5	1.0, 1.5, 2.0, 2.5, 3.0
Glyfosate (mg L^{-1})	900.0, 925.0, 9_{50}.0, 975.0	900.0, 925.0, 9_{50}.0, 975.0	900.0, 925.0, 9_{50}.0, 975.0	900.0, 925.0, 9_{50}.0, 975.0
Surfactant (mg L^{-1})	8.0, 9.0, 10.0, 11.0, 12.0	3.0, 4.0, 5.0, 6.0, 7.0, 8.0	4.0, 6.0, 8.0, 10.0, 12.0, 14.0	7.0, 8.0, 9.0, 10.0, 11.0
*GF+0.5% (mg L^{-1})	900.0, 925.0, 9_{50}.0, 975.0	900.0, 925.0, 9_{50}.0, 975.0	900.0, 925.0, 9_{50}.0, 975.0	900.0, 925.0, 9_{50}.0, 975.0
*GF+1.0% (mg L^{-1})	475.0, $_{50}$0.0, 525.0, 5_{50}.0, 575.0	900.0, 925.0, 9_{50}.0, 975.0	400.0, 425.0, 4_{50}.0, 475.0	900.0, 925.0, 9_{50}.0, 975.0

*GFT+0.5% glyphosate + 0.5% surfactant; GFT+1.0% glyphosate + 1.0% surfactant

lower selectivity, post-emergence application, systemic action and does not represent environmental risks to the water bodies (Shiogiri et al., 2010), and has a large potential to control aquatic weeds. The macrophyte present countless benefits to the environment, however they grow in an accelerated rate under eutrophic conditions, causing negative impacts to the use of the water body, like fishing, recreation, and power generation.

The herbicide application on the aquatic environment needs a higher scientific base, due to the lack of knowledge about the molecules for non-target organisms, morph-physiological effects, and control effectiveness (Botelho et al., 2009) and residues on water. Another issue is the toxicity of the other formulation components, since the inert components, such as the surfactants, may be more toxic than the active ingredient (IA) for non-target organisms (Carraschi et al., 2011). For Amarante Jr et al. (2002), Tsui and Chui (2003), and Navarro and Martinez (2014), the surfactants from the glyphosate formulations are more toxic for fish than the molecule itself.

At this context, the acute and chronic toxicity assays may be performed to characterize the effects and to evaluate dangerousness and environmental risks of the formulations (Schmitt-Jansen et al., 2008). Thus, the organism selection is based on a series of criteria, such as sensibility, short reproduction cycle, operation easiness, and optimization costs (Cruz et al., 2008).

The neotropical species may be used as bioindicators for xenobiotics presence at the aquatic environment. In Brazil, the study about the application of this method for environmental monitoring is scarce (Glusczak et al., 2006; Shiogiri et al., 2012).

Thus, the aim of this study was to evaluate the sensitivity to a reference substance (potassium chloride) and the acute toxicity (LC_{50};96 h) of the glyphosate and the Aterbane® BR surfactant (alkylphenolpolyglycol ether) alone and in association with glyphosate (GFT + 0.5 and 1.0% surfactant) for the neotropical fish *Piaractus mesopotamicus*, *Hyphessobrycon eques* and *Phallocerus caudimaculatus* and the comparison with the standard species *Brachydanio rerio* and to evaluate possible histological effects caused by the glyphosate on gills, liver and kidney after acute exposure.

MATERIALS AND METHODS

The potassium chloride with 99.9% purity was used as reference substance, the Rodeo® herbicide was the glyphosate source (480.0 g L^{-1}) and Aterbane® BR was used as the surfactant alkylphenolpolyglycol ether source (466.0 g L^{-1}).

The 50% lethal concentration values were estimated by the Trimmed Spearman-Karber (Hamilton et al., 1977).

Sensitivity and acute toxicity tests

To perform the tests, the fish (*P. mesopotamicus*, *H. eques*, *B. rerio* and *P. caudimaculatus*) were acclimatized during seven days, under bioassay room conditions (25.0 ± 2°C temperature and 12 h photoperiod).

The fish were exposed to potassium chloride, glyphosate, surfactant and GFT + surfactant associations (Table 1), with three repetitions per treatment, three fish per repetition, at maximum density of 1.0 g L^{-1}, with 96 h duration (ABNT, 2011).

The substances concentrations on the definitive assays were previously adjusted by preliminary tests, to find the concentrations interval which caused zero and 100% mortality.

Histological analysis after glyphosate exposure

After 96 h exposure, three representative animals from each treatment and each species (n=3) were euthanized through anesthesia (1.0 g L^{-1} benzocaine). Liver, kidney, and gills were collected. The samples were immersed in buffered formaldehyde solution (0.1 m; pH 7.3) for 24 h. After the fixation, the pieces were dehydrated in alcohol, diaphanized on xylene and included in Histosec® (Merck).

Table 2. Potassium chloride lethal concentration (LC_{50}, 96 h) for fish.

Parameter	*P. mesopotamicus*	*H. eques*	*P. caudimaculatus*	*B. rerio*
Upper limit	2.26	1.22	0.91	1.89
LC_{50}, 96 h	1.86	1.98	0.55	1.58
Lower limit	1.72	1.49	0.34	1.33

Table 3. Lethal concentration (CL_{50}, 96 h) of the glyphosate, surfactant and their associations (mg L^{-1}) for fish.

Parameter	Glyphosate	Surfactant	Glyphosate + 0.5% Surfactante	Gly + 1.0% Aterbane®
B. rerio	>975.00	8.61	>975.00	>975.00
P. mesopotamicus	>975.00	9.45	929.69	528.$_{50}$
H. eques	>975.00	8.21	922.62	411.91
P. caudimaculatus	>975.00	5.81	>975.00	>975.00

*****Surfactant: alkylphenolpolyglycol ether (Aterbane®BR)

Then, the microtomy was carried out in an automatic microtome (RM2155 - Leica®), performing cuts ranging from 3.0 to 5.0 μm thickness. The pieces were placed in glass slides, which were colored with hematoxylin-eosin and PAS (Schiff's periodic acid) (Behmer et al., 1976).

The histological alterations were transformed into effect index, and then classified according to each tissue, as described in the results and discussion. For the gills, the following indexes were evaluated: 0 - similar to the control - normal; 1 - Hypertrophy and hyperplasia of primary and secondary lamellae; 2 - Epitelial lifting; 3 - Lamelar tip fusion; 4 - secondary lamella disarrangement; 5 - secondary lamella congestion; 6 - decrease of the interlamellar epithelium; and 7 - sub epithelial edema on the secondary lamella. For the liver, the indexes were as follows: 0 - similar to the control - normal; 1 - capillaries congestion; 2 - hepatocytes hypertrophy with nucleus displacement to the periphery; 3 - hepatocytes fusion; 4 - disarrangement of cordonal structure; 5 - picnotic nucleus; and 6 - necrosis. And the indexes for the liver were the following: 0 - similar to the control - normal; 1 - Bowman's capsule release; 2 - epithelium disarrangement from the proximal tubules; and 3 - light increase of the proximal and distal tubules.

RESULTS AND DISCUSSION

Fish sensitivity

The most sensitive fish to the potassium chloride (KCl) was *P. caudimaculatus* and the most tolerant was *H. eques*; however, the sensitivity between species was similar (Table 2). Thus, the potassium chloride was classified as moderately toxic for the fish ($1.0<CL_{50}<1.0$ mg L^{-1}), except for *P. caudimaculatus*, which was highly toxic ($0.1<CL_{50}<1.0$ mg L^{-1}) (Zucker, 1985).

Based upon the sensitivity and in comparison with the standard fish *B. rerio*, the tested species may be standardized for ecotoxicological assays in Brazil, except for *P. mesopotamicus*, because it does not fill the requirements for a test organism. This fish has low availability, because it is a rheophilic species, with only one spawning per year. The other species have a higher availability, due to a short reproduction cycle, as shown by Cruz et al., (2008) on the sensitivity test for *H. eques* exposure to potassium dichromate.

Neotropical fish sensitivity is similar to *B. rerio*, with higher sensibility to potassium chloride than *Pimephales promelas* (7.62 g L^{-1} LC_{50};96 h) (USEPA, 2001). The fish sensibilities were similar as described for *H. eques* (1.5 g L^{-1}) (Fujimoto et al., 2012) and for *P. mesopotamicus* (1.33 g L^{-1}) (Carraschi et al., 2012).

According to USEPA (2002), the advantage on the use of potassium chloride over potassium dichromate is the presence of hexavalent chromium on the potassium dichromate composition, which is highly toxic for the environment and may cause severe morphological and functional effects on neotropical fish as *P. mesopotamicus* (Castro et al., 2014).

Acute toxicity for glyphosate, surfactant and the associations

The lethal concentration 50% (LC_{50}; 96 h) of glyphosate for the tested species was >975.0 mg L^{-1} (Table 3). The mortality ranged from 10 to 40%, regardless of the tested concentration. The lethal concentration was not estimated precisely, due to a lack of pattern regarding the ratio concentration/mortality.

The most sensitive species to the surfactant exposure was *P. caudimaculatus*, although the LC_{50};96 h was similar for all tested species (Table 2). No mortality occurred for *B. rerio* under 7.0 mg L^{-1} exposure; however, 100% mortality occurred with 11.0 mg L^{-1}. For *P. mesopotamicus*, no mortality occurred with 8.0 mg L^{-1}, and 100% mortality occurred with 12.0 mg L^{-1} surfactant exposure. For *H. eques*, no mortality occurred with 4.0 mg L^{-1} and 100% mortality was achieved with 14.0 mg L^{-1}.

For *P. caudimaculatus* no mortality occurred at 3.0 mg L^{-1} and 100% mortality was obtained with 8.0 mg L^{-1} surfactant exposure.

The LC_{50};96 h for the glyphosate + 0.5% surfactant association was > 975.0 mg L^{-1} for *B. rerio* and *P. caudimaculatus*. For *P. mesopotamicus* and *H. eques* the LC_{50};96 h was > 900.0 mg L^{-1} (Table 3). For *P. mesopotamicus,* 22% mortality occurred with 875.0 mg L^{-1} and 100% mortality was achieved with 975 mg L^{-1}. For *H. eques*, no mortality occurred at 875.0 mg L^{-1} exposure and 100% mortality occurred at 975 mg L^{-1}. *B. rerio* and *P. caudimaculatus* displayed no mortality during association exposure.

The LC_{50};96 h observed for the glyphosate + 1.0% surfactant association were as follows: *P. caudimaculatus* and *B. rerio* > 975.0 mg L^{-1}; *H. eques* > 400.0 mg L^{-1}; *P. mesopotamicus* > $_{50}$0 mg L^{-1} (Table 3). With 400.0 mg L^{-1} exposure, 22.2% mortality occurred for *H. eques;* 100% mortality occurred with 475 mg L^{-1} exposure. No *P. mesopotamicus* mortality occurred with 475.0 mg L^{-1} exposure, whereas 100% mortality occurred with 575.0 mg L^{-1}. No mortality occurred for *B. rerio* and *P. caudimaculatus* during the association exposure.

The glyphosate (Rodeo®) is practically non toxic (LC_{50} > 100.0 mg L^{-1}) for all studied species, different from found for *Prochilodus lineatus* exposed to Roundup® (LC_{50};96 h = 13.69 mg L^{-1}) (Langiano and Martinez, 2008). Other authors also found different results for Roundup Ready® and *P. mesopotamicus* (LC_{50};96 h = 3.74 mg L^{-1}) (Shiogiri et al., 2012); other glyphosate formulations and *Rhinella arenarum* (LC_{50};96 h = 6.8 to 72.8 mg L^{-1}) (Brodeur et al., 2014); and Roundup Original® and *Pseudoplatystoma* species (LC_{50};96 h = 15.0 mg L^{-1}) (Sinhorin et al., 2014).

The glyphosate toxicity observed in this study was similar to the values found for *Salmo gairdneri* and *Oncorhynchus tshawytscha* (LC_{50};96 h = 1070.0 to 1440.0 mg L^{-1}) (Mitchell et al., 1987); *Hybognathus amarus* and *P. promelas* (LC_{50};96 h > 1000.0 mg L^{-1}) (Beyers, 1995) and *P. promelas* and *Polonichthys macrolepidoyus* (LC_{50};96 h = 1154.0 to 1132.0 mg L^{-1}) (Riley and Finlayson, 2004). The commercial formulations type (inert compounds and surfactant) has an important role for the glyphosate toxicity characterization. The lower toxicity of the formulation studied in this paper (Rodeo®) may be due to the absence of surfactants. Thus, the knowledge about the formulations toxicity is fundamental at the moment of decision making, concerning the monitoring of possible glyphosate environmental effects.

The fish evaluated for this study were more tolerant to the alkylphenolpolyglycol ether than *S. gairdneri, P. promelas, Ictalurus punctatus* and *Lepomis macrochirus* exposed to the MON0818 surfactant (amine ethoxylate) (LC_{50} = 1.4 to 3.0 mg L^{-1}) (Wan et al., 1989). *Hypomesus transpacificus, P. promelas* and *P. macrolepidoy* were also less tolerant when exposed to the surfactants alkylphenolpolyethoxylate and alcohol polyalkoxylate acid (LC_{50} = 0.7 to 3.9 mg L^{-1}) (Riley and Finlayson, 2004). And the surfactant Ammoeng 130® was also classified as more toxic for *P. promelas* (LC_{50};96 h = 0.51 to 1.80 mg L^{-1}) (Versteeg et al., 2006). However, the toxicity observed for *Onchorynchus mykiss* exposed to alkylphenolpolyethoxylate was similar to the toxicity observed in this study, with 5.18 to 6.57 mg L^{-1} LC_{50} (Curran et al., 2004).

The surfactant presence in any concentration has increased the formulation toxicity in about $_{50}$% for *H. eques* and *P. mesopotamicus*, indicating that the surfactant addition interfere on the glyphosate toxicity, regarding some non-target organisms. According to Giesy et al. (2000), the polyethoxylate amine surfactant (POEA) may be responsible for making the Roundup® formulation more toxic for aquatic organisms. Riley and Finlayson (2004) observed that the LC_{50};96 h for *H. transpacificus* was 5.5 and 3.9 mg L^{-1} for *P. promelas*, after the exposure to Rodeo® formulation with the addition of R-11® surfactant, while the LC_{50} of the commercial formulation without the surfactant was 270.0 and 1132.0 mg L^{-1} for the fish, respectively, showing the connection between the surfactant presence and glyphosate toxicity.

Based on the classification by Zucker (1985) and Giesy et al. (2000), the glyphosate and the associations with 0.5 and 1.0% surfactant (recommended doses for herbicide application) can be considered practically non-toxic and the alkyl phenolpolyglycol ether is classified as moderately nontoxic (1>LC_{50}<10 mg L^{-1}).

Glyphosate histopathological effects for fish

Gills

The pacu (*P. mesopotamicus*) gills consist of four branchial arches, supported by two rows of primary lamellae, which are covered with stratified epithelium. The secondary lamellae consist of epithelial, lining, pillar, chloride, and mucous cells (Figure 1A), as described by Severi et al., (2000). The observed histological changes are described below according to each concentration (Figure 1), on Table 4.

The epithelium increase and the blood congestion at the secondary lamellae also occurred on *O. mykiss* exposed to methiocarb insecticide (Altonok et al., 2006) and on *Oreochromis niloticus* exposed to diquat herbicide (Henares et al., 2008). The epithelium increase works as a barrier to decrease the xenobiotics absorption (Mallatt, 1985), similar as described for *Cyprinus carpio* exposed to GFT (Neskovic et al., 1996).

The presence of sub epithelial edema and the disarrangement of the secondary lamellae was reported for *C. carpio* (Neskovic et al., 1996) and for *O. niloticus* exposed to GFT (Jiraungkoorskul et al., 2002). The blood

Table 4. Histological alterations on fish gills after glyphosate exposure.

Concentration (mg L^{-1})	*P. mesopotamicus*	*P. caudimaculatus*	*H. eques*
0.0	0	0	0
900.0	0	0	0
925.0	1	1 and 7	3, 4 and 7
9_{50}.0	1	1 and 7	5 and 6
975.0	2	-	5 and 6

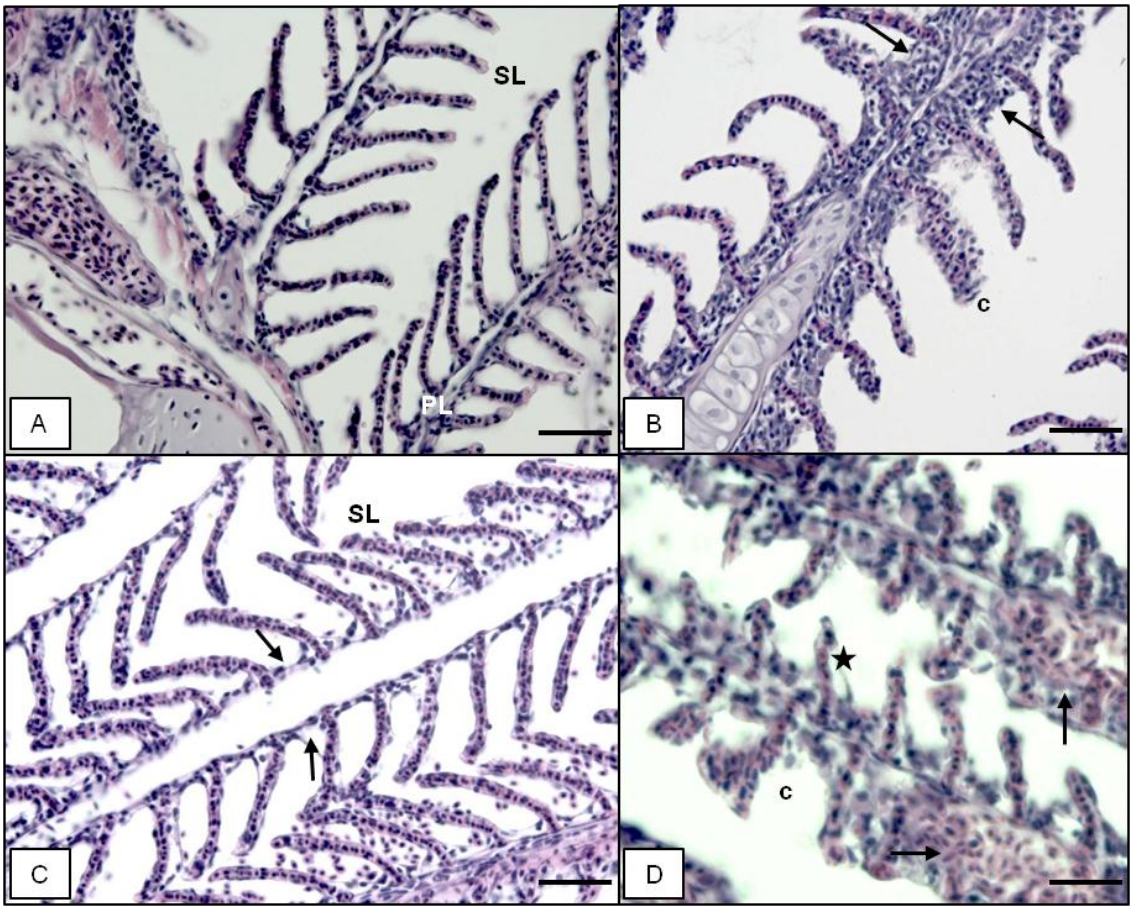

Figure 1. Gills photomicrography. A. Primary and secondary lamellae (PL and SL) from *P. mesopotamicus* with no exposure (control). B. Hypertrophy and hiperplasia of the primary lamellae (arrow) and congestion in the secondary lamellae (c) (925.0 mg L^{-1}) on *H. eques*. C. Decrease of the interlamellar epithelium height (arrow) (975.0 mg L^{-1}) on H. eques. D. Sub epithelial edema (star) and hypertrophy and hyperplasia of primary and secondary lamellae (arrow) (925 mg L^{-1}) on *P. caudimaculatus*. Bar =12.5 µm. Color HE.

congestion caused by the blood flow increase leads to the sub epithelial edema and the lamellae disarrangement. According to Shiogiri et al. (2012), *P. mesopotamicus* exposed under acute conditions to Roundup Ready® also presented pillar cells hyperplasia and lamellar epithelium hypertrophy. Although, such alterations are reversible (Henares et al., 2008; Shiogiri et al., 2012) and after some days of exposure, the tissue can remake its common histomorphology, different from paraquat herbicide, which caused cells disarrangement, uncommon regeneration of the epithelial cells and deformation of the branchial cartilage cells from *Colossoma macropomum*, exposed to 10.0 mg L^{-1} (Salazar-Lugo et al., 2011).

H. eques was the most sensitive bioindicator to GFT, with several histological changes at the gills. Despite the changes not being specific to the GFT toxicity, this kind of biomarkers indicates the presence of xenobiotics on the

Table 5. Histological alterations on the fish liver after glyphosate exposure.

Concentration (mg.L^{-1})	*P. mesopotamicus*	*P. caudimaculatus*	*H. eques*
0.0	0	0	0
900	0 and 1	2 and 4	2, 3, and 5
925	2	4 and 6	2 and 2
9_{50}	2, 3 and 4	4 and 6	2 and 3
975	2, 3 and 4	4 and 6	2 and 3

environment. This fact is important to environmental monitoring and identification of contaminants on the aquatic environment.

P. caudimaculatus displayed branchial structure similar as observed for *P. mesopotamicus* and *H. eques* on the control and under 900.0 mg L^{-1} exposure. The sub epithelial edema on the secondary lamellae and the increase of the interlamellar epithelium was similar to a report with *O. niloticus* exposed to 36.0 mg L^{-1} glyphosate as the Roundup® formulation (Jiraungkoorskul et al., 2002).

Liver

The evaluated fish liver (Control) showed cordonal organization of the hepatocytes in direct contact with the capillary sinusoids. The hepatocytes showed a hexagonal shape, with central nucleus, basophil, decondensed chromatin, a visible nucleolus and rosy cytoplasm, indicating high acidophilia (Figure 2A).

The main histological changes occurred after acute exposition were as follows: capillary sinusoids congestion (hyperemia), hypertrophy and fusion of the hepatocytes and cordonal structure disarrangement of the hepatocytes, with 900.0 and 925.0 mg L^{-1} exposure (Table 5), followed by tissue necrosis with 925.0 to 975.0 mg L^{-1} exposure for *P. caudimaculatus* (Table 5) (Figure 2C, D, E, F).

The cellular nucleus displacement to the periphery and the hepatocytes hypertrophy was also observed for *O. niloticus* exposed to GFT (Jiraungkoorskul et al., 2002). The displacement indicates the increase of the organelles and of the enzymes which are responsible for the xenobiotics metabolism, whereas the blood congestion may be a signal of the pressure decrease of the hepatic circulation, similar to *O. mykiss* exposed to 5.0 mg L^{-1} GFT (Topal et al., 2015). The cordonal disarrangement also occurred with *Spaurus aurata* exposed to imazapyr, terbutrin and triasulfuron (Arufe et al., 2004) and with *Trichogaster trichopterus* exposed to paraquat (Banaee et al., 2013). According to Shiogiri et al. (2012), the *P. mesopotamicus* liver showed hepatocytes vacuolization and cells hypertrophy when exposed to 3.0 or 4.0 mg L^{-1} glyphosate as Roundup Ready®. The vacuolization may work as storage of compounds which are difficult to be metabolized.

The liver, used as GFT exposure biomarker, was the tissue which exhibited the highest histological index, reaching tissue necrosis during *P. caudimaculatus* exposure to Rodeo®. This alteration was also described by Ayoola (2008) on *C. gariepinus*, same for the fibrosis which occurred on *O. mykiss* exposed in a chronic manner to 5.0 or 10.0 mg L^{-1} GFT (Topal et al., 2015). The necrosis probably occurred due to an excessive work performed by the hepatocytes, in attempt to eliminate the herbicide during the detoxification process.

Kidneys

The fish kidneys are formed by a renal corpuscle containing the glomerulus constituted by capillaries and the Bowman's capsule. Around the renal corpuscle are also found the hematopoietic tissue with basophilic cells, proximal and distal tubules, as observed on the control for all studied species on this research (Figure 3A).

The alterations were the shrinkage of the Bowman's capsule and disarrangement of the proximal tubules epithelium in all assessed concentration for *P. caudimaculatus* and *H. eques*, whereas did not occurred alterations of the tissue structure on *P. mesopotamicus* in any tested concentration (Table 6 and Figure 3B).

The glomerulus capsule release, the disarrangement of the proximal tubules epithelium and the proximal and distal tubule light increase was also observed on *O. mykiss* exposed to linuron herbicide (Oulmi et al., 1995) and on *O. niloticus* exposed to Roundup® (Jiraungkoorskul et al., 2002). The Bowman's capsule space increase, or the capsule release, and the disarrangement of the tubules cells was also observed on *C. gariepinus* exposed to GFT, which leads to the kidneys physiological functions loss (Ayoola, 2008).

Conclusions

The fish species *H. eques, P. caudimaculatus* and *P. mesopotamicus* present great potential to be used as standard organisms for ecotoxicological assays for herbicides monitoring, for they present an excellent answer to the tested reference substance. The addition of

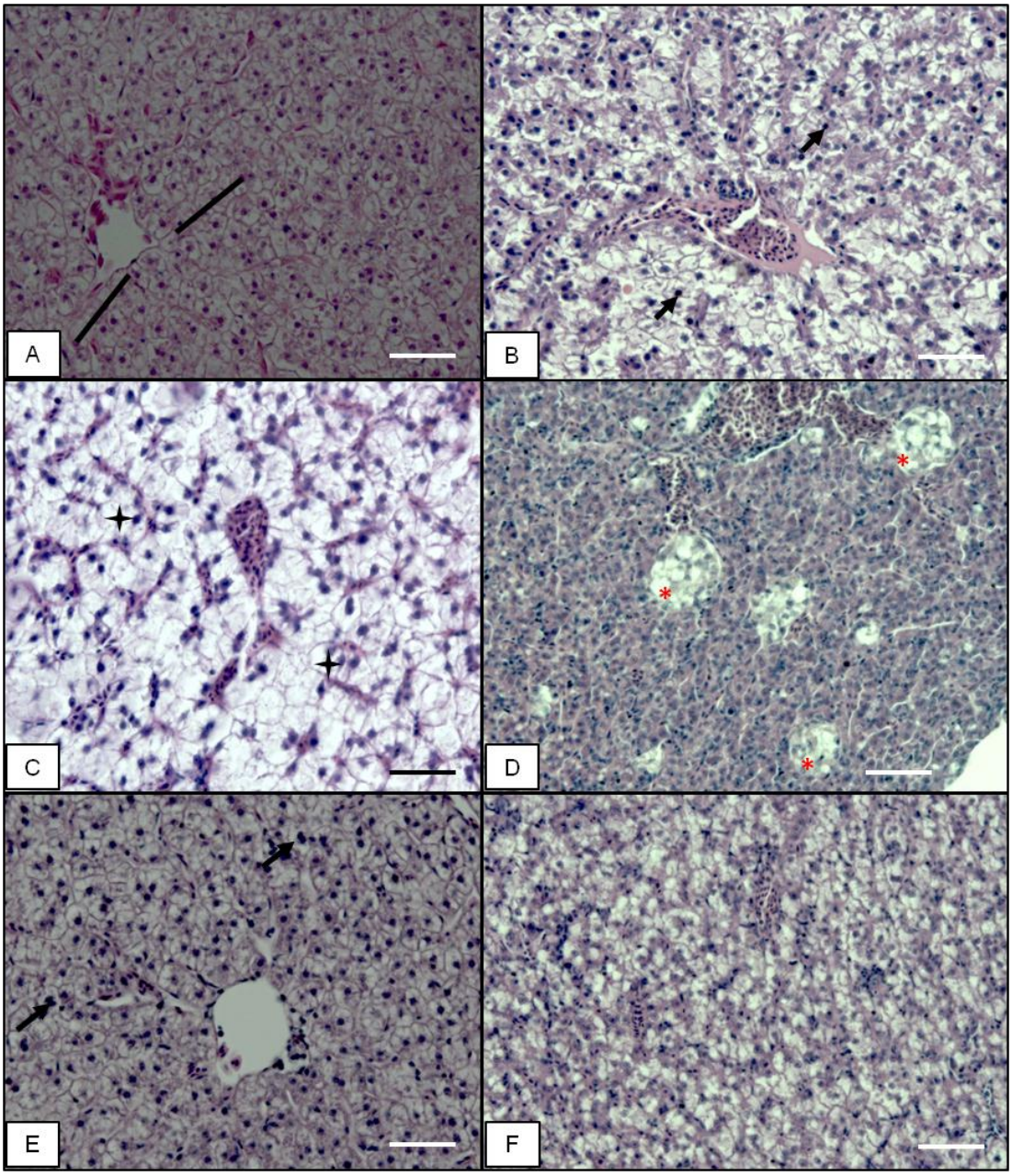

Figure 2. A. Cordonal organization of the hepatocytes (line) from *H. eques* (control). B. Picnotic nucleus (Arrow) (900.0 mg L^{-1}) on *H. eques*. C. Hepatocyte hypertrophy (star) (925.0 mg L-*1*) on *H. eques*. D. Necrosis spots (asterisk) (975.0 mg L^{-1}) on *P. caudimaculatus*. E. Cell fusion (arrow) (950.0 mg L^{-1}) on *P. mesopotamicus*. F. Disarrangement of the hepatocytes cordonal structure (900 mg L^{-1}) on *P. caudimaculatus*. Bar = 12.5 µm. Color HE.

Table 6. Histological alterations on fish kidneys after glyphosate exposure.

Concentration (mg.L^{-1})	*P. mesopotamicus*	*P. caudimaculatus*	*H. eques*
0.0	0	0	0
900	0	1, 2 and 3	1, 2 and 3
925	0	1, 2 and 3	1, 2 and 3
9_{50}	0	1, 2 and 3	1, 2 and 3
975	0	1, 2 and 3	1, 3 and 3

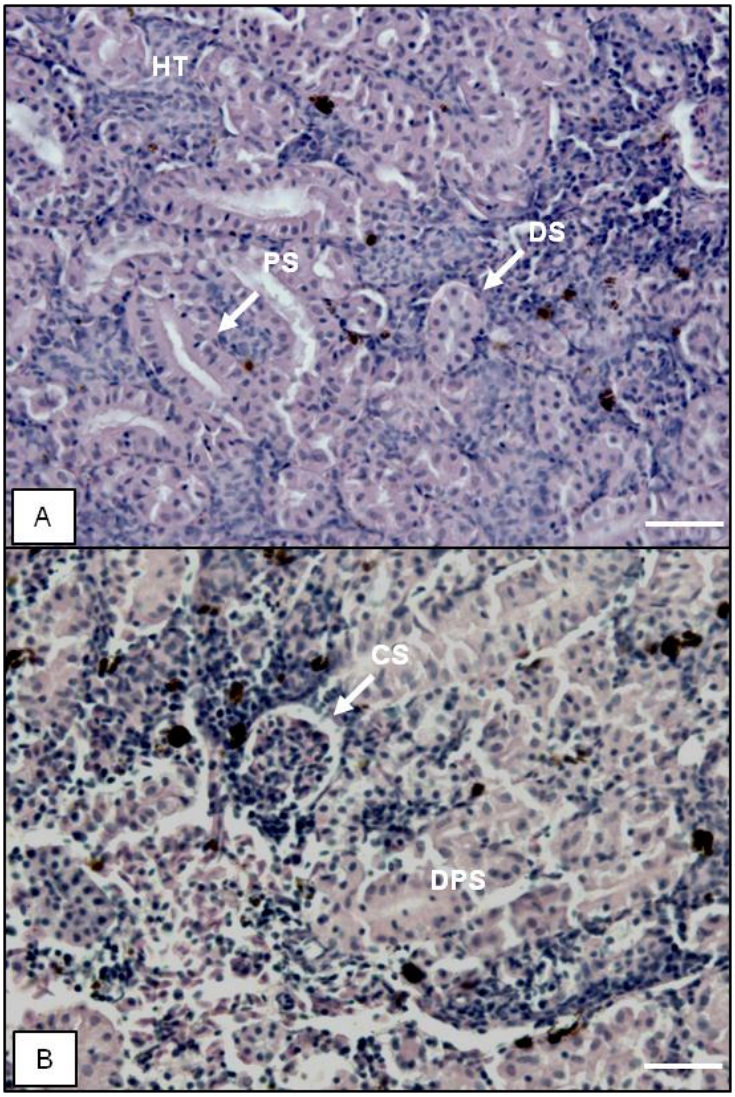

Figure 3. Kidneys photomicrography. A. Proximal segment (ps), distal segment (ds) (control), hematopoietic tissue (HT) on *P. mesopotamicus.* B. Shrinkage of the Bowman's capsule (cs) and disarrangement of the epithelium proximal segment (dps) on 975 mg L^{-1} on *H. eques*, Bar: 12.5 µm. Color HE.

surfactants to glyphosate formulations may change the herbicide acute toxicity pattern.

The use of glyphosate without surfactant addition is enough to cause histological alterations on *H. eques* and *P. caudimaculatus*, featuring specially the kidneys and liver as the xenobiotics presence biomarkers, which makes them possible to be applied on environmental monitoring studies.

Conflict of interests

The authors have not declared any conflict of interests.

REFERENCES

ABNT (2011). Brazilian Association of Techicnal Standards.NBR 12713. Aquatic Ecotoxicology. Fish Sao Paulo, Brazil. 19pp.

Arufe MI, Arellano J, Moreno MJ, Sarasquete C (2004). Toxicity of a commercial herbicide containing terbutryn and triasulfuron to seabream (*Sparusaurata* L.) larvae: A comparison with the microtextest. Ecotoxicol. Environ. Saf. 59(2):209-216.

Ayoola SO (2008). Histopathological effects of Glyphosate on Juvenile African Catfish (*Clariasgariepinus*). Am. Euras. J. Agric. Environ. Sci. 4(3):362-367.

Altonok I, Capkin E, Karahan S, Boran M (2006). Effects of water quality in fish size on toxicity of methiocarb: A carbamate pesticide, to rainbow trout. Environ. Toxicol. Pharmacol. 22(1):20-26.

Amarante Jr OP, Santos TCR, Brito NM, Ribeiro ML (2002). Glyphosate: Properties, toxicities, uses and legislation. New Chem. 25(4):589-593.

Banaee M, Davoodi MH, Zoheiri F (2013). Histopathological changes induced by paraquat on some tissues of Gourami fish (*Trichogaster trichopterus*). Open Vet. J. 3(1):36-42.

Behmer AO,Tolosa EMC, Feritas-Neto AG (1976). Manual of techniques for normal and pathological histology.1ª Edition, Edart, Editora da USP, Sao Paulo. P 239.

Beyers DW (1995). Acute toxicity of Rodeo® herbicide to Rio Grande silvery minnow as estimated by surrogate species: Plains minnow and fathead minnow. Arch. Environ. Contam. Toxicol. 29:24-26.

Botelho RG, Santos JB, Oliveira TA, Braga RR, Byrro ECM, (2009). Acute toxicity of herbicides to Tilapia (*Oreochromis niloticus*). Weed Plant 27(3):621-626.

Brodeur JC, Poliserpi MB, D'Andrea MF, Sánchez M (2014). Synergy between glyphosate- and cypermethrin-based pesticides during acute exposures in tadpoles of the common South American Toad *Rhinella arenarum*. Chemosphere 112:70-76.

Castro MP, Moraes FR, Fujimoto RY, Cruz C, Andrade Belo MA, Moraes JRE (2014). Acute Toxicity by Water Containing Hexavalent or Trivalent Chromium in Native Brazilian Fish, *Piaractus mesopotamicus*: Anatomopathological Alterations and Mortality. Bull Environ. Contam.Toxicol. 92:213-219.

Carraschi SP, Cubo P, Schiavetti BL, Shiogiri NS, Cruz C, Pitelli RA (2011). Efeitos tóxicos de surfactantes fitossanitários para o peixe mato grosso (*Hyphessobryconeques*). Acta Sci. Biol. Sci. 33(2):191-196.

Carraschi S, Luna L, Neto A, Gírio A, Cruz C, Pitelli R, (2012). Ecotoxicity of agricultural surfactants for guaru *Phalloceros caudimaculatus*. Ecotoxicol. Environ. Contam. 7(1):27-32.

Cruz C, Cubo P, Gomes GR, Venturini FP, Guilherme PE,Pitelli RA (2008). Sensibilidade de peixes neotropicais ao dicromato de potássio. J. Bra. Soc. Ecotoxicol. 3(1):53-55.

Curran CA, Grassley JM, Grue CE (2004). Toxicity of R-11® surfactant to juvenile rainbow trout: Does size matter? Bull. Environ. Contam. Toxicol. 72:401-404.

Giesy JP, Dobson S, Solomon KR (2000). Ecotoxicological risk assessment for Roundup®herbicide. Rev. Environ. Contam. Toxicol. 167:35-120.

Fujimoto RY, Gabbay MI, Anjos ECS, Carraschi SP, Cruz C (2012). Toxicidade e risco ambiental da oxitetraciclina e efeito em leucócitos de mato grosso (*Hyphessobryconeques*). Ecotoxicol. Environ. Contam. 7(2):11-15.

Glusczak L, Miron DS, Crestani M, Fonseca MB, Pedron FA, Duarte MF, VieiraVLP (2006). Effect of glyphosate herbicide on acetylcholinesterase activity and embolic and hematological parameters in piava (*Leporinusobtusidens*). Ecotoxicol. Environ. Saf. 65(2):237-241.

Hamilton MA, Russo RC, Thurston V (1977). Trimmed Spearman-Karber method for estimating medial lethal concentrations in toxicoty bioassays. Environ. Sci. Technol. 7:714-719.

Henares MNP, Cruz C, Gomes GR, Pitelli RA, Machado MRF (2008). Acute toxicity and histopathological effects of herbicide diquat in gill and liver of tilapianile (*Oreochromisniloticus*). Acta Sci. Biol. Sci. 30:77-81.

Jiraungkoorskul W, Upatham ES, Kruatrachue M, Sahaphong S, Vichasri-Grams S, Pokethitiyook P (2002). Histopathological effects of roundup, a glyphosate herbicide, on nile tilapia (*Oreochromisniloticus*). Sci. Asia 28:121-127.

Langiano VC, Martinez CBR (2008).Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochiloduslineatus.* Comp. Biochem. Physiol. C. 147:222-231.

Mallatt J (1985). Fish gill structural changes induced by toxicants and other irritants: A statistical review. Can. J. Fish Aquat. Sci. 42(4):630-648.

Mitchell DG, Chapman PM, Long TJ (1987). Acute toxicity of Roundup® and Rodeo® herbicides to rainbow trout, Chinook and coho salmon. Bull. Environ. Contam. Toxicol. 39:1028-1035.

Navarro CD, Martinez CB (2014). Effects of the surfactant polyoxyethylene amine (POEA) on genotoxic, biochemical and physiological parameters of the freshwater teleost *Prochilodus lineatus*. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 165:83-90.

Neskovic NK, Poleksic V, Elezovic I, Karan V, Budimir M (1996). Biochemical and histopathological effects of glyphosate on carp, *Cyprinus carpio* L. Bull. Environ. Contam. Toxicol. 56:295-302.

Oulmi Y, Negele RD, Braunbeck T (1995). Cytopathology of liver and kidney in rainbow trout (*Oncorhynchus mykiss*) after long-term exposure to sublethal concentrations of linuron. Dis. Aquat. Org. 21:35-52.

Riley F, Finlayson S (2004). Acute toxicities of herbicides used to control water hyacinth and Brazilian *Elodea* on larval delta smelt and sacramentosplittail. Office of Spill Prevention and Response 9pp.

Salazar-Lugo R, Mata C, Oliveros A, Rojas LM, Lemus M, Villarroel ER (2011). Histopathological changes in gill, liver and kidney of neotropical fish *Colossoma macropomum* exposed to paraquat at different temperatures. Environ. Toxicol. Pharm. 31:490-495.

Severi W, Rantin FT, Fernandes MN (2000). Structural and morphological features of *Piaractus mesopotamicus* (Holmberg, 1887) gills. Rev. Braz. Biol. 60(3):493-501.

Schmitt-Jansen M, Veit U, Dudel G, Altenburger R. (2008). An Ecological perspective in aquatic ecotoxicology: Approaches and challenges. Basic Appl. Ecol. 9:337-345.

Shiogiri NS, Carraschi SP, Cubo P, Schiavetti BL, Cruz C, Pitelli RA (2010). Ecotoxicity of glyphosate aterbane®br surfactant on guaru (*Phallocerus caudimaculatus*). Acta Sci. Biol. Sci. 32(3):285-289.

Shiogiri NS, Paulino MG, Carraschi SP, Baraldi FG, Cruz C, Fernandes MN (2012). Acute exposure of a glyphosate-based herbicide affects the gills and liver of the Neotropical fish, *Piaractus mesopotamicus.* Environ. Toxicol. Pharmacol. 34:388-396.

Sinhorin VDG, Sinhorin AP, Santos Teixeira JM, Miléski KML, Hansen PC, Moreira, PSA, Kawashita NH, Baviera AM, Loro VL (2014). Effects of the acute exposition to glyphosate-based herbicide on oxidative stress parameters and antioxidant responses in a hybrid Amazon fish surubim (*Pseudoplatystoma* sp). Ecotoxicol Environ. Saf. 106:181-187.

Topal A, Atamanalp M, Uçar A, Oruç E, Kocaman EM, Sulukan E, Akdemir F, Beydemir S, Kilinç N, Erdogan O, Ceyhum SB (2015). Effects of glyphosate on juveline rainbow trout (*Oncorhynchus mykiss*): Transcriptional and enzymatic alayses of antioxidant defence system, histopathological liver damage and swimming performance. Ecotoxicol. Environ. Saf. 111:206-214.

Tsui M, Chu L (2003). Aquatic toxicity of glyphosate-based formulations: Comparison between different organisms and the effects of environmental factors. Chemosphere 52:1189-1197.

USEPA - United States Environmental Protection Agency. (2001). Final report: Interlaboratory study of EPA short-term chronic and acute whole effluent toxicity test methods. v. 1. Office of Water, Washington, D.C. 20460EPA/821/B-01/004.

USEPA - United States Environmental Protection Agency. (2002). Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms.5ª ed. October. 275pp.

Versteeg DJ, Rawlings J, Bozso E, Shi J (2006). The acute and chronic of hexadecyl and heptadecyl sulfate to aquitic organisms. Arch. Environ. Contam. Toxicol. 51:43-53.

Wan MT, Watts RG, Moul DJ (1989). Effects of different dilution water types on the acute toxicity to juvenile pacific salmonids and and rainbow trout of glyphosate and formulated products. Bull. Environ. Contam. Toxicol. 4:378-385.

Zucker E (1985). Hazard evaluation division.Standard evaluation procedure: Acute toxicity test for freshwater fish.USEPA publication 540/9-85-006, Washington. 17pp.