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Toxic effects of the herbicide Roundup in the guppy *Poecilia vivipara* acclimated to fresh water

Cyntia Ayumi Yokota Harayashiki[a], Antonio Sergio Varela Junior[b], Anderson Abel de Souza Machado[c], Liziara da Costa Cabrera[d], Ednei Gilberto Primel[d], Adalto Bianchini[b], Carine Dahl Corcini[e,*]

[a] *Programa de Pós-Graduação em Ciências Fisiológicas – Fisiologia Animal Comparada, Universidade Federal do Rio Grande, Av. Itália km 8, 96203-900 Rio Grande, RS, Brazil*
[b] *Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Av. Itália km 8, 96203-900 Rio Grande, RS, Brazil*
[c] *Institute of Biological Sciences, Federal University of Rio Grande, Av Italy 8 km, 96203-900 9Rio Grande, RS, Brazil*
[d] *Escola de Química e Alimentos, Universidade Federal do Rio Grande, Av. Itália km 8, 96203-900 Rio Grande, RS, Brazil*
[e] *Departamento de Patologia Animal, Faculdade de Veterinária, Universidade Federal de Pelotas, Campus Universitário, Caixa Postal 354, 96001-970 Pelotas, RS, Brazil*

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ABSTRACT

Although it is believed that glyphosate-based herbicides are relatively nontoxic to humans, its broad use in agriculture and consequent contamination of aquatic systems is a concern. In the present study, reproductive (sperm quality) and biochemical parameters (acetylcholinesterase and glutathione S-transferase activity, lipoperoxidation, and antioxidant capacity against peroxyl radicals) were evaluated in adult guppies (*Poecilia vivipara*) acclimated to fresh water and exposed (96 h) to environmentally realistic concentrations of glyphosate (130 and 700 $\mu g\,L^{-1}$) as the commercial formulation Roundup. Male guppies exposed to Roundup showed a poorer sperm quality, measured as reduced plasmatic membrane integrity, mitochondrial functionality, DNA integrity, motility, motility period and concentration of spermatic cells, than those kept under control condition (no Roundup addition to the water). Most of the spermatic parameters analyzed showed strong association to each other, which may help to understand the mechanisms underlying the observed reduction in sperm quality. Exposure to Roundup did not alter the biochemical parameters analyzed, though differences between genders were observed and deserve further investigations. Findings from the present study suggest that exposure to environmentally relevant concentrations of Roundup may negatively affect at long-term the reproduction of *P. vivipara*, with consequent changes in fish populations inhabiting environments contaminated with the herbicide.

1. Introduction

Roundup is a commercial formulation of glyphosate presented as the isopropylamine salt added by surfactants, usually polyoxyethylene amine (POEA), and inert compounds (WHO, 1994). Despite the potential effects of the other chemicals present in the Roundup formulation, glyphosate itself is a broad-spectrum post-emergent, systemic and non-selective herbicide. These characteristics led to a fast increase in the use of this herbicide in both agricultural and non-agricultural areas around the world (WHO, 1994, 2005). The indiscriminate use of Roundup associated with careless handling, accidental spillage, or discharge of untreated effluents into natural waterways has caused harmful effects on aquatic life and may have contributed to long-term biological effects (Jiraungkoorskul et al., 2002).

In a recent review, concentrations of Roundup, measured as glyphosate acid equivalents, in natural water bodies were reported to range between 0.01 and 0.7 $mg\,L^{-1}$, reaching the maximum value of 1.7 $mg\,L^{-1}$ in extreme situations after direct application of the herbicide into the water (Guilherme et al., 2010). Although it is believed that glyphosate-based herbicides are relatively nontoxic to humans (WHO, 1994; Zouaoui et al., 2013), the contamination of aquatic systems associated with the wide and indiscriminate use of these chemicals is now of great ecotoxicological concern (Lushchak et al., 2009).

In light of the described above, the identification of appropriated biomonitors and biomarkers related to the toxic effects

* Corresponding author. Tel.: +55 5332759161.
E-mail addresses: cyayumi@uol.com.br (C.A.Y. Harayashiki), corcinicd@gmail.com (C.D. Corcini).

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of glyphosate-based herbicides is needed. In this context, fish species are described as suitable monitors of the effects of noxious compounds because of their ecological and economical relevance (Jiraungkoorskul et al., 2002). Additionally, changes at cellular and biochemical levels are among the most sensitive biological responses reported after fish exposure to aquatic pollutants (Glusczak et al., 2007; Sandrini et al., 2013). Therefore, studies focused on the biochemical and physiological effects of glyphosate-based herbicides in fish can provide not only useful toxicological information, but also help to select adequate biomonitors and biomarkers of glyphosate exposure and effect.

In Brazil, glyphosate has been used since 1978 (Galli and Montezuma, 2005). According to the National Health Surveillance Agency (ANVISA) (2010), since 2008 Brazil is the largest consumer of agrichemicals in the world. In fact, Silva et al. (2003) detected high concentrations of glyphosate in samples collected in water bodies near to areas of intense plantation in southern Brazil.

Although the intense use of glyphosate in Brazil, there is a lack of investigations using Brazilian native species to investigate the sublethal effects of this herbicide (Albinati et al., 2009).

With this background in mind, the sublethal effects of Roundup, a commercial formulation of glyphosate, were evaluated on a suite of physiological and biochemical parameters in the Brazilian guppy *Poecilia vivipara* (Bloch and Schneider, 1801). This fish species has been described as a promising biomonitor of aquatic pollution that has been employed recently in ecotoxicological studies with both inorganic and organic contaminants (Ferreira et al., 2012; Machado et al., 2013). Endpoints evaluated were selected based on their association with the short and long-term stability of fish populations as well as on the information they could provide to improve our knowledge on the mechanism involved in biological disturbances induced by glyphosate exposure and fish ability to counteract the effects and to detoxify the contaminant.

Considering the aspects above, the first endpoint evaluated in the present study was related to the response of sperm cell quality after fish exposure to sublethal concentrations of Roundup. In fact, reproduction is considered one of the most relevant biological functions related to long-term stability of fish populations. In this context, the effects of Roundup on several indicators of sperm quality, an early warning biomarker of reproductive disturbance, were evaluated. The potential impact of the herbicide exposure on fish behavior associated with change in neurotransmission function, measured through the brain and muscle acetylcholinesterase (AChE) activity, was also evaluated. Finally, some aspects related to the fish ability to detoxify the herbicide and its capacity to deal with the exposure to the contaminant were also considered. Therefore, the activity of glutathione S-transferase (GST), an enzyme involved in detoxification of organic contaminants, was analyzed as a measurement of detoxification ability. In turn, the total antioxidant capacity against peroxy-radicals (ACAP) and lipid peroxidation (LPO) were used as measurements of fish tissue capacity to protect against the oxidant effect of Roundup and the potential oxidative damage induced by exposure to the herbicide. The effects of Roundup on sperm quality and biochemical parameters (AChE, GST, ACAP and LPO) were evaluated in *P. vivipara* acclimated to fresh water and exposed to environmentally realistic concentrations of glyphosate (130 and 700 $\mu g\,L^{-1}$) as the commercial formulation Roundup.

2. Material and methods

2.1. Fish biology, collection and acclimation

P. vivipara is a guppy that belongs to the Poeciliidae family, being characterized as benthopelagic and non-migratory fish, behavior that allow its environmental exposure to several substances. It is euryhaline, being found in fresh water and estuarine environments along the coast of South America, from Venezuela to Argentina (Froese and Pauly, 2011). Indeed, it is one of the most common species of fish found in small ponds, rivers and coastal lagoon ecosystems of Brazil (Santos et al., 2011). For that reason, the National Institute of Science and Technology-Aquatic Toxicology (INCT-TA) recently has pointed *P. vivipara* as one of the priority species to access environmental health in Brazilian aquatic environments. Thus the findings of present work contribute simultaneously to better understand glyphosate effects on fishes as well as to establish standards biomonitors to South America.

Adults of *P. vivipara* were collected at the Gelo Creek (Cassino Beach, Rio Grande, RS, Southern Brazil) with nets and minnow traps. They were transferred to the animal care room of the Institute of Biological Sciences at the Federal University of Rio Grande (FURG) and acclimated for at least 7 days in continuously aerated and dechlorinated tap water. Room photoperiod (12L:12D) and temperature (28 °C) were fixed. Fish were daily fed with commercial food until apparent satiation. Feeding was stopped 24 h prior to the beginning of the experiments. Fish were fastening during the experimental period.

2.2. Fish exposure to Roundup

Due to the sexual dimorphism, 24 males [body length (mean ± standard deviation): 3.8 ± 1.2 cm; body weight: 0.54 ± 0.06 g; $n = 8$ fish per treatment] and 21 females (body length: 3.5 ± 0.9 cm; body weight: 0.41 ± 0.03 g; $n = 7$ fish per treatment) were individually kept under control condition (no Roundup addition into the water) or exposed (96 h) to Roundup (130 and 700 $\mu g\,L^{-1}$ of glyphosate). Both concentrations tested can be found in natural water bodies. According to Guilherme et al. (2010), the concentration of 700 $\mu g\,L^{-1}$ was the highest concentration detected in the environment. Considering that the lowest concentration found in natural water bodies (10 $\mu g\,L^{-1}$) was considered too low to induce a significant biological effect, an intermediary concentration (130 $\mu g\,L^{-1}$) potentially capable of inducing such effect, but considerably lower than the maximum concentration reported in the environment was tested. Other experimental conditions were kept as described above for the acclimation period (Section 2.1).

Every 24 h, exposure media were completely renewed. Before and after fish transfer to the experimental tank, water samples ($n = 24$) from control and treatments were collected, filtered (0.2 μm-mesh filter; Millipore, Merck; São Paulo, SP, Brazil), and stored at 4 °C in glass bottles until analysis.

After exposure, fish were euthanized by decapitation (AVMA, 2001) and tissue (brain, muscle, gills and liver) were dissected and stored at −80 °C for biochemical assays (Section 2.4). In males, testes were also dissected and immersed in Hanks balanced-salt solution (HBSS; 0.137 M NaCl, 5.4 mM KCl, 0.25 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$ and 4.2 mM $NaHCO_3$) for sperm analysis (Section 2.3).

The present work was approved by the Ethics Committee on Animal Use of Federal University of Rio Grande (CEUA – FURG; reference # P054/2011).

2.3. Sperm analysis

Testis samples were placed in 1.5 mL bullet tubes containing HBSS and shaken for the release of spermatozeugmatas (sperm bundles). Sperm was released by gently and repeatedly disrupting spermatozeugmatas with a 10-μL pipette tip (Sun et al., 2010). The sperm suspension was used for analyses described below.

For estimation of sperm motility and motility period, 10 μL of sperm suspension was placed on a glass microscope slide with a