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Effects of sub-lethal exposure of rats to the herbicide glyphosate in drinking water: Glutathione transferase enzyme activities, levels of reduced glutathione and lipid peroxidation in liver, kidneys and small intestine

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ABSTRACT

Glyphosate (GLP), the active ingredient of many weed killing formulations, is a broad spectrum herbicide compound. Wistar rats were exposed during 30 or 90 days to the highest level (0.7 mg/L) of GLP allowed in water for human consumption (US EPA, 2011) and a 10-fold higher concentration (7 mg/L). The low levels of exposure to the herbicide did not produce histomorphological changes. The production of TBARS was similar or tended to be lower compared to control animals not exposed to the herbicide. In rats exposed to GLP, increased levels of reduced glutathione (GSH) and enhanced glutathione peroxidase (GPx) activity may act as a protective mechanism against possible detrimental effects of the herbicide. Overall, this work showed certain biochemical modifications, even at 3–20-fold lower doses of GLP than the oral reference dose of 2 mg/kg/day (US EPA, 1993). The toxicological significance of these findings remains to be clarified.

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1. Introduction

Pesticides are economically important in agriculture and their use is relevant for increasing crop production worldwide. Crop protection using chemical compounds such as insecticides and herbicides has contributed significantly to increase productivity since 1950s (Beddington, 2010). However, the persistence of agricultural products in the environment may impose risk hazards to humans and animals (Dallegrave et al., 2007).

Glyphosate [N-(phosphonomethyl) glycine] (GLP), the active ingredient of Roundup® and many other weed killing formulations, is a broad spectrum herbicide compound. It is worldwide used for the protection of soybean, cotton and maize GLP-resistant crops (Dill, 2005). Its mode of action is related to the inhibition of an enzyme involved in the shikimate metabolic pathway, found only in plants and certain bacteria but not in animals. Therefore, GLP is considered a safe herbicide for non-target organisms living in the natural environment. Moreover, the low environmental impact of GLP is due to its relative rapid degradation into

Abbreviations: GLP, glyphosate; GSH, reduced glutathione; GST, glutathione-S-transferase; GPx, glutathione peroxidase; RNS, reactive nitrogen species; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substance.

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aminomethylphosphonic acid (AMPA) by soil microbes but also by its low mobility as consequence of a strong adsorption to soil particles (Vereecken, 2005; Borggaard and Gimsing, 2008; Cerdeira and Duke, 2010). Consequently, it has been shown that GLP does not bioaccumulate, biomagnify, or persist in a biologically available form in the environment (Solomon and Thompson, 2003). However, under certain circumstances, the herbicide compound may be leached into drainage/surface water and groundwater. For instance, heavy rain conditions or irrigation just after its application on soil surface, or the presence of phosphate in soil profiles may enhance GLP mobility into surface and groundwater (Vereecken, 2005; Borggaard and Gimsing, 2008; Peruzzo et al., 2008; Zhao et al., 2009; Candela et al., 2010). Hence, non target organisms may be exposed to the herbicide through the drinking water. In this regard, the highest level of GLP allowed in water for human consumption is 0.7 mg/L (US EPA, 2011). Interestingly, a study carried out within the nucleus area of soybean sowing in Argentina revealed concentrations of GLP in surface water between 0.1 and 0.7 mg/L (Peruzzo et al., 2008). Notwithstanding the available information on this issue, monitoring studies on the occurrence of GLP in surface and groundwater of sowed areas around the world are still limited (Vereecken, 2005; Borggaard and Gimsing, 2008).

Tissue damage due to excessive and unregulated generation of reactive oxygen and nitrogen species (ROS, RNS) has been shown after exposure to certain pesticides (Astiz et al., 2009a,b,c; El-Shenawy, 2009; Remor et al., 2009). Fortunately, organisms have evolved many protective mechanisms from these reactive intermediates. These defensive mechanisms include the activity of various enzymes such as superoxide dismutase, glutathione peroxidase (GPx), catalase, glutaredoxins, peroxiredoxins, and glutathione transferases (GSTs), as well as certain water- and lipid-soluble antioxidant substances such as reduced glutathione (GSH) and α-tocopherol that scavenge ROS and RNS. Under normal conditions, these antioxidant defense systems minimize cellular damage, but oxidative stress occurs when the generation of these reactive substances increases to an extent overcoming the capacity of cellular antioxidant systems. Most investigations on the ability of GLP to generate oxidative stress in non-target species were conducted in aquatic organisms. In this respect, it has been shown that low concentrations of GLP may cause oxidative stress in fish when the herbicide is used for the control of emergent aquatic weeds in wetlands and margins of water bodies (Slaninova et al., 2009; Lushchak et al., 2009). As far as mammalian species are considered, for example, increased levels of malondialdehyde (MDA) was observed in the liver of Albino rats treated during two weeks with sub-lethal doses of either GLP parent compound or its herbicide formulation Roundup® (El-Shenawy, 2009). A further work showed increased MDA levels in brain, kidney, liver and plasma in Wistar rats injected with low doses of GLP by the intraperitoneal route using a sub-chronic administration scheme (Astiz et al., 2009a). These authors also observed changes in antioxidant enzymatic functions, being a decrease in superoxide dismutase activities in brain and liver the most relevant findings in GLP-exposed animals. The present research is based on these previous findings and on the possible occurrence of this herbicide in surface and groundwater. We hypothesized that, even without neither macroscopic nor histological alterations on the target tissues studied, subchronic exposition to sublethal concentrations of GLP may induce changes on the antioxidant defensive mechanisms. In the current work, rats were exposed during 30 or 90 days to the highest level (0.7 mg/L) of GLP allowed in water for human consumption (US EPA, 2011) and a 10-fold higher concentration. The investigation was aimed to the study of GPx and GST enzymatic activities in cytosolic fractions obtained from liver, kidney and small intestine of rats exposed to GLP through the drinking water. Levels of GSH and MDA were also measured in subcellular fractions obtained from each tissue.

2. Materials and methods

2.1. Chemicals

Glyphosate, 1-chloro-2-4-dinitrobenzene (CDNB), 1,2-dichloro-4-nitrobencene (DCNB), ethacrynic acid, cumene hydroperoxide, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), glutathione reductase from yeast, L-glutathione reduced (GSH), malondialdehyde (MDA) and 2-thiobarbituric acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Buffer salts (KCl, $NaHCO_3$, Na_2HPO_4, NaH_2PO_4, K_2HPO_4 and KH_2PO_4) were purchased from Baker Inc. (Phillipsburg, USA).

2.2. Animals and treatments

Wistar rats weighting 265 ± 35 g were inspected prior to the experimental period and judged to be healthy by a licensed veterinarian. They were maintained under temperature controlled conditions (25 °C), and a normal photoperiod of 12 h of darkness and 12 h of light. Animals were randomly divided into 5 groups of 4 (four) male and 4 (four) female rats each other. The first group was maintained untreated throughout all the experimental period and received water *ad libitum* without GLP (control group). The second and third groups received drinking water *ad libitum* with GLP at 0.7 mg/L during 30 and 90 days, respectively. The two groups remaining received water *ad libitum* with GLP at 7 mg/L for 30 and 90 days, respectively. Water consumption was monitored in each experimental group every day.

Animal procedures and management protocols were carried out according to the Animal Welfare Policy of the Faculty of Veterinary Medicine, Universidad Nacional del Centro de la Provincia de Buenos Aires, Tandil, Argentina (Academic Council Resolution 087/02, http://www.vet.unicen.edu.ar) and internationally accepted animal welfare guidelines (American Veterinary Medical Association, 2007). At the end of the experimental period, rats were starved overnight and then killed under slight ether anesthesia in agreement with these institutional and internationally accepted animal welfare guidelines.

2.3. Sample collection and preparation of subcellular fractions

After sacrifice, the abdomen was opened and the liver, kidneys and small intestine removed. Liver and kidneys were

rinsed with ice-cold KCl 1.15%, covered with aluminium foils and immediately frozen and stored in liquid N_2 during 1 or 2 days until used for preparation of subcellular fractions. The gut lumen was slowly flushed with ice-cold KCl 1.15% and the whole small intestine was stored in an aluminium foil, chilled in ice and transported to the laboratory. All subsequent operations were performed between 0 and 4 °C. Gut segments (10–15 cm) were everted, the mucosa was washed again with ice-cold KCl 1.15%, blotted dry and thereafter scraped using a microscope glass slide.

All tissue samples were weighted and placed into vessels filled with two volumes ice cold homogenization buffer (Tris 20 mM, KCl 150 mM, EDTA 1 mM, pH 7.5). Tissue homogenization was performed with a Potter–Elvenjem tool (4–6 passes). Homogenates were filtered through a hydrophilic gauze, centrifuged at 10,000 × *g* for 20 min, and the resulting supernatant at 100,000 × *g* for 65 min. Aliquots of supernatants (cytosolic fractions) were frozen in liquid N_2 and stored at −70 °C. Pellets (microsomal preparations) were suspended in Tris 10 mM (containing 1 mM of EDTA and 20% of glycerol), frozen in liquid N_2 and stored at −70 °C until used for incubation assays. An aliquot of each subcellular fraction was used to determine protein content using bovine serum albumin as a control standard (Lowry et al., 1951).

2.4. *Histopathological analysis*

Small (~25 mm^2) pieces of fresh hepatic, renal and intestinal tissues were fixed in formalin 10% immediately after removing from the abdominal cavity. After 24 h, tissue specimens were washed, dehydrated with ethanol and embedded in paraffin wax. Paraffin blocks were cross-sectioned (5 μm thickness) using a rotary microtome. Cross sections were rehydrated in distilled water, stained with hematoxylin and eosin (H-E), and then examined by light microscopy OLYMPUS B-40.

2.5. *Enzyme assays*

The activities of cytosolic total GST and of GST isoenzymes μ and π were monitored by a continuous spectrophotometric method (Habig et al., 1974) using the following substrates: 1-chloro, 2,4-dinitrobenzene (CDNB) (non specific substrate), 1,2-dichloro-4-nitrobenzene (DCNB) for GSTμ, and ethacrinic acid for GSTπ. Selenium-independent GPx activity, also referred to as GSTα, was measured using cumene hydroperoxide as substrate following the method described by Nebbia et al. (1993).

2.6. *Determination of GSH content and lipid peroxidation*

Reduced glutathione levels were estimated by the method of Ellman (1959) with slight modifications. Briefly, hepatic cytosolic fractions were diluted (1:10 v/v) with 5% trichloroacetic acid for protein precipitation and centrifuged (3000 × *g*) during 25 min at 4 °C. Then, 200 μL of the supernatant were combined with 25 μL of 1 mM DTNB in a 0.1 M potassium phosphate buffer (pH 8) to reach a final volume of 2500 μL. Under these conditions, DTNB is reduced by GSH and the thiolate anion production was measured at 412 nm. A standard curve was constructed with known GSH concentrations and results are expressed as nmol/mg of cytosolic protein. Lipid peroxidation was assessed in microsomal fractions by the thiobarbituric acid reactive substances (TBARS) method (Ohkawa et al., 1979) and results are expressed as nmol of MDA per mg of protein.

2.7. *Statistical analysis*

A total number of 8 animals were sampled in each experimental group (control and GLP-exposed) and data are expressed as mean ± standard deviation (±SD). A normality test was performed for testing if the data was sampled from populations that follow Gaussian distributions. This assumption was tested using the Kolmogorov and Smirnov method. Statistical analysis of data was performed by means of parametric (one-way) ANOVA. Where significant overall differences ($p < 0.05$) were observed, further analysis among experimental groups was performed using Tukey multiple-range test.

3. Results

The presence of GLP in the drinking water did not modify the pattern of water intake in rats exposed to the herbicide. Thus, control rats consumed 32.4 ± 2.73 mL of water per animal per day. Similarly, water intakes in groups exposed to GLP at 0.7 and 7 mg/L were 33.2 ± 6.4 and 35.4 ± 5.29 mL/animal/day, respectively. The estimated daily doses of GLP, based on animal's body weight and the total amount of water ingested, were 0.09 ± 0.02 mg/kg/day (GLP 0.7 mg/L) and 0.9 ± 0.13 mg/kg/day (GLP 7 mg/L).

The microscopical examination of H–E stained sections of liver, kidney and small intestine revealed the absence of histomorphological changes in these tissues after GLP exposure through the drinking water. A classical hexagonal arrangement of hepatocytes within hepatic lobules was observed in both control and GLP-exposed rats. No signals of cellular vacuolization, necrosis, or chromatin condensation were observed in hepatic histological preparations from animals receiving GLP (Fig. 1A). Both control and GLP-exposed rats showed no evidences of cellular damage in Bowman's capsules and arterioles, as well as in the cuboidal epithelium of renal tubules (Fig. 1B). Similarly, the microscopical observation of intestinal villi revealed no evidences of tissular damage in GLP-exposed rats (Fig. 1C).

Table 1 shows metabolic rates for the cytosolic GST-dependent conjugations of CDNB, DCNB, and ethacrinic acid in liver, kidney and intestinal mucosa from control and GLP-exposed rats. Compared to control animals, rats exposed to GLP at 7 mg/L for 90 days showed a 62% lower ($p < 0.01$) GST-dependent conjugation of CDNB in the kidney. No other changes in this metabolic activity were observed in liver, kidney and gut mucosa as consequence of GLP exposure. The hepatic conjugation of DCNB was higher after 90 days of GLP ingestion at both 0.7 mg/L (+96%, $p < 0.001$) and 7 mg/L (+58%, $p < 0.01$) compared to that observed in control rats. There were no statistically significant differences in the conjugation of ethacrinic acid in kidney and small intestine between control animals and those exposed to GLP, except for a 43% increase ($p < 0.001$) of this enzyme activity in the intestinal mucosa after

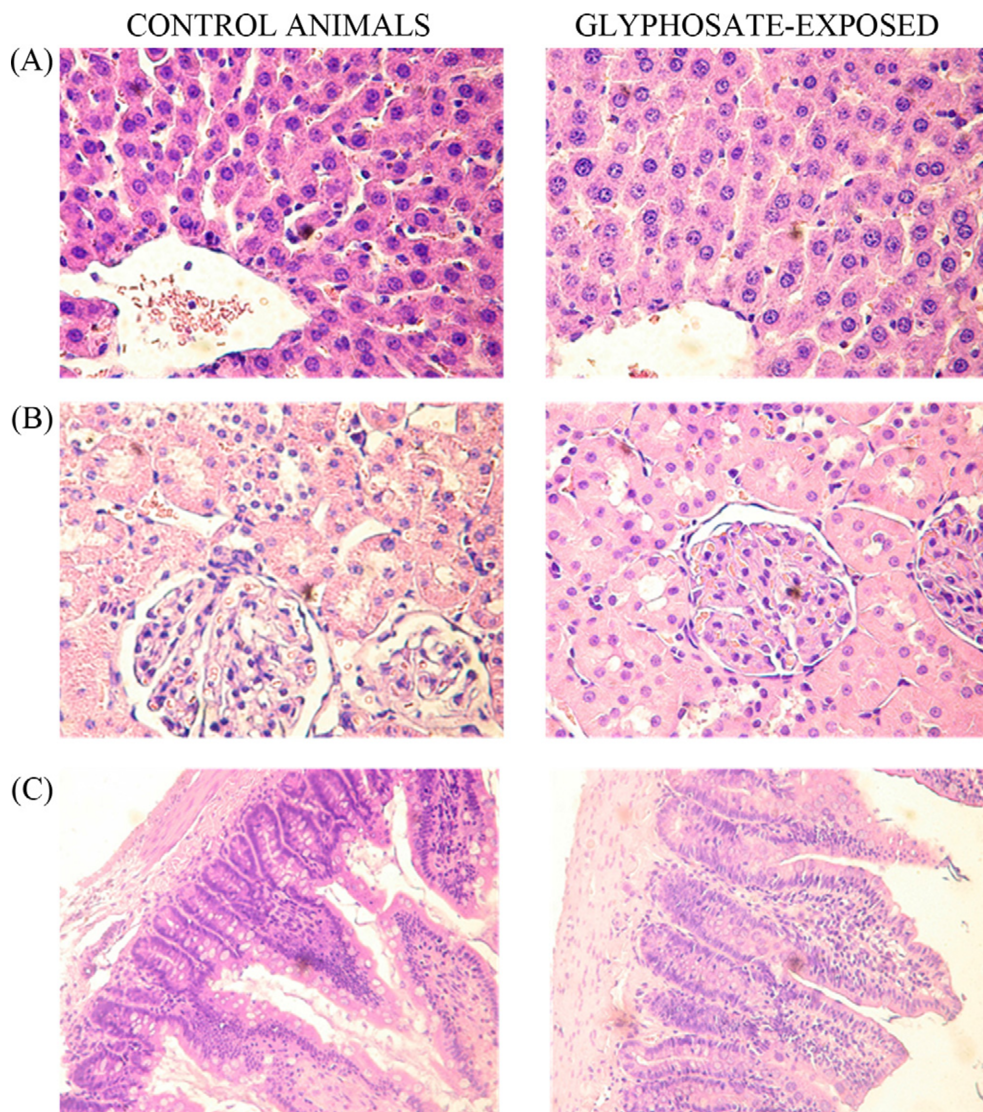

Fig. 1 – Histology of liver (A), kidney (B) and small intestine (C) sections obtained from control and glyphosate-exposed rats (for 90 d at 7 mg/L of drinking water). Samples were stained with Hematoxylin-Eosin and photomicrographs were taken using 400× (liver and kidney) and 200× (small intestine) magnifications.

Table 1 – Comparative cytosolic glutathione S-transferase (GST) activities measured with the substrates 1-chloro-2,4-dinitrobenzene (CDNB), 3,4-dichloronitrobenzene (DCNB), and ethacrinic acid in liver, kidney and small intestinal mucosa from control and glyphosate-exposed rats.

Tissue/substrate		Control group	Glyphosate 0.7 mg/L		Glyphosate 7 mg/L	
			30 days	90 days	30 days	90 days
Liver	CDNB	519 ± 67	524 ± 81	573 ± 61	543 ± 111	540 ± 118
	DCNB	12.0 ± 3.8	10.2 ± 1.7	23.6 ± 7.9***	10.8 ± 1.9	18.9 ± 5.2**
Kidney	CDNB	140 ± 97.0	92.2 ± 46.4	70.3 ± 13.2	93.8 ± 43.1	53.3 ± 39.1**
	Ethacrinic acid	52.0 ± 29.2	32.3 ± 9.8	34.3 ± 9.5	33.0 ± 10.9	35.3 ± 10.4
Small intestine	CDNB	167 ± 66	111 ± 23	154 ± 30	115 ± 39	158 ± 43
	Ethacrinic acid	21.2 ± 4.5	16.0 ± 4.2	17.8 ± 4.5	21.3 ± 3.9	30.3 ± 6.0***

Enzyme activities (mean ± SD) are expressed in nmol/min per mg of cytosolic protein.

** Values are significantly different ($p < 0.01$) than that observed in the control group.

*** Values are significantly different ($p < 0.001$) than that observed in the control group.

the administration of the herbicide at 7 mg/L in the drinking water during 90 days.

An increased GPx-dependent reduction of cumene hydroperoxide in liver, kidney and small intestinal mucosa was observed in rats exposed to GLP in the drinking water (Fig. 2). Compared to control animals, this enzyme activity increased in the liver (49%, $p < 0.05$), in kidneys (96%, $p < 0.001$) and in the small intestine (130%, $p < 0.001$) of rats receiving GLP at 7 mg/L during 90 days. Also, renal cytosolic fractions obtained from rats exposed to GLP at 7 mg/L (30 days) and 0.7 mg/L (90 days) showed 59% and 69% higher ($p < 0.05$) GPx-activities, respectively, in comparison to control animals. Additionally, in the small intestinal mucosa, a 97% higher ($p < 0.01$) GPx activity was only observed in rats receiving GLP at 0.7 mg/L during 90 days.

Exposure to GLP at both 0.7 mg/L and 7 mg/L in the drinking water during 30 and 90 days enhanced the hepatic GSH concentrations (Fig. 3). Table 2 shows TBARS levels in liver, kidney and small intestinal mucosa of control and GLP-exposed rats. A lower production of TBARS was observed in the liver of rats receiving GLP at 7 mg/L during 90 days, and also in kidneys in all experimental groups receiving the herbicide.

4. Discussion

In healthy aerobic organisms, the production of free radicals, as well as other ROS and RNS, is continuously regulated by the antioxidant defense system. Certain pesticides may increase the production of ROS/RNS and, therefore, induce oxidative stress in non target species. For instance, the administration of 2,4-dichlorophenoxyacetic acid (2,4-D) in the drinking water *ad libitum* during 25 days induced oxidative stress (Celik et al., 2006) and hepatotoxicity (Tayeb et al., 2010) in rats. Similarly, organophosphate pesticides such as chlorpyrifos, parathion and malathion, either alone or in mixture, caused oxidative stress in Wistar rats exposed to one fourth their LD50s during 1 or 2 days (Ojha and Srivastava, 2012). In addition, a mixture of pesticides (alachlor, captan, diazinon, endosulfan, maneb and mancozeb) normally ingested through the intake of fruits and vegetables, induced specific gender-linked variations in the level of hepatic metabolites involved in oxidative stress and in the regulation of glucose metabolism in mice (Merhi et al., 2010).

Because GLP is an organophosphate herbicide, some investigations reported on its ability to induce oxidative stress and/or an impairment of the antioxidant defensive mechanisms. Thus, increased hepatic TBARS production was observed in pregnant rats and their fetuses when the animals received a GLP formulation in the drinking water during the gestational period (Beuret et al., 2005). Furthermore, the intraperitoneal administration of GLP in rats, at 270 and 135 mg/kg every 2 days during one or two weeks, elevated serum enzyme activities of hepatic function, increased the lipid peroxidation and depleted GSH in the liver (El-Shenawy, 2009). In the same way, increased TBARS levels in brain, liver, kidney and plasma were observed in rats treated with GLP at 10 mg/kg 3 times a week during 5 weeks by the intraperitoneal route (Astiz et al., 2009a). These authors also showed, in rats injected with GLP, a significant increment in the

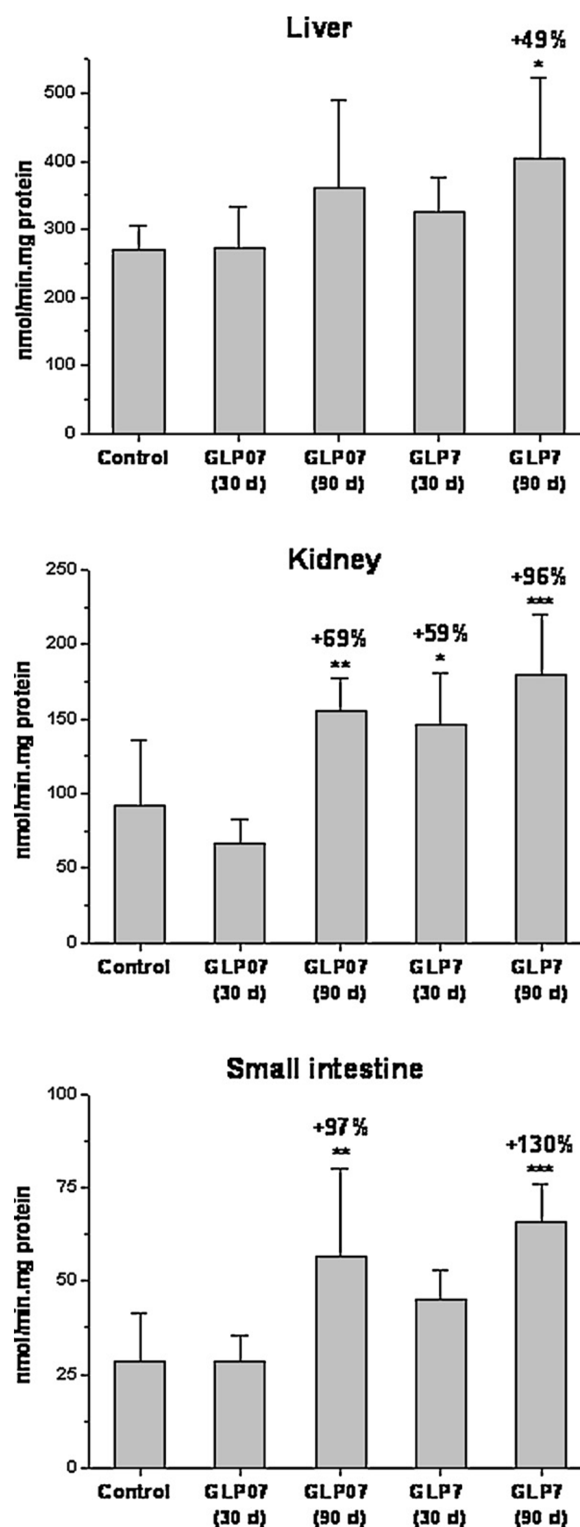

Fig. 2 – Glutathione peroxidase (GPx) activity in liver (A), kidney (B) and small intestinal mucosa (C) cytosolic fractions obtained from control and glyphosate-exposed rats. The herbicide was added to drinking water at 0.7 mg/L during 30 (GLP07 30d) and 90 (GLP07 90d) days and at 7 mg/L during the same time periods (GLP7 30d and GLP7 90d). Data (mean ± SD) are expressed as nmol of cumene hydroperoxide metabolized per min mg of cytosolic proteins. Significantly different (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$) from control values.

Table 2 – Concentrations of thiobarbituric acid reactive substances (TBARS) in liver, kidney and small intestinal mucosa from control and glyphosate-exposed rats.

Tissue	Control group	Glyphosate 0.7 mg/L		Glyphosate 7 mg/L	
		30 days	90 days	30 days	90 days
Liver	0.32 ± 0.26	0.48 ± 0.24	0.13 ± 0.06	0.17 ± 0.06	0.10 ± 0.05*
Kidney	0.82 ± 0.43	0.45 ± 0.28*	0.15 ± 0.01***	0.33 ± 0.07**	0.32 ± 0.07***,a
Small intestine	0.94 ± 0.59	0.71 ± 0.49	0.46 ± 0.14	0.61 ± 0.42	0.50 ± 0.13

Data (nmol of malondialdehyde per mg of microsomal protein) are expressed as mean ± SD.
[a] Significantly different ($p < 0.001$) compared to the value observed in the kidney of rats exposed to glyphosate 0.7 mg/L during 90 days.
* Value is significantly different ($p < 0.05$) than that observed in the control group.
** Value is significantly different ($p < 0.01$) than that observed in the control group.
*** Value is significantly different ($p < 0.001$) than that observed in the control group.

levels of end-metabolic products of nitric oxide and peroxinitrite anion (as biomarkers for RNS) in brain and plasma. Moreover, marked changes in these parameters of oxidative stress were observed when GLP was combined with other pesticides such as zineb and dimethoate (Astiz et al., 2009a). Overall, these previous investigations were carried out as an approach to a real situation in which non-target species could be exposed to sub-lethal doses of pesticides for longer periods. A similar approach, based on the possible occurrence of GLP in the drinking water, was employed in the current work. Indeed, different animal species, or even human beings, could be exposed to traces of GLP dissolved in surface or groundwater. We observed that the presence of GLP in the drinking water did not modify the pattern of water intake in rats exposed to the herbicide. In addition, the estimated daily dose of GLP in animals receiving 0.7 mg/L (the tolerance limit for the herbicide in water) was 0.09 mg/kg. Interestingly, the estimated daily dose was 10-fold higher (0.9 mg/kg) in rats receiving GLP at 7 mg/L. In addition, a poor bioavailability of GLP was observed after its oral administration in rats (Anadón et al., 2009). Therefore, a combination of reduced daily doses and poor bioavailability may indicate a much lower level of exposure to the herbicide in the current work.

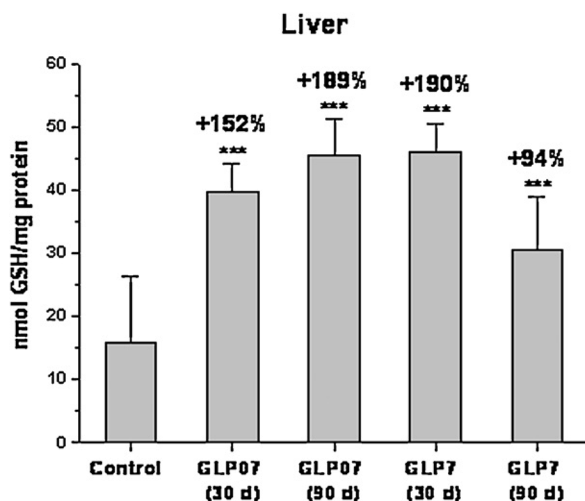

Fig. 3 – Levels of reduced glutathione (GSH) in liver cytosolic fractions obtained from control and glyphosate-exposed rats. The herbicide was added to drinking water at 0.7 mg/L during 30 (GLP07 30d) and 90 (GLP07 90d) days and at 7 mg/L during the same time periods (GLP7 30d and GLP7 90d). Data (mean ± SD) are expressed as nmol of GSH per mg of cytosolic proteins. Significantly different (: $p < 0.01$; ***: $p < 0.001$) from values measured in control cytosol.**

In contrast to the above mentioned investigations, our results show that GLP exposure through the drinking water did not produce marked modifications in lipid peroxidation levels. Moreover, the production of TBARS tended to be lower, rather than higher, in rats receiving the herbicide. On the other hand, tissue GSH is often depleted after a short period of oxidative stress, but elevated after long-term oxidant exposures (Slaninova et al., 2009). In the case of GLP, GSH tissue levels may depend on the administered dose, as well as on the extent of exposure to the herbicide. Therefore, GSH increased in plasma but not in brain when rats were treated with GLP alone or in combination with zineb or dimethoate (Astiz et al., 2009a). Conversely, a time-dependent depletion of GSH was observed in rat liver after administration of GLP during 2 weeks by the intraperitoneal route (El-Shenawy, 2009). Increased GSH synthesis, as an adaptive response during a moderate oxidative stress, has been reported in aquatic organisms (Slaninova et al., 2009) and this fact agrees with the increased GSH levels in the liver of rats exposed to GLP in the current work. It has been suggested that oxidative stress may trigger a compensatory mechanism through the induction of hepatic γ-glutamyl-cysteine synthetase, the enzyme that controls the biosynthesis of glutathione (Sharma et al., 2005; Dringen, 2005). This fact may have accounted for the increased GSH levels observed here and is in agreement with previous findings observed in the plasma of rats exposed to GLP (Astiz et al., 2009a).

The GSTs catalyse nucleophilic attack by GSH on a wide array of nonpolar compounds that contain an electrophilic carbon, nitrogen or sulfur atom such as plant phenols, mycotoxins (including aflatoxin B1), many chemical carcinogens, insecticides, herbicides, quinones as well as certain reactive oxygen species (Eaton and Bammler, 1999; Hayes et al., 2005). Four main classes of cytosolic GSTs (α, μ, π and θ) have been identified in humans, rats and mice, each of them containing different isoenzymes (Sherratt and Hayes, 2002). The regulation and function of GSTs have implications in cell growth, oxidative stress as well as disease progression and prevention (Raza, 2011). Marker substrates for cytosolic GSTs include CDNB (a general non specific substrate), DCNB, and ethacrinic acid (for GSTμ and GSTπ, respectively) (Hayes et al.,

2005; Gusson et al., 2006). On the other hand, GPxs constitute a family of isoenzymes that catalyse the reduction of H_2O_2 or organic hydroperoxides to water or their corresponding alcohols using reduced glutathione (GSH) as an electron donor and/or other reducing equivalents (Khün and Borchert, 2002; Margis et al., 2008). There are selenium- and non-selenium-containing (selenium-independent) GPxs; the later also referred to as GSTα catalyzing the reduction of cumene hydroperoxide (Nebbia et al., 1993). Generally, exposure to GLP and to other pesticides caused a reduction of GST-dependent activities in rats in concordance to GSH depletion (Sharma et al., 2005; El-Shenawy, 2009). Our results showed not relevant changes on GSTμ and GSTπ enzyme activities. Conversely, an enhanced metabolic activity of selenium-independent GPx (GSTα) was observed principally after 90 days of GLP exposure, and the most relevant increments were observed in kidneys and the small intestine (see Fig. 2). Elevated GSTs/GPx enzyme activities have been associated with tolerance of non-target species to deleterious effects caused by different insecticides and herbicides (Ranson et al., 2001; Edwards and Dixon, 2004; Hayes et al., 2005). This fact is in agreement with the increased GPx activities observed in the current work.

In conclusion, the current work was carried out in rats exposed to GLP in the drinking water. The low levels of exposure to the herbicide did not produce histomorphological changes in liver, kidneys nor in the small intestine. The production of TBARS in rats receiving GLP was similar or tended to be lower compared to control animals not exposed to the herbicide. In rats exposed to GLP, increased GSH levels and enhanced GPx activity may act as a protective mechanism against possible detrimental effects of the herbicide. It has been shown that GLP is a safe herbicide, and no evidences of adverse reproductive or developmental effects at realistic exposure concentrations were found in the available literature (Williams et al., 2012). However, our work showed certain biochemical modifications, even at 3–20-fold lower doses of GLP than the oral reference dose of 2 mg/kg/day (US EPA, 1993). The toxicological significance of these findings remains to be clarified.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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