Arch Toxicol (2010) 84:309–317
DOI 10.1007/s00204-009-0494-z

REPRODUCTIVE TOXICOLOGY

Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology

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Received: 1 July 2009 / Accepted: 30 November 2009 / Published online: 12 December 2009

Abstract Glyphosate is a herbicide widely used to kill weeds both in agricultural and non-agricultural landscapes. Its reproductive toxicity is related to the inhibition of a StAR protein and an aromatase enzyme, which causes an in vitro reduction in testosterone and estradiol synthesis. Studies in vivo about this herbicide effects in prepubertal Wistar rats reproductive development were not performed at this moment. Evaluations included the progression of puberty, body development, the hormonal production of testosterone, estradiol and corticosterone, and the morphology of the testis. Results showed that the herbicide (1) significantly changed the progression of puberty in a dose-dependent manner; (2) reduced the testosterone production, in semineferous tubules' morphology, decreased significantly the epithelium height ($P < 0.001$; control = 85.8 ± 2.8 μm; 5 mg/kg = 71.9 ± 5.3 μm; 50 mg/kg = 69.1 ± 1.7 μm; 250 mg/kg = 65.2 ± 1.3 μm) and increased the luminal diameter ($P < 0.01$; control = 94.0 ± 5.7 μm; 5 mg/kg = 116.6 ± 6.6 μm; 50 mg/kg = 114.3 ± 3.1 μm; 250 mg/kg = 130.3 ± 4.8 μm); (4) no difference in tubular diameter was observed; and (5) relative to the controls, no differences in serum corticosterone or estradiol levels were detected, but the concentrations of testosterone serum were lower in all treated groups ($P < 0.001$; control = 154.5 ± 12.9 ng/dL; 5 mg/kg = 108.6 ± 19.6 ng/dL; 50 mg/dL = 84.5 ± 12.2 ng/dL; 250 mg/kg = 76.9 ± 14.2 ng/dL). These results suggest that commercial formulation of glyphosate is a potent endocrine disruptor in vivo, causing disturbances in the reproductive development of rats when the exposure was performed during the puberty period.

Keywords Glyphosate · Roundup · Endocrine disruption · Prepubertal exposure · Testosterone · Testicular morphology

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Introduction

The agricultural chemical industry manufactures many different products every year (Solomon and Schettler 2000). In some of these products, substances are found that mimic hormones (endocrine disruptors), especially concerning estrogen activity and thyroid hormones (Solomon and Schettler 2000; Colborn et al. 1996; Scott 2005). Glyphosate is a herbicide used to control weeds in several agricultures, being very effective (Cerdeira et al. 2007; Hayes and Laws 1991). The development of genetically modified seeds tolerant of glyphosate allowed the product can be safely applied in any stage of growth of plant (Cerdeira et al. 2007). Its use still contemplates the control of pernicious water plants, and its effect over biodiversity is still questionable (Brausch and Smith 2007; Tsui and Chu 2003).

Glyphosate inhibits the development of plants by interfering with the production of essential aromatic amino acids and inhibiting the enzyme enolpyruvylshikimate phosphate synthase (EPSPS), which is responsible for the biosynthesis of chorismate, an intermediate in the biosynthesis of phenylalanine, tyrosine and tryptophan. This pathway to the biosynthesis of aromatic amino acids is not expressed by any member of the animal kingdom, making this mechanism of action exclusive to plants (Cerdeira et al. 2007; Williams et al. 2000).

The detection of pesticide residues in the home of agricultural workers (Curwin et al. 2005), and the presence of these residues and their metabolites in the urine of families living near country areas (Curwin et al. 2007) shows that there is a risk of environmental exposure, which makes it extremely important to ascertain the toxic effects of low doses applied over extended periods (Curwin et al. 2005, 2007). In addition, the presence of residues in food has also been identified (Cox and Surgan 2006).

Glyphosate in low non-toxic concentrations causes the disruption of the aromatase enzyme in human placental cells in vitro. From the moment that glyphosate penetrates the cells, as is facilitated with Roundup formulations with adjuvants (Monsanto Co.), it reduces the aromatase enzyme activity responsible for the synthesis of estrogens (Richard et al. 2005).

This effect over the aromatase enzyme has been demonstrated in vitro in cellular culture of the tumor Leydig MA-10 subjected to different concentrations of Roundup, in a procedure that also identified an expressive diminution of STAR protein expression (Steroidogenic Acute Regulatory Protein) (Walsh et al. 2000).

The reduction in the aromatase activity was also observed in cultures of embryonic human cells, in which there was a greater sensitivity than in placental cells. The disruption of aromatase was more intense in the presence of Roundup, but was also observed when using glyphosate without adjuvants. Cytotoxic effects were also detected and were elevated after long-term exposure in both dose- and time-dependent manners (Benachour et al. 2007).

However, studies about this herbicide exposure in rats from prepubertal until puberty period is completed were not performed at this moment. Thus, the main objective of this study was to evaluate the effects of exposure to commercial formulation of glyphosate (Roundup Transorb®, Monsanto) in a protocol previously developed and validated (Stoker et al. 2000; Parker 2006). The progression of puberty, body development, the testosterone, estradiol and corticosterone serum levels as well as the morphology of the testis using the germinative epithelium of the seminiferous tubules and the adrenal gland were performed.

Materials and methods

Chemicals

The product used was Roundup Transorb (Monsanto Co., St. Louis, MO; Monsanto of Brazil Ltda, São Paulo, Brazil), in a commercial formulation with a base of glyphosate; this formulation was composed of 480 g/L of glyphosate, 648 g/L of isopropylamine salt and 594 g/L of inert ingredients.

Animals

Sixty-eight newly weaned male Wistar rats were used, coming from female rats that were followed from the 17th day of pregnancy in order to determine the exact days of birth. On the fourth-day post-natal (PND 4), the litters were culled to eight pups per female and were kept at this proportion until weaning (PND21). During development, the pup rats were fed with a commercial balanced mixture for rats, with mineral water available ad libitum, and were kept under a photoperiod of a 12:12 hour dark/light cycle and in a controlled room temperature (23 ± 1°C). All the procedures were performed according to the ethical principles in animal research adopted by the bioethical Commission of Faculty of Veterinary Medicine and Zootechny of University of São Paulo (http://www.fmvz.usp.br).

Experimental design and treatment

The experimental design was composed of random blocks, with the formation factor of these blocks as the body weight at the PND23. All the animals were weighed, and the average and standard deviation were calculated. The animals having body weights lower or higher than two standard deviations from the average were removed from the experiment. Thereafter, the animals were randomly divided into four treatment groups.

The animals were submitted to experimental treatments from the PND23 until the PND53. The glyphosate-Roundup Transorb was diluted in a watery suspension and administered once a day, per os (gavage) in a volume of 0.25 mL/100 g of body weight, between 7 and 8 a.m. Based on the NOAEL (no observed adverse effect level) or NEL (no effect level) for glyphosate (50 mg/kg according to Lu 1995) and the study of Benedetti et al. (2004) that observed toxic effects on the liver at the doses from 4.87 mg/kg of glyphosate-Biocarb, we chose the doses of 5, 50 or 250 mg/kg of body weight of glyphosate-Roundup Transorb. The control group was treated in the same form but with deionized water.

Preputial separation

To determinate the puberty age, an evaluation of the balanopreputial separation was made, which consists of the separation of the preputial membrane and the externalization from the glands of the penis (Parker 2006). This method was performed from the PND33 and was completed once a day at the time of the balanopreputial separation, with gentle tissue manipulation. During this period, the animals were also weighed.

Organ weights

The testes and the adrenal glands were weighed in absolute values and then transformed to relative weights as mg/100 g of body weight at PND53.

Hormone measurements

The serum collected via cardiac puncture in 53-day-old animals between 07:30 and 08:30 a.m. was stored and freezed at −18°C for subsequent hormonal analysis. The serum dosages were accomplished by radioimmunoassay (RIA) from commercial kits (Testosterone Total Coat-A-Count, Estradiol Coat-A-Count and Coat-A-Count Corticosterone in rats, DPC, Los Angeles, CA, USA). All the samples were analyzed in duplicate. The coefficients of variation both intra- and inter-assay were <6% for testosterone, 3, 9% for estradiol, respectively, and <5% for corticosterone.

Histology and morphometry

The testes and the adrenal glands of all animals ($n = 68$) were fixed in Bouin's solution for 8 h, treated with alcohol, embedded in paraffin and prepared as stained laminas with hematoxylin and eosin. The laminas were observed initially with a magnification of 40×, for the general observation of the organ architecture. Next, a magnification of 100× was used for a more detailed analysis of the seminiferous tubules' architecture. This included analyzing a linear morphometry from the seminiferous tubules by determining the tubular diameter (measured from the basal lamina to the basal lamina in the opposite direction), seminiferous epithelium (from the basal lamina to the neck of the elongated spermatids) and luminal diameter. Ten fields per cut per animal were selected within histological cuts in the transverse direction of the tubules. For each tubule, the averages were calculated for the measurements indicated and, then, the average of each field was also calculated. The measurement for each animal was obtained through measure of all the analyzed fields. These measurements were performed using the software tpsDig2 version 2.10 (Available from http://life.bio.sunysb.edu/morph).

Statistical analysis

The variables under study were first submitted to tests of normality from Kolmogorov–Smirnov and homocedasticity by the test of Bartlet. When some of the premises of parametric testing were not obtained, non-parametric tests were chosen for subsequent averages and tests. Statistical differences were considered significant when the value of P was lower than 0.05. The values were expressed in mean (x) and standard error of the mean (±SEM). For all analyses, the software (Statistica 6) was used.

Data analysis of daily weights was performed through the two-way analysis of variance for repeated measures (MANOVA) by a general linear model (GLM). The weights were compared between different groups and different ages, considering the evolution expected by the body growth. The day and the weight of the complete balanoprepucial separation were compared among the groups using non-parametric analyses by the Kruskal–Wallis method followed by the post hoc Dun test. The testis and the adrenal weights were analyzed by the Kruskal–Wallis followed by the post hoc Dunn test, or by using a one-way analysis of variance (ANOVA) followed by the post hoc Tukey test. The testis measures of tubular diameter and epithelium depth, as well as the serum concentrations of testosterone, estradiol and corticosterone, were analyzed by the ANOVA followed by the Tukey test.

Results

Body weight

The evaluation of the daily weight from weaning to puberty was performed with the goal of checking possible effects in the development caused by the herbicide. There were no significant differences in the animals' weights among the groups studied, with different treatments of glyphosate-Roundup Transorb [$F(3, 65) = 0.4$; $P > 0.05$]. Only the expected variation relative to age was observed, showing that the body evolution was not affected by the concentrations used [$F(90, 1950) = 1.8$; $P < 0.001$], and its development was not jeopardized. Otherwise, there was a distinct unity in the development among the groups, with low variability from the averages.

Age and weight at preputial separation

The daily exposure to the herbicide glyphosate-Roundup Transorb caused a significant delay in the pubertal age [KW (3, 69) = 35.5 (corrected for ties); $P < 0.001$] (Fig. 1). On the other hand, relative to the controls, the body weight of the experimental rats was not significantly different [KW(3, 69) = 2.1; $P > 0.05$] (Fig. 2).

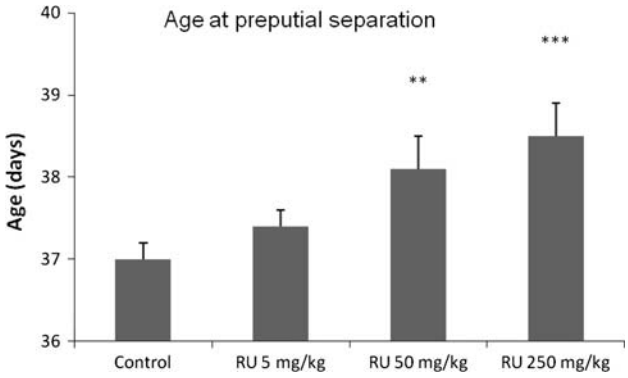

Fig. 1 Effects of the herbicide glyphosate-Roundup on age at preputial separation. Values are mean ± SEM, $n = 18$ (control), 17 (RU 5 mg/kg), 16 (RU 50 mg/kg), 18 (RU 250 mg/kg), *RU* Roundup, $^{**}P < 0.01$, $^{***}P < 0.001$

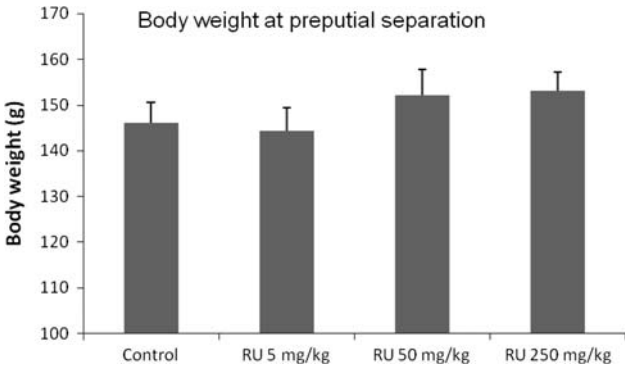

Fig. 2 Effects of the herbicide glyphosate-Roundup on weight at preputial separation. Values are mean ± SEM, $n = 18$ (control), 17 (RU 5 mg/kg), 16 (RU 50 mg/kg), 18 (RU 250 mg/kg), *RU* Roundup, $P > 0.05$

Testicular and adrenal weights

There were significant differences in the relative testicular weights analyzed between the control and 250 mg/kg groups [KW (3, 69) = 9.2; $P < 0.05$], such as for the one-sided left and right measurements, and for the average of the weight of both testis (Table 1).

The analysis of the average weights of the adrenals showed significant differences among the groups ($P < 0.05$), in the one-sided analyses and in the average. There were differences between the groups of 50 and 250 mg/kg for the right adrenal [KW (3, 69) = 9.2; $P < 0.05$], and between the groups of 0 and 250 mg/kg for the adrenal average [KW (3, 69) = 11.3; $P < 0.05$] and for the left adrenal [$F(3, 69) = 4.6$; $P < 0.01$].

Histology

The sections observed did not present pathologic alterations like degeneration, fibrosis or the accumulation of strange substances in the testicular tissue or still pathological cells, as determined by the results of the tissue processing technique (Fig. 3).

The histopathological study of the adrenal glands also did not show pathological alterations by the results of the tissue processing technique used, in spite of the macroscopic alterations observed.

Morphometry

The photomicrography study of the histological cuts from the testicular seminiferous tubules of the control and treated groups demonstrated that modifications occurred among them. A statistical analysis of the tubular epithelium demonstrated significant differences among the three treated groups and the control group [$F(3, 63) = 8.5$; $P < 0.001$], with the epithelium lengths (μm) reduced in the groups of 5, 50 and 250 mg/kg by 16, 20 and 24%, respectively, denoting a reduction in the amount of germ cells in these treated animals. Among the groups treated with different concentrations of glyphosate-Roundup Transorb, there were no significant differences ($P > 0.05$), indicating that the maximum effect for this variable could be seen at the lowest dosage of 5 mg/kg (Table 2; Fig. 3).

Table 1 Effects of the herbicide Roundup on testicular and adrenal weights

Groups	*n*	Right testis (mg/100 g BW)	Left testis (mg/100 g BW)	Testicular weight mean (mg/100 g BW)	Right adrenal (mg/100 g BW)	Left adrenal (mg/100 g BW)	Adrenal weight mean (mg/100 g BW)
Control	18	528.6 ± 12.4[a]	534.3 ± 13.5[a]	531.4 ± 12.8[a]	11.4 ± 0.6	11.1 ± 0.5[c]	11.3 ± 0.5[c]
RU 5 mg/kg	17	534.6 ± 12.8	542.4 ± 13.9	538.5 ± 13.2	12.9 ± 0.4	12.8 ± 0.6	12.8 ± 0.4
RU 50 mg/kg	18	548.6 ± 12.4	556.5 ± 13.5	552.6 ± 12.8	11.5 ± 0.4[a]	13.0 ± 0.5	12.3 ± 0.3
RU 250 mg/kg	16	578.4 ± 13.1[b]	582.2 ± 14.3[b]	580.3 ± 13.6[b]	15.2 ± 1.1[b]	14.0 ± 0.6[d]	14.6 ± 0.8[d]

The herbicide significantly increased the testicular and adrenal weights in animals treated at a dose of 250 mg/kg

Values are mean ± SEM, *RU* Roundup, *n* animals per group, *BW* body weight

[a, b] Differ in the column ($P < 0.05$)

[c, d] Differ in the column ($P < 0.01$)

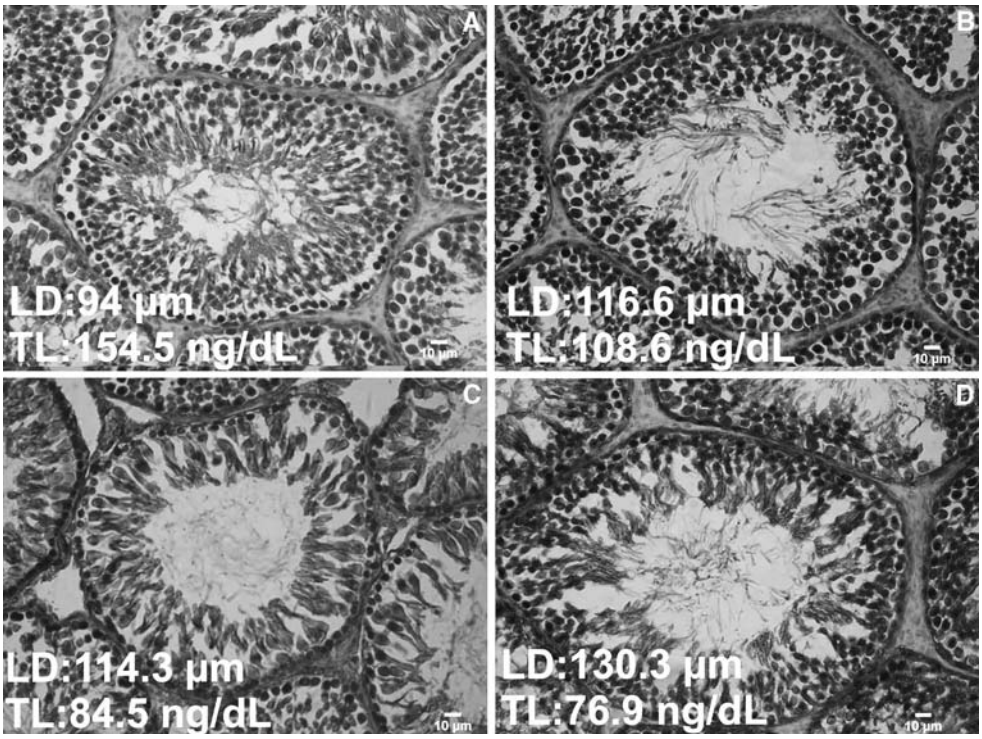

Fig. 3 Effects of the herbicide glyphosate-Roundup on testicular morphology of the control group (**a**) and treated groups at the doses of 5 mg/kg (**b**), 50 mg/kg (**c**) or 250 mg/kg (**d**). All seminiferous tubules of treated groups (**b**, **c**, **d**) presented increase in luminal diameter (LD) and reduction in seminiferous epithelium in relation to control group (**a**). The testosterone level (TL) is also shown in the picture. *RU* Roundup. *Scale bar* = 10 µm. Hematoxilin and eosin stain

Table 2 Effects of the herbicide Roundup on seminiferous epithelium, and luminal and tubular diameters

Groups	*n*	Epithelial height (µm)	Luminal diameter (µm)	Tubular diameter (µm)
Control	18	85.8 ± 2.8^{a}	94.0 ± 5.7^{a}	265.7 ± 4.8
RU 5 mg/kg	16	71.9 ± 5.3^{b}	116.6 ± 6.6^{b}	260.5 ± 7.8
RU 50 mg/kg	18	69.1 ± 1.7^{b}	$114.3 \pm 3.1^{b,c}$	252.6 ± 5.4
RU 250 mg/kg	15	65.2 ± 1.3^{b}	$130.3 \pm 4.8^{b,d}$	260.7 ± 4.9

The herbicide significantly decreased the seminiferous epithelium and significantly increased the luminal diameter in all treated groups

Values are mean ± SEM, *n* animals per group, *RU* Roundup

[a,b] Differ in the column ($P < 0.001$)

[c,d] Differ in the column ($P < 0.05$)

The diameters from the tubular lumen were also different, with higher values among the treated groups compared to control [$F(3, 63) = 8.3$; $P < 0.001$], by 24, 22 and 32% for dosages of 5, 50 and 250 mg/kg, respectively, related to the control of 0 mg/kg (Table 2; Fig. 3).

Besides the variations in the epithelium length and the tubular lumen, the total tubular diameter remained unchanged among the groups [$F(3, 63) = 0.9$; $P > 0.05$], indicating that the alterations occurred only with the reduction in germinal epithelium, while the total diameter remained unchanged (Table 2; Fig. 3).

Hormone levels

The concentrations of testosterone serum were significantly different between the control and treated groups [$F(3, 52) = 6.4$; $P < 0.001$]. The concentrations were reduced by 30, 45 and 50%, respectively, in the 5, 50 and 250 mg/kg treated groups, in relation to the control group (Table 3; Fig. 4). The relative analysis of corticosterone did not show significant differences among the control and treated groups [$F(3, 62) = 1.8$; $P > 0.05$] (Table 3). The analysis of estradiol also did not show significant differences between the groups [$F(3, 31) = 1.6$; $P > 0.05$] (Table 3).

Discussion

The present results are the evidence that glyphosate-Roundup Transorb has toxic effects on the endocrine reproductive system of rats. The specificity in the action of glyphosate salt over the enzyme EPSPS is a technological advance in the production of pesticides that are safe to human beings. However, the toxicity of its inert ingredients alters, in a significant way, the toxicity of the final formulation (Brausch and Smith 2007; Cox and Surgan 2006; Walsh et al. 2000; Marc et al. 2002; Peixoto 2005).

Table 3 Serum concentrations of testosterone, estradiol and corticosterone

Groups	*n*	Testosterone (ng/dL)	Estradiol (pg/mL)	Corticosterone (ng/mL)
Control	17	154.5 ± 12.9[a,c]	31.5 ± 1.2	103.4 ± 22.8
RU 5 mg/kg	16	108.6 ± 19.6[b]	37.3 ± 2.9	177.2 ± 39.8
RU 50 mg/kg	18	84.5 ± 12.2[d]	36.0 ± 1.8	104.5 ± 24.1
RU 250 mg/kg	15	76.9 ± 14.2[d]	37.6 ± 2.8	104.5 ± 14.8

The herbicide Roundup decreased significantly the testosterone production in all treated groups. The corticosterone and estradiol levels were maintained unaltered

Values are mean ± SEM, *n* animals per group, *RU* Roundup

[a,b] Differ in the column ($P < 0.05$)

[c,d] Differ in the column ($P < 0.001$)

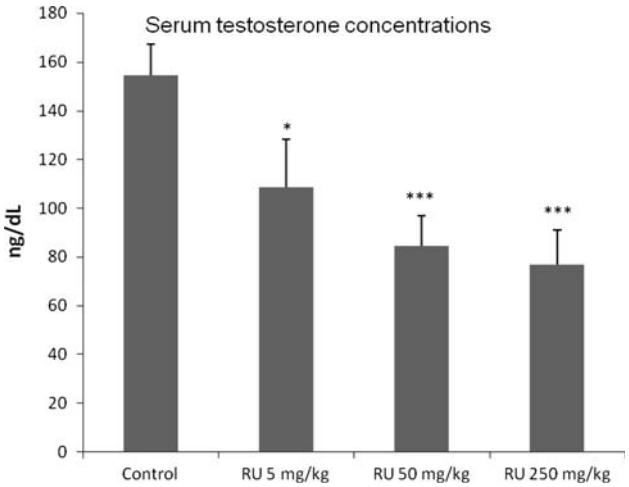

Fig. 4 Serum testosterone levels in animals treated with the herbicide Roundup during the prepubertal period. The testosterone production was affected in all treated groups, with reductions of 30, 45 and 50% in the groups of 5, 50 and 250 mg/kg, respectively. Values are mean ± SEM, $n = 18$ (control), 17 (RU 5 mg/kg), 16 (RU 50 mg/kg), 18 (RU 250 mg/kg), *RU* Roundup, $*P < 0.05$, $***P < 0.001$

Currently, the importance of glyphosate a an herbicide is very high due to the development of plants resistant to its active principle (Cerdeira et al. 2007; Silva et al. 2003), and it is expected that the product will be frequently used in the transgenic cultures of soy, corn, cotton or any other new plants derived through genetic manipulation.

However, the safety of processes must be evaluated relative to the environment, since the consequent contamination of superficial and subterranean waters (Goldsborough and Brown 1993) may cause direct and indirect impacts on biodiversity (Brausch and Smith 2007).

The experimental period of 30 days used in this study can be considered relevant to the effects of environmental contamination, because the degradation of the active principle by the microorganisms present in soil (Haney et al. 2002) occurs between 30 and 90 days (Abreu et al. 2003). During this period, the degradation of glyphosate is not influenced by external environmental factors, such as the soil pH and solar light (Getenga and Kengara 2004), and the organisms present in such an ecosystem are in contact with the product and subjected to the toxic effects.

During the experimental period, the evolution and development of the animals were considered normal and was not observed any negative effects of the daily manipulation or ingestion of the herbicide in the general metabolism of the animals. These were also observed in our previous studies (Romano et al. 2008). Alterations in the growing process were observed for higher doses than that used in this study (FAO and WHO 2004), and the results suggest that the inert ingredients of Roundup Transorb do not alter the toxicity of this salt, considering the doses and periods used in this experiment.

The delay of preputial separation suggests that a hormonal disturbance occurred in this phase. The puberty is a development period where profound hormonal, physical, behavioral and psychological alterations occur that make the individual capable of reproduction (Ojeda and Urbanski 1994). Accordingly, disturbances during this period can result in subsequent adult reproductive problems.

However, this skin separation from the penis gland, evaluated according to protocols to check reproductive toxicity and of the effect of hormonal alterations (Stoker et al. 2000; Parker 2006), which includes a procedure that requires delicate manipulation and a personal interpretation (subjective analysis). This could be reflected in the lack of delay for the observation of this variable in the group receiving the dose of 5 mg/kg, although the associated modification in hormonal production was significant.

It is extremely important for future toxic analyses of the herbicide-Roundup Transorb that the observations from the delay of puberty especially consider the dose of 50 mg/kg, because this dose limits the consideration of the deleterious effects of glyphosate salt on reproduction (Lu 1995).

It is likely that the direct action of this salt was not the major cause of the puberty delay, since some studies on glyphosate salt did not present harmful effects on fertility, but instead showed effects for its adjuvant components. Studies in vitro observed a higher toxicity of glyphosate commercial formulations in relation to the pure salt, indicating that the toxicity from inert ingredients is higher than from active ingredients and that their presence produces a larger toxic effect (Brausch and Smith 2007; Cox and Surgan 2006; Walsh et al. 2000; Marc et al. 2002; Peixoto 2005).

Although the relative testicular weights differed among the treated groups, histopathological modifications that could explain this weight difference were not observed in the group of 250 mg/kg. The endocrine disruption mechanism elucidated in vitro links most modifications with a

lack of production from the protein StaR (Walsh et al. 2000). The lack of actuation of this protein is associated with the accumulation of esters of cholesterol in the cellular cytoplasm, causing cells to grow. An expected final result would be an organ size increase if the accumulation was high. However, the histological procedure used in this study did not maintain the specificity required for observing fat droplets and, therefore, any subtle modifications that may have happened were not detected. Another study has confirmed the presence of cytoplasmic fat droplets, for all groups treated with Roundup, in the histological laminas studied with appropriate pigments (Oliveira et al. 2007).

The seminiferous tubule morphology was studied to check the effects provoked by the hormonal synthesis' modifications, as verified on drakes (Oliveira et al. 2007) and in cellular cultures (Richard et al. 2005), with toxic modifications directed over the cellular function (Peixoto 2005).

Those studies used photomicrography of the histological cuts of the seminiferous tubules, with modifications among the treated groups related to the control groups. The epithelium depth was reduced in the groups of 5, 50 and 250 mg/kg by 16, 20 and 24%, respectively. The epithelium germinative depth is an indicator for the process of spermiogenesis (Hafez and Hafez 2000; Norman and Litwack 1997) and for the hormonal modifications associated with problems in its architecture (Atanassova et al. 2005; Goyal et al. 2003; Akingbemi 2005), explaining the lesser epithelial height for all the treated groups, with such a phenomenon also observed in drakes (Oliveira et al. 2007).

The diameter from the tubular lumen was larger by 24% in the group of 5 mg/kg, by 22% in the group of 50 mg/kg and by 32% in the group of 250 mg/kg. It should be noted, again, that even the lower doses allowed the observation of the harmful effects of the herbicide; other researchers have also observed harmful effects, described as a reduction in the spermatic concentrations by around 20%, in animals exposed to oral doses of 25,000 and 50,000 ppm (Walsh et al. 2000; Peixoto 2005). The associated alterations in the tubular diameter are also related to hormonal problems with the germinative epithelium.

Aside from the variations of the epithelium height and the tubular lumen, the total tubular diameter remained unchanged among the study groups, indicating that alterations occur only with the reduction in the germinative epithelium. However, for example, the total diameter and the anatomic integrity remained unchanged, just as described, for the seminiferous tubules of drakes (Oliveira et al. 2007).

The serum concentration of testosterone was significantly different between the control and the treated groups, being reduced by 30, 45 and 50% in the groups of 5, 50 and 250 mg/kg, respectively. This may explain the alterations observed in the seminiferous tubule morphology. The reduction in testosterone production also jeopardizes the appearance of secondary sexual characteristics, like the preputial separation, and the spermatic production. The interstitial cell of Leydig produces the testosterone from intracellular cholesterol, and the reduction observed in this study follows other authors' observations in drakes (Oliveira et al. 2007), rats exposed in pregnancy and lactation (Dallegrave et al. 2007) and in cellular culture (Walsh et al. 2000). However, the former author observed a reduction of 90% of the testosterone production.

The serum concentration of estradiol was not statistically different between animals in the treatment groups, in spite of the reduction in testosterone production. This can be related to the fact that other tissues also produce estradiol, like adipose and that the serum levels of estradiol are not exclusively dependent on testosterone steroidogenesis (Akingbemi 2005).

Although testosterone is a precursor of estradiol (Norman and Litwack 1997), this experiment did not exhibit disturbances in serum estradiol, even in the presence of a reduction in testosterone levels. The inhibition of the aromatase enzyme was observed in vitro (Richard et al. 2005; Walsh et al. 2000), but in vivo, there was no observed reduction in serum estradiol (Oliveira et al. 2007). These results suggest differences between the in vitro and in vivo assays.

An analysis of the relative weights of the adrenals showed significant differences among the groups studied. The adrenal gland was included in this study because it also produces steroid hormones, using cholesterol for the substratum as in the testis. The intracellular transport of cholesterol is facilitated by the same sub-type of StaR protein as that in testicular transport, $StARD_1$ (Epand 2006). For this reason, alterations in the tissue morphology and in the production of steroids were expected to occur. However, none of these alterations were confirmed. Just like in the testis case, the histological procedure did not confirm the abnormal accumulation of fat, since there was a normal accumulation in the cortical region. The non-observation of alterations in the production of corticosterone indicates that the intracellular transport of cholesterol was not affected in a significant way. It is possible that the appearance of a deficiency in the hormonal status of this organ requires a more severe interruption of the protein StaR, like the one described for the congenital lipoid adrenal hyperplasia in humans (Caron et al. 1997).

It is possible to suggest that the glyphosate-Roundup Transorb is a potent endocrine disruptor, causing alterations in the production of testosterone as well as morphological testis alterations in rats. Since, residues of this pesticide were detected in the home of agricultural workers (Curwin et al. 2005), in the urine of families living near country areas (Curwin et al. 2007), as well as in the food, the

present results could contribute to evaluate the risk of human being environmental exposure. Therefore, people exposed to pesticides may have their fertility compromised over the years (Clementi et al. 2008; Foster et al. 2008; Roeleveld and Bretveld 2008), but we must consider what interactions that may be occurring between other chemicals also utilized in these areas. It would be interesting to evaluate the effect of the interaction of time, type and concentrations of pesticides used to assert the possible participation of glyphosate in these populations. In this study, the short period of treatment allowed the observation of the effects of endocrine disruption of glyphosate-Roundup Transorb. However, chronic studies should be conducted to check for other possible damage.

Acknowledgments The authors would like to thank the Brazilian National Council of Scientific and Technological Development for financial support of this research (Edital MCT/CNPq 02/2006-Universal/process 471651/2006-0), the CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for scholarship.

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