International Journal of Pure and Applied Zoology
Volume 4, Issue 2, pp: 142-148, 2016
http://www.ijpaz.com

ISSN (Print) : 2320-9577
ISSN (Online): 2320-9585

Research Article

EFFECTS OF THE EXPOSURE TO ATRAZINE AND GLYPHOSATE THROUGHOUT INCUBATION ON BONE DEVELOPMENT OF *PODOCNEMIS EXPANSA* (TESTUDINES, PODOCNEMIDIDAE)

Juliana dos Santos Mendonça1, Lucélia Gonçalves Vieira1, Sady Alexis Chavauty Valdes2*, André Luiz Quagliatto Santos1

[1]Wild Animal Research and Teaching Laboratory (LAPAS), Universidade Federal de Uberlândia (UFU), Uberlândia, MG, Brazil
[2]Centro Universitário de Patos de Minas (UNIPAM), Patos de Minas, MG, Brazil

Article History: Received 08th January 2016; Accepted 12th February 2016; Published 18th February 2016

ABSTRACT

Brazil is considered to be one of the largest consumers of pesticides in the world, and herbicides represent the most commonly used class of these products. Atrazine and glyphosate, the most important herbicides, contaminate the waters of several Brazilian rivers and are involved in organ and bone malformations in different species. Among the possible target organisms, reptiles may be indirectly affected by pesticide use once their natural habitats are rivers and streams. Therefore, the objective of this study was to assess the possible effects of exposure to the herbicides atrazine and glyphosate in bone ontogeny of *Podocnemis expansa*. Eggs were artificially incubated in sand moistened with water contaminated with atrazine at concentrations equal to 0, 2, 20, or 200 µg/L, and glyphosate at 65, 650 or 6,500 µg/L. In the control group, the substrate was moistened with distilled water. Two eggs were collected from each incubator every ten days until hatching. For the analysis of bone development, soft tissues were diaphanized, and bones and cartilages were stained with Alizarin red S and Alcian blue, respectively. Specimens were analyzed by stereomicroscopy. Morphological characteristics of the cartilages and bones of the embryos were compared with descriptions of normal ontogeny available in the literature for *P. expansa* embryos. No abnormalities were observed in bone ontogeny of any of the experimental groups.

Keywords: Ecotoxicology; Skeleton; Herbicides; Reptiles; South American River Turtle

INTRODUCTION

Technological progress enabled the evolution of several areas related to food production, health, and agriculture. However, these improvements are intimately connected with the use of chemical compounds that still have unknown properties (Oga, 2003). Nowadays, pesticides are the most important environmental contaminants from human activities that cause severe problems to living organisms. Given the characteristics of these compounds and their persistence in the biosphere, this kind of pollutant may affect natural communities, causing impacts and deleterious effects at tissue and molecular levels in animals (Bueno-Guimarães *et al*., 2001; Berti *et al*., 2009).

The use of herbicides in weed control has been recognized all over the world as a useful agricultural practice. However, its indiscriminate use may impact non-target organisms, mainly aquatic organisms (Nwani *et al*., 2010). Brazil is considered the largest consumer of pesticides in the world (ANDEF, 2012), and herbicides, mainly atrazine and glyphosate, are the most used class of pesticides in the country (Cox, 2001; IBAMA, 2010b).

Atrazine (2-chloro-4-ethylamine-6-isopropylamine-s-triazine) is more commonly used in rural areas, mainly in corn, sorghum, and sugarcane crops. Although it is classified as moderately toxic for aquatic species, this herbicide is one of the most common contaminants detected in streams, rivers, lakes, dams, and underground waters (Battaglin *et al*., 2003; Scrubner *et al*., 2005; Battaglin *et al*., 2008). On the other hand, glyphosate (N-(phosphonomethyl) glycine) may be the most important herbicide ever developed (World Health Organization, 1994). Due to its low persistence in the soil and biodegradability, glyphosate is applied repeatedly in weed control of several crops. As, glyphosate sales represent 76% of the total trade of herbicides in Brazil (IBAMA, 2009), large amounts of this pesticide may reach non-target organisms (Mitchell *et al*., 1987; Servizi *et al*., 1987).

In 2006, the legal regulations of the herbicide glyphosate started to be reviewed in Brazil. Today, this review request is under analysis, according to the resolution RDC no. 10/2008 (ANVISA, 2014). After eight years of study, the Genetics and Environmental Mutagenesis Research Group (GEMA) made up by researchers from the Universidade Nacional de Río Cuarto (UNRC), issued a report in which they linked the use of glyphosate with genetic changes that may lead to spontaneous miscarriage, fetal malformation, and cancer (Torres *et al*., 2006). However, the greatest obstacle

*Corresponding author e-mail: sadyzola@uol.com.br

to this review of glyphosate use is posed by lawsuits from manufacturers of the products that attempt to stop the review process.

Wild fauna is exposed to a wide array of conditions and synthetic chemical products in the environment. Ecotoxicological studies have demonstrated that many of these compounds may affect wild animal survival, interfering with the individual functioning of organisms, causing disease, and affecting reproduction (Guillette and Crain, 2000). Although aquatic environments may be contaminated by different pollutants, little is known about the real effects of these compounds, their toxicological properties, and the risks they pose to the fauna, especially to reptiles. Among these animals, the order Testudines has shown to be of great ecotoxicological importance. Representatives of this group are useful indicators of chemical and radioactive contamination because of their wide geographical distribution, variety of habitats, and longevity (Meyersschöne and Walton, 1994).

The Amazon aquatic community of Testudines is one of the most diverse in the world (Mittermeier, 1978). The species *Podocnemis expansa* is one of the main representatives of this order, which is considered the largest freshwater species of Testudines in South America, reaching up to 107 cm in shell length and weighting 90 kg. These animals are subjected to environmental influences (temperature, water, and gas exchanges) that interfere both with their embryonic development and sex determination (Malvasio, 2001; Pough *et al*., 2008).

Due to the use of their eggs and meat (Pearse *et al*., 2006) by riverside communities, *P. expansa* population has been drastically reduced (Moll and Moll, 2004), and became regulated by Appendix II of the Convention on International Trade in Endangered Species (CITES). These animals were also listed by the IUCN as a low risk, conservation-dependent species (IUCN 2011). Nowadays, the Centro Nacional de Pesquisa e Conservação de Répteis e Anfíbios, an unit of Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) is responsible for conservation and management projects involving Testudines, with *P. expansa* as one of the most protected and studied species.

Aquatic organisms are successively exposed to a variety of contaminants, mainly from agricultural activities, such a heavy metals, hydrocarbons, organic compounds, and pesticides (Schnurstein and Braunbeck, 2001). Given the scarcity of studies related to the exposure of Testudines, and the possible effects that these products may cause to these animals, the objective of this study was to assess the effects of the herbicides atrazine and glyphosate in bone development of *P. expansa* embryos exposed to different concentrations of these pesticides during artificial incubation.

MATERIAL AND METHODS

Egg collection

In October 2013, a total of 140 eggs of *P. expansa* were collected in an environmental protection area Meandros do Rio Araguaia, Brazil (13º 20' 38" S and 50º 38' 05" W; SISBIO/ICMBio license no. 36957-1/2012). All procedures in the study were approved by the Research Ethics Committee from Universidade Federal de Uberlândia (CEUA/UFU 055/12).

Eggs were removed from the nests, placed in plastic bags with vermiculite moistened with water 2:1 v/v, and sent to the Wild Animal Research and Teaching Laboratory at Universidade Federal de Uberlândia (LAPAS/UFU) for artificial incubation. Artificial incubation and exposure to the pesticides

Eggs were artificially incubated in seven trays placed in the incubators according to the method by Verdade *et al*. (1992), and maintained at 28-31°C and 80-100% relative humidity throughout the incubation.

Sand from the site of egg collection was used as the incubation substrate. This substrate was contaminated with atrazine or glyphosate-based commercial herbicides in predetermined concentrations based on the maximum limit of 2 µg/L e 65 µg/L, respectively, for atrazine and glyphosate contamination in superficial waters determined by the Conselho Nacional do Meio Ambiente (CONAMA) resolutions no. 357/2005 and no. 20/1986. Substrates were moistened daily with pure distilled water or distilled water contaminated with either Atrazine PROOF® at concentrations equal to 2, 20 and 200 parts per billion (ppb) or Glyphosate Roundup Original®, at concentrations equal to 65, 650, and 6500 ppb, making up a control group and six experimental groups. A total of 20 eggs of *P. expansa* were incubated in each group.

Handling of the embryos

Eggs were placed in the incubation trays on Day 0. From this day on, two eggs were collected from each incubator every ten days until hatching. A total of 10 eggs were collected per treatment, in average. After eggs were collected, embryos were killed humanely following specific technical guidelines and regulations. Eggshells were cut with surgical scissors, embryos were removed and immediately received sodium pentobarbital (Close *et al*., 1997) in a dose greater than 60 mg/Kg by intracoelomic route (Reilly *et al*., 2001). This procedure ensured overdose by anesthesia, with quick unconsciousness followed by death by respiratory arrest. After animals were euthanized, embryos were preserved in formaldehyde 3.7%. Diaphanization of soft tissues and staining of cartilage and bones. Embryos soft tissues were diaphanized with potassium hydroxide (KOH), and bones and cartilages were stained with Alizarin red S and Alcian blue, respectively, according to the methods by Davis and Gore (1936) and Dingerkus and Uhler (1977), with some modifications.

Data record and analysis

The specimens were analyzed in a stereomicroscope (Leica, DM 1000). Morphological characteristics of the cartilages and bones of the embryos exposed to atrazine were compared with the results obtained by Vieira (2008), who described bone ontogeny in *P. expansa* embryos.

RESULTS AND DISCUSSION

After tissue analysis by diaphanization and staining, embryos

were evaluated for the expected bone development based on Vieira (2008). No changes were observed in bones and cartilages of the embryos exposed to atrazine and glyphosate, in none of the concentrations analyzed. Figure 1 shows ventral view of some embryos in different develpment stages exposed to some of the herbicide concentrations analyzed. Dorsal views are shown in Figure 2.

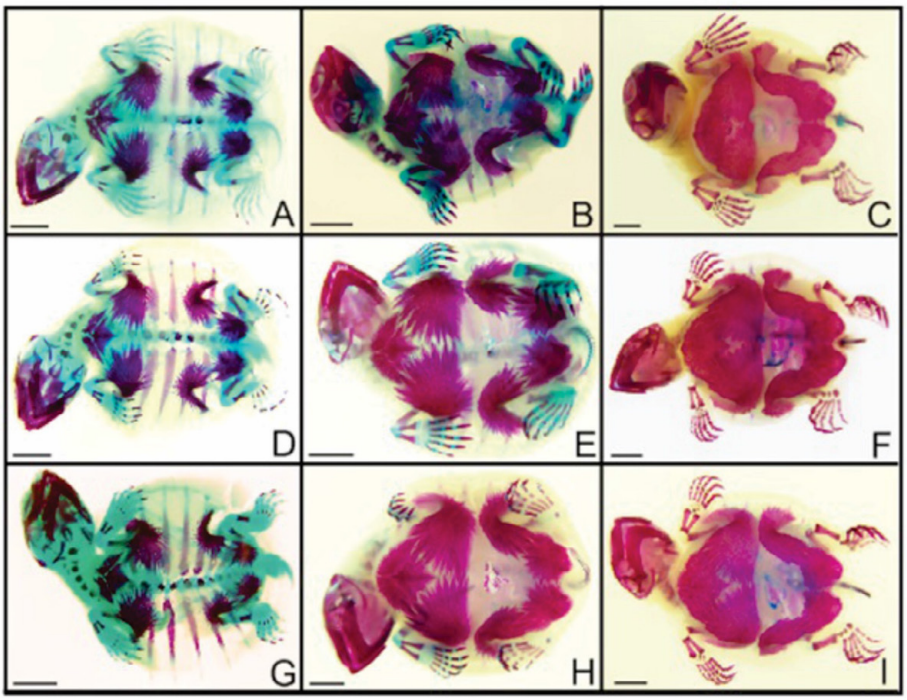

Figure 1: *Podocnemis expansa* embryos, ventral view. (A) control, stage 17; (B) control, stage 21; (C) control, stage 23; (D) substrate contaminated with atrazine 200 µg/L, stage 17; (E) substrate contaminated with atrazine 2 µg/L, stage 21; (F) substrate contaminated with atrazine 2 µg/L, stage 23; (G) substrate contaminated with glyphosate 65 µg/L, stage 17; (H) substrate contaminated with glyphosate 650 µg/L, stage 21; (I) substrate contaminated with glyphosate 6 5 µg/L, stage 23. Diaphanization by KOH; staining of the cartilages with Alcian blue and bones with Alizarin red. Scale: 5 mm.

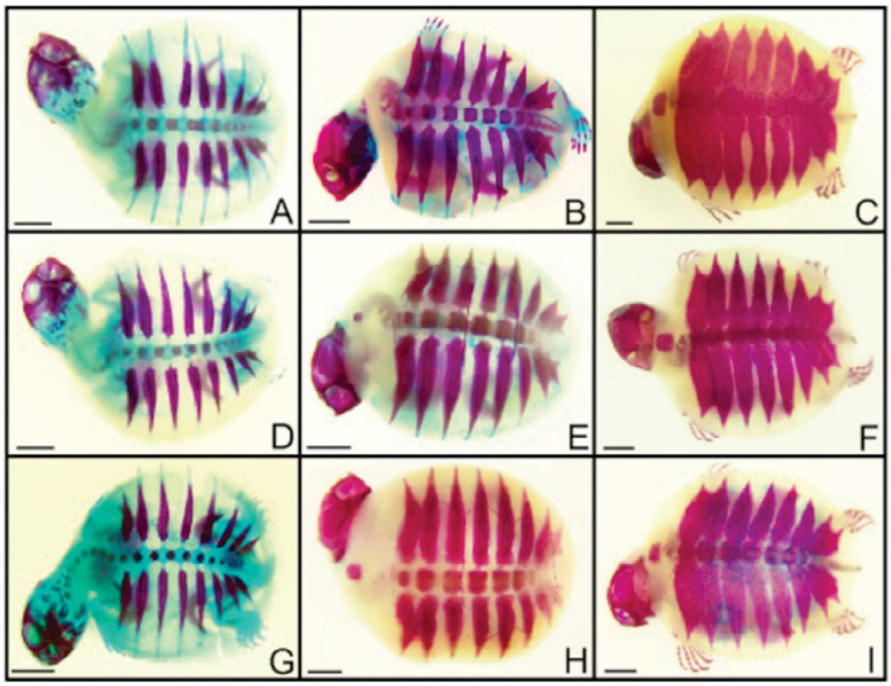

Figure 2: *Podocnemis expansa* embryos, dorsal view. (A) control, stage 17; (B) control, stage 21; (C) control, stage 23; (D) substrate contaminated with atrazine 200 µg/L, stage 17; (E) substrate contaminated with atrazine 2 µg/L, stage 21; (F) substrate contaminated with atrazine 2 µg/L, stage 23; (G) substrate contaminated with glyphosate 65 µg/L, stage 17; (H) substrate contaminated with glyphosate 650 µg/L, stage 21; (I) substrate contaminated with glyphosate 65 µg/L, stage 23. Diaphanization by KOH; staining of the cartilages with Alcian blue and bones with Alizarin red. Scale: 5 mm.

As for the herbicides used in the present study, it has to be emphasized that both atrazine and glyphosate have been widely studied, mainly in relation to exposure of living organisms. The absence of adverse effects in animals exposed to these contaminants has also been described in other species. Le Mer *et al*. (2013) contaminated eggs of a fish species (*Gasterosteus aculeatus*) in laboratory, during 42 days of incubation, using four concentrations of atrazine and glyphosate (0.1; 1; 10 and 100 µg/L). After they hatched, alevins were evaluated in relation to their mass (length, wet weight), and biochemical and histological analyses were carried out (genotype and phenotype analysis). According to these tests, no significant effect of these pesticides was observed in the initial development phases of these alevins.

Studies carried out with reptiles using a glyphosate-based compound, Roundup®, showed a decrease in white cell counts, an increase in heterophile antibodies, changes in plasma proteins, besides a negative effect on the development of juvenile *Caiman latirostris* alligators (Latorre *et al*., 2013). In those studies, juvenile individuals were exposed to concentrations of the herbicide equal to 11 and 21 mg/L for two months, and were compared with a control group. The herbicide concentration was progressively decreased throughout the exposure period to simulate glyphosate degradation in water. It should be emphasized that, in this study, different from the present one, animals were directly exposed to the pesticide.

Several studies have been carried out on the pesticide toxicity, mainly with amphibians, given the fact that pesticide absorption is quicker in these animals than in other vertebrates (Quaranta *et al*., 2009). Relyea (2005) sprayed glyphosate directly on the animals using the doses recommended in the USA, and observed that 79% of the population of juvenile frogs and toads died in the first 24 hours of the experiment. Another study with anurans showed that 30% of the animals died (Bernal *et al*., 2009). However, it is known that not all glyphosate-based herbicides present immediate risk to amphibians in field conditions, as mortality may range from 0% to 80%, depending on the formula (Dinehart *et al*., 2009). Therefore, similar to what is observed with mortality rates, adverse effects of exposure may vary depending on the formula, including in *P. expansa*.

As for atrazine exposure, Storrs and Semlitsch (2008) demonstrated that as amphibians have a shorter larval period and, consequently, quicker development, they are more susceptible to atrazine contamination. Other authors reported that the exposure to this herbicide may cause hermaphroditism, demasculinization, and gonad malformation in African clawed frogs (*Xenopus laevis*) (Hayes *et al*., 2002; Hayes *et al*., 2003). As for the effects of atrazine in reptiles, there are few reports in the literature. De Solla *et al*. (2006) observed changes in gonad development with different atrazine concentrations in *Chelydra serpentine*. Exposure to atrazine during embryonic development of *Graptemys ouachitensis* and *Graptemys pseudogeographica* turtles inhibited flight response and reduced post-hatching survival (Neuman-Lee and Janzen, 2011). However, physical aptitude parameters have not been evaluated for *P. expansa*.

Other studies demonstrated physiological and biochemical effects of herbicides on living organisms. Glyphosate and atrazine affected phagocytosis efficiency in immunological cells of fish (*Rhamdia quelen*), and animals showed greater susceptibility to bacterial infections (Kreutz *et al*., 2010). After exposure to sublethal concentrations of these pesticides for 96 hours, the authors reported a significant decrease in the number of cells and in intracoelomic phagocytosis rates. These fish were also more susceptible to pathogenic bacteria.

Studies on the evaluation of embryo and fetal toxicity, and of the teratogenic potential of atrazine in rats and rabbits showed results that are also different from those of the present study. Atrazine was administered by oral route in doses equal to 0, 10, 70 or 700 mg/kg to groups of 6 to15 day pregnant rats; and in doses equal to 0, 1, 5 or 75 mg/kg to 7 to 19 day pregnant rabbits (Infurna *et al*., 1988). Maternal and fetal toxicity were observed both in rats and rabbits at the highest concentrations. However, no teratogenic effect was observed in any of the species.

In studies on reproductive toxicity in rats, mothers were exposed to 500, 750 or 1000 mg/kg of a glyphosate formula by oral route and animals showed incomplete ossification of cranial bones, increased size of the fontanels, bipartite interparietal and supraoccipital bones, absence of tail vertebrae, undulation in ribs, and absence of metatarsal bones and phalange ossification in pelvic limbs. Changes were observed in all concentrations (Dallegrave, 2003), demonstrating the teratogenic power of glyphosate, in spite of the absence of bone effects in *P. expansa*, as observed in this study. In these animals, the eggshell may have functioned as a vital protective barrier.

The eggshell in Testudines is highly important for the embryo, once it controls gas exchanges through the pores, is a source of minerals for the embryo, and functions as a protective barrier (Kitimasak *et al*., 2003; Kusuda *et al*., 2013). In terms of chemical composition, other studies showed that the eggshell in Testudines is an extremely good barrier: only in extreme conditions may external moisture influence embryo development (Lesem and Dmi'el, 1986). Exposure of *P. expansa* eggs to technical grade atrazine for only one day of artificial incubation caused changes in the chemical composition of the eggshell in terms of phosphorus concentrations, fat content, and thickness. However, no teratogenic effect was observed in bone ontogeny (Souza, 2013). Given the methodology and results of the present study, it may be suggested that the eggshell was an important barrier, preventing herbicides from reaching the interior of the eggs. However, the study was not designed to evaluate the ability of the eggshell to function as a barrier.

As for the absence of macroscopic changes in bone ontogeny of *P. expansa* after exposure to atrazine and glyphosate, the present study showed different results compared with literature reports on other pesticides. In studies with juvenile *Alligator mississippiensis* females living in Apopka lake in Florida, which is contaminated with DDT, Lind *et al*. (2004) observed greater trabecular density in long bones in computed tomography, suggesting that bone resorption in these animals was compromised by the inhibition of osteoclastic activity.

Studies with malformations in amphibians and fish also reported teratogenic effects. *Rana perezi* tadpoles kept for 14 weeks in water containing two lethal concentrations of the insecticides ZZ-Aphox (Carbamate) or Folydol (Organophosphate) (0.25 and 1 mg/L, respectively) showed malformations in the spine and/or limbs, besides differences in bone matrix and abnormal vascularization of the periosteum (Alvarez, 1995). Wells and Cowan (1982) showed vertebral dysplasia in fish exposed to the herbicide Trifluralin. In an experiment carried out in fish tanks, the species *Salmon parr* was exposed to three doses of the contaminant (0.5, 0.25 and 0.01 mg/L) for 16 hours during 10 days. X-ray analysis showed clear signs of vertebral damage in these animals, mainly in those that were exposed to the highest concentrations. In these studies, different from what happened to *P. expansa*, the animals were in direct contact with pesticides and showed changes in morphology.

Bone malformations were also observed in recently hatched chicks. Uggini *et al*. (2010) injected commercial insecticides at different concentrations in chicken eggs on day zero of incubation. After hatching, live and dead chicks were analyzed. The specimens were stained with Alizarin and Alcian blue for the analysis of the cartilages. Embryo malformations were found in the axial and appendicular skeleton at all concentrations. In the lowest concentration (0.01 µg), the morphologic effects were not obvious, but increasing concentrations showed more evident effects, such as bent legs and distorted phalanges, as well as deformities in the beak, sternum, ribs, and other sites. Other trials with birds corroborated these findings (Rao *et al*., 1992; Anwar, 2003; Ahmad and Asmatullah, 2007).

In spite of the daily contamination using Atrazine PROOF® and Glyphosate Roundup Original®, no changes were observed in *P. expansa* bone development. However, it cannot not be stated that exposure may not have changed other parameters, such as physiology, neurology, or behavior of the individuals, as observed and discussed by other authors.

CONCLUSION

Exposure to commercial herbicides atrazine and glyphosate in concentrations up to 100 times greater than those allowed by current regulations did not cause any bone abnormality in *P. expansa* embryos exposed daily to the pesticides during their ontogeny.

ACKNOWLEDGMENTS

We would like to thank RAN/ICMBio and CEUA/UFU for allowing this study to be carried out. We would also like to thank Omar Teodoro da Silva Júnior for providing the herbicides, and to Lilian Freitas Bastos for the aid in egg collection. We also thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial aid and support for the study. The Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) provided financial support for publication.

REFERENCES

Ahmad, K. R. 2007. Teratological effects of Chlorpyrifos in mice. *Iranian Jr Toxicol*., 1: 91-99.

Alvarez, R., Honrubia, M. P. and Herráez, M. P. 1995. Skeletal Malformations Induced by the Inseticides ZZ-APhox® and Folidol® During Larval Development of *Rana perezi*. *Arch Environ Contam Toxicol*, 28: 349-356.

ANDEF - Associação Nacional de Defesa Vegetal. 2012. Electronic Database accessible at http: //www. andef. com.br/defensivos. Captured on 24 november 2014.

ANVISA – Agência Nacional de Vigilância Sanitária. 2014. Reavaliação de Agrotóxicos - Resolução RDC nº 10/2008. Electronic Database accessible at

http://portal.anvisa.gov.br/wps/content/Anvisa+Portal/Anvisa/Inicio/Agrotoxicos+e+Toxicologia/Assuntos+de+Interesse/Reavaliacoes+de+Agrotoxicos/W+Reavaliacao+de+Agrotoxicos++Resolucao+RDC+n+10+2008> Captured on 26 november 2014.

Anwar, K., 2003. Cypermethrin, a Pyrethroid induces teratological and biochemical changes in young chick embryos. *Pakistan J Biol Sci*, 6: 1698-1705.

Battaglin, W. A., Thurman, E. M., Kalkhoff, S. J. and Porter S. D. 2003. Herbicides and transformation products in surface waters of the Midwestern United States. *J. Am. Water Res. Assoc,* 39: 743-756.

Battaglin, W. A., Rice, C. K., Foazio, M. J., Salmons, S. and Barry, R. X. 2008. The occurrence of glyphosate, atrazine, and other pesticides in vernal pools and adjacent streams in Washington, DC, Maryland, Iowa and Wyoming 2005–2006. *Environ. Monit. Assoc*, 155: 281-307.

Bernal, M. H., Solomon, K. R., and Carrasquilla, G. 2009. Toxicity of formulated glyphosate (Glyphos) and Cosmo-Flux to larval and juvenile Colombian frogs 2. Field and laboratory microcosm acute toxicity. *J Toxicol Env Health A*, 72: 966-973.

Berti, A. P., Düsman, E., Soares, L. C. and Grassi L. E. A. 2009. Efeitos da contaminação do ambiente aquático por óleos e agrotóxicos. *Sabios: Rev Saúde e Biol* 4: 45-51.

Bueno-Guimarães, H. M., Ferreira, C. M., Garcia, M. L. B. and Saldiva, P. H. N. 2001. Tadpole epithelium test: potential use of *Rana catesbeiana* histopathologic epithelial changes to evaluate aquatic pollution. *Bull. Environ. Contam Toxicol,* 67: 202-209.

Close, B., Banister, K., Baumans, V., Bernoth, E., Bromage, N., Bunyan, J., Erhardt, W., Flecknell, P., Gregory, N., Hackbarth, H., Morton, D. and Warwick C. 1997. Recommendations for euthanasia of experimental animals: Part 2. Laboratory Animals 31: 1-32.

Cox, C. 2001. Atrazine: environmental contamination and ecological effects. *J. Pestic. Reform*, 2: 12-20.

Dallegrave E. 2003. Toxicidade reprodutiva do herbicida Glifosato-Roundup® em ratos Wistar. Tese (Doutorado) – Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Ciências Veterinárias. 200f.

Davis, D. D. and U. R. Gore. 1936. Clearing and staining skeleton of small vertebrates. Field Museum of Natural History, 4: 3-15.

De Solla, S. R., Martin, P. A., Fernie, K. J., Park, B. J. and Mayne, G. 2006. Effects of environmentally relevant concentrations of atrazine on gonadal development in snapping turtles (*Chelydra serpentina*). *Environ Toxicol Chem*, 25: 520-526.

Dingerkus, G. and L. D. Uhler, 1977. Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. *Stain Technol*, 52: 229-232.

Dinehart, S. K., Smith, L. M., McMurry, S. T., Anderson, T. A., Smith, P. N. and Haukos, D. A. 2009. Toxicity of a glufosinate- and several glyphosatebased herbicides to juvenile amphibians from the Southern High Plains, USA. *Sci Total Environ*, 407: 1065-1071.

Guillette, L. J. JR. and Crain, D. A. (ed.) 2000. Environmental Endocrine Disrupters: An Evolutionary Perspective. New York:Taylor & Francis. 400pp.

Hayes, T. B., Collins, A., Lee, M., Mendoza, M., Noriega, N., Stuart, A. A. and Vonk, A. 2002. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. *Ecology*. 99: 5476-5480.

Hayes, T., Haston, K., Tsui, M., Hoang, A., Haeffele, C. and Vonk, A. 2003. Atrazine induced hermaphroditism at 0.1 ppb in American Leopard Frogs (*Rana pipiens*): Laboratory and field evidence. *Environ Health Persp.,* 111: 568-575.

IBAMA - Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. 2010. Produtos agrotóxicos e afins comercializados em 2009 no Brasil: Uma abordagem ambiental. Rebelo,R.M.(Coord.),Vas concelos, R.A., B.D.M.C. Buys, J.A. Rezende, K.O.C. Moraes, R.P Oliveira. IBAMA, Brasília.

Infurna, R., Levy, B., Meng, C., Yau, E., Traina, V. Rolofson, G., Stevens, J. and Barnett, J. 1988. Teratological evaluations of atrazine technical, a triazine herbicide, in rats and rabbits. *J Toxicol Environ Health A*, 24: 307-319.

IUCN - International Union for Conservation of Nature and Natural Resources .2011. IUCN Red List of Threatened Species. Electronic Database accessible at http: <http:\\ www.redlist.org> Captured on 10 november 2014.

Kitimasak, W., Thirakupt, K. and Moll. D. L. 2003. Eggshell structure of the Siamese narrow-headed softshell turtle *chitra chitra* nutphand, 1986 (Testudines: Trionychidae). *Science Asia*, 29: 95-98.

Kreutz, L. C., Barcellos, L. J. G., Marteninghe, A., Santos, E. D. and Zanatta, R. 2010. Exposure to sublethal concentration on glyphosate or atrazine-based herbicides alters the phagocytic function and increases the susceptibility of silver catfish fingerlings (*Rhamdia quelen*) to *Aeromonas hydrophilia* challenge. *Fish Shellfish Immunol,* 29: 694-697.

Kusuda, S., Yasukawa, Y., Shibata, H., Saito, T. and Yoshizaki, N. 2013. Diversity in the Matrix Structure of Eggshells in the Testudines (Reptilia) *Zool Sci,* 30: 366-374.

Latorre, M. A., González, E. C. L., Larriera, A., Poletta, G. L. and Siroski, P. A. 2013. Effects of *in vivo* exposure to Roundup® on immune system of *Caiman latirostris*. *J Immunotoxicol,* 10: 349-354.

Le Mer, C., Roy, R. L., Pellerin, J., Couillard, C. M., Maltais, D. 2013. Effects of chronic exposures to the herbicides atrazine and glyphosate to larvae of the threespine stickleback (*Gasterosteus aculeatus*). *Ecotoxicol Environ Saf*, 89: 174-181.

Lesem, A. and Dmi'el, R. 1986. Water loss from *Trionyx triunguis* eggs incubating in natural nests. *Herpetol. J*., 1: 115-117.

Lind, P. M., Milnes, M. R., Lundberg, R., Bermudez, D., Örberg, J. and Guillette, JR. L. J. 2004. Abnormal Bone Composition in Female Juvenile American Alligators from a Pesticide-Polluted Lake (Lake Apopka, Florida). *Environ Health Perspect.*, 112: 359-362.

Malvasio, A. 2001. Aspectos do mecanismo alimentar e da biologia reprodutiva em *Podocnemes expansa* (Schweigger, 1812), *Podocnemes unifilis* (Troschel, 1848) *e P.sextuberculata* (Cornalia, 1809)(Testudines, Pelomedusidae). 199p. Tese (Doutorado em Ciências Biológicas)- Faculdade de Zoologia, Instituto de Biociências da Universidade de São Paulo, São Paulo.

Meyers-Schöne, L. and Walton, B. T. 1994.Turtles as monitors of chemical contaminants in the environment. *Rev Environ Contam Toxicol*, 135: 93-153.

Mitchell, D. G., Chapman, P. M. and Long, T. L. 1987. Acute toxicity of Roundup and Rodeo herbicides to rainbow trout, chinook and coho salmon. *Bull Environ Contam Toxicol*, 39: 1028-1035.

Mittermeier, R. A. 1978. South America's River Turtles: Saving Them by Use. Oryx 14: 222-230.

Moll, D. and Moll, E. O. 2004. The ecology, exploitation and conservation of river turtles. New York: Oxford University Press.420pp.

Neuman-Lee, L. A. and Janzen F. 2011. Atrazine exposure impacts behavior and survivorship of neonatal turtles. *Herpetologica*, 67: 23-31.

Nwani, C. D., Lakra, W. S., Nagpure, N. S., Kumar, R., Kushwaha, B. and Srivastava, S. K. 2010. Toxicity of the Herbicide Atrazine: Effects on Lipid Peroxidation and Activities of Antioxidant Enzymes in the Freshwater Fish *Channa Punctatus* (Bloch). *Int. J. Environ. Res. Public Health,* 8: 3298-3312.

Oga, S. 2003. Fundamentos da Ecotoxicologia, 2 ed. São Paulo: Atheneu Editora, 474pp.

Pearse, D. E., Arndt, A. D., Valenzuela, N., Miller, B. A., Cantarelli, V. and Sites, JR J. W. 2006. Estimating population structure under non-equilibrium conditions in a conservation context: continent- wide population genetics of the giant Amazon river turtle *Podocnemis expansa* (Chelonia; Podocnemidae). *Mol Ecol,* 15: 985-1006.

Pough, F. H., Heiser, J. B. and Janis, C. M. 2008. A vida dos vertebrados. 4.ed. São Paulo: Atheneu Editora. 684pp.

Quaranta, A., Bellantuono, V., Cassano, G. and Lippe, C. 2009. Why amphibians are more sensitive than mammals to xenobiotics. *PLoS One,* 4: e7699.

Rao, J. V., Swamy, A. N., Yamin, S., Rao, S. H. and. Rahman, M. F. 1992. Teratism induced in the developing chick by RPR-V, an organophosphate. *Food Chem Toxic,* 3: 945-951.

Relyea, R. A. 2005. The lethal impact of Roundup on aquatic and terrestrial amphibians. *Ecol Appl.* 15: 1118-1124.

Reilly, J. S. 2001. Reptiles. In: _____. Euthanasia of animals used for scientific purposes. Adelaide, Australian and New Zealand Council for the Care of Animals in Research and Teaching. p. 83-89.

Servizi, J. A., Gordon, R. W. and Martens, D. W. 1987. Acute toxicity of Garlon 4 and Roundup herbicides to salmon, daphnia and trout. *Bull Environ Contam Toxicol*, 39: 15-22.

Schnurstein, A. and Braunbeck T. 2001. Tail moment versus tail lengh application of an vitro version of the comet assay in biomonitoring for genotoxicity in native surface waters using primary hepatocytes and gill cells from zebrafish (*Danio rerio*). *Ecotoxicol Environ Saf*, 49: 187-196.

Scrubner, E. A., Thurman, E. M., Goolsby, D. A., Meyer, M. T., Battaglin, W. A. and Kolpin D. W. 2005. Summary of significant results from studies of atrazine herbicides and their degradation products in surface water, groundwater, and precipitation in the Midwestern United States during the 1990s. In U.S. Geological Survey Scientific Investigations Report; USGS: Lawrence, KS, USA, pp. 2005-5094.

Souza, R. R. 2013. Efeitos da atrazina na composição química e morfologia de cascas de ovos de *Podocnemis Expansa* (Testudines, Podocnemididae) incubados artificialmente. Dissertação (mestrado) - Universidade Federal de Uberlândia, Programa de Pós-Graduação em Ciências Veterinárias. 52 pp.

Storrs, S. and Semlitsc, R. 2008. Variation in somative and ovarian development: Predicting susceptibility of amphibians to estrogenic contaminants. *Gen Comp Endocrinol*, 156: 524-530.

Torres, F. M., Urroz, M. B. G. C., Ovando, H. G., Anchordoqui, I. W., Vera, L. U., Hand, I. B. L. H. and Abrate, N. G. 2006. Evaluation of genotoxicity of the herbicide glyphosate quantitatively measured by the comet assay and micronucleius formation in treated mice. *Theoria*, 15: 53-60.

Uggni, G. K., Patel, P. V. and Balakrishanan, S. 2010. Embryotoxic and Teratogenic Effects of Pesticides in Chick Embryos: A Comparative Study Using Two Commercial Formulations. *Environ toxicol*. 27: 166-174.

Verdade, L. M., Michelotti, F., Rangel, M. C., Cullen, JR L., Ernandes, M. M. and Lavorenti. A. 1992. Manejo de ovos de jacaré-de-papo-amarelo (Caiman latirostris) no CIZBAS/ESALQ/USP, in: Verdade LM, Lavorenti A (Eds.) In Anais do I workshop sobre conservação e manejo do jacaré-de-papo-amarelo (*Caiman latirostris*). ESALQ/USP, Piracicaba, pp 92-99.

Vieira, L. G. 2008. Ontogenia dos ossos do esqueleto da tartaruga-da-amazônia *Podocnemis expansa* Schweigger, 1812 (Testudines, Podocnemididae). 2008. 152 f. Dissertação (Mestrado) - Universidade Federal de Uberlândia, Uberlândia, 2008.

Wells, D. E. and Cowan. A. A. 1982. Vertebral dysplasia in salmonids caused by the herbicide trifluralin. *Environ Pollut A,* 29: 249-260.

World Health Organization. 1994. Glyphosate. Environmental Health Criteria. Publication No 159, Geneva, Switzerland.