Volume 12 – No. 4 – 2003
REPRINT pp. 364 - 367

COMPARATIVE ACUTE TOXICITY OF THE COMMERCIAL HERBICIDES GLYPHOSATE TO NEOTROPICAL TADPOLES *Scinax nasicus* (ANURA: HYLIDAE)

R. Lajmanovich - E. Lorenzatti - Ma. I. Maitre - S. Enrique - P. Peltzer

Angerstr. 12
85354 Freising - Germany
Phone: ++49 - (0) 8161-48420
Fax: ++49 - (0) 8161-484248
e-mail: info@psp-parlar.de
http://www.psp-parlar.de

COMPARATIVE ACUTE TOXICITY OF THE COMMERCIAL HERBICIDES GLYPHOSATE TO NEOTROPICAL TADPOLES *Scinax nasicus* (ANURA: HYLIDAE)

R. Lajmanovich[1,3], E. Lorenzatti[2], Ma. I. Maitre[2], S. Enrique[2] and P. Peltzer[1]

[1]Instituto Nacional de Limnología - INALI, José Macias 1933 (3016), Santo Tomé, (Santa Fe), Argentina
[2]Instituto de Desarrollo Tecnológico para la Industria Química - INTEC, Güemes 3450, (3000) Santa Fe, Argentina
[3]Escuela Superior de Sanidad, (FABICIB-UNL), Paraje el Pozo s/n (3000), Santa Fe, Argentina

SUMMARY

We investigated the effect of glyphosate (GLY) to *Scinax nasicus* tadpoles and the influence of GLY degradations in the variation of LC_{50} values. These results showed that 96-h LC_{50} for *S. nasicus* tadpoles exposed to continuous applications of GLY in the renewal tests (RT) was 3.13 mg GLY/L, compared to an estimated LC_{50} in static tests (ST) of 5.27 mg GLY/L. These data indicate large differences in toxicity between RT and ST, *S. nasicus* tadpoles did not die when the GLY exposure was not continuous.

KEYWORDS:
Glyphosate, amphibians, *Scinax nasicus*, tadpoles, toxicity.

INTRODUCTION

Glyphosate, (GLY) or N-phosphonomethyl glycine, is a systemic herbicide used for non-selective weed control on 'rights-of-way', forestry plantations, sites prepared for plantings, and as a foliage desiccant for selected crops [1, 2]. It is considered to have low toxicity to bees, fish, and other aquatic organisms [3, 4]. However, some of the surfactants used in agricultural formulations have been found to be significantly more toxic to fish, amphibians, and aquatic invertebrates than the herbicide itself [5-7].

The pesticide degradation phenomenon implies the possibility to decrease toxic concentrations of xenobiotics in the environment. In water, GLY is rapidly dissipated, and has under laboratory conditions a half-life for degradation ranging from a few days to approximately 20 days [2]. Moreover, it has been found to dissipate from the sediment of a farm pond with a half-life of 120 days, and still be present in pond sediment at a concentration of 0.1 ppm one year later [8]. The presence of GLY in surface water is most likely to occur as a result of heavy rainfall after recent application on neighboring land, with subsequent rapid displacement into stream sediment [2]. The presence of an environmental contaminant can decrease larval amphibian activity [9, 10] and alter swimming and feeding behavior [11]. Alternatively, it is possible for contaminants to increase the anuran larval activity and, therefore, predation rates [12]. The objective of this study was to evaluate and compare the acute toxicity of glyphosate formulations (GLY-F) on *Scinax nasicus* tadpoles. Furthermore, the behavioral responses are described.

Scinax nasicus was selected for this study because of its wide Neotropical distribution (i.e. present in Argentina, Paraguay, Uruguay, Bolivia, and Brazil) [13]. This anuran is common in forests, wetland, riparian areas, urban, and agricultural lands [14]. In addition, another study indicated that natural *S. nasicus* populations could be affected by herbicide application [15].

MATERIALS AND METHODS

Two hundred and fifty *S. nasicus* tadpole individuals (Gosner Stage 25-26) [16] were collected from an unpolluted temporary pond in the floodplain of the Paraná River (31º 42´S; 60º 34´O, Santa Fe, Argentina) and maintained under laboratory conditions. The tadpoles were acclimatized over 7 days to a 12 h light/12 h dark cycles in glass tanks with artificial pond water (APW) (pH: 8.2 ± 0.3, conductivity: 237 ± 25 µmhos/cm^{-1}, dissolved oxygen: 8.4 ± 0.1 mg/L, and hardness: 56 ± 12 mg $CaCO_3$/l, at 22 ± 2 ºC).

96-h acute toxicity test was conducted according to ASTM Standard Methods [17], with 26-27 Gosner Stage larvae. Glass tanks (35 cm diameter and 60 cm high), with 4 L of APW and 10 tadpoles (average weight: 0.025 ± SE

0.006 g) per tank, were used in the experiments. The tested product was Glyfos®, a commercial herbicide containing 48 % GLY, that was measured by high performance liquid chromatography (HPLC). In a renewal test (RT), solutions were renewed at 24-h intervals during the test period and tadpoles that had been taken out were transferred into the new solution. Simultaneously, a static test (ST) was conducted, in which solutions were not renewed during exposure. In both acute toxicity tests, the concentrations used were: 0, 3.07, 3.84, 4.8, 6.0, and 7.5 mg of GLY-F/L. Tests were conducted at 22 ± 2 ºC and 12-h / 12-h light-dark. The mortality was recorded every 24-h and the dead animals were removed at each observation time. The LC_{50s} with confidence limits ($P \leq 0.05$) were estimated using the probit analysis [18]. The criterion of non-overlapping 95% confidence intervals[19] was used to determine the significant differences ($P \leq 0.05$) between LC_{50} values in RT and ST. Moreover, bobbing (swimming to the surface for air) rates were measured as well as the number of times that larvae broke the water surface with the anterior part of their body in a 10 minute interval was recorded.

GLY was identified and quantified by Water 600 HPLC system with IC-Pack ion-exclusion 50 Å-7µm (7,8 x 150 mm) column, mobile phase phosphoric acid: 0.05% at 55 °C, Water® postcolumn derivatization system at 38 ºC, fluorescence spectrophotometer detector at 339-nm excitation and 345-nm emission wavelengths by Milenium[32] manager data processor. This method was adapted from Winfield [20].

RESULTS AND DISCUSSION

The 96-h LC_{50} for *S. nasicus* exposed to GLY-F in the RT was 3.13 mg GLY-F/L, compared to an estimated higher LC_{50} in ST (where solutions were not renewed during exposure) of 5.27 mg GLY-F/L. Although at 24-h LC_{50} the RT and ST did not differ statistically ($P > 0.05$), differences was observed at 48, 72, and 96-h ($P < 0.05$) (Figure 1). In RT, we observed a decrease in LC_{50} values at all time during the bioassay (Figure 1 A). In ST, mortality stabilized at 24-h, which was related to the GLY degradation. Nevertheless, we found that the half-life of GLY was approximately 96-h (Figure 1B).

After 24-h of exposure in RT, cephalic edemas and bent tail occurred in 3.84 mg/L of GLY and there were mouth deformities at 4.8 mg/L. These manifestations were increased with the time and intestinal vacuity was detected at 48-h exposure, although these tadpoles showed different behaviors at differing GLY concentrations with the control. The rates of the tadpoles coming to the surface to gulp air is lower without herbicides, Chi-square statistic (with Yates correction) = 26.34; $P < 0.01$. In contrast, in the ST test, the alterations were not so marked and they were only poorly observed at the highest concentration, 7.5 mg/L. The specific behavioral differences may also be important, many temporary aquatic environments are highly eutrophic and have low levels of dissolved oxygen. Clearly, the ability of larvae to meet their respiratory requirements, unduly favors their exposure to predation risk. To what extent amphibians are presently limited in their range by the contamination of potential breeding sites is unknown.

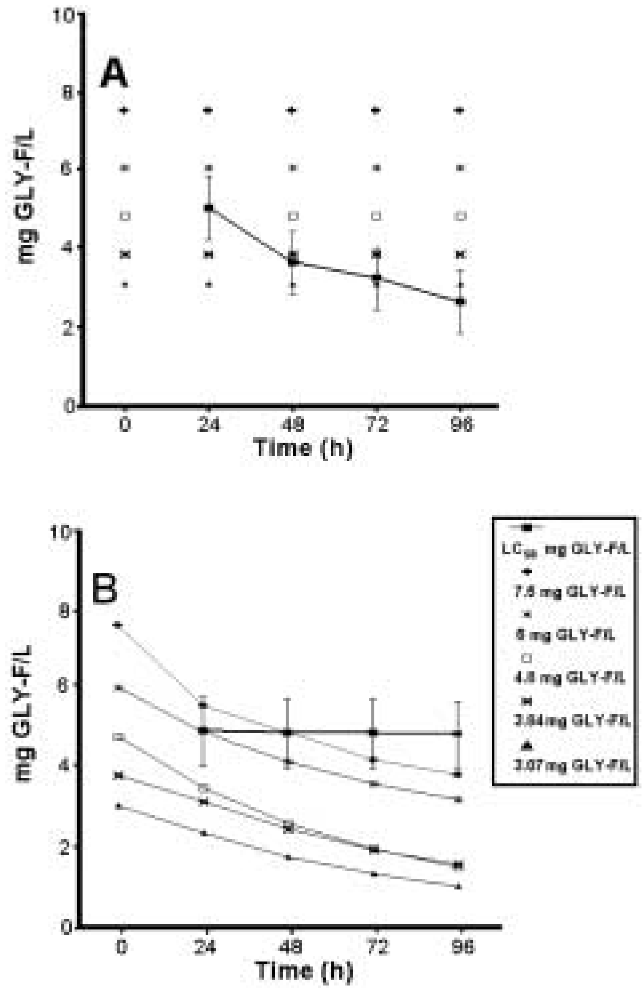

FIGURE 1 - LC_{50} (95% confidence limits) values of *Scinax nasicus* tadpoles in RT (A) and ST (B) (see the dynamics of the disappearance of the glyphosate in water). Observe the stabilization of the LC_{50} values when the expositions are not continuous.

Mann and Bidwell [21] found, in test solutions renewed after 24-h with freshly made solutions, several LC_{50} values for tadpoles of Australian frogs (*Crinia insignifera*, *Heleioporus eyrei*, *Limnodynastes dorsalis*, and *Litoria moorei*) (48-LC_{50}: 8.1-32.2 mg GLY/L). An Australian study confirmed that Roundup® herbicides are toxic to frogs, especially to tadpoles, because of the effect of inert ingredients on tadpole gills [22]. The herbicide Glyphos® contains polyethoxylated tallowamine (POEA) [23], a surfactant bound to suspended material that can have toxic effects in water systems. The same surfactant is included in Roundup® formulations because it aids to counteract the surface tension on leaf surfaces, but it may also interfere with gill respiration in tadpoles [24].

In this context, transgenic soya (resistant to Roundup-Ready) is the most extensively cultivated, compared to others crops, in Argentina agricultural lands and during 2000-2001 it had occupied 19 million hectares [25]. Moreover, the GLY-F use in Argentina increased from 1 to 58 million liters from 1991 to 2000 [26]. The validity of extrapolating laboratory based acute toxicity data to the field situation is contentious [21]. However, our results indicated a variability of toxicity of GLY-F according to the type of exposure. Specifically, we found that non-target tadpoles did not die when the GLY exposure was not continuous. Moreover, we argue that the GLY-F pulse can affect native populations of neotropical tadpoles. Also, we suggest that prevention of possible contamination of lentic, or ephemeral water bodies where most of the anuran breed, should be considered. On the other hand, the impacts and the costs of the required alternatives to the GLY formulations should be minimized for uses away from aquatic situations.

ACKNOWLEDGMENTS

We thank Richard Wassersug for reviewing a draft of manuscript.

REFERENCES

[1] Worthing, C. R. (1983) The pesticide manual. A world compendium. 7th edition. The British Crop Protection Council. Lavenham Press, Lavenham, U.K.

[2] WHO, (1994) Environmental Health Criteria 159: Glyphosate. Geneva

[3] U.S. EPA, (1986) Pesticide Fact Sheet: Glyphosate. Office of Pesticide Programs, EPA Publication No. 540/FS-88-124, Washington, DC

[4] Water and River Commission, (2001)Water notes for rivers management -Advisory Notes for Land Managers on River and Wetland Restoration. Western, Australia

[5] Mitchell, D. G., Chapman, P. M. and Long, T. J. (1987) Acute toxicity of Roundup ® herbicides to rainbow trout, chinnok and coho salmon. Bull. Environ. Contam. Toxicol. 39, 1028-1035.

[6] 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.

[7] Wan, M. T., Watts, R. G. and Moul, D. J. (1989) Effects of different dilution water types on the acute toxicity to juvenile pacific salmonids and rainbow trout of glyphosate and formulated products. Bull. Environ. Contam. Toxicol. 43, 378-385.

[8] U.S. EPA, (1993) Registration Eligibility Document. Glyphosate. Office of Prevention, Pesticides and Toxic Substances, Washington DC

[9] Kutka, F. J. (1994) Low pH effects on swimming activity of Ambystoma salamander larvae. Environ. Toxicol. Chem. 13, 1821-1824.

[10] Bridges, C. M. (1997) Effect of an agricultural pesticide on tadpole activity and swimming performance. Environ. Toxicol. Chem. 16, 1935-1939.

[11] Freda, J. and Taylor, D. H. (1992) Behavioural response of amphibian larvae to acidic water. J. Herpetol. 26, 429-433.

[12] Cooke, A. S. (1971) Selective predation by newts on frog tadpoles treated with DDT. Nature 229, 275- 276.

[13] Frost, D. R. (1985) Amphibian species of the world a taxonomic and geographical reference, Allen Press, Inc. and the Association of Systematic Collections, Kansas

[14] Ordano, M., Collins, P. and Lajmanovich, R. (2000) *Scinax nasicus* (Habitat). Herpetol. Rev. 31, 171.

[15] Lajmanovich, R. C., Izaguirre, M. F. and Casco, V. H. (1998) Paraquat tolerance and Alteration of internal gill structures of *Scinax nasica* tadpoles (Anura: Hylidae). Arch. Environ. Contam. Toxicol. 34, 364-369.

[16] Gosner, K. L. (1960) A simplified table for staging amphibian embryos and larvae with notes on identification. Herpetologica 16, 183- 190.

[17] ASTM, (1993) Standard practice for conducting acute tests with fishes, macroinvertebrates and amphibians. Designation E 729-88a$^{\epsilon 1}$. In: ASTM standards on aquatic toxicology and hazard evaluation. Philadelphia, PA, pp. 102-121.

[18] Finney, D. J. (1971) Probit analysis 3º Ed. Cambridge Univ. Press, New York

[19] APHA, (1989) American Water Works - Association and Water Pollution Control Federation. Standard Methods for the Examination of Water and Wastewater. 17th Edition, Washington, DC

[20] Winfield, T. W. (1990) Determination of glyphosate in drinking water by direct-aqueous- injection HPLC, post - column derivatization, and fluorescence detection EPA Method 547, Ohio

[21] Mann, R. M. and Bidwell, J. R. (1999) The toxicity of glyphosate and several glyphosate formulations to four species of southwestern Australian frogs. Arch. Environ. Contam. Toxicol. 36, 193- 199.

[22] Bidwell, J. R. and Gorrie, J.R. (1995) Acute toxicity of a herbicide to selected frog species. Final Report, Western Australia Department of Environmental Protection, Perth, Western

[23] Environmental Standards & Procedures (2001) Vegetation Management - List of Approved Herbicides ESP # E-VGM-004

[24] Tyler, M. J. (1997) Herbicides Kill Frogs, University of Adelaide, South Australia. Froglog 21, 11.

[25] Arregui, M. C. (2001) Manejo de agroquímicos en cultivos extensivos. Universidad Nacional del Litoral, Santa Fe, Argentina

[26] Morales, C. (2001) Las nuevas fronteras tecnológicas: promesas, desafíos y amenazas de los transgénicos. Nº 101 - Serie desarrollo productivo. Naciones Unidas. Santiago de Chile

Received for publication: November 29, 2002
Accepted for publication: January 08, 2003

CORRESPONDING AUTHOR

R. Lajmanovich
Instituto Nacional de Limnología – INALI
José Macias 1933 (3016)
Santo Tomé, (Santa Fe) - ARGENTINA

e-mail: lajmanovich@hotmail.com