Environmental & Analytical
Toxicology

Jofré et al., J Environ Anal Toxicol 2013, 4:1
http://dx.doi.org/10.4172/2161-0525.1000199

Research Article Open Access

Fish Toxicity of Commercial Herbicides Formulated With Glyphosate

Diego Martín Jofré, María José Germanó García, Rodrigo Salcedo, Mirta Morales, Maria Alvarez, Daniel Enriz and Fernando Giannini*

Departamento de Química, Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis, Chacabuco, San Luis-Argentina

Abstract

We report here the acute and chronic toxicity of two commercial formulations of herbicides whose active ingredient is glyphosate (Glacoxan® and Estrella®). The acute toxicity was tested toward two fish species *Danio rerio* and *Poecilia reticulata* by evaluating the mortality. The chronic toxicity was assessed in *D. rerio* by measuring the biochemical parameters glutamic pyruvic transaminase (AST) and glutamic oxaloacetic transaminase (ASL). In addition the analysis of haematological parameters (morphological study) was carried out.

The results indicate that both herbicides produce acute toxicity toward the two tested species. In addition the biochemical parameters displayed high values as a sign of chronic toxicity. Thereby, indicating that both herbicides may produce environmental damage.

Keywords: Fish toxicity; Commercial herbicides; Glyphosate; *Danio rerio; Poecilia reticulata*; Glutamic pyruvic transaminase; Glutamic oxaloacetic transaminase; Haematological parameters

Introduction

The concept "sustainable development" is used worldwide since 1984, this represents the connection between development and several issues such as human resources, food, species, ecosystems, energy and industry. Argentina produces raw materials and agricultural inputs, being soy one of the most important. Much of this production is exported as grain and the Asian countries are the main buyers. Transgenic varieties, such as glyphosate resistant soy, are used to increase the yields and facilitate the agricultural practices; these particular advantages have significantly increased the use of glyphosate formulations in order to control weeds in the soybean crop cycle. This massive and uncontrolled use of this herbicide causes concern in urban and mainly rural population regarding the toxicological risks.

Glyphosate is a highly water soluble substance (10,500 mg/L) with a half-life in water fluctuating between 3.5 and 70 days. Its herbicidal action is due to its ability to inhibit 5-enolpyruvylshikimate-3-phosphatesynthase [1], an enzyme involved in the biosynthesis of aromatic amino acids in plants. Although this substance is an acid, it is commonly used as a salt, such as the isopropylamine salt (IPA) of N-(phosphonomethyl) glycine or the glyphosate isopropylamine salt. There are many commercial formulations commonly characterized by containing 480 g/l glyphosate IPA salt (48%) and the surfactant POEA (polyoxyethylamine), the latter belongs to the family of alkyl polyethoxylated compounds which are synthesized from fatty acids of animals and is thought to be the primarily responsible for the toxic effects of the formulations [2-4]. The strong risk that glyphosate and adjuvants contaminate surface water bodies and groundwater in areas of application constitutes a serious threat to the ecological balance of the regions affected because of their toxic potential to alter the food chain from the first link.

The fish have been used for a long time as experimental biological models to measure the environmental impact of different substances [5,6]. Our research group has used fish to assess the potential toxicity of different natural and/or synthetic novel drugs [7-9].

The main objective of this study was to determine the acute and chronic toxicity toward fish of two commercial herbicides formulated with glyphosate. Two fish species frequently used in these kinds of tests [10], *Poecilia reticulata* "guppies" and *Danio rerio* "zebrafish" were selected as model organisms.

Materials and Methods

Formulations and solutions

We selected two commercial formulations of herbicides present in the local market whose active ingredient is glyphosate, the solution A (Glacoxan®) refers to an herbicide used in gardening; whereas solution B (Estrella®) is an herbicide used in extensive farming practices. Both products were purchased in agrochemistry shops in the province of San Luis, Argentina. The characteristics of these solutions are summarized in Table 1.

Acute toxicity assays

The technique recommended by the U.S. Fish and Wildlife Service [11] has been modified in order to employ a smaller amount of testing compounds as it was already reported by our group [12-14]. Organisms and solutions were placed in a 1 L capacity container. The water level was maintained during the measurements. The essay begins with an initial exposure to potentially toxic agents and continues for 96 h. Every 24 h the number of dead specimens in each container were counted and then removed. The mortality rate was documented at 96 h. The toxic effect was evaluated toward two fish species, *D. rerio* and *P. reticulate*.

Property	Herbicide A	Herbicide B
Gliphosate concentration	48 %	48 %
Active compound	Salt isopropilamine	Salt isopropilamine
Excipients	non informed	non informed
pH	5.5	6
Color	Amber	Amber
Transparency	Transparent	Transparent

Table 1: Characteristics of tested commercial formulations of glyphosate.

***Corresponding author:** Fernando Giannini, Departamento de Química, Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis, Chacabuco 917. (5700) San Luis-Argentina; E-mail: fagian3@gmail.com

Received October 11, 2013; **Accepted** November 28, 2013; **Published** December 02, 2013

Citation: Jofré DM, García MJG, Salcedo R, Morales M, Alvarez M, et al. (2013) Fish Toxicity of Commercial Herbicides Formulated With Glyphosate. J Environ Anal Toxicol 4: 199. doi: 10.4172/2161-0525.1000199

Specimens were bred in our laboratory reaching a maximum size of 1 cm and 15 to 20 days old. Ten fish of each species were exposed to a solution of the tested compound starting from a maximum value of 100 µL/L. The lowest concentration of commercial formulation that produces 100% mortality (LC-100%M) and the maximum concentration that does not produce mortality (MC-0%M) were determined.

Chronic toxicity testing

Specimens of *D. rerio* of 5-6 months were placed in 6 L containers for a period of 21 days. Three specimens were placed in a container (1 specimen per 2 L of water) and kept at room temperature with controlled aeration and standardized food. Each group was exposed to sublethal doses considering as subletal doses the maximum concentration that does not produce mortality (MC-0%M) obtained from acute toxicity tests conducted previously.

A control group no exposed to herbicides was also tested. At the end of the evaluation period 3 specimens of each group were taken for biochemical testing. Fishes were sacrificed with scalpel and their wet weight was determined in precision analytical balance KERN 770. For each specimen a smear of fresh blood at the time of the sacrifice was made and then proceeded to carry out a dilution of 50% p/p (sample fish / saline solution). After that, a macerated in porcelain mortar Coors was performed and the macerated product was centrifuged for 10 min at 1500 rpm in a centrifuge ("Arcane" Macro TDL80-2B). The supernatant was sampled and the following studies were conducted:

a) Biochemical parameters: a colorimetric method was used to determine the activity of glutamic pyruvic transaminase (AST) and glutamic-oxaloacetic transaminase (ASL) in serum using commercial enzymatic kits (transaminases 200, Wiener).

b) Haematological parameters: a morphological study was performed from a blood smear cells using the coloration of May Grunwald-Giemsa [15].

In all cases the results were compared with the control group.

Statistical analyzes

The chi-square method was used in order to compare the mortality rates depicted in the acute toxicity tables, meanwhile the nonparametric Student test Mann-Whitne was used for comparisons between the LC-100%M and MC-0%M.

A nonparametric statistics using the Kruskal Wallis test was used to contrast chronic toxicity results (transaminases); a subsequent comparison using the Dunn's test for a significance level of 95% was also applied. The statistical software used was Statistx 8.0.

Results

Acute toxicity

We have previously shown that differential effects in acute toxicity of commercial formulations of glyphosate and its pure salt against *P. reticulata* occurred [12].

In the present study we observed that for the two fish species, the lowest concentration of the commercial formulation of herbicide A that produce 100% of mortality (LC-100%M) was 100 µL/L and that 50 µL/L was the maximum concentration that does not produce mortality (MC-0%M) (Table 2).

Values of 50 µL/L for the lowest concentration of commercial formulation producing 100% mortality (LC-100%M) and 25 µL/L for the maximum concentration that produce no mortality (MC-0%M) were observed when the herbicide B was tested toward *D. rerio* specimens.

It is evident that there is a differential sensitivity to the herbicide B in both species, being *D. rerio* the most affected; this result are in agreement with previous reports about the effect of other formulations toward different fish species [2, 12].

Statistical analyzes

The acute toxicity difference is significant when compared the concentrations of 100 µL/L and 50 µL/L obtained for the two solutions tested with a $p \geq 0.0001$. It is also significant ($p \geq 0.0022$) the difference observed in the acute toxicity for both species *D. rerio* and *P. reticulata* (LC-0%M= 25 µL/L and 50 µL/L respectively).

Chronic toxicity

These studies were performed taking into account that various toxic effects in organisms do not occur immediately; but rather alter their biology, conditioning their survival chance.

Glutamic pyruvic transaminase (AST) and glutamic oxaloacetic transaminase (ASL)

Transaminases (AST and ASL) are enzymes widely distributed in the body. Their normal activity in serum is low or absent. The destruction of the tissues in which they are present leads to an increase of their presence in serum. In addition these enzymes are inducible by toxicity due to herbicides [16]. In particular, the pyruvic transaminase is an enzyme used to assess liver function, so its high concentration in blood confers some specificity in the diagnosis of toxic liver damage [17].

According to the data of Table 3 it can be concluded that, considering the time and concentration of herbicide exposure, both transaminase values are increased compared to controls although the values corresponding to the specimens treated with the solution of herbicide B are considerably higher than the ones obtained after treatment with herbicide A.

Since the levels of both enzymes can be considered as indicators of toxicity, it is clear that the exposure for a period of 21 days at sublethal doses (MC-0%M) produces chronic toxicity effects toward *D. rerio*, being the herbicide B the most toxic formulation in agreement with the results of acute toxicity tests above described.

Evaluation of herbicides A and B on *Danio rerio*					
Sample	**Solution conc.**	**Percent of mortality**			
		24 hs.	**48 hs.**	**72 hs.**	**96 hs.**
A-100	100 µL/L	100	-	-	100
A-50	50 µL/L	0	0	0	0
B-100	100 µL/L	100	-	-	100
B-50	50 µL/L	100	-	-	100
B-25	25 µL/L	0	0	0	0
Control	Control	0	0	0	0
Evaluation of herbicides A and B on *Poecilia reticulata*					
Sample	**Solution conc.**	**Percent of mortality**			
		24 hs.	**48 hs.**	**72 hs.**	**96 hs.**
A-100	100 µL/L	100	-	-	100
A-50	50 µL/L	0	0	0	0
B-100	100 µL/L	0	25	100	100
B-50	50 µL/L	0	0	0	0
Control	Control	0	0	0	0

Table 2: Acute toxicity tests results.

Specimen	Exposed to	AST (UI/L)	ASL (UI/L)
Control 1	---	42	35
Control 2		50	40
Control 3		45	42
Danio rerio 1	Herbicide A	80	72
Danio rerio 2		103	91
Danio rerio 3		92	87
Danio rerio 1	Herbicide B	158	146
Danio rerio 2		129	130
Danio rerio 3		138	121

Table 3: Biochemical values chronic toxicity assessment.

Statistical analyzes

Significant differences ($p \geq 0.001$) were observed in transaminase activity values. By subsequent comparisons using the Dunn's test, we found differences between the values of the solution prepared with the formulation A and B respect to the control.

Blood smear

This parameter has been reported by several authors as an indicator of toxic effects of the action of herbicides formulated with glyphosate. The study involves morphology analysis of blood cells through microscopic observation of colored preparations, in order to distinguish cytoplasmic structures such as granular appearance, as vacuoles and lysosomes [17].

The analysis of the blood smear performed from *D. rerio* specimens exposed to solutions of the herbicides A, B compared to the controls without herbicides do not show any significative celular morphological change. However, this observation contrasts with other authors reports about experiences performed with other commercial herbicides toward *Cyprinus carpio haematopterus* [12] *and Carassius auratus* [17].

Conclusions

Both tested herbicides formulations, Glacoxan® and Estrella®, present acute toxic effects toward *P. reticulata*, since the lowest concentrations that produce 100% of mortality and the maximum concentration that does not produce mortality are of 100 µL/L and 50 µL/L respectively. Estrella® formulation shows a higher acute toxicity toward *D. rerio* since the lowest concentration that produces 100% mortality was 50 µL/L.

Subletal doses of both formulations accuse chronic liver damage in *D. rerio* without morphological alterations at hematological level. In this case the formulation Estrella® is also more toxic than Glacoxan® in agreement with the differences observed for the acute toxicity.

Considering fish as experimental biological models to measure the environmental impact of different substances, these results indicate that both tested herbicides may produce potential environmental damage.

References

1. Mallory-Smith CA, Ratzinger EJ Jr. (2003) Revised classification of herbicides by sites of action for weed resistance management Strategies. Weed Technol 17: 605-619.
2. Servizi JA, Gordon RW, Martens DW (1995) Acute toxicity of Garlon 4 and Roundup herbicides to salmon, daphnia and trout. Environ Contam Toxicol 33: 355-361.
3. Folmar LC, Sanders HO, Julin AM (1979) Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. Arch Environ Contam Toxicol 8: 269-278.
4. Domínguez Cortinas G, Mejía Saavedra J, Santos Medrano GE, Martínez RR (2008) Analysis of the toxicity of glyphosate and Faena® using the freshwater invertebrates Daphnia magna and Lecane quadridentata. Environ Toxicol Chem 90: 377-384.
5. Ballesteros ML, Wunderlin DA, Bistonia MA (2009) Oxidative stress responses in different organs of Jenynsia multidentata exposed to endosulfan. Ecotox Environ Safe 72: 199-205.
6. Lushchak OV, Kubrak OI, Storey JM, Storey KB, Lushchak VI (2009) Low toxic herbicide Roundup induces mild oxidative stress in goldfish tissues. Chemosphere 76: 932-937.
7. Bisogno F, Mascotti L, Sanchez C, Garibotto F, Giannini F, et al. (2007) Structure-antifungal activity relationship of cinnamic acid derivatives. J Agric Food Chem 55: 10635-10640.
8. Mascotti ML, Enriz RD, Giannini FA (2008) Acute toxicity study of commercial antifungal drugs using Poecilia reticulata. Lat Am J Pharm 27: 904-905.
9. Garibotto FM, Garro AD, Masman MF, Rodríguez AM, Luiten PGM, et al. (2010) New small-size peptides possessing antifungal activity. Bioorg Med Chem 18: 158-167.
10. Slooff W, De Zwart D, Van de Kerkhoff, J (1983) Monitoring the rivers Rhine and Meuse in the Netherlands for toxicity. Aquat Toxicol 4: 189-198.
11. Johnson WW, Finley MT (1980) Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Department of the Interior Fish and Wildlife Service. Resource Publication 137. Washington D.C. United States.
12. Alvarez M, Gimenez I, Saitua H, Enriz RD, Giannini FA (2012) Toxicidad en peces de herbicidas formulados con glifosato. Acta Toxicológica Argentina 20: 5-13.
13. Germano GMJ, Devia C, Morales M, Juarez A, Enriz RD, et al. (2011) Ecotoxicidad de herbicidas comerciales formulados comn glifosato utilizando como modelo experimental Rinhella arenarum. Revista Argentina de Ecotoxicología y Contaminación Ambiental 2: 37-46.
14. Saitua H, Giannini FA, Padilla AP (2012) Drinking water obtaining by Nanofiltration from waters contaminated with glyphosate formulations: process evaluation by means of toxicity tests and studies on operating parameters. Journal of Hazardous Materials 227-228: 204-210.
15. Ióvine Selva- Ióvine (1990) El laboratorio en el diagnóstico de la enfermedad. 6° ed. Editorial Médica Panamericana. Buenos Aires. Argentina.
16. Rip JW, Cherry JH (1976) Liver Enlargement Induced by the Herbicide 2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T). J Agric Food Chem 24: 245-250.
17. Cavas T, Konen S (2007) Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (Carassius auratus) exposed to a glyphosate formulation using the micronucleus test and the comet assay. Mutagenesis 22: 263-268.

Citation: Jofré DM, García MJG, Salcedo R, Morales M, Alvarez M, et al. (2013) Fish Toxicity of Commercial Herbicides Formulated With Glyphosate. J Environ Anal Toxicol 4: 199. doi: 10.4172/2161-0525.1000199