



# Comparative Study of Acute Toxicity of Herbicides Used in South America



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## Abstract

Fish are a reliable biological model to measure the environmental impact of different substances. Argentina is an agricultural producer country where its main crops being grains such as soybeans, wheat and corn; To increase the yield of the crops a large amount of herbicides are used. Among these the most used has been glyphosate, but others such as 2,4D and atrazine are also used. We carried out in this work two types of acute toxicity comparative studies for three commercial herbicides formulated with glyphosate, 2-4D and atrazine. Firstly, the acute toxicity evaluation was carried out against *Poecilia reticulata*, measuring the mortality of juveniles species in an exposure period of up to 96 hours; determining for glyphosate that a dose of 12mg/L produced 100% mortality and that 6mg/L did not cause mortality, thus differing from 2, 4D and atrazine, which at a concentration of 116.8mg/L and 48mg/L respectively did not cause mortality. In the second place, an embryo hatching study was carried out in *Danio rerio* following methodologies proposed by the OECD (Guidelines for the Testing of Chemicals) for sublethal doses of the herbicides (6mg/L), none of them having any effect on the hatching of the eggs.

**Keywords:** Herbicides; Glyphosate; 2,4D; atrazine; Fish; Acute toxicity

**Abbreviations:** 2,4 D: Ácido 2,4-Diclorofenoxiacético

## Introduction

Fish have been used in toxicity studies since 1950 [1,2], demonstrating over time a reliable model for measuring the environmental impact of different substances [3], our work group has used an adaptation of the proposed technique by Johnson et al. [4] in several studies [5-7]. Argentina is a producer of agricultural commodities, its main crops being grains such as soybeans, wheat and corn. To increase the yield of the crops a large amount of herbicides are used. Among these the most used has been glyphosate, but others such as 2,4D and atrazine are also used; the amounts of herbicides discharged into the environment are alarmant [8] and has led to the need for realization studies of the impact of the same using different bioindicators. We present in this work two types of acute

toxicity studies evaluating three commercial herbicides formulated with glyphosate, 2,4D and atrazine, using as experimental models fish of the species *P. reticulata* and *D. rerio*.

## Discussion

First, the acute toxicity evaluation of the commercial formulations of the three herbicides against *Poecilia reticulata* was carried out, according to the Johnson and Finley adapted technique that measures the mortality of the specimens in a period of 96 hours; The evaluated forms were: Round-Up (glyphosate); *Atrazine* 90WG Equipagro (*Atrazine*) and Herbifen Amina TD (2,4 D). The results obtained are presented in Table 1.

**Table 1:**

Sample	Concentration of Solution	Concentration of Herbicide	% of Mortality 96Hs.
Glifosato	25µL/L	12mg/L	100
Glifosato	12.5µL/L	6mg/L	0
atrazine	2.4µL/L	48mg/L	0
2,4D	200µL/L	116.8mg/l	0
Control	-	-	0

The data of the initial values to be evaluated for the solution of Round UP (glyphosate) were taken from previous studies carried out by our working group [9], determining that a dose of 12mg/L of active ingredient produced 100% mortality and that 6mg/L did not cause mortality. For the other two herbicides, it was started to be evaluated at equal concentrations of active principle (12mg/L and 6mg/L) and thus it was increased until reaching a value of 116.8mg/L for 2,4D and 48mg/L for atrazine, concentrations at which no mortality was observed, demonstrating a large acute toxicity difference of the commercial glyphosate formulation compared to the other two. As an additional observation it can be men-

tioned that at concentrations of 24mg/L and 48mg/L of atrazine produced a decrease in the mobility of the specimens. Next, an embryo hatching study was carried out in *D. rerio* following methodologies proposed by the OECD (Guidelines for the Testing of Chemicals) for sublethal doses of the herbicides, evaluating for all cases a concentration of 6mg/L [10]. the eggs were observed from the time 0 of harvest until 96 hours, at which time they hatched (Figure 1a,1b& 1c) showing none of the herbicidal effects in the hatching of the eggs but in the viability of the eggs. fry, producing the mortality of the same in the glyphosate atrazine, both in the original and in the two replicas.



Figure 1a: Embryo of 0 to 24hrs.



Figure 1b: Embryo of 48 to 72hrs.



Figure 1c: Embryo of 96hrs.

### Conclusion

Of the three evaluated herbicides, the commercial formulation that possesses glyphosate as an active ingredient is the most toxic against *P. reticulata* according to the applied methodology, the other two herbicides did not produce mortality even at much higher values, but for the case of atrazine it could be observed that high doses decreased the mobility of the fish, being this a limitation for the survival of the same in the natural environment. In the case of *D. rerio* egg hatching, it was observed that no herbicide is prevented from hatching at the evaluated dose; but the glyphosate produces the mortality of the fry once hatched. These results warn about the effects of the application of herbicides on bioindicator organisms and justify the need for a redirection of crops towards more sustainable methodologies.

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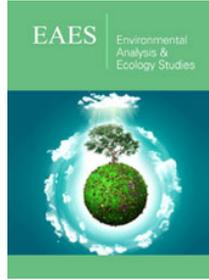
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