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Effects of Technical Grade and a Commercial Formulation of Glyphosate on Algal Population Growth

M. E. Sáenz, W. D. Di Marzio, J. L. Alberdi, M. del Carmen Tortorelli

Ecotoxicology Research Program, Department of Basic Sciences, National University of Luján, P.O. Box 221 (6700) Luján (B), Argentina

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Glyphosate [N-(phosphonomethyl) glycine] (Gly) is a broad spectrum, non-selective, post-emergence herbicide. The isopropylamine salt of glyphosate is the active ingredient of Ron-do®, a formulation employed extensively for aquatic grasses, broadleaf weeds and brush control.

The specific site of action of Gly at the molecular level has been identified. Glyphosate inhibits the activity of 5-enolpyruvyl shikimic acid-3-phosphate (EPSP) synthase, an enzyme of the shikimic acid pathway, in a wide variety of plants and organisms (Duke 1988). This results in the cessation of aromatic amino acid synthesis, followed by reduced protein synthesis, growth, and premature cellular death (Lydon and Duke 1988).

Glyphosate is biodegraded aerobically and anaerobically by microorganisms present in soil and water, so it is considered not to be an environmentally persistent herbicide (Duke 1988). The minimum half-life observed in aquatic environments has been two weeks, while in non-flowing natural water systems it has been from seven to ten weeks (Reinert and Rodgers 1987).

Glyphosate may enter into aquatic systems from treated terrestrial land through surface runoff movements, spray drift, or direct overspray applications. Similarities between the physiology of higher plants and phytoplankton in aquatic environments surely will cause adverse effects on the latter non target primary producers. Phytoplankton in streams and ponds form an integral part of the aquatic food web providing food for larger organisms such as zooplankton and fish. Several studies indicate that this herbicide is moderately toxic to aquatic flora and fauna (Becerril et al. 1989; Lockhart 1989; Goldborough and Brown 1988; Sullivan et al. 1981; Peterson et al. 1994).

The present paper reports the results of a study about the effects of technical grade glyphosate and the commercial formulation Ron-do®, on population growth and chlorophyll "a" content of the common freshwater green algae, *Scenedesmus acutus* and *Scenedesmus quadricauda*.

Correspondence to: M. E. Sáenz

MATERIALS AND METHODS

Toxicity test of 96 hr of exposition (USEPA 1989) was conducted using as test organisms *S. acutus* (SAG N° 276-3a) and *S. quadricauda*. *Scenedesmus acutus* was an axenic strain which was obtained from the Culture Collection of Algae, University of Göttingen, Germany. *Scenedesmus quadricauda* was isolated from water samples of Luján river, before the input of the effluent from the sewage treatment plant of Luján city. It is one of the largest rivers in the West of Buenos Aires Province, running across cities which have important industrial establishments. There is also agricultural activity, which deals with the use of great amounts of pesticides for weeds and pests control.

Algal stock cultures were initiated in aseptic conditions. They were maintained in autoclaved modified Detmer's nutrient medium (Accorinti 1962) (pH: 7.5, conductivity: 410 mμhos/cm, total hardness: 80.1 mg $CaCO_3$/L and dissolved oxygen at saturation) under controlled conditions in a climatized room at 22 °C ± 1 °C, 3000 lux/cm^2 of continuous "cool-white" fluorescent lighting and at 100 excursions/min on a shaker (Sáenz et al. 1992; 1993). The inoculum of both species was prepared from a five-days stock culture, with enough cells in order to provide an initial cell density of 5 x 10^4 cell/mL in control and treated flasks. The test solutions consisted in modified Detmer's nutrient medium with the addition of different concentrations of toxicants. The toxic substances were Gly technical grade (isopropilamine salt with 99.5 % of purity) and Ron-do formulation (48 % Gly as active ingredient and 15 % of surfactant oxide-coco-amide-propyl dimethyl amine). The concentrations used in Gly technical grade toxicity test were for *S. acutus* 276-3a: 6, 8, 10, 12, 14, 16, 18 and 20 mg Gly/L and for *S. quadricauda:* 3.1, 6.2, 12.5, 25, 50 and 100 mg Gly/L. The concentrations used in the toxicity assessment of Ron-do formulation, expressed as mg of active ingredient, were for *S. acutus* 276-3a: 5.1, 6.4, 8, 10 and 12.5 mg Gly/L and for *S. quadricauda:* 2.5, 5, 10, 20 and 40 mg Gly/L. Control and treated cultures were grown under the same conditions of temperature, photoperiod and shaking that of the stock cultures and were done in duplicate. Cell counts were correlated with absorbance (A 750 nm) on a Shimadzu MUV 240 spectrophotometer (Walsh 1988; Lorenzetti 1989).

Control cultures with the same cellular density as the treated ones were prepared, in order to determine the effect of Gly on chlorophyll "a" content. The concentrations of toxicant were chosen according to the inhibition effect observed. These were 8, 12 and 16 mg Gly/L in the case of *S. acutus* 276-3a, corresponding to 10, 76 and 89 % inhibition of growth with respect to the control. For *S. quadricauda* these concentrations were 3.2, 12.5, 25 and 50 mg Gly/L, corresponding to 15, 60, 75 and 80 % inhibition of growth. Content of chlorophyll "a" was determined in each control and its respective treated cultures as described in Sáenz et al. (1993). The differences in pigment concentration between each control and its treated culture was statistically evaluated through t- test ($p < 0.05$). The observed differences between control and treated flasks were evaluated through one-way statistical analysis of variance ($p < 0.05$) in conjunction with

Tuckey's multiple range test (Steel and Torrie 1960). These analyses were done with TOXSTAT® version 3.5 (West Inc and Gulley 1996). The EPA probit analysis version 1.4 was employed to calculate the EC_{50} values and 95 % confidence intervals. Sperman - Karber's method was employed when the probit analysis couldn't be performed. Dunnett's procedure was used to calculate the NOEC (highest concentration of toxicant in which the values for the observed parameters are not statistically significantly different from the control) and LOEC (lowest concentration of toxicant in which the values for the observed parameters are statistically significantly different from the control) values. Chronic values (ChV) and MATC (toxicant concentration that may be present in a receiving water without causing significant harm to productivity or other uses) were calculated according U.S. Environmental Protection Agency (USEPA 1989) and American Public Health Association (APHA 1992). The EPA program version 1.61 was used to determine statistical differences between the 96 hr EC_{50} values

RESULTS AND DISCUSSION

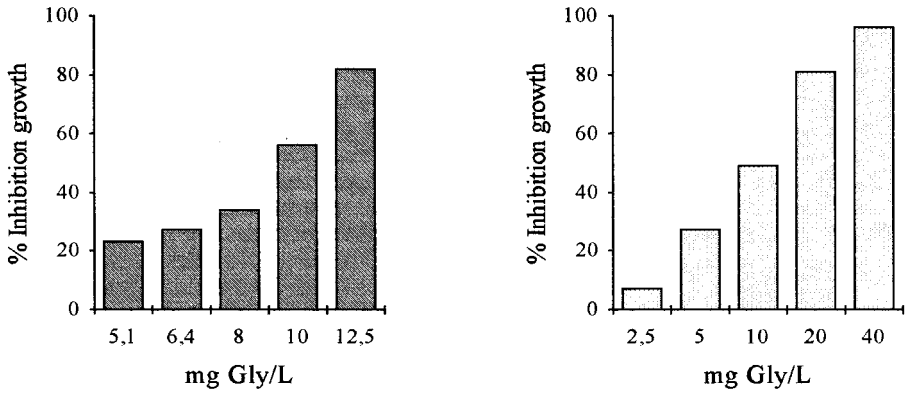
The assessment of the toxic effects of Gly on *S. quadricauda* indicated a significant decrease in population growth, with respect to the control in the cultures exposed at all assayed concentrations at 48 hr. At this time, there wasn't a significant difference between the growth of the cultures exposed at 12.5 and 25 mg Gly/L. By 72 hr, all concentrations of toxicant including the lowest, exhibited significant reduction in culture growth, with respect to the control. The cultures exposed to 100 mg Gly/L showed a total inhibition growth along the test duration. The 96 hr EC_{50}, NOEC, LOEC and ChV endpoints of Gly are shown in Table 1.

Table 1. 96 hr toxicity endpoints (mg Gly/L) of Gly and Ron-do formulation to both *Scenedesmus* species. (in brackets confidence limits).

	S. acutus		*S. quadricauda*	
	Gly	Ron-do	Gly	Ron-do
NOEC	2	3.2	0.77	1.25
LOEC	4	4.08	1.55	2.5
ChV	2.82	3.61	1.09	1.76
96 hr EC_{50}	10.2 * (10.4 - 11.2)	9.08 (8.4 - 9.7)	7.2* (4.4 - 8.9)	9.09 (8.06 - 10.2)

* significant difference (see explanation on the text).

The experiences carried out with Ron-do formulation showed that, at 48 hr of exposition, there was a significant inhibition with respect to the control of the culture exposed from 20 mg Gly/L and higher. At the end of the test, all



Scenedesmus acutus 276-3a *Scenedesmus quadricauda*

Figure 1. Response of 96 hr % inhibition growth with respect to the control to different concentrations of Ron-do of both species.

concentrations caused a significant inhibition of the culture growth with respect to the control, except those exposed to 2.5 and 5 mg Gly/L. The cultures exposed to 40 mg Gly/L showed a notable inhibition of growth at 96 hr of exposure (Fig 1). The 96 hr EC_{50}, NOEC, LOEC and ChV endpoints of Ron-do are shown in Table 1.

Scenedesmus acutus 276-3a showed a different behavior in the presence of Gly respect to *S. quadricauda*. A very closed interval was used between the concentrations assayed. At 48 hr, the cultures exposed from 6 to 10 mg Gly/L did not show a significant inhibition of growth with respect to the control. The growth of the cultures exposed from 12 to 18 mg Gly/L, was significantly different from the control, but there weren't differences between them. At this time, the culture exposed to 20 mg Gly/L showed a total growth inhibition. At the end of the test, all the exposed cultures showed a significant difference in growth with respect to the control (Fig 2). The culture exposed at 20 mg Gly/L showed a slight growth, which began after the 72 hr. The 96 hr EC_{50}, NOEC, LOEC and ChV endpoints for Gly are shown in Table 1.

In Ron-do experiences, an increase in toxicity was noticed as the exposition time increased. At 96 hr, all the assayed concentration exerted a significant inhibition of growth, with respect to the control. The cultures exposed to the highest concentration (12.5 mg Gly/L) showed a notable decrease on growth since the beginning, being more than 80 % respect to the control growth at the end of the test (Fig 1). The 96 hr EC_{50}, NOEC, LOEC and ChV endpoints of Ron-do are shown in Table 1.

It is documented that glyphosate active ingredient has relatively strong effects on chlorophyll synthesis. Glyphosate inhibits the synthesis of the chlorophyll precursor

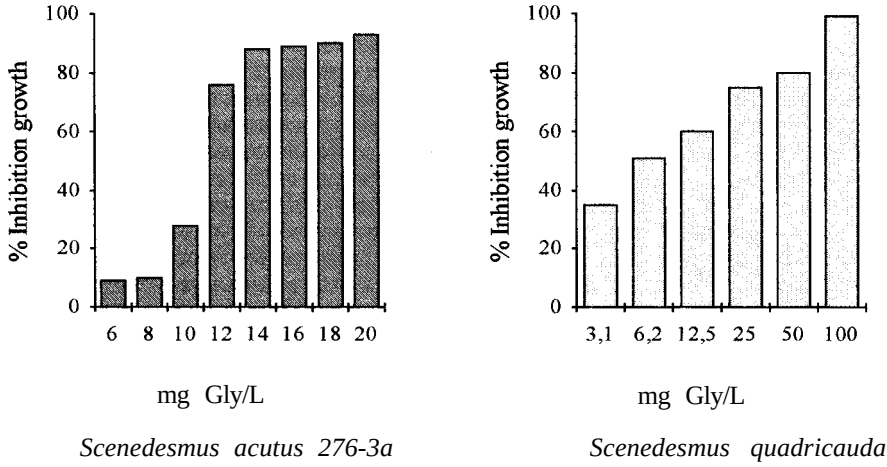


Figure 2. Response of 96 hr % inhibition growth with respect to the control to different concentrations of Gly of both species.

5-aminolevulinic acid (ALA) (Duke 1988). The concentrations chosen in the assessment of the effects of Gly on chlorophyll "a" content had exerted, as mentioned above, adverse effect on algal population growth of both species. In *S. quadricauda* was registered a decrease in amount of chlorophyll "a" between each control and cultures exposed to 3.2, 12.5, 25 mg Gly/L respectively, but this difference was not statistically significant. The cultures exposed to 50 mg Gly/L showed a significant decrease in chlorophyll "a" content, with respect to its control value (Fig. 3). In the case of *S. acutus*, the chosen concentrations of Gly that is 8, 12 and 16 mg Gly/L, exerted a slight decrease in chlorophyll "a" content when compared each control culture with the treated ones, but they weren't statistically significant (Fig 3).

There was a significant difference between Gly 96 hr EC_{50} values of both species, indicating a major sensitivity to *S. quadricauda.* In this case, there was a different response in the presence of Gly between both species, since a close interval between concentration assayed was needed to know the effects of Gly upon *S. acutus* (Fig 2). In spite of the differences observed about the percentage inhibition of growth with respect to the control from both species exposed to concentrations of Ron-do (Fig l), there wasn't a significant difference between the respective 96 hr E C_{50} values (Table 1). In the case of *S. acutus,* 96 hr EC_{50} values from Gly and Ron-do were statistically different, which indicated that Ron-do formulation was more toxic than the active ingredient as it has been found for other organisms such as microcrustaceans and fish (Alberdi et al. 1996). According to chronic toxicity endpoints from Table 1, Gly would be more harmful for the population development of these species than Ron-do.

Peterson et al. (1994) have estimated the Expected Environmental Concentrations (EEC) for 23 pesticides. For glyphosate formulations the EEC result was to be 2.848

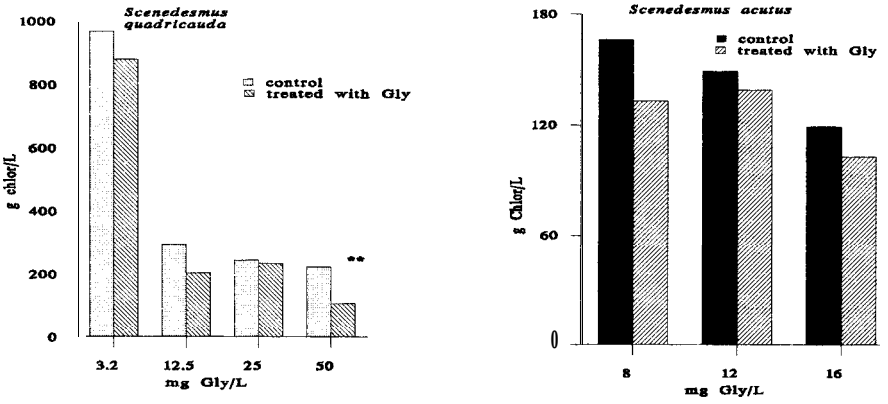


Figure 3. Effect of the chosen concentrations of Gly on chlorophyll "a"content of both species. ** significantly different ($p < 0.05$). (Control cultures with the same cellular density of the treated ones).

mg Gly/L. The 96 hr EC_{50} found for both species were higher than this value, but the NOEC and LOEC of Ron-do to *S. quadricauda* are under the EEC (Table 1). The concentration of glyphosate formulation found in the aquatic environments wouldn't cause acute effect to both species, but it could be important to consider the chronic and sublethal effects of glyphosate formulation on *S. quadricauda* natural populations, by virtue of the ChV values (Table 1).

We conclude that there was a difference in sensitivity between species for Gly. On the contrary, in Ron-do experiments, there was not a difference in sensitivity between species.

About the effect of Gly on chlorophyll "a" content, only in the case of *S. quadricauda* cultures exposed at 50 mg Gly/L was found a significant decrease.

With regard to the ecotoxicological risk assessment for the development of *S. quadricauda* under environmental conditions, the NOEC, LOEC and ChV values are under the EEC, so the use of glyphosate formulation in aquatic environments may cause harmful effects on long-term development of *S. quadricauda* natural populations. These endpoints would not indicate harmful chronic effects in the case of *S. acutus* 276-3a, although NOEC (3.2) is very close to EEC (2.848).

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