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Ecotoxicity of the herbicide glyphosate on four chlorophyte sweet water algae.

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ABSTRACT

Ecotoxicity of herbicide Glyphosate to four chlorophyceaen freshwater algae

The increasing use of glyphosate in Argentina is directly related to the increase of areas cultivated with a glyphosate-tolerant transgenic variety of soybean. That has raised concern about the effects of this herbicide in the pampean aquatic ecosystems. Hence, the ecotoxicity of pure (active ingredient) and commercial grade (Roundup) of the herbicide glyphosate was evaluated towards four green freshwater algae: *Scenedesmus acutus*, *Scenedesmus quadricauda*, *Chlorella vulgaris*, and *Raphidocelis subcapitata*. Toxic effects of glyphosate were assessed on both short-term (photosynthetic rates, determined as oxygen production) and at long-term (growth of the populations, determined as number of cells). A stimulation of the photosynthetic rate was observed at low herbicide concentrations (hormesis). Pure grade short-term effects on the photosynthetic rates appeared at concentrations between 50 and 166 mg L^{-1} , whereas long-term effects on growth appeared in the 1.55-4 mg L^{-1} range. The commercial grade resulted more toxic than the pure grade; its long-term effects appeared at concentrations between 0.1-3.7 mg L^{-1} . These concentrations are clearly below the expected environmental concentrations (EEC) for this herbicide. Recovery experiments showed that both the pure grade and the commercial grade had algistatic and not algicidal effects. Possible effects and implications at algal community level regarding competitiveness are discussed.

Key words: Toxicity, herbicides, algae, *Scenedesmus* sp, *Chlorella vulgaris*, *Raphidocelis subcapitata*, photosynthesis, hormesis.

SUMMARY

Ecotoxicity of the herbicide Glyphosate on four freshwater chlorophyte algae

The increase in the use of glyphosate in Argentina is directly related to the increase in areas cultivated with a glyphosate-tolerant transgenic soybean variety. This fact has increased concern about the effects of this herbicide on the aquatic ecosystems of the Pampas region. Thus, the ecotoxicity of the herbicide glyphosate in its pure form (active ingredient) and its commercial formulation (Roundup) was evaluated towards four freshwater green algae: Scenedesmus acutus*,* Scenedesmus quadricauda*,* Chlorella vulgaris *and* Raphidocelis subcapitata*. The toxic effects of glyphosate were evaluated for both short-term toxicity (photosynthetic rate, determined as oxygen production) and long-term toxicity (population growth, determined as cell number). A stimulation of photosynthetic rate was observed at low concentrations of the herbicide (hormesis). The short-term effects of the active ingredient*

The long-term effects on photosynthetic rate appeared at concentrations between 50 and 166 mg L^{-1} while the long-term effects on growth appeared in the range of 1.55-4 mg L^{-1} . The commercial formulation was more toxic than the pure active ingredient; its long-term effects appeared at concentrations between 0.1 and 3.7 mg L^{-1} . These concentrations are clearly below the Expected Concentration in the Environment (CEA) for this herbicide. The experiences of recovery of the herbicide

The results of this study showed that both the pure active ingredient and the commercial formulation exerted algaecidal and non-algaecidal effects. Possible effects and implications at the algal community level in relation to competitive ability are discussed.

Key words: *toxicity, herbicides, algae,* Scenedesmus sp, Chlorella vulgaris, Raphidocelis subcapitata, *photos'ınthesis, hormesis.*

INTRODUCTION

Agricultural and livestock activities are the most important in Argentina both historically and economically. Due to the increase in agricultural activity in recent years, especially related to the increase in the areas destined to the cultivation of transgenic soybeans, significant quantities of different phytosanitary chemicals are being released into the environment. Among herbicides, glyphosate is the most widely used, with 170 and 146 million liters/kg in 2006 and 2007, respectively. Based on these data, the study of their impact on aquatic ecosystems is of fundamental importance. This study focuses on the short- and long-term effects that exposure to glyphosate will have on the primary producers of phytoplankton components. This aspect is remarked in the US EPA glyphosate registration manual (1993), where it concludes that further studies on this group of organisms are necessary since adverse effects are to be expected.

The routes of entry of this compound into water systems can occur by direct application on the ground, with subsequent drift influenced by local characteristics related to wind orientation and intensity, and by aerial applications, in the case of large extensions. In the latter case, the magnitude of drift to water systems is greater. On the other hand, there is transport to aquatic environments by drainage and runoff from treated soils.

Glyphosate (N-(phosphonomethyl) glycine) (molecular formula: $C\ H_{38}\ NO_5\ P$) is the most widely used active ingredient in broad-spectrum, fast-acting, low-cost herbicides. It is applied by spraying on the leaf surface. According to CASAFE (2007), the most used formulation is 48% of active ingredient, occupying the first place among the 30 most used phytosanitary products (82.3%).

By virtue of the water solubility and the ionic characteristics of glyphosate, no bioaccumulation phenomena are likely to occur. This has been confirmed by studies conducted in field conditions on fish, crustaceans and mollusks (WHO, 1994). Glyphosate is considered to dissipates rapidly in aquatic environments (WHO, 1994). The site of action of the herbicide is the inhibition of the enzyme EPSP (5-enolpyruvylsikimate-3-phosphate synthetase), an enzyme of the sik'ımic acid cycle, one of the two metabolic pathways in the synthesis of aromatic amino acids. This enzyme is found in plants and bacteria, but is not found in animals. Although it is not considered a herbicide that exerts direct action on photosynthesis reactions, there are some studies that report that it will inhibit electron flow in photosystem II (Duke, 1988). It has been shown to exert important effects on chlorophyll synthesis by inhibiting the synthesis of the precursor of chlorophyll synthesis, 5-aminolevulinic acid (ALA) (Duke, 1988). This would cause secondary effects such as pigment bleaching, leading to altered chloroplast formation. Although the effects on protein synthesis would be indirect, they would be responsible for the development of subsequent damage caused by this herbicide. Similarly, the observed effects on nucleic acid synthesis would be due to a general dysfunction of the cell associated with the inhibition of protein synthesis. In this same context, abnormal mitosis and ultrastructural effects such as rupture of the chloroplast envelopes, disintegration of the mitochondria and separation of the plasmalemma from the cell wall have been observed (Duke, 1988).

Studies have been conducted on the effect of glyphosate active ingredient on the growth of *Cyanophyte* species such as *Anabaena-flos-aquae*, on Chlorophytes such as *Raphidocelis sub- capitata* and *Chlorella pyrenoidosa* and on Dia- tomeas such as *Skeletonema costatum* and *Navicula pelliculosa*. These studies have shown differences in sensitivity between the different groups, with EC $values_{50}$ ranging from 1.2 to 7.8 mg glyphosate/l and NOEC values ranging from 0.3 to 34 mg glyphosate/l (Pipe, 1992; WHO, 1994).

One way to study the direct and indirect effects of glyphosate together is the analysis of its effects on growth, since this is an integrative process that combines different cellular reactions. Although

glyphosate is not considered an inhibitor of photosynthesis reactions, some studies have shown its [illegible]on electron transport at the photosystem II level (Duke, 1988). Evaluations were carried out to establish the effect of different concentrations of the pure herbicide product on the photosynthetic rate of the selected algal species. In order to know the environmental risk associated with terrestrial applications, one of its commercial formulations (Round-up ®) was also evaluated, determining the
[illegible]n algal growth and algae capacity
[illegible]f the treated populations.

MATERIALS AND ME[illegible] ALL

Organizations

The four algal species used belonged to the D[illegible] Chlorophyta. These were: *Scenedesmus quadricauda* (isolated from samples collected at the headwaters of the Luja'n River by isolation and p[illegible] techniques [illegible] to Sa'enz, 2000), *Scenedesmus acutus* (SAG 273-3a), *Chlorella vulgaris* (Companhia de Saneamento Ambiental do Estado de Sa˜o Paulo, Brazil, CETESB) and *Raphidocelis subcapi- tata* (ESE L1, Metz, France). The mentioned strains are currently kept in the Cepario of the Ecotoxicology Research Program of the National University of Luja'n, Province of Buenos Aires, Argentina.

Chemical substances

Technical grade glyphosate (95 % glyphosate) in the form of isopropylamine salt and the commercial formulation Round-up® containing 48 % glyphosate as active ingredient and a polyethoxylated amine (POEA) as surfactant were used.

Effects on algal growth

Algal growth assessments were conducted following US EPA (1996) protocols. The tests consisted of 96 hours of [illegible] to an initial algal density of
50,000 cells/ml at various concentrations of the active ingredient and the commercial formulation of glyphosate. In the case of the active ingredient, the following exposure concentrations were used: 1; 2.5; 6.2; 15.6; 39 and 97 mg/l for *Raphi- docelis subcapitata* and *Chlorella vulgaris* and 0.77;
1.55; 3.1; 6.2; 12.5; 25; 50 and 100 mg/l for *Scene- desmus quadricauda* and *Scenedesmus acutus*. For the commercial formulation, the concentrations of 0.3; 1.1; 3.7; 12.3 and 41.4 mg/l were used for all species. Cultures were incubated under the same conditions in the absence of toxicant, which were considered as controls. The incubations were carried out in a Forma incubation chamber with programmable temperature, photoperiod and [illegible]lgal biomass was determined by measuring chlorophyll *a* concentration *in vivo* by fluorometry using a Turner model TD 700 fluorometer (New York, USA). The determinations were carried out every 24 hours. The assays were performed twice and three replicates of each [illegible]nd the controls were performed. At the end of the incubations, observations were made under an optical microscope with phase contrast.

The procedure of Payne & Hall (1979) was used to differentiate algaecidal (growth [illegible] from algaecidal (cell death) effects. In summary, after the 96 h contact period, the exposed cultures were centrifuged (10 min, 1000 x g) and the pellet resuspended in nutrient medium in the absence of toxicant. Algal growth was observed during a 10-day [illegible]eriod that was performed under the same experimental conditions as the contact period. During this period, the number of cells was counted on days 3, 5, 7 and 10 (Rand, 1995; US EPA, 1996; Sa'enz, 2000).
Algal effects were considered to be those that prevented a net change in the number of algal cells during the contact period, but allowed a [illegible] and return to exponential growth in the [illegible]eriod. The algistatic concentration was the concentration of herbicide that caused a temporary inhibitory effect at the cellular level, but did not cause permanent or irreversible damage to the algal [illegible]t was determined by performing a [illegible]between the logarithm of the quotient between the

The algae cell numbers at the end of the contact period and the initial and logarithm of the herbicide concentrations tested [illegible]to Payne & Hall (1979) and Ricker (1973). Hall (1979) and Ricker (1973) Algaecidal effects were considered as those that prevented a net change in the number of algal cells during the contact period, and did not allow a [illegible]and return to exponential growth in the [illegible]

Effects on photosynthetic rate

The action on photosynthetic rate was evaluated from the analysis of the change in oxygen concentration using Clark oxygen microelectrodes (Walker, 1987). These effects were studied only for the active principle. The algal cultures used in these studies were maintained in a climate-controlled chamber (Walker, 1987).
da at 20° C ± 0.5° C, with a luminous intensity of 50 Watt/m^2 under a 14-hour photoperiod.
light/10 hours of darkness and in an orbital shaker at 100 rpm continuously. Previously, the light intensity, cell density and age of the optimal cultures for [illegible] evaluations were determined. Light [illegible]were obtained for each of the species used (Hall & Rao, 1992). A Licor model LI 185 [illegible]meter was used for irradiance determinations. The photosynthetic rate of control cultures (in the absence of [illegible] was determined and the percentage of inhibition or stimulation of the photosynthetic rate was calculated in order to obtain the dose-response curves. The exposure was 40 to 60 minutes. The experiments were carried out in duplicate. A detailed [illegible]f the methods and procedures used in the [illegible] the experimental conditions are described in Sa'enz (2000) where the methodology and measurement intervals are included. The [illegible]f this methodology in the [illegible]f the effects of herbicides on green algae is detailed in Mingazzini *et al.*

Data analysis

The analysis of the differences observed between the treatments and the controls was carried out using the following methods a one-factor ANOVA analysis of variance combined with Dunnet's test. The criteria of normality (chi-square) and homogeneity of variances (Bartlett) were met. The [illegible]of the different concentrations of the [illegible]ubstances was evaluated with Tuckey's multiple comparisons post ANOVA test. Both analyses were performed with the TOXSTATVersion program.
3.5 (West Inc & Gulley, 1996). The variables used were: chlorophyll *a* [illegible] the case of the evaluations on algal growth and millimoles of oxygen produced per minute in the case of the evaluations on photosynthetic rate. Differences were considered significant at a 95 % confidence level ($p < 0.05$). The values of EC_{50} (effective concentration) were estimated by probit analysis. Statistical comparisons between$_{50}$ -96 hr ECs were performed by StudentÕs t-tests (effects on photosynthetic rate) while comparisons between$_{50}$ -96 hr ECs from more than two species (effects on algal growth) were performed by analysis of variance (Sprague, 1990).

The Dunnet test (EPA, 1996) was used to [illegible]e LOEC (lowest effective concentration) and NOEC (non-effective [illegible]The chromatic value (ChV) was estimated as the geometric mean between the NOEC and LOEC (EPA, 1996).

RESULTS

Effects on algal growth

Effects of glyphosate active ingredient

At the end of the evaluations, it was observed that *Scenedesmus quadricauda* populations exposed to 0.77 mg Gli/l did not show significant growth inhibition compared to the controls. However, those exposed to concentrations greater than and equal to 1.55 mg Gli/l experienced significant growth inhibition. In the case of *Scenedesmus acutus,* glyphosate concentrations of 8 to 20 mg Gli/l caused a significant inhibition of algal growth compared to controls. The glyphosate

Toxicity indices (mg Glyphosate/l) estimated on the basis of population growth after 96 hours of exposure to the active ingredient glyphosate. * 95% confidence intervals. *Toxicity indexes (mg Gli/l) estimated based on population growth after 96 hrs of exposure to the active ingredient glyphosate. * 95 % confidence intervals.*

	Scenedesmus quadricauda	*Scenedesmus acutus (SAG 276-3a)*	*Chlorella vulgaris*	*Raphidocelis subcapitata*
EC_{50} - 96 hours	7.2 (4.4 - 8.9)*	10.2 (9.5 - 11.4)	13.1 (11.4 - 15.0)	11.66 (9.9 - 13.6)
NOEC	0.77	2	2.5	1
LOEC	1.55	4	5	2.5
ChV	1.09	2.82	3.53	1.58

phosphate similar effects on *Chlore-*
exercise'.
lla vulgaris. Concentrations above 5 mg Gli/l caused significant growth inhibition. *Raphidocelis subcapitata* responded similarly to concentrations higher than 2.5 mg Gli/l. At 97 mg Gli/l and above, an almost total inhibition of growth was observed in the exposed populations. The algae species studied showed different sensitivities to the [illegible]f glyphosate in the form of active ingredient. Table 1 shows the indices for each of the species mentioned, indicating a higher sensitivity (significant difference ANOVA $p < 0.05$) of *Scene- desmus quadricauda*, while *Scenedesmus acutus*, *Chlorella vulgaris* and *Raphidocelis subca- pitata* showed a similar sensitivity (non-significant differences ANOVA $p < 0.05$).

Effects of commercial formulation

The results obtained in the evaluations carried out with the commercial formulation Round-up○R determined a more severe action on algal growth than the active ingredient. *Scenedes- mus quadricauda* was severely inhibited at higher and equal [illegible]oncentrations.

The inhibition was significant in populations exposed to 0.1 mg Gli/l in the form of a commercial formulation, at 96 hours of [illegible] *Scenedesmus acutus* presented a significant inhibition in the populations exposed to concentrations higher than and equal to 0.9 mg Gli/l at the end of the same period. In the case of *Chlorella vulgaris*, it was observed that the concentrations that exerted a significant inhibition of growth were higher than and equal to 1.1 mg Gli/l. *Raphidocelis subcapitata* was significantly

Inhibited in the presence of higher concentrations of

3.7 mg Gli/l or more.

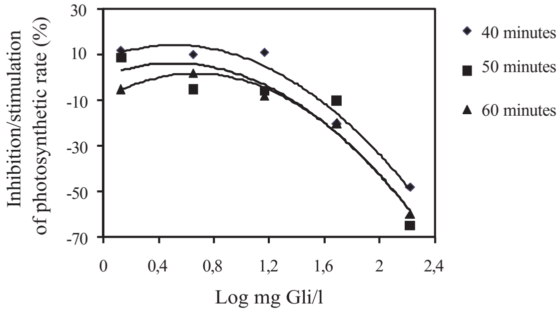
The estimation of toxicity indices indicated a higher sensitivity of *Scenedesmus* species, compared to the other two species used, for both short-term and long-term toxicity (Table 2).

Growth recovery

Glyphosate as active ingredient and the commercial formulation Round-up exerted algistatic effects on the species *Scenedesmus qua-dricauda*, *Scenedesmus acutus*, *Chlorella vulga- ris* and *Raphidocelis subcapitata*. H[illegible]r the populations that were severely inhibited by the [illegible]n of the different concentrations of the herbicide, both in the form of active principle and commercial formulation, recovered their exponential growth after ten days of the [illegible]eriod. Estimates of algal concentrations for glyphosate active ingredient were found to be between 10.07 and 43.2 mg Gli/l, while for the commercial Round-up formulation this range was between 3.36 and 62.5 mg Gli/l.

Toxicity indices (mg Gli/l) estimated after 96 hours of exposure, for the commercial formulation Round-up□R . * 95% confidence intervals. *Toxicity inde- xes (mg gli/1) estimated after 96 hrs of exposure, for the Round-up□R Commercial formulation. * 95 % confidence intervals*

	Scenedesmus quadricauda	*Raphidocelis subcapitata*
EC_{50} -50 minutes	120 (100 -141)*	154 (122 -194)
NOEC	15	50
LOEC	50	166
ChV	27.3	91.10



Effect of glyphosate on the photosynthetic rate of *Scenedesmus quadricauda*. *Effect of glyphosate on* Scenedes- mus quadricauda *photosynthetic rate.*

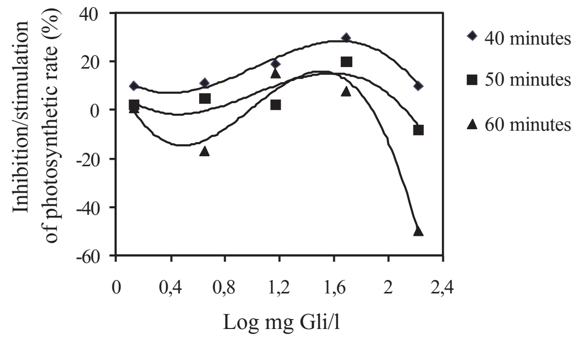
Effects on photosynthetic rate

The active ingredient glyphosate exerted a stimulating effect on the photosynthetic rate of *Scenedesmus quadricauda* populations after 40 minutes of [illegible]t concentrations lower or equal to 15 mg Gli/l. With increasing [illegible]ime, against the same range of concentrations, the [illegible]ffect was not observed but, on the contrary, an inhibition of 5 % was produced, not significant with respect to the controls.

A significant inhibition in photosynthetic rate was observed, compared to controls, in those populations exposed to concentrations between 50 and 166 mg Gli/l after 40 and 60 minutes of [illegible](Fig. 1). When the effects produced on *Raphidoce- lis subcapitata* were considered, it was observed that herbicide concentrations between 1.35 and 50 mg Gli/l produced a higher photosynthetic rate than those of the controls (Fig. 2).

significant stimulation (hormesis) in the photosynthetic rate compared to the controls at 40 and 50 minutes of [illegible] After 60 minutes of [illegible]an inhibition of the photosynthetic rate was observed.

significant i[illegible]n the photosynthetic rate with respect to the



Effect of glyphosate on photosynthetic rate of *Raphidocelis subcapitata*. *Effect of glyphosate on* Raphidocelis subcapitata *photosynthetic rate.*

of the controls, when the populations were exposed to 166 mg Gli/l (Fig. 2).

The toxicity indices estimated on the basis of photosynthetic rate are shown in Table 3. Both species showed similar sensitivity to the [illegible]of glyphosate in the form of active ingredient, since no significant differences were found between the EC values$_{50}$ of both species (Student's t-test $p < 0.05$). Considering the NOEC, LOEC and ChV values, they were lower for the *Scenedes- mus quadricauda* species, indicating a greater sensitivity to the herbicide evaluated.

DISCUSSION

Glyphosate proved'. toxico for the populations of the The most notable effects were observed on population growth and photosynthetic rate. The most notable effects were observed on the growth and photosynthetic rate.

Toxicity indices (mg Gli/l) after 50 minutes of exposure for the active ingredient glyphosate estimated on the basis of photosynthetic rate. * 95% confidence intervals. *Toxicity indexes (mg gli/1) after 50 minutes of exposure for the active ingredient glyphosate estimated on the base of the photosynthetic rate. * 95 % confidence intervals.*

	Scenedesmus quadricauda	*Scenedesmus acutus (SAG 276-3a)*	*Chlorella vulgaris*	*Raphidocelis subcapitata*
EC_{50} - 96	2.9	2.7	6.2	4.4
hours	(1.44- 6.18)*	(2.2-3.5)	(4.2-9.7)	(3.1-6.2)
NOEC	0.01	0.27	0.3	1.1
LOEC	0.1	0.9	1.1	3.7
ChV	0.03	0.49	0.57	2.01

algal growth, since the highest concentrations where toxic effects were observed were found between 1.55 and 5 mg Gli/l. Algal populations exposed to concentrations higher than 1.09 mg Gli/l could show chemical effects on their growth. Taking into [illegible]the NOEC, LOEC and ChV index values, the *Scenedesmus quadricauda* species isolated from the source of the Luja'n river was the most sensitive species to the [illegible]f the active ingredient glyphosate on algal growth.

The evaluations carried out on the photo-synthetic rate determined the existence of an [illegible] this parameter in *Scenedesmus qua-dricauda* and *Raphidocelis subcapitata* against low concentrations of the herbicide, while higher concentrations exerted in-hitory effects. This phenomenon was described more than a century ago as the Arndt-Schulz law (Cala-brese, 2005), and the term "hor- mesis" was introduced in 1943 by Southam and Erlich. It would be an adaptive response of organisms to a [illegible]f homeostasis due to an induced environmental stress. Overcompensation would occur as a result of a process of transferring slightly excess resources to restore homeostasis.

A recent study (Cedergreen *et al.*, 2007) conducted with terrestrial and aquatic vascular plants and algae exposed to low doses of 10 herbicides has shown that there are some herbicides that cause less mild hormesis [illegible] while others provoke more marked responses describing less biphasic [illegible](Calabrese, 2005). Among the latter are glyphosate and metsulfuron-methyl, herbicides that affect the synthesis of aromatic amino acids. In the case of the effects observed on algae, an increase in the photo-synthetic rate would occur, which would correspond to an increase in the growth rate. The results obtained by us in the present study on photosynthetic rate correspond to the biphasic dose-response behavior described in Calabre- se (2005) as type A corresponding to the most [illegible] form of inverted U curve and corroborates the findings of Cedergreen *et al* (2007) for glyphosate, as mentioned above.

Glyphosate was found to be among the herbicides that elicited the greatest response in several groups of aquatic and algal plants and in terrestrial plants (Duke *et al.*, 2006), initiating an interesting debate about the ability of this herbicide, widely used worldwide, to stimulate the growth of a wide range of plant species. Another fundamental aspect is the [illegible]f the sustained effect of the stimulation over time, with possible effects on reproduction, linked to the competitive ability of affected plants with n[illegible] plant communities (Cedergreen, 2008b). Goldsborough and Brown (1988) evaluated the toxicity of glyphosate on photosynthesis of periphytic algae, finding EC_{50} estimated between 69.7 and 35.4 mg Gli/l. Duke (1988) estimated an EC_{50} of 118 mg/l after 60 to 90 minutes of [illegible]o the active ingredient glyphosate for a species of the genus *Scenedesmus*. These values are similar to those estimated in this work.

Previous studies (Sa'enz *et al.*, 1997a) showed that glyphosate active ingredient did not produce a [illegible] the protein content of the species studied after 96 hours of [illegible]t concentrations between 2.5 and 50 mg Gli/l. Although the direct site of action of the active principle glyphosate is at the level of inhibition of the synthesis of aromatic amino acids, the effect on the [illegible] the synthesis of proteins containing these amino acids may not have been detected at the time [illegible]ur evaluations. The evaluations performed on population growth proved to be the most sensitive, yielding lower toxicity indices. The species *Scenedesmus quadricauda* presented lower NOEC, LOEC and ChV values in both the evaluations performed on population growth and on the photo-synthetic rate, indicating its greater sensitivity to prolonged herbicide exposure.

The active ingredient glyphosate is a moderately toxic herbicide (EC_{50} -96 hours between 7.2 and 13.1 mg/l) [illegible]electron transport inhibitor herbicides, such as [illegible]ro'n and Atrazine (EC_{50} -96 hours between 0.011 and 0.09 mg/l) and the electron transport deviator herbicides, which have a lower EC (EC -96 hours between 0.011 and 0.09 mg/l) and a higher EC.

photosynthetic energy, such as Paraquat (EC_{50} - 96 hours between 0.047 and 0.67 mg/l) (Sa'enz *et al.*, 1997b). It is possible to reach the same conclusion if we consider the EC_{50} calculated from the effects on the photosynthetic rate, where we find EC values$_{50}$ between 50 and 166 mg Gli/l in the case of the active ingredient glyphosate and between 0.047 and 0.67 mg/l in the case of Paraquat.
0.013 and 0.082 mg/l, in the case of Atrazine and Diuron (Sa'enz, 2000), or in the case of Simazine with EC values$_{50}$ of 0.8 mg/l (Goldborough and Robinson 1988). These results confirm the US EPA classifying moderate toxicity of glyphosate to aquatic organisms, with EC values$_{50}$ between 1 and 10 mg/l on algal growth (Giesy *et al.,* 2000).

The comparison between the traditional 96-hour exposure methodology (algal growth) and the use of Clark's oxygen electrodes (photosynthetic rate) yielded similar results for those herbicides that exert direct action on photosynthetic activity (Min- gazzini *et al.*, 1997; Sae'nz 2000). Regarding herbicides with different modes of action, such as glyphosate, the results obtained with both methods showed wide differences, since it is a herbicide that affects several cellular processes. The high EC_{50} estimated at 50 minutes would indicate that the herbicide evaluated has a low toxicity to the algal populations exposed. It should be noted that the aforementioned toxicological information on the effects of glyphosate on algal growth, based on the performance of traditional ecotoxicological tests, allows similar conclusions to be reached. The advantage of the application of Clark's oxygen electrodes lies in the speed in obtaining significant toxicological responses, allowing a first approximation of the toxicity of a substance to be obtained quickly. This can be useful even in the case of evaluating toxic substances that do not exert their direct action on the photosynthesis process.

The studies carried out with the commercial Round-up formulation showed that it exerted adverse effects on the growth of the four species studied, with no differences in sensitivity among the algae species considered.

by calculating the EC_{50} 96 hours. Analyzing the NOEC, LOEC and ChV values, *Scenedesmus quadri- cauda* was the most sensitive species.

The higher toxicity of Roundup on algal growth was reflected in lower toxicity indices than those of the public product. These results are in agreement with those found by other authors on the effects of Roundup on population growth (WHO, 1994). The results of risk evaluations carried out taking into account microorganisms, amphibians, invertebrates, fish and aquatic macrophytes, show the same conclusions, increasing the risk in shallow systems (0.15 m), a very frequent in lagoons of Buenos Aires (Giesy *et al.,* 2000). Hernando *et al.* (1989) studied the effects of Round-up on photosynthesis of isolated chloroplasts exposed to concentrations greater than and equal to 55 mg Round-up/l, and found a greater inhibition of photosystem II with respect to photosystem I. The authors concluded that this greater inhibition of photosystem I was due to the presence of Round-up. The authors conclude that this higher inhibition is due to the polyoxyethyleneamine surfactant (POEA) present in the formulation, as indicated in materials and methods. Studies conducted independently on the toxicity of the active ingredient and the POEA surfactant showed that the following results were not available.
Toxicity indices of 120-140 mg glyphosate/l and 0.65-7.4 mg POEA/l, respectively, indicating the greater toxicity of the latter (US EPA, 1993). The effects produced by the commercial for- mulate corresponded to a synergetic effect caused by the herbicidal properties of the active ingredient and by the adverse effects caused by the surfactants.

In risk assessments conducted with the commercial formulation Round-up®R , it has been considered that according to its properties and the rates and manner of application in agricultural practices, the EEC (expected concentration in the environment) would be between 1.7 and 2.88 mg Gli/l (WHO, 1994; Peterson *et al.*, 1994; Giesy *et al.,*
2000) depending on the depth of the aquatic systems. Comparison between these environmental herbicide concentrations and estimated toxicity levels suggests that long-term adverse actions would be exerted on aquatic ecosystems.

algal components in the recommended range of pH for this compound.

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