The Impact of Eskoba®, a Glyphosate Formulation, on the Freshwater Plankton Community

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ABSTRACT: This study analyzed the acute effects of a glyphosate-based herbicide (Eskoba®) on the microalgae *Chlorella vulgaris*, the cladoceran *Simocephalus vetulus*, and the copepod *Notodiaptomus conifer*, and evaluated the recovery ability of the surviving microcrustaceans. Survival, age of first reproduction, and fecundity were used as endpoints for *S. vetulus*, while survival and time to reach the adult stage were used as endpoints for *N. conifer*. The registered order of sensitivity was *S. vetulus* (48-hour effective concentration [EC_{50}]: 21 mg/L) > *C. vulgaris* (72-hour EC_{50}: 58.59 mg/ L) > *N. conifer* (48-hour EC_{50}: 95 mg/L). Despite the growth of *C. vulgaris* stimulated after 24 hours of exposure to the commercial formulation of glyphosate Eskoba®, it was inhibited after 48 hours by all the concentrations tested. In postexposure experiments, microcrustaceans reduced their life expectancy, *S. vetulus* decreased its fertility, and *N. conifer* inhibited its sexual maturity. In summary, it was demonstrated that these species lost their recovery ability. *Water Environ. Res.*, **86,** 2294 (2014).

KEYWORDS: glyphosate, *Chlorella vulgaris, Simocephalus vetulus, Notodiaptomus conifer,* acute toxicity, recovery ability.

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Introduction

In Argentina, the amount of pesticides associated with soybean production has increased considerably over the last 10 years. Within these pesticides, herbicides containing glyphosate (*N*-(phosphonomethyl) glycine) (GLY) are mainly used to control annual and perennial plants and different kinds of weeds (WHO, 1994). The use of different GLY formulations increased from 2 to 180 million liters between 1990 and 2007 (Binimelis et al., 2009).

In plants, the mode of action of the active ingredient (i.e., main ingredient or key component) in such formulations implies the inhibition of the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase involved in the synthesis of aminoacids such as phenylalanine, tyrosine and tryptophan that are essential for the formation of many proteins (Duke, 1988; Sierra et al., 2008). Studies have tested glyphosate (Demetrio, 2012; Di Fiori et al., 2012; Tsui and Chu, 2004; Vedrell et al., 2009), but little is known about the toxicity of commercial formulations with additives (Puglis and Bonne, 2011; Tsiu and Chu, 2003). The Council of Agricultural Health and Fertilizers of Argentina (2011) has cited 32 products with glyphosate as active ingredients at various concentrations and types of chemical formulae (potassium, isopropylamine, ammonium, monoammonium, monopotassium, and dimethylamine). While the issue has received some attention worldwide, most researchers have only evaluated the effects of Raundup® (Cuhra et al., 2013; Dutra et al., 2011; Lipok et al., 2010; Papchenkova et al., 2009; Raipulis et al., 2009; Romero et al., 2011; Vera et al., 2010).

Pesticides enter aquatic systems via accidental application in rivers, streams, or lakes; by washing sprayers and pesticide containers; or via runoff after strong rainfalls (Jergentz et al., 2004, 2005; Peruzzo et al., 2008; Romero et al., 2011). These events make herbicide concentrations widely variable in environments because they are generally below the threshold value for resident organisms. Nevertheless, the occurrence of these specific discharges could increase pesticide concentrations in a short lapse of time, thus exposing organisms to higher concentrations than those considered innocuous. Among aquatic systems, the planktonic community is the most sensitive to changes in water quality and its alterations could affect the adjacent trophic levels through "top down" and "bottom up" effects (Hanazato, 2001; McCormick and Cairns, 1997). Considering the lack of information on the toxicity of commercial formulations, the active ingredient of which is GLY, and the significant ecological role of plankton, this work aimed to analyze the effects of the glyphosate commercial formulation Eskoba® on three main components of the plankton community: the microalgae *Chlorella vulgaris*, the cladoceran *Simocephalus vetulus*, and the copepod *Notodiaptomus conifer*. These species were selected because they are good representatives of many shallow lakes of the Paraná River's alluvial valley (Argentina) and because, at present, no information exists about their sensitivity to the aforementioned commercial GLY formulation. In addition, considering the occurrence of short acute events in nature, the authors aimed to evaluate the recovery ability of microcrustaceans (*S. vetulus* and *N. conifer*) that survived herbicide exposure. In this sense, the authors hypothesized that organisms exposed to the highest concentrations would exhibit low recovery ability and that the studied biological parameters would be seriously affected.

Eskoba® was selected because its formulation is one of the most widely used in Argentina's Pampas (CONICET, 2009; UNL, 2010). In spite of this, to the authors' knowledge, there is no information about its toxicity on nontarget aquatic organisms of the southern hemisphere.

Materials and Methods

Chemicals. The commercial formulation of glyphosate Eskoba® (Ciagro S.A., Buenos Aires, Argentina) used in this

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study is composed of 48% (w/v) isopropylamine (N-phosphonomethyl glycine salt) and 52% inert ingredients and coadjuvants or additives of analytical grade that are not specified on the product label.

The stock solution was prepared by diluting the commercial formulation of technical grade (95%) provided by Ciagro SRL (Santa Fe, Argentina) in sterile, bidistilled water to obtain a concentration of 1000-mg/L acid equivalent. It was kept in darkness at −4 °C until its analytical determination using a Dionex DX-100 ion chromatograph equipped with a conductivity detector (Waters 430; Waters Corporation, Milford, Massachusetts), a suppressor column (Dionex ASRS300; Dionex Corporation, Sunnyvale, California), a column (Dionex Ion Pack AS4A-SC), and a precolumn (Ion Pack AG4ASC; Dionex Corporation.

The eluent was NaOH 3.2 mM/Na_2CO_3 7.2 mM. The measured GLY concentration was 1067.5 (± 38.48) mg/L; this stock solution was used to prepare each test concentration for the experiments with the three species.

Test Organisms and Culture Conditions. The strain of *C. vulgaris* (CLV2) was provided by the Scientific Research and Superior Education Center of Ensenada (Baja California, Mexico). It was cultivated in 2000-mL Erlenmeyers under sterile conditions using Bold Basal Medium (BBM) (Sager and Granik, 1953). The culture was maintained at constant temperature (23±1 °C) with uniform and continuous aeration, constant light intensity (approximately 8000 Lux), and continuous stirring using a 100-rpm magnetic plate.

Microcrustaceans. *Simocephalus vetulus* and *Notodiaptomus conifer* specimens were collected with a plankton net (100 μm) from lentic unpolluted waterbodies of the Paraná River's alluvial valley. After being collected, the animals were carried to the laboratory for acclimation. Cladocerans and copepods were raised in different culture media because of their different nutritional requirements.

Cladocerans were individually maintained in glass beakers with 30 mL of synthetic medium (APHA et al., 1998) comprising 2.4 g Mg SO_4, 3.84 g $NaHCO_3$, 0.16 g KCl, and 2.4 g $CaSO_4.2H_2O$ dissolved in 20 L of distilled water. Copepods were maintained in dechlorinated tap water, the physicochemical characteristics of which were similar to that of the Paraná River, as follows: nitrates, <0.1 mg/L; nitrites, 0.01 mg/L; ammonium, 0.29 mg/L NH_3; chlorides, 3.5 mg/L; sulphates, 8.3 mg/L; total alkalinity, 77 mg/L $CaCO_3$; bicarbonates, 94 mg/L; sodium, 7.7 mg/L; magnesium, 6.8 mg/L; calcium, 12.9 mg/L; potassium, 1.8 mg/L; chemical oxygen demand, 10 mg/L; and biological oxygen demand, 0.08 mg/L.

Both media were changed weekly and oxygenated by bubbling air for at least 24 hours before using them. The significant water quality parameters, which were maintained constant, were as follows: pH 7.6 to 7.75; dissolved oxygen, 7.32 to 7.89 mg/L; temperature, 25 ± 2 °C; and photoperiod 16:8 (light:darkness). Animals were fed three times a week with a drop of suspension of algae (*C. vulgaris*; absorbance = 1.5 λ, 650 nm) for each culture chamber.

Toxicity of Glyphosate (N-(phosphonomethyl) glycine) to *C. vulgaris*. The experimental treatments of algal growth inhibition with *C. vulgaris* were carried out according to the standard protocol of the Organisation for Economic Co-Operation and Development (1984), that is, microalgae were harvested in the exponential growth phase and then centrifuged and resuspended in sterile, ultrapure water. All the experiments started with the same initial cell density: 10^4 cells/mL. The algal density estimation was carried out directly using a Neubauer chamber (1.02 × 106 cells/mL) and, indirectly, by spectrophotometry (Abs 1.5 λ; 650 nm). Control and treatments were triplicated; they were conducted in 150-mL flasks containing 100 mL of BBM medium in the following GLY concentrations: 0.5, 1, 2, 4, 8, and 16 mg/L. The controls were carried out in BBM medium without GLY.

The vessels were maintained in an incubation chamber under the same controlled conditions as those for the stock culture. Three replicates of 100 μL each were taken at 24, 48, and 72 hours for cells counting with a 400 x Olympus light microscope in a Neubauer chamber. In all instances, at least 25 squares were counted to ensure errors lower than 10% (Venrick, 1978). The considered endpoints were the effective concentration (EC_{50}) (72-hour EC_{50}) and the growth rate (μ). The 72-hour EC_{50} was estimated using the linear interpolation method (U.S. EPA, 2002) and μ was estimated according to the following equation (Guillard, 1975):

$$\mu = \mathrm{Log}_2(N_1/N_0)/t_1 - t_0 \quad (1)$$

Where

N_1 = final cell density (number of cells/mL),
N_0 = initial cell density (number of cells/mL),
t_1 = final time, and
t_0 = initial time.

The pH values (mean ± standard deviation) at the start 6.15 (±0.13) and the end 6.18 (±0.11) of each assay were maintained practically constant (Wilcoxon matched pairs test, $p = 0.3125$). Differences between control and treatments ($\log_{10}$ [x] transformed values) were analyzed using repeated measures analysis of variation (ANOVA) ($\alpha = 0.05$).

Toxicity of (*N*-(phosphonomethyl) glycine) to Microcrustaceans. For microcrustaceans, acute (48-hour) toxicity assays were started with neonates (<24 hours) of *S. vetulus* (U.S. EPA, 2002) and with copepodites of *N. conifer* at the fifth instar (Copepodite 5). Five GLY concentrations plus the control (without GLY) were used for each species: 3.2, 6.4, 12.8, 25.6 and 51.2 mg/L for *S. vetulus* and 20, 40, 80, 160, and 320 mg/L for *N. conifer*. The assays were carried out using glass beakers with 10 mL of the culture medium/specimen. Culture conditions were identical to those used for the stock culture. The number of replicates for *S. vetulus* and *N. conifer* assays were 30 and 20, respectively, per each concentration tested, placing one specimen per replica. As indicative of the toxic effect, the authors considered the complete immobilization of the organisms and the absence of response after to be stimulated or prodded by a metal rod.

At 48 hours, the number of live and dead organisms was recorded. Results were considered acceptable when mortality in the control group was ≤10%. Levels of pH and oxygen were recorded during all the experiments and ranged from 7.6 to 8 mg/L and 6 to 8 mg/L, respectively. The lethal concentration 50 (LC_{50}) (e.g., the dose required to kill half the members of the population tested) was determined at 48 hours; the 48-hour LC_{50} values and their 95% confidence limits were estimated using Probit analysis (Finney, 1971).

Table 1—Effective concentration (72-hour EC_{50} and 48-hour LC_{50}) of the glyphosate-based herbicide Eskoba® to the alga *C. vulgaris*, the cladoceran *S. vetulus,* and the copepod *N. conifer*.

Studied species	LC_{50} (mg/L)	EC_{50} (mg/L)	Exposure time (h)	Confidence interval (95%)
Simocephalus vetulus	21.5	–	48	13.9–30.8
Chlorella vulgaris	–	58.59	72	16.47–70.42
Notodiaptomus conifer	95.2	–	48	71.8–128.2

To evaluate the resilience (recovering capacity after the toxicant exposure) of both microcrustaceans, the following procedure was performed: survivors of acute trials were moved to new containers with their respective culture media without GLY for 15 days under identical conditions to those used for the stock culture. Animals were fed three times a week with a drop of a suspension of *C. vulgaris* per chamber (absorbance = 1.5 λ, 650 nm). Both pH values and oxygen concentrations were recorded at the beginning and end of each assay, within the limits established by *Standard Methods for the Examination of Water and Wastewater* (APHA et al., 1998). As endpoints for *S. vetulus,* mortality, age of first reproduction and fecundity (neonates/female) were considered. For *N. conifer,* the mortality and the time requested to achieve the adult stage (Copepodite 5 to Copepodite 6) were evaluated, because the latter has been suggested as a suitable trait for detecting the effects of pollutants (Brown et al., 2002; Gutierrez et al., 2010).

Differences between control and treatments in each one of the aforementioned endpoints were tested using one-way ANOVA, followed by a Tukey-Kramer multiple comparisons post Test (95% confidence level) (Sokal and Rohlf, 1969). Before each analysis, the normal distribution of data was verified with the Kolmogorov-Smirnov test. All statistical analyses were carried out using the package GraphPad InStat (InfoStat, 2004).

Results

(*N*-(phosphonomethyl) glycine) Acute Toxicity. The sensitivity of organisms varied among species. According to the acute toxicity test results, the order of sensitivity of the three species to GLY was: *S. vetulus* > *C. vulgaris* > *N. conifer* (Table 1).

Toxicity of (*N*-(phosphonomethyl) glycine) to *C. vulgaris*. The increase of the herbicide concentration and the extension of the exposure time reduced the *C. vulgaris* growth rate (μ). After 24 hours of exposure, a slight tendency to stimulation of cell proliferation was produced in the higher GLY concentrations (ANOVA repeated measures, $F = 3.94$; $p < 0.05$). Conversely, no significant differences were found between the lower one and the control (Figure 1). After 48 hours of exposure, 16 mg/L of GLY inhibited the division of *C. vulgaris* cells (ANOVA repeated measures, $F = 6.09$; $p < 0.01$) (Figure 1). After 96 hours, this inhibition was very significant in the five higher concentrations (ANOVA repeated measures, $p < 0.01$) (Figure 1).

Recovery Experiments with Microcrustaceans. For *S. vetulus*, the mortality in the control was 10%; in other words, 27 organisms began the recovery test. On the other hand, after acute exposure to 3.2, 6.4, 12.8, 25.6 and 51.2 mg/L of GLY, the number of remaining survival organisms that entered the recovery test was 24, 21, 20, 17 and 9 respectively. At the end of the recovery tests without GLY, mortality was 100% in all the concentrations tested, with the exception of the lower concentration (3.2 mg/L), for which mortality was 86.7%.

For organisms that have been exposed to 3.2 mg/L, their fecundity and age at first reproduction did not show significant differences with the control (ANOVA, $p > 0.05$). However, in GLY concentrations of 6.4 and 12.8 mg/L, the age at first reproduction was delayed 2 to 4 days (ANOVA, $p < 0.05$) (Table 2) and the average number of neonates/females was significantly reduced (ANOVA, $p < 0.05$). In the highest concentrations (25.6 and 51.2 mg/L of GLY), no reproduction was recorded (Figure 2).

For copepods, the mortality in the control was 20%; in other words, 16 organisms began the recovery test. On the other hand, after acute exposure to 20, 40, 80, 160, and 320 mg/L of GLY, the number of remaining survival organisms that entered the recovery test was 20, 16, 12, 7, and 1, respectively. At the end of the recovery tests, the mortality for each one of the concentrations tested was 45, 12, 25, 57, and 0%, respectively. As can be seen, in the recovery tests, the mortality was strongly dependent on the number of organisms that could survive after the acute exposure tests.

After being exposed to GLY concentrations of 160 and 320 mg/L, *N. conifer* could not reach the adult stage. In copepods exposed to 20 and 40 mg/L, this endpoint was not different from that of the control group (ANOVA, $p > 0.05$). Conversely, 80 mg/L of GLY significantly delayed the copepods' sexual maturity (ANOVA, $p < 0.05$) (Figure 3).

Discussion

After exposing the microalgae *C. vulgaris*, the cladoceran *S. vetulus*, and the copepod *N. conifer* to the commercial formulation of glyphosate Eskoba®, the results obtained in this

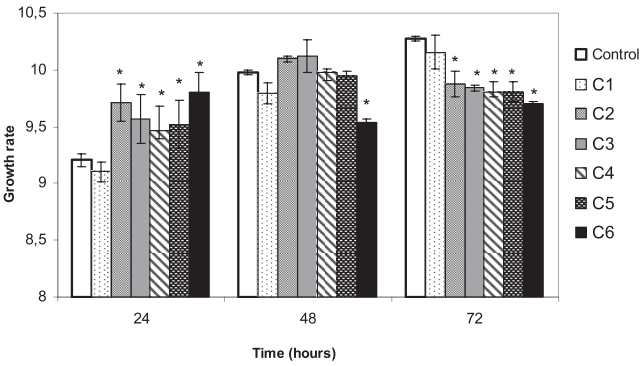

Figure 1—Growth rate (μ) of *C. vulgaris* exposed to six concentrations of GLY and the control (without GLY): 0 mg/L (Control), 0.5 mg/L (C1), 1 mg/L (C2), 2 mg/L (C3), 4 mg/L (C4), 8 mg/L (C5), and 16 mg/L (C6) during 24, 48, and 72 hours. Error bars indicate the (±) standard deviation (three replicates per treatment). An asterisk ("*") denotes significant differences of the control (one-way ANOVA, $p < 0.05$).

Table 2—Age at first reproduction of the cladoceran *S. vetulus* after being exposed to five concentrations of GLY and the control (without GLY) for 48 hours. The table shows the mean value and, in parenthesis, the (±) standard deviation. A dash ("–") indicates that the organism did not produce neonates.

GLY concentrations (mg/L)	Age at first reproduction (days)
0	9.3 (±0.57)
3.2	9.6 (±2.88)
6.4	11 (±0.44)
12.8	13 (±0.44)
25.6	–
50.2	–

work were highly variable and dependent on the species analyzed. This is in accordance with previous studies in different countries (e.g., Cuba, Poland, Russia, and Argentina) that also showed significant variations in glyphosate toxicity on planktonic organisms (Albarracín et al., 2011; Lipok et al., 2010; Raipulis et al., 2009; Romero et al., 2011; Vendrell et al., 2009). Notoriously, such variations showed up to two orders of magnitude among them, depending on whether the active ingredient was isolated or constituting commercial formulations, hence the importance of considering the concentration of the active component when comparing toxicities to different organisms.

The results of the present study indicate that 24-hour exposure to GLY stimulated *C. vulgaris* cell division. This pattern of early stimulation by low concentrations of toxic substances (also called "hormesis") is similar to that reported by other researchers (Calabrese et al., 1999; Sáenz and Di Marzio, 2009; Sáenz et al., 2012; Spoljaric et al., 2011). However, further investigations are necessary to clearly understand the mechanisms of such stimulation. Despite this early effect, after 48 hours of exposure all Eskoba® concentrations tested finally inhibited *C. vulgaris* growth, being 58.59 mg/L the measured effect level at 72 hours.

By comparing the sensitivity of *C. vulgaris* with that of other Chlorophyceae exposed to GLY commercial formulations, it is noticeable that the effective concentration obtained in the present study was higher than the one obtained by other reserarchers; for instance, Tsui and Chu (2003) obtained 96-hour EC_{50} values of 5.81 mg/L for *Selenastrum capricornutum* exposed to Roundup® and Romero et al. (2011) registered values of 55.62 mg/L after exposing *C. kessleri* for 96 hours to the formulated Atanor®. Conversely, studies carried out by Sáenz and Di Marzio (2009) showed higher 96-hour EC_{50} values (ranging from 120 to 154 mg/L) in several microalgal species exposed to Roundup®. However, the order of sensitivity reported by these authors (*Scenedesmus quadricauda* > *S. acutus* > *C. vulgaris* > *Raphidocelis subcapitata*) also suggest a relatively high tolerance of *C. vulgaris* to GLY.

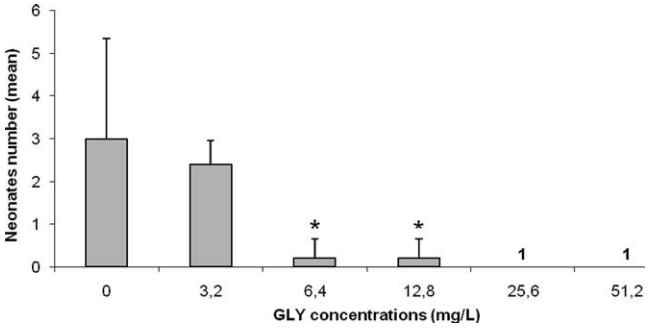

Figure 2—Fecundity (mean number of neonates) of the cladoceran *S. vetulus* after being exposed to five concentrations of GLY and the control (without GLY) for 48 hours. Vertical bars indicate each (±) standard deviation. An asterisk ("*") denotes significant differences of the control (one-way ANOVA, $p < 0.05$). A "1" indicates that the organisms did not produce neonates during 15 days of the experiment.

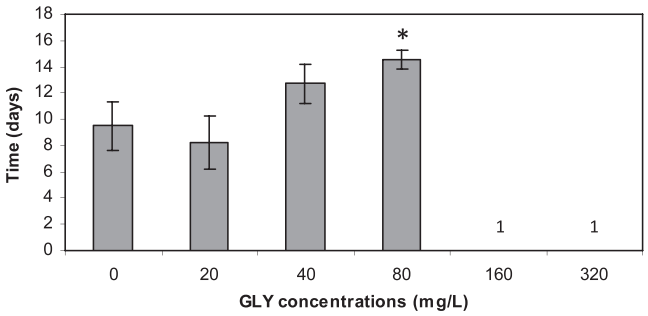

Figure 3—Time (in days) at which the copepod *N. conifer* reaches the adult stage (from copepodite 5 to copepodite 6) after being exposed to five concentrations of GLY and the control (without GLY) for 48 hours. Vertical bars indicate the (±) standard deviation. An asterisk ("*") denotes significant differences with the control (one-way ANOVA, $p < 0.05$). A "1" indicates that the organisms did not reach the adult stage.

All these results reflect that sensitivity of microalgae to GLY herbicides strongly differs among species and is markedly dependent on the chemical composition of the formulations tested. Glyphosate formulations with different compositions, which, as previously mentioned, generally are not reported on the label of the products sold, have been manufactured after the expiration of Roundup® herbicide's patent in 2000, without caring for the complete composition of the formulae and their possible toxicity to nontarget species (Cox, 2004). This situation conducted to important difficulties in ecotoxicological assessments, aimed to determine and compare the degree of sensitivity of different species.

For microcrustaceans, as expected, *S. vetulus* (48-hour LC_{50} of 21.59 mg/L) was much more sensitive than *N. conifer* (48 hour-LC_{50} of 95.23 mg/L); this is consistent with field work in polluted environments, where diversity and abundance of cladocerans were much lower than those of copepods (Gagneten and Paggi, 2009).

Compared to species universally used as "test organisms" (i.e., frequently having holartic distribution), native species used in this study showed different sensitivity to GLY. For example, *Daphnia magna* registered 48-hour LC_{50} values of 7.9 mg/L exposed to Faena®, 61.72 mg/L exposed to Ron-do®, and 190 mg/L exposed to Roundup®. Toxicity values in *Ceriodaphnia dubia* ranged from 5.39 (Roundup®) to 415 mg/L (Rodeo®) (Alberdi et al., 1996; Domínguez-Cortinas et al., 2008; Raipulis et al., 2009; Tsui and Chu, 2003).

Table 3—Comparison of the toxicity of glyphosate (*N*-phosphonomethyl glycine) and the most common commercial formulations and surfactants.

Active ingredient	Species	48-hour LC_{50} and 96-hour EC_{50} (mg/L)	Authors
N-fosfonometilglicina	*Daphnia magna*	146	Domínguez-Cortinas et al. (2008)
N-fosfonometilglicina	*Ceriodaphnia dubia*	35.3	Tsui and Chu (2003)
N-fosfonometilglicina	*Acartia tonsa*	147	Tsui and Chu (2003)
N-fosfonometilglicina	*Daphnia magna*	199.61	Demetrio (2012)
N-fosfonometilglicina	*Chlorella pyrenoidosa*	590	Maule and Wright (1984)
Commercial formulation			
Roundup®	*Ceriodaphnia dubia*	5.39	Tsui and Chu (2003)
Roundup DuraMax®	*Daphnia magna*	0.1	Appendices to Glyphosate (2010)
Roundup UltraMax®	*Daphnia magna*	11	Appendices to Glyphosate (2010)
Faena®	*Daphnia magna*	7.9	Domínguez-Cortinas et al. (2008)
Roundup®	*Acartia tonsa*	1.77	Tsui and Chu (2003)
Atanor®	*Chlorella kessleri*	55.62	Romero et al. (2011)
Roundup 360 SL®	*Chlorella vulgaris*	118.17	Lipok et al. (2010)
Roundup®	*Scenedesmus quadricauda*	120	Sáenz and Di Marzio (2009)
Surfactants or additives			
POEA	*Ceriodaphnia dubia*	1.15	Tsui and Chu (2003)
POEA	*Acartia tonsa*	0.57	Tsui and Chu (2003)
POEA	*Daphnia magna*	0.097	Brausch and Smith (2007)
Ethoxylated alcohols $C_{9–15}$ EO_9	*Daphnia magna*	1.13	Dorn et al. (1993)
POEA	*Selenastrum capricornutum*	3.91	Tsui and Chu. (2003)
Ethoxylated alcohols C_{12}-$_{15}$ EO_9	*Selenastrum capricornutum*	0.7	Dorn et al. (1993)

The differences found between the acute toxicities registered in this work and others, could be attributed to the composition of commercial formulations (i.e., differences regarding chemical associations of the active ingredient and the proportion and nature of the coadyudants or additives used in the commercial formulation, which could be more toxic than the active ingredient alone [Table 3]). Tsui and Chu (2003) found that the Roundup® and the surfactant polyoxyethylene amine (POEA) were always more toxic than the active ingredient (e.g., 48-hour LC_{50} = 5.39, 1.77 mg/L, for the commercial formulation, but 1.15 and 0.57 mg/L for *C. dubia* and *Acartia tonsa,* respectively, after being exposed to POEA). These values could be compared with 48-hour LC_{50} for glyphosate acid = 35.3, 147 mg/L, for cladoceran and the copepod, respectively. Other research yielded the same results, assuming that the Roundup® surfactant, and no glyphosate itself, caused increased toxicity (Kitulagodage et al., 2008; Pereira et al., 2009).

In the postexposure experiments, as hypothesized, after 48-hour exposure to the GLY formulation, both the cladoceran and the copepod species tested were severely affected, at least in one trait of their life cycle: both species reduced their life expectancy and, while *S. vetulus* decreased its fertility, *N. conifer* inhibited or delayed its sexual maturity. These results show strong evidence that these species lost their resilience, that is to say, their recovey capacity, according to the results obtained on recovery experiments. Therefore, to establish protection limits, life history traits should be taken into account because short-term expositions (e.g., 24 or 48 hours) are not enough to detect postexposure damages that could alter the organisms' *fitness*.

The results of the present work suggest that the glyphosate formulation tested (48-hour LC_{50}: 21.5 and 95.2 mg/L for *S. vetulus* and *N. conifer,* respectively; 72-hour EC_{50}: 58.59 mg/L for *C. vulgaris*) promotes harmful effects on native nontarget species. To the authors' knowledge, this is the first work recording and comparing the changes in the life cycle of planktonic species that belongs to different trophic levels, caused by short-term exposure (48 hours) to a glyphosate formulation. These results are particularly important considering that the concentrations often found in nature may eventually reach higher peaks after intense anthropogenic activities on natural environments, such as spraying directly on waterbodies adjacent to the fields, or by involuntary inputs after washing drums and agricultural machinery (Battaglin et al., 2009; Feng et al., 1990; Peruzzo et al., 2008; Vera et al., 2010).

This work also highlights the importance of assessing the effects of commercial formulations of GLY on aquatic species and the need to clearly indicate the additives of glyphosate-based herbicide commercial formulations. Finally, this study demonstrates the importance of increasing information on the ranges of tolerance to GLY of organisms belonging to the planktonic community, and emphasizes the urgency of knowing its effects on wild species.

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