

# Effects of Herbicide Glyphosate and Glyphosate-Based Formulations on Aquatic Ecosystems

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## 1. Introduction

Public awareness of worldwide increase herbicides use and their adverse effects on ecosystems has been growing over the past decades. Herbicides may reach water bodies via agricultural runoff and leaching processes, as well as by direct applications to control noxious aquatic weeds. Once in the aquatic ecosystems, herbicides may reduce environmental quality and influence essential ecosystem functioning by reducing species diversity and community structures, modifying food chains, changing patterns of energy flow and nutrient cycling and changing the stability and resilience of ecosystems. The aim of this chapter is to provide a general notion of the current knowledge concerning the direct and indirect effects of glyphosate and commercial formulations of glyphosate on aquatic ecosystems. Glyphosate based products are the leading post-emergent, systemic and non-selective herbicides for the control of annual and perennial weeds in the world. Here, we present a revision of their toxicity to non-target species of algae, aquatic plants, protozoa, crustaceans, molluscs, fish and amphibians. In addition, we describe the importance of each group of organisms in the functioning and health of aquatic ecosystems. With this information, a conceptual framework can be developed contributing to enhance our attention and concern about human impacts on ecosystems.

## 2. The scenario where glyphosate appeared on stage

The transition from biologically based to intensive-chemical based agricultural production systems advanced in North America and Europe soon after World War II. This change was supported by growing availability of inorganic fertilizers and organically synthesized pesticides. Afterwards, this type of agriculture has been adopted by other major crop production areas throughout the world during the 1960s and 1970s. The intensive cropping systems are characterized as large-scale production enterprises that utilize high inputs of chemical fertilizers and pesticides. Little emphasis is given to managing soil organic matter through use of traditional crop rotations, cover crops, or organic soil amendments that are central to maintaining the biological activity and allowing the long-term preservation of

agroecological systems in biologically based cropping systems (Yamada et al., 2009). One of the most significant inputs necessary for successful intensive crop production are herbicides for management of the variety of weed infestations especially encountered in row cropping systems. This technology was rapidly adopted because most weeds could be controlled when matched with selective herbicides, which were compatible with the crop, and was considered more cost-effective than cultural methods of weed management. In this scenario the herbicide glyphosate appeared on stage.

Glyphosate under the trade name Roundup® was introduced in the marked by Monsanto Company during the 1970s. It was initially registered as a broad-spectrum, non selective, systemic herbicide for certain non-crop and plantation crop uses (fallowed fields, orchards, vineyards and timber plantations) and for the control of annual and perennial weeds before the emergence of agronomic crops (Folmar et al., 1979; Woodburn, 2000). The development of minimum and no-tillage cultivation systems (zero-till) for row-cropping systems greatly expanded the use of herbicides, such as glyphosate, as it became standard practice to apply herbicides to growing weeds in fields prior to planting. This “burndown” application eliminated the need for traditional tillage (such as plow tillage) and allowed farmers to plant crop seeds directly into soil beneath a mulch of dead plant residues. The no-till practice was rapidly adopted around the world and really booming in some countries of South America like Brazil, Argentina, Paraguay and Uruguay (Altieri & Pengue, 2006). The reasons for the rapid growth of this practice are manifold, but the most important aspects are mainly economical (less work for field preparation, few expenses on fuel and machinery and higher profits). Important ecological aspects have been also pointed out. Non-till practice improves soil quality avoiding organic matter lost (Bayer et al., 2006) and water evaporation, despite of an increment in the use of herbicides. In addition, this cultivation system protects soil from erosion. For example in the southern of Brazil, no-till practice was adopted to reduce extensive soil erosion resulting from intensive row-cropping (Bolliger et al., 2006).

However, glyphosate became the most widely used herbicide worldwide with the introduction of genetically modified (GM) glyphosate-resistant (GR) crops (Woodburn, 2000). Monsanto’s glyphosate-tolerant Roundup Ready (RR) soybean was the first GR crops to be commercialized (Dill et al., 2008). In 1996, RR soybean was commercially available for the first time in the USA. These crops greatly improved conventional farmers' ability to control weeds, since glyphosate could be applied before seeding and sprayed several times during growth without damage the crop. Nowadays, glyphosate has established itself as the leading herbicide for the control of annual, perennial weeds and volunteer crops in a wide range of different situations (Woodburn, 2000). The arrival of GR soybean was followed by GR cotton, maize, canola, alfalfa and sugarbeet (Dill et al., 2008). These transgenic solutions (GR seeds + glyphosate) lead a sharp increase of worldwide areas under GR crops with concomitant increase of glyphosate use. The worldwide GR hectares planted during 1998 to 2008, increased from about 15 millions to more than 130 millions (Dill et al., 2008; James, 2008). Under these circumstances, only in USA, glyphosate usage increased from 3 10<sup>6</sup> kg of a.i. (active ingredient) in 1987 to more than 54 10<sup>6</sup> kg of a.i. in 2007 (Fig. 1). The two other countries with large areas under GR crops are Argentina and Brazil, however data set concerning glyphosate use in these countries are scarce.

The reported substantial increase in the global use of glyphosate has been also related with other items like herbicide price cuts and aggressive marketing, as well as the increased reliance on herbicides for weed control (Pengue, 2005). The latter issue is represented in the occurrence of weed population shifts toward less sensitive species and the evolution of

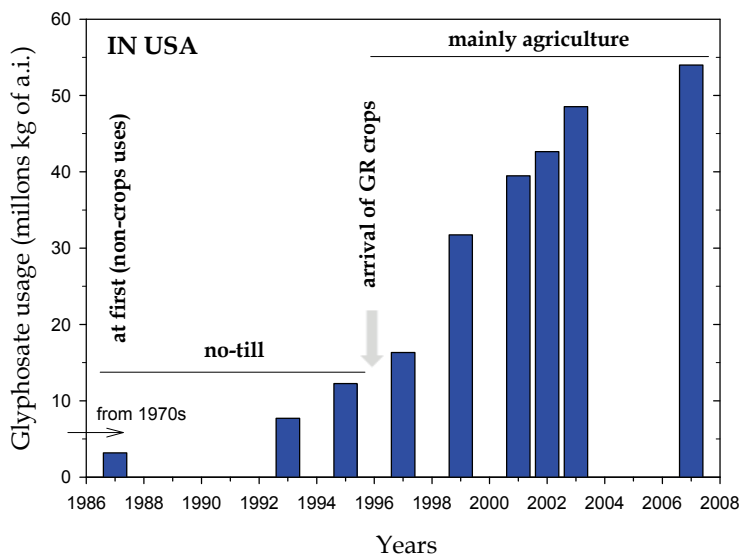


Fig. 1. Evolution of glyphosate usage in USA. Sources: USEPA, USDA.

herbicide-resistant weed populations. Glyphosate has been used worldwide since 1974 and, despite its widespread and long-term use, no case of evolved resistance to glyphosate under field conditions had been identified by 1993 (Holt et al., 1993). However, in 1996 the first case of weed resistance to glyphosate was documented in two accessions of the rigid ryegrass *Lolium rigidum*, from an orchard in Australia (Powles et al., 1998; Pratley et al., 1999). Since then, an increasing number of cases of glyphosate resistant biotypes have been reported. Currently, 14 GR weeds have been documented worldwide (Van Gessel, 2001; Pérez & Kogan, 2002; Powles, 2008; Binimelis et al., 2009; among others). Consequently, the average of glyphosate application per Ha showed a marked global increase associated with the appearance of a growing number of tolerant or resistant weeds. Bonny (2008) pointed out that the amount of glyphosate spread over the total US soybean land raised from less than 0.1 Kg/Ha in 1990 to more than 1.4 kg/Ha in 2006. Higher application rates (up to 5.6 a.i. kg/Ha) have been reported by Giesy et al. (2000). Regarding the use of other herbicides, at first the rapid growth in the use of glyphosate was accompanied by a decrease in the consumption of other former herbicides. However, for example in Argentina the consumption of the herbicides atrazine and 2,4-D, have risen again during the growing seasons of (2005-2006) (Binimelis et al., 2009). These observations coincide with Bonny (2008) who concluded that the total amount of herbicides applied per Ha in USA decreased initially between 1996 and 2001, but tended to rise afterwards.

### 3. Glyphosate (the molecule)

#### 3.1 Chemistry

The chemical (technical-grade) name of glyphosate is N-(phosphonomethyl) glycine (IUPAC), an acid that belongs to chemical group of Phosphonoglycine or more generic: Organophosphonate herbicides (Fig. 2). Its main degradation product is the metabolite aminomethyl phosphonic acid (AMPA).

Glyphosate is an aminophosphonic analogue of the natural amino acid glycine and the name is a contraction of *glycine*, *phos-*, and *-ate*. The molecule has several dissociable hydrogens, especially the first hydrogen of the phosphate group. Technical-grade glyphosate has relatively low solubility in water (1.2 % at 25 ° C), and is insoluble in other solvents. Strong intermolecular hydrogen bonds stabilize the crystal lattice, causing the low water solubility. Various salts of glyphosate have much higher solubility, and do not lose any of the herbicidal properties of the parent compound (Franz, 1985). Glyphosate is commonly formulated in its form of isopropylamine salt (IPA salt), though other related chemical form are also commercialized. Glyphosate concentration is commonly expressed as mg a.i./L or mg a.e./L, where: a.e. (acid equivalents). Glyphosate is an unusual herbicide, in that essentially no structurally related compounds show any herbicidal activity (Hollander & Amrhein, 1980; Franz, 1985), with the exception of glyphosine, which has reduced herbicidal effects but shows some interesting plant growth regulatory effects, such as enhancing ripening of sugar cane (Franz, 1985). The herbicidal properties of glyphosate were reported in 1971 (Baird et al., 1971). The compound was found during a study of the herbicidal effects of tertiary aminomethylphosphonic acids derived from various primary and secondary amines (Moedritzer & Irani, 1966). Only two of the compounds produced showed some herbicidal activity, but both had very low unit activities. Attempts to find other tertiary aminomethylphosphonic acids with improved herbicidal activity failed. As a last resort, it was suggested that degradation of the two compounds might give rise to a common, active metabolite (contrary to the general trend that metabolism reduces toxicity). Glyphosate was among the possible metabolites of the two compounds, and was found to have extremely high herbicidal activity (Franz, 1985).

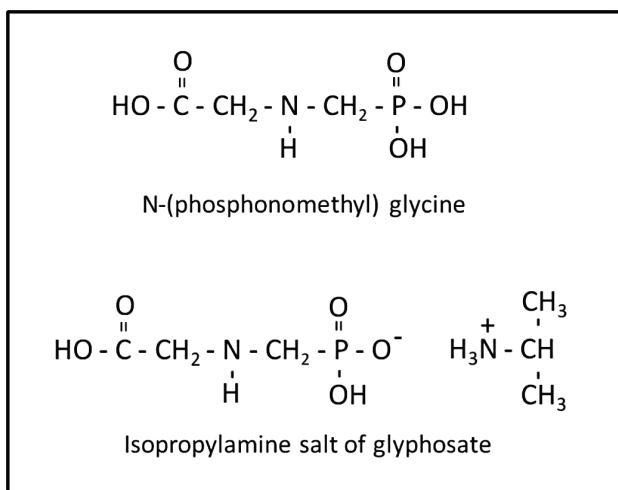


Fig. 2. Glyphosate molecule (as an acid and IPA salt).

### 3.2 Mode of action and biochemistry

Glyphosate is a systemic herbicide that is phloem mobile and is readily translocated throughout the plant. From the leaf surface, glyphosate molecules are absorbed into the plant cells where they are symplastically translocated to the meristems of growing plants

(Laerke, 1995). Unlike many contact herbicides, phytotoxic symptoms of glyphosate injury often develop slowly. Visible effects on most annual weeds occur within two to four days and may not occur for 7 days or more on most perennial weeds. Visual symptoms are a gradual wilting and yellowing of the plant which advances to complete browning and finally with the total deterioration and death of the plant.

Although glyphosate may ultimately perturb a variety of biochemical processes including protein synthesis, nucleic acid synthesis, photosynthesis and respiration, the primary mode of action of glyphosate was localized to the shikimate pathway of aromatic amino acid biosynthesis, a pathway that links primary and secondary metabolism. Its mode of action is the competitive inhibition of the enzyme 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase, a chloroplast-localized enzyme in the shikimate pathway (Steinrücken & Amrhein, 1980; Bode et al., 1984a; Rubin et al., 1984). This inhibition prevents the production of chorismate, which is the last common precursor in the biosynthesis of numerous aromatic compounds in bacteria, fungi and plants. Essential aromatic amino acids are used by plants in protein synthesis and to produce many secondary plant products (e.g. growth promoters, growth inhibitors, lignin precursors, flavonoids, tannins, and other phenolic compounds). The major end products are the amino acids phenylalanine, tyrosine, and tryptophan.

Evidence for the involvement of this pathway in glyphosate toxicity has come from a variety of studies in a wide range of microorganisms, plant cell cultures and plants (e.g. Jaworsky, 1972; Haderlie et al., 1977; Gresshoff, 1979; Duke et al., 1980; Hollander & Amrhein, 1980; among others). Glyphosate caused a massive accumulation of shikimate in treated cells and tissues (Amrhein et al., 1980; Berlin & Witte, 1981; Bode et al., 1984; Rubin et al., 1984). These studies narrowed the possible site of action to three enzymes, involved in the conversion of shikimate to chorismate. The enzyme that was finally implicated was EPSP synthase; its inhibition by glyphosate is competitive with phosphoenolpyruvate (PEP), and non-competitive with respect to shikimate-3-phosphate with a single glyphosate binding site on the enzyme.

### 3.3 Commercial formulations of glyphosate

When the toxicity of herbicides is discussed, the focus is mostly on the active compound (in this case glyphosate acid or glyphosate salts). However, herbicides are formulated to increase their efficacy against target plants. Commercial glyphosate based herbicides contain other components, which are called inert ingredients. These inert ingredients are mainly surfactants, solvents and antifoam compounds. Shortly, surfactant refers to chemicals that have pronounced surface activity in aqueous solutions that can decrease surface tension and perturb membrane permeability or transport function of membranes including permeability to glyphosate (Riechers et al., 1994). Antifoam compounds are chemical additives that reduce and hinder the formation of foam in industrial process liquids. Numerous contributions have demonstrated that inert ingredients in glyphosate formulations have several folds higher toxicity on non-target organisms than glyphosate alone (e.g. Folmar et al. 1979; Wan et al. 1989; Cedergreen & Streibig, 2005; among others). Therefore, glyphosate formulations are chemical mixtures and must be considered as mixtures in toxicity assessments. In this context, studies regarding specific toxicity or generalization about toxicology of inert ingredients (e.g. surfactants) must be conducted on glyphosate, inert ingredient and commercial formulation separately. The lack of such data will render any predictions about the effects of the formulations on glyphosate highly uncertain (Diamond & Durkin, 1997).

Glyphosate concentration, as well as nature and concentration of inert additives, depend on commercial formulations; though available information about inert ingredients in glyphosate products is commonly not listed on the label. The commercial formulation of glyphosate, Roundup®, is a most popular brand name for glyphosate herbicides. Roundup® contains IPA salt of glyphosate (35-50 %) plus inert ingredients. Roundup® concentration is commonly expressed as mg a.i./L or mg a.e./L of glyphosate, while also as mg of Roundup® (whole product) per L. We considered 1 mg a.i./L equal to 0.75 mg a.e./L (Relyea, 2006).

The surfactant in Roundup®, as well as in some other glyphosate based products, is the highly toxic polyethoxylated tallowamine compound (Smith & Oehme, 1992; Giesy et al., 2000). This material is referred to in the literature as MON0818, or polyoxyethyleneamine (POEA), present at about 15% in Roundup®. Presumably POEA is a derivative of tallow, a complex mixture of fat from the fatty tissue of cattle or sheep. Other trade names of glyphosate based herbicides include Aquamaster®, Filedmaster®, Touchdown®, Glyphos®, Duramax®, Durango®, Glyphomax®, Fosato®, Ron-do®, Vision®, Rodeo®, Sulfosato®, etc.

## 4. Glyphosate in aquatic environments

### 4.1 Offsite movement and direct applications

The use of herbicides and other chemical agents in agriculture may result in accidental introduction into waters. When it is applied as post emergence spray, herbicides may enter aquatic systems through accidental offsite movement in herbicide spray drift, or through transport in leaching and surface run-off.

Particularly, under field conditions, glyphosate is usually assumed to be rapidly and tightly adsorbed to soil. Consequently, glyphosate is unlikely to leach into ground waters or runoff significantly into surface waters following application. In several laboratories (Rueppel et al., 1977; Crisanto et al., 1994; among others) and some field studies (Roy et al., 1989), the immobility of glyphosate in soils has been demonstrated. In contrast, other field studies showed detectable concentrations of glyphosate in flume and streams after applications. Even though it was only restricted to a relatively brief window of time (about 1 day post application), due to the fast dissipation kinetics of glyphosate in the field. In natural waters, glyphosate dissipate rapidly (half lives < 4 days) being removed from water due to adsorption to suspended particulates followed by subsequent sedimentation and or biodegradation. However, longer half lives were reported in hard waters, where glyphosate residues could be measured after 11 days post application (Pérez et al., 2007).

Edwards et al. (1980) reported important glyphosate concentrations in runoff from natural rainfall following early springtime treatments in no-tillage agriculture soils. The highest concentration of glyphosate in runoff waters (5.2 mg/L) was found in runoff occurring 1 day after treatment at the highest rate (8.6 Kg/Ha of Roundup®) (Edwards et al., 1980). The maximum amount of glyphosate transport by runoff was 1.85% of the amount applied, most of which occurred during a single storm on the day after application. In addition, Feng et al. (1990) found in a treated watershed, a dramatic increase of glyphosate concentrations (about 1.1 and 1.5 mg/L) in two oversprayed streams in response to first rainfall event 27 h post application. Authors attributed their observations to several source of input mobilization of residues in ephemeral stream channels feeding the tributary; wash off of unabsorbed residues from overhanging vegetation, surface runoff and subsurface flow. Regarding POEA residues, based on adsorption and degradation data, leaching and runoff potential is

expected to be small. POEA strongly adsorb to soil (Giesy et al., 2000), although little information about POEA offsite movement is nowadays available.

Offsite movement of glyphosate is also possible through spray drift (e.g. Payne et al., 1990; Payne, 1992). Although the spray drift of pesticides is not compound specific, this is relevant when non-target effects of glyphosate based herbicides are considered, and several studies have specifically addressed the issue. Some studies reported that the spray deposition decreased to around 10 % of the application rate in the first 30 m and less to 5 % at a distance of 200 m (Payne et al., 1990; Riley et al., 1991). Other studies suggested that drift rates would be greater. For instance, residues have been measured 400 m downwind from applications sites (Yates et al., 1978; Payne & Thompson, 1992).

Considering offsite movement of glyphosate from treated soils through drift and run-off, Giesy et al. (2000) estimated an acute scenario considering worst-case exposure conditions. The estimate was based on two assumptions, (a) that runoff (2%) from 10 Ha field treated at the maximum single use rate of Roundup® entered to 1 Ha pond (2 m deep) and (b) that 10% of maximum single application rate per hectare entered the pond through drift, assuming aerial application. Based on these assumptions, maximum concentrations of Roundup® in natural water would range from 0.27 to 0.41 mg/L (Giesy et al., 2000). However, clearly higher concentrations in surface waters could be expected if assumptions are changed. For instance, some authors have reported that glyphosate can be readily desorbed from soil and has the potential to be extensively mobile in the soil environment. Adsorption of glyphosate to soil particulates is determined by chemical and physical characteristic of soils, which in turn affect the potential for off-target movement of the herbicide through water runoff or subsurface flow. Interestingly, given that glyphosate is bound to soil through its phosphonic acid moiety, the addition of inorganic phosphorus could potentially release glyphosate from soil particles through competition for sorption sites (Franz et al. 1997; Pechlaner, 2002). Piccolo et al. (1994) reported in an experimental study with some European soils that desorption varied from around 15 to 80% of the absorbed herbicide according to the soil characteristic. These observations, as well as supposing higher rates of terrestrial uses and higher spray drift due to weather conditions, could elicit elevated off-target movements of glyphosate formulations in to water ecosystems. Particularly, these impacts will be more important in ponds, ephemeral streams and ditchbank areas of irrigation canals due to their low water volume, and higher perimeter and area /volume proportions.

On the other hand, some glyphosate based herbicides (e.g. Rodeo® and AquaMaster®) were specially formulated to be used as aquatic herbicides, and have been employed extensively to control noxious aquatic weeds and algal blooms (Seddon, 1981; Diamond & Durkin, 1997; Siemering et al., 2008). For this purpose, glyphosate based herbicides are directly applied in aquatic ecosystems and their residues can be expected to be higher than that resulting from agricultural and other non aquatic uses. Furthermore, glyphosate can move considerable distances in canal or stream waters affecting undesired places (Duke, 1988). Fifty-eight percent of applied glyphosate was detected at distances 8 and 14.4 Km downstream from sites of introduction (Comes et al., 1976). Regardless herbicide sources, it is very important to set up the amount of glyphosate that have been measured in the field. Unluckily, there are few relevant field data on the concentration of glyphosate in aquatic habitats. The highest concentrations that have been observed in nature were: 1.24 mg a.e./L (Newton et al., 1994); 1.54 mg a.e./L (Couture et al., 1995); 2.8 mg a.e./L (Legris & Couture, 1989) and 5.2 mg a.e. /L (Edwards et al., 1980).

## 4.2 Toxicity of glyphosate based herbicides in aquatic environments

### 4.2.1 Toxicity assessment

In this chapter, we extensively reviewed published contributions about glyphosate, glyphosate formulations and surfactants effects on non-target aquatic organisms. Different parameters (lethal and sublethal effects) were evaluated in reviewed studies to characterize the hazard of chemicals (e.g. mortality, growth, biomass,  $^{14}\text{C}$  uptake, weight, density, length, pigments, mobility, reproduction, metabolism, etc). Results were expressed as LC (concentration lethal to 10% and 50% of test organisms), EC (effective concentration causing specified effects in 10% and 50% of test organisms) and IC (inhibition concentration to specified effects in 10% and 50% of test organisms). In addition, when available, values of NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) were pointed out.

When dose-response curves were not available or were not calculated due to experimental design (e.g. field studies carried out in micro- and mesocosms), contributions were described as concentrations of treatments and obtained outcomes (generally in % of control values). A complete resume of published outcomes in acute and chronic tests and field experiments were listed in tables (from Table 1 to Table 6). Concentrations were preferentially expressed as were originally reported in each reviewed contribution.

In order to relate the aquatic toxicity of the herbicide to realistic exposure levels, the expected environmental concentration (EEC) was taken as a reference value. We considered a EEC of 2.6 mg a.i./L, (following Relyea, 2005). Similar values were estimated by other authors: 1.87 mg a.i./L (Chen et al., 2004) and 3.73 mg a.i./L (Perkins et al., 2000); though higher values were also evaluated (e.g. 10.13 mg a.i./L; Mann and Bidwell, 1999).

### 4.2.2 Effects on non-target aquatic plants and algae

Herbicides are mainly designed to kill unwanted terrestrial plants. Consequently the most sensitive group of aquatic non-target organisms is expected to be aquatic plants and algae. Aquatic plants and algae play a pivotal role for the function of aquatic ecosystems (Scheffer et al., 1993). Aquatic plants aid in stabilizing the sediment both in lakes and running waters, and their presence affects sedimentation rates, flow velocity, nutrient uptake and recirculation. In addition, they provide refuges for insects, crustaceans and fish, and act as substrates for surface-living microorganisms, snails and other epiphyte grazers. Microalgae (phytoplankton and periphyton communities) provide the basis for a range of food-webs in the aquatic environment and are therefore fundamental to the functioning of aquatic ecosystems (Wetzel, 2001).

Single species test in algae and aquatic plants treated with glyphosate alone (i.e. technical grade acid or salts of glyphosate), showed a wide range of EC and IC values; indicating different sensibilities. Microalgae presented  $\text{EC}_{50}$  values for glyphosate treatments ranging from 0.68 mg a.e./L in the diatom *Skeletonema costatum* (Malcolm Pirnie, 1987) to around 600 mg a.e./L in the green algae *Chlorella pyrenoidosa* (Maule & Wright, 1984) (Table 1). It is important to clarify that these values indicate the concentration that elicited the 50 % of reduction in the evaluated parameter. Some works showed that 10 % of reduction ( $\text{EC}_{10}$ ) could be reached between 3 to 16 folds lower concentrations than  $\text{EC}_{50}$ . For instance, 10% growth inhibition in the green algae *Scenedesmus subpicatus* was observed in treatments with 1.6 mg/L of glyphosate acid (Vedrell et al., 2009). In addition, Christy et al. (1981) reported a 10% growth inhibition in *Chlorella sorokiniana* at the concentration of 2 mg a.e./L. Regarding macrophytes, generally lower values of EC and IC were reported, indicating a higher



sensibility. For example,  $IC_{50}$  and  $EC_{50}$  values ranged from 0.22 mg a.i./L for *Myriophyllum aquaticum* (Turgut & Fomin, 2002) to 46.9 mg/L for *Lemma minor* (Cedergreen & Streibig, 2005) (Table 1).

The relative toxicity of glyphosate itself vs. commercial formulations and surfactants only can be evaluated in studies specially designed to this purpose. In general, commercial formulations (e.g. Ron-do® and Roundup®) were more toxic than glyphosate alone. For example, Tsui & Chu (2003) observed a 7 folds higher toxicity of Roundup® than the IPA salt of glyphosate in the green algae *Selenastrum capricornutum* (Table 1). Alike results were reported for *Selenastrum capricornutum* and the macrophyte *Lemma minor*, showing 4 folds higher toxicity of Roundup® than glyphosate (Cedergreen & Streibig, 2005). Lower differences in toxicity were registered by other authors (e.g. Sáenz et al., 1997; Sobrero et al., 2007), reporting between 1.2 to 1.8 folds higher toxicity of commercial formulations than active ingredient (Table 1). POEA itself contributed to Roundup® toxicity with values ranged from about 45% for *Skeletonema costatum* to 85% for *Selenastrum capricornutum* (Tsui & Chu, 2003).

Numerous studies have been published about pesticide toxicity assessment on microalgae, using single species tests. However, Bérard et al. (1999) demonstrated that single-species tests may fail to predict indirect or system responses to toxicants, such as changes in population competition or succession. According to these authors, studies focusing on the whole natural community provide more reliable predictions about herbicide safety in aquatic environments. In studies assessing communities, significant direct and indirect effects of commercial glyphosate formulations have been reported. For example, Schaffer & Sebetich (2004) reported an increment of 161% in net primary production for phytoplankton community treated with 0.13 mg a.i./L of Rodeo® (commercial formulation without POEA). In contrast, Goldsborough & Brown (1988), registered a 50% of reduction in periphyton primary production at values varying from 35.4 to 69.7 mg a.i./L of Roundup® (Table 1). However, in this contribution, 4 of the 6 treated ponds showed a reduction in the mean values of primary production with much lower concentrations (a dosage of 0.89 mg a.i./L). In microcosms experiments with natural marine microbial community, significant effects in species number and relative abundance of phytoplankton were observed at 10 µg a.i./L of Roundup® (Stachowski-Haberkorn et al., 2008). Comparable results were obtained by Pesce et al. (2009), reporting changes in riverine algal communities exposed to about 10 µg/L of glyphosate alone, in a microcosms experiment. In addition, mesocosms studies showed remarkable results with a single pulse application of Roundup® at concentrations of 6 and 8 mg a.i./L (Pérez et al., 2007; Vera et al., 2010). Even if these two contributions assessed herbicide effects in worst case scenarios, glyphosate concentration at the end of the experiments (11 and 14 days respectively), were around 2 mg a.i./L and effects were still clearly observed. At day 11, significant differences were observed in chemical and biological variables (Pérez et al., 2007). For example, we observed changes in phytoplankton assemblage fractions, with a reduction of micro and nanophytoplankton densities (2.5 folds) and a concomitant increase of picocyanobacteria (PICY) densities (40 folds) accompanied by an increase of primary production. These results can be expected by either direct effect of herbicide (differences in sensibility among species) or indirect effects duo to interspecific competition. In addition, Vera et al. (2010) found that Roundup® produced a clear delay in periphytic colonization in treated mesocosms. The periphytic mass variables: dry weight (DW), ash-free dry weight (AFDW) and chlorophyll a, were always higher in control mesocosms. Despite the mortality of algae, (mainly diatoms), cyanobacteria was favoured in treated mesocosms. We also observed that Roundup® produced a long term shift in the typology of mesocosms, "clear" turning to "turbid" state due to an eutrophication process.

AQUATIC ALGAE & PLANTS	ASSESSED CHEMICAL	STUDY TYPE	ASSESSED PARAMETER	EFFECTS CONCENTRATION (mg/L) ##	REFERENCES
<b>- Phytoplankton and Periphyton</b>					
<i>Chlorococcumhynposporum</i>	Gly. (acid)	SST (7 d.)	Growth	96h EC <sub>50</sub> = <b>68.0</b>	Maule & Wright, 1984
<i>Chlorella pyrenoidosa</i>				96h EC <sub>50</sub> = <b>590.0</b>	
<i>Skeletonema costatum</i>	Gly. (acid)	SST (7 d.)	Biomass	EC <sub>50</sub> = <b>0.64</b> ; NOEC = <b>0.28</b>	Malcolm Pirnie, 1987
(Periphyton community)	Roundup®	LTNC (4 h.)	NPP	4h EC <sub>50</sub> = (between <b>35.4 to 69.7</b> )	Goldsbrough & Brown, 1988
<i>Scenedesmus acutus</i>	Gly. (IPA salt)	SST (4d.)	Density	96h EC <sub>50</sub> = <b>10.2</b> ; NOEC = <b>2.0</b>	Sáenz et al., 1997
	Ron-do®			96h EC <sub>50</sub> = <b>9</b> ; NOEC = <b>3.2</b>	
<i>Ankistrodesmus sp</i>	Rodeo®	SST (10 d.)	Density	96h EC <sub>50</sub> = <b>74.0</b>	Gardner et al., 1997
(Phytoplankton community)	Rodeo®	LTNC (6 h.)	NPP	<b>0.13 mg/L</b> elicited NPP increment	Schaffer & Sebetich, 2004
<i>Selenastrum capricornutum</i>	Gly. (IPA salt)	SST (4 d.)	Growth	96h EC <sub>50</sub> = <b>41.0</b>	Tsui & Chu, 2003
	Roundup®			96h EC <sub>50</sub> = <b>5.81</b>	
	POEA			96h EC <sub>50</sub> = <b>3.91</b>	
<i>Skeletonema costatum</i>	Gly. (IPA salt)	SST (4 d.)	Growth	96h EC <sub>50</sub> = <b>5.89</b>	Tsui & Chu, 2003
	Roundup®			96h EC <sub>50</sub> = <b>1.85</b>	
	POEA			96h EC <sub>50</sub> = <b>3.35</b>	
<i>Selenastrum capricornutum</i>	Gly. (n.c.)	SST (2 d.)	Growth	48h EC <sub>10</sub> = <b>95.5</b> ; 48h EC <sub>50</sub> = <b>270.0</b>	Cedergreen & Streibig, 2005
	Roundup®			48h EC <sub>10</sub> = <b>13.6</b> ; 48h EC <sub>50</sub> = <b>64.7</b>	
(Phytoplankton & periphyton community)	Roundup®	MES (11 d.)	Density #	6 mg/L elicited changes in community structure	Pérez et al., 2007
(Microbial community)	Roundup®	MIS (7 d.)	Community structure #	<b>10 µg/L</b> elicited a reduction in species number.	Stachowski-Haberkorn et al., 2008
(Microbial community)	Gly. (n.c.)	MIS (14 d.)	Community structure #	~ <b>10 µg/L</b> elicited changes in algal community structure.	Pesce et al., 2009
<i>Chlorella saccharophila</i>	Gly. (acid)	SST (3 d.)	Growth	72h EC <sub>10</sub> = <b>3.0</b> ; 72h EC <sub>50</sub> = <b>46.6</b>	Vedrell et al., 2009
<i>Scenedesmus subspicatus</i>				72h EC <sub>10</sub> = <b>1.6</b> ; 72h EC <sub>50</sub> = <b>26.0</b>	
(Periphyton community)	Roundup®	MES (42 d.)	Density #	8 mg/L elicited changes in community structure	Vera et al., 2010
<b>- Macrophytes</b>					
<i>Myriophyllum sibiricum</i>	Gly. (acid)	SST (14d.)	Root length #	14d IC <sub>10</sub> = <b>0.59</b> ; 14d IC <sub>50</sub> = <b>0.84</b>	Roshon et al., 1999
	Roundup®			14d IC <sub>50</sub> = <b>1.22</b>	
<i>Myriophyllum aquaticum</i>	Gly. (n.c.)	SST (14d.)	Growth & chl <i>a</i> #	14d EC <sub>50</sub> = <b>0.22</b> (for growth)	Turgut & Fomin, 2002
				14d EC <sub>50</sub> = <b>0.22</b> (for chl <i>a</i> )	
<i>Lemma minor</i>	Gly. (n.c.)	SST (7 d.)	Growth	7d EC <sub>10</sub> = <b>3.8</b> ; 7d EC <sub>50</sub> = <b>46.9</b>	Cedergreen & Streibig, 2005
	Roundup®			7d EC <sub>10</sub> = <b>3.5</b> ; 7d EC <sub>50</sub> = <b>11.2</b>	
<i>Myriophyllum spicatum</i>	Roundup®	SST (21 d.)	Weight & length #	21d IC <sub>50</sub> = <b>1.0</b> (for weight) 21d IC <sub>50</sub> = <b>2.8</b> (for length)	Sánchez et al., 2007
<i>Lemma gibba</i>	Gly. (acid)	SST (10 d.)	Growth #	10d IC <sub>10</sub> = <b>4.6</b> ; 10d IC <sub>50</sub> = <b>20.5</b>	Sobrero et al., 2007
	Roundup®			10d IC <sub>10</sub> = <b>2.5</b> ; 10d IC <sub>50</sub> = <b>11.6</b>	

Abbreviations and acronyms: gly. (glyphosate); n.c. (not clarified); n.a (not available); d. (days); SST (single species laboratory tests); LTNC (laboratory tests with natural communities), MES (mesocosms studies); (MIS) microcosms studies; SSFE (single species field experiments); chl *a* (chlorophyll *a*); NPP (net primary production). Notes: (##) Effects concentrations were expressed as mg/L of formulation, mg a.i./L or mg a.e./L, see bibliographic references to clarify. (#) Several parameters were assessed in these contributions; remarkable examples of the reported outcomes were listed in tables.

Table 1. Effects of glyphosate, different commercial formulations of glyphosate and POEA on algae and aquatic plants

#### 4.2.3 Effects on non-target aquatic bacteria and protozoa

The majority of the available pesticide data regarding aquatic microorganisms is for algae. Far fewer pesticide studies exist for aquatic bacteria and protozoa. Aquatic bacteria and protozoa (e.g. amoeboids, flagellates, ciliates and sporozoans) have key roles in the functioning of aquatics environments. Shortly aquatic bacteria occupy an important position

in the aquatic food web since they are major actors in the decomposition of dead material, and thereby in the recycling of nutrients and carbon. They are extremely important in “Lake metabolism”, being involved in mineralization processes and in the chemical transformation of elements between reduced and oxidized forms. Protozoans are ecologically important as key links in food chains. Ubiquitous in aquatic environments, protozoans prey upon algae, bacteria, and other organisms and are themselves consumed by animals such as microinvertebrates. Thus, the ecological role of protozoa in the transfer of bacterial and algal production to successive trophic levels is very important (in the traditional food web and in the microbial loop). On the other hand, some protozoa are important as parasites and symbionts of multicellular animals.

Concentration effects of glyphosate itself on bacteria and protozoa varied widely and seem to indicate low sensibility (Table 2). For instance, EC<sub>50</sub> values obtained in treatments with glyphosate ranging from 18.2 mg/L for the bacteria *Vibrio fischeri* (Bonnet et al., 2007) to 386.0 mg a.e./L for the ciliate *Tetrahymena pyriformis* (Tsui & Chu, 2003) (Table 2). However, lower concentrations have been reported to produce observable effects (Everett & Dickerson, 2003). These authors registered a LOEC value of 5 mg/L for the parasite ciliate *Ichthyophthirius multifiliis* treated with glyphosate acid.

Roundup® showed higher toxicity than glyphosate for bacteria and protozoa in the revised bibliography. Tsui & Chu (2003) reported 6 folds higher sensibility of *Vibrio fischeri* to

MICROORGANISMS	ASSESSED CHEMICAL	TYPE OF STUDY	ASSESSED PARAMETER	EFFECTS CONCENTRATION (mg/L) ##	REFERENCE
<b>-Bacteria and Protozoa</b>					
<i>Ichthyophthirius multifiliis</i>	Gly. (acid)	SST (5 h)	Mortality	NOEC = <b>2.5</b> , LOEC = <b>5.1</b>	Everett & Dickerson, 2003
	Roundup®			NOEC = n.a., LOEC = <b>0.07</b>	
<i>Tetrahymena thermophila</i>	Gly. (acid)	SST (24 h)	Mortality	NOEC = <b>10.1</b> , LOEC = n.a.	Everett & Dickerson, 2003
	Roundup®			NOEC = n. a., LOEC = <b>0.31</b>	
<i>Brachionus calyciflorus</i>	Gly. (n.c.)	SST (24 h)	Growth #	24h EC <sub>50</sub> = <b>28.0</b>	Xi & Feng, 2004
	Gly. (IPA salt)			5min EC <sub>50</sub> = <b>162.0</b>	
<i>Vibrio fischeri</i>	Roundup®	SST (5 min.)	Growth	5min EC <sub>50</sub> = <b>24.9</b>	Tsui & Chu, 2003
	POEA			5min EC <sub>50</sub> = <b>10.2</b>	
	Gly. (IPA salt)			40h EC <sub>50</sub> = <b>386.0</b>	
<i>Tetrahymena pyriformis</i>	Roundup®	SST (40 h.)	Growth	40h EC <sub>50</sub> = <b>29.5</b>	Tsui & Chu, 2003
	POEA			40h EC <sub>50</sub> = <b>4.96</b>	
	Gly. (IPA salt)			48h EC <sub>50</sub> = <b>64.1</b>	
<i>Euplotes vannus</i>	Roundup®	SST (48 h.)	Growth	48h EC <sub>50</sub> = <b>23.5</b>	Tsui & Chu, 2003
	POEA			48h EC <sub>50</sub> = <b>5.0</b>	
	Gly. (IPA salt)			48h EC <sub>50</sub> = <b>64.1</b>	
<i>Euglena gracilis</i> *	Roundup®	SST (7 d.)	Velocity #	NOEC = <b>0.05</b> , LOEC = <b>0.1</b>	Pettersson & Ekelund, 2006
	Avans®			NOEC = <b>0.05</b> , LOEC = <b>0.1</b>	
<i>Vibrio fischeri</i>	Gly. (acid)	SST (15 min.)	Bioluminescence	15min EC <sub>50</sub> = <b>18.2</b>	Bonnet et al., 2007
	AMPA			15min EC <sub>50</sub> = <b>53.4</b>	
<i>Tetrahymena pyriformis</i>	Gly. (acid)	SST (45 min.)	Enzyme activities #	45min EC <sub>50</sub> = <b>87.9</b>	Bonnet et al., 2007
	AMPA			45min EC <sub>50</sub> = <b>166.5</b>	

Abbreviations and acronyms: see Table 1. Notes: (\*) *Euglena gracilis* is a mixotrophic green flagellated; although in this resume was grouped with protozoa; see Table 1 for other notes.

Table 2. Effects of glyphosate, different commercial formulations of glyphosate, AMPA and POEA on bacteria and protozoa

Roundup® than to glyphosate acid. In addition, these authors observed 2.7 and 13 folds higher sensibility of the ciliates *Euplotes vannus* and *Tetrahymena pyriformis* to Roundup®, respectively. In addition, the ciliate parasite *Ichthyophthirius multifiliis* showed an accentuated response, being several times more sensible to Roundup® (Everett & Dickerson, 2003) (Table 3). Values of EC<sub>50</sub> obtained in Roundup® treatments ranged from 23.5 to 29.5 mg a.e./L (Tsui & Chu, 2003); though lower values produced observable effects. For example, Everett & Dickerson (2003) registered LOEC values of 0.07 and 0.31 mg a.e./L for two ciliates. Besides, values of 0.1 mg a.i./L of Roundup® and Avans® (other glyphosate commercial formulation) elicited reduction of at least 50% in the swimming velocity of *Euglena gracilis* (Pettersson & Ekelund, 2006).

AQUATIC INVERTEBRATES	ASSESSED CHEMICAL	STUDY TYPE	ASSESSED PARAMETER	EFFECTS CONCENTRATION (mg/L) ##	REFERENCES
<b>-Crustaceans (copepods, cladocerans and amphipods)</b>					
<i>Daphnia magna</i>	Roundup®	SST (48h)	Immobilization	48h EC <sub>50</sub> = 3.0	Folmar et al., 1979
<i>Gammarus pseudolimnaeus</i>	Roundup®	SST (48h)	Mortality	48h LC <sub>50</sub> = 62.0	Folmar et al., 1979
<i>Daphnia pulex</i>	Roundup®	SST (96 h.)	Immobilization	96h EC <sub>50</sub> = 8.5	Servizi et al., 1987
	POEA			96h EC <sub>50</sub> = 2.0	
<i>Daphnia magna</i>	Ron-Do®	SST (48h)	Immobilization	48h EC <sub>50</sub> = 61.7	Alberdi et al., 1996
<i>Daphnia spinulata</i>	Ron-Do®	SST (48h)	Immobilization	48h EC <sub>50</sub> = 66.2	Alberdi et al., 1996
<i>Simocephalus vetulus</i>	Vision®	SST (8 d)	Survival and reproduction #	0.75 mg/L elicited survival and reproduction reduction	Chen et al., 2004
	Rodeo®			48h LC <sub>50</sub> = 415.0	
<i>Ceriodaphnia dubia</i>	Roundup bioactive®	SST (48 h.)	Mortality#	48h LC <sub>50</sub> = 81.5	Tsui & Chu, 2004
	Roundup®			48h LC <sub>50</sub> = 5.7	
	Rodeo®			48h LC <sub>50</sub> = 225.0	
<i>Hyalella azteca</i>	Roundup bioactive®	SST (48 h.)	Mortality#	48h LC <sub>50</sub> = 120.0	Tsui & Chu, 2004
	Roundup®			48h LC <sub>50</sub> = 1.5	
	Gly. (IPA salt)			48h LC <sub>50</sub> = 415.0	
<i>Ceriodaphnia dubia</i>	Roundup®	SST (48 h.)	Mortality	48h LC <sub>50</sub> = 5.4	Tsui & Chu, 2003
	POEA			48h LC <sub>50</sub> = 1.2	
	Gly. (IPA salt)			48h LC <sub>50</sub> = 49.3	
<i>Acartia tonsa</i>	Roundup®	SST (48 h.)	Mortality	48h LC <sub>50</sub> = 1.77	Tsui & Chu, 2003
	POEA			48h LC <sub>50</sub> = 0.57	
	POEA (5:1)			48h LC <sub>50</sub> = 0.18	
<i>Daphnia magna</i>	POEA (10:1)	SST (48 h.)	Mortality	48h LC <sub>50</sub> = 0.097	Brausch et al., 2007
	POEA (15:1)			48h LC <sub>50</sub> = 0.85	
<b>-Molluscs (snails and mussels)</b>					
<i>Pseudosuccinea columella</i>	Gly. (n. c.)	SST (12 d.)	Growth and hatching #	1 mg/L elicited growth increment and 10 mg/L inhibited hatching.	Tate et al., 1997
<i>Pseudosuccinea columella</i>	Gly. (n. c.)	SST (12 d.)	Metabolism	0.1 mg/L elicited an increment in free amino acids.	Tate et al., 2000
<i>Utterbackia imbecillis</i>	Roundup®	SST (24 h.)	Mortality #	48h LC <sub>50</sub> = 18.3	Connors & Black, 2004
<b>-Others (insects and worms)</b>					
<i>Chironomus plumosus</i>	Gly. (acid)	SST (48h)	Immobilization	48h EC <sub>50</sub> = 55.0	Folmar et al., 1979
	Roundup®			48h EC <sub>50</sub> = 18.0	
	POEA			48h EC <sub>50</sub> = 13.0	
<i>Lumbriculus variegatus</i>	Gly. (acid)	SST (2-4 d.)	Bioaccumulation & enzyme action	0.05 mg/L elicited increase in sGST activity.	Contardo-Jara et al., 2009
	Roundup Ultra®			0.05 mg/L elicited an increase in sGST activity.	

Abbreviations, acronyms and notes: see Table 1

Table 3. Effects of glyphosate, different commercial formulations of glyphosate and POEA on aquatic invertebrates

Surfactant POEA itself resulted more toxic to bacteria and ciliates (between 2.4 to 5.8 folds) than Roundup® (Tsui & Chu, 2003); though the degradation product of glyphosate (AMPA) resulted less toxic than glyphosate acid (Bonnet et al., 2007).

#### 4.2.4 Effects on non-target aquatic invertebrates

Invertebrates comprise a large group of aquatic species with a wide variety in shape and size, and evolved to utilize different habitats and resources (e.g., insects, worms, snails, hydroids, crustacean, etc.). They can be practically divided in micro and macro groups. Micro-invertebrates (zooplankton) are keystone species (e.g. as food for predators and top-down controller of phytoplankton, periphyton and detritus) (Montenegro Rayo, 2004). The micro-invertebrates (rotifers and copepods) are usually more abundant in number of individuals, but their smaller size limit their impact to size discrimination of phytoplankton rather than a reduction of total algal biomass (Scheffer, 1998). Large cladocerans (e.g., *Daphnia spp.*) can feed efficiently on a wide range of particle types and sizes. Moreover, cladocerans are also important as a major food source for many fish species and predatory invertebrates. Some crustacean species (e.g., shrimps, crabs and crayfish) are important as food resource for men. Insects are foraging on zooplankton, periphytic algae, and detritus and themselves prey for fish and waterfowl. In addition, aquatic molluscs (i.e. snails and mussels) have also a significant importance in aquatic environments and in human life (e.g. parasite vectors; invasive species; top-down controller of phytoplankton, periphyton and detritus; source of food to higher trophic levels, as well as human food resources) (Brönmark & Hansson, 2005).

Aquatic invertebrates seem to have low sensibility to glyphosate itself (Table 3). Values of LC<sub>50</sub> obtained in treatments with glyphosate ranged from 49.3 mg a.e./L for the marine copepod *Acartia tonsa* to 415 mg a.e./L for the cladoceran *Ceriodaphnia dubia* (Tsui & Chu, 2003). However, Tate et al. (1997 and 2000), reported remarkable results in the snail *Pseudosuccinea columella* (an intermediate snail host of *Fasciola hepatica*) treated with lower glyphosate concentrations (0.1, 1 and 10 mg/L). Concentrations of 1 mg/L elicited an increment in the growth in the third-generation of snails, as well as 0.1 and 10 mg/L elicited an inhibition of eggs hatching, abnormalities and polyembryony (Tate et al., 1997). Same authors observed significant differences in the metabolism of *P. columella*. Glyphosate concentrations of 0.1 mg/L induced about 2 folds increment in five free amino acids (Tate et al., 2000). In addition, Contardo-Jara et al. (2009) reported significant increment in the enzymes activities (sGST and SOD) of worm *Lumbriculus variegatus* at 0.05 and 0.1 mg a.i./L of glyphosate.

On the other hand, invertebrates showed higher sensibility to commercial formulations. For instance, Roundup® showed between 3 folds to 76 folds higher toxicity than glyphosate itself. Values of LC<sub>50</sub> obtained in Roundup® treatments ranged from 1.5 mg a.e./L for the amphipod *Hyalella azteca* (Tsui & Chu, 2004) to 62.0 mg a.i./L for other amphipod *Gammarus pseudolimnaeu* (Folmar et al., 1979) (Table 3). In addition, 0.7 mg a.e./L of Vision® (a commercial formulation containing POEA) elicited a 100 % of mortality and more than 50 % reduction in total neonates per female in the cladoceran *Simocephalus vetulus* at values of pH = 7.5 (Chen et al., 2004).

Sublethal effects were observed at much lower concentration of Roundup (1.1 µg/L) in the Clam, *Ruditapes decussates*, showing histological alterations (Abdel-Nabi, et al., 2007; El-Shenawy, et al., 2009).

The surfactant POEA was several times more toxic for invertebrates than glyphosate itself and Roundup®. For example, Folmar et al. (1979) reported almost 1.4 folds higher toxicity of POEA relative to Roundup® in the midge larvae *Chironomous plumosus*; contributing with

about 66 % of the Roundup® toxicity. Higher values were reported by Servizi et al. (1987) and Tsui & Chu (2003), being POEA 3 and 5 folds more toxic than Roundup®, respectively. POEA can contribute with more than the 90% of Roundup® toxicity (Tsui & Chu, 2003) On the other hand, commercial formulations without POEA (e.g. Ron-Do®, Rodeo® and Roundup bioactive®) showed lower toxicity (Alberdi et al., 1996; Tsui & Chu, 2004) than other formulations (Table 3).

#### 4.2.5 Effects on non-target aquatic vertebrates

Fishes are well appreciated in human societies in many ways (e.g., economical, recreational, ecological). In many countries, commercial fishing has a large economic importance as national food supply and as an export product. Fishes are one of the most demanded pets and there are a lot of people who enjoy sport fishing. This vertebrate group has an amazing diversity in morphology, size and color, which reflects their life history adaptations (e.g., feeding behavior reproduction and habitat selection). It is well known that fish populations have both direct and indirect effects on ecosystem function and structure in general (e.g., nutrient dynamics and cycling, zooplanktonic community composition), and especially in freshwater ecosystems where they are top consumers on lower trophic levels (e.g., piscivore, planktivore, benthivore fish) (Scheffer, 1998; Montenegro Rayo, 2004; Brönmark & Hansson, 2005).

The major groups of amphibians found in lakes and ponds are frogs, toads and salamanders. Some species live their whole life in freshwater whereas other species are completely terrestrial and depended on water for their reproduction. Most tadpoles have a feeding apparatus that allows them to trap bacteria, phytoplankton, and other small particles suspended in the water. Many species also graze on periphytic algae and some species even have mouth parts adapted for a predatory feeding mode. Salamanders start to feed on zooplankton but as they grow they include larger invertebrates in their diet and some species even prey on tadpoles. Tadpoles, frogs, toads and salamanders are important source of food for fish and birds.

Aquatic fish and amphibians appear to have low sensibility to glyphosate itself (Table 4 and 5). Values of LC<sub>50</sub> in glyphosate treatments ranged from 130 mg a.i./L for the channel catfish *Ictalurus punctatus* (Folmar et al., 1979) to 620 mg a.i./L for the carp *Cyprinus carpio* (Neškovic et al., 1996) (Table 4). In amphibians, values of LC<sub>50</sub> obtained with glyphosate (IPA salt) treatments, varied from 340 to 460 mg a.e./L in four tadpoles of Australian frogs (Table 5). However, lower values were obtained in treatments with glyphosate as an acid, reporting values of LC<sub>50</sub> from 82 to 121 mg a.e./L (Mann & Bidwell, 1999). Similar toxicity was registered for Roundup® Biactive, a commercial formulation without surfactant POEA (Mann & Bidwell, 1999).

Wide differences were observed in the toxicity of glyphosate itself and commercial formulations. In fish, values of LC<sub>50</sub> obtained with Roundup® treatments ranged from 2.3 mg a.i./L for the fathead minnows *Pimephales promelas* (Folmar et al., 1979) to 14.5 mg /L for *Ictalurus punctatus* (Abdelghani et al., 1997). Treatments with Vision® showed middle LC<sub>50</sub> (10.42 mg a.i./L) for the rainbow trout *Oncorhynchus mykiss* (Morgan & Kiceniuk 1992). In addition, values of 4 and 12 mg a.i./L of Eskoba III Max® (a commercial formulation with unknown surfactant) elicited the 25 % and 20% of mortality in juveniles of Pejerrey *Odontesthes bonariensis* and adults of Tosquero *Jenynsia lineata* respectively; though not lethal effects were observed in glyphosate treatments (Pérez & Miranda unpublished) (Fig. 3). However, much lower concentrations of Roundup® have shown to cause effects in the biometry, metabolism and enzyme activities of fish. For instance, Gluszczak et al. (2007) reported a significant decrease in AChE activity (enzyme presents in cholinergic synapses and motor end plates) and

TBARS levels (a measure of oxidative stress) in the brain of silver catfish *Rhamdia quelen* exposed to 0.2 and 0.4 mg/L. Besides, significant reduction in the biometry of Piava *Leporinus obtusidens* was observed in treatments with 1 mg/L, eliciting a reduction of length (15%) and weight (50%) (Salbego et al., 2010). Other recent study, indicated molecular responses for the flounder *Platichthys flesus* treated with low doses of herbicide cocktail (< 10 µg/L of glyphosate) during a long-term contamination (62 days) (Evrard et al., 2010).

VERTEBRATES	ASSESSED CHEMICAL	STUDY TYPE	ASSESSED PARAMETER	EFFECTS CONCENTRATION (mg/L) ##	REFERENCE
<i>Fish</i>					
<i>Oncorhynchus mykiss</i>	Gly. (acid)	SST (96h)	Mortality	96h LC <sub>50</sub> = <b>140.0</b>	Folmar et al., 1979
	Roundup®			96h LC <sub>50</sub> = <b>8.3</b>	
	POEA			96h LC <sub>50</sub> = <b>2.0</b>	
<i>Pimphales promelas</i>	Gly. (acid)	SST (96h)	Mortality	96h LC <sub>50</sub> = <b>97.0</b>	Folmar et al., 1979
	Roundup®			96h LC <sub>50</sub> = <b>2.3</b>	
	POEA			96h LC <sub>50</sub> = <b>1.0</b>	
<i>Ictalurus punctatus</i>	Gly. (acid)	SST (96h)	Mortality	96h LC <sub>50</sub> = <b>130.0</b>	Folmar et al., 1979
	Roundup®			96h LC <sub>50</sub> = <b>13.0</b>	
	POEA			96h LC <sub>50</sub> = <b>13.0</b>	
<i>Oncorhynchus mykiss</i>	Roundup®	SSFE (96h)	Mortality	96h LC <sub>50</sub> = <b>52.0</b>	Hildebrand et al., 1982
<i>Oncorhynchus mykiss</i>	Roundup®	SST (96h)	Mortality	96h LC <sub>50</sub> = <b>8.5</b>	Servizi et al., 1987
	POEA			96h LC <sub>50</sub> = <b>3.2</b>	
<i>Oncorhynchus nerka</i>	Roundup®	SST (96h)	Mortality	96h LC <sub>50</sub> = <b>8.1</b>	Servizi et al., 1987
	POEA			96h LC <sub>50</sub> = <b>2.6</b>	
<i>Oncorhynchus mykiss</i>	Vision®	SST (96h)	Mortality	96h LC <sub>50</sub> = <b>10.2</b>	Morgan & Kiceniuk, 1992.
<i>Cyprinus carpio</i>	Gly. (acid)	SST (96h)	Mortality #	96h LC <sub>50</sub> = <b>620.0</b>	Neškovic et al., 1996
<i>Ictalurus punctatus</i>	Roundup®	SST (96h)	Mortality	96h LC <sub>50</sub> = <b>14.5</b>	Abdelghani et al., 1997
<i>Lepomis macrochirus</i>	Roundup®	SST (96h)	Mortality	96h LC <sub>50</sub> = <b>13.0</b>	Abdelghani et al., 1997
<i>Rhamdia quelen</i>	Roundup®	SST (96h)	Metabolism & enzyme activity#	<b>0.2 mg/L</b> elicited a decrease in AChE and TBARS.	Glusczak et al., 2007
<i>Prochilodus lineatus</i>	Roundup®	SST (96h)	Mortality & physiology #	96h LC <sub>50</sub> = <b>13.7</b>	Carmo Langiano & Martinez, 2008
<i>Leporinus obtusidens</i>	Roundup®	SST (90 d.)	Biometry & enzyme activity#	<b>1 mg/L</b> elicited a decrease in length and weight and AChE	Salbego et al., 2010
<i>Platichthys flesus</i>	Roundup® + AMPA	SST (62 d.)	Molecular and physiology	<b>0.16 µg/L</b> elicited liver injury	Evrard et al., 2010
<i>Odontesthes bonariensis</i>	Gly. (IPA salt)	SST (96h.)	Mortality	Not observed lethal effect	Pérez & Miranda (unpublished)
	Eskoba III Max®			<b>4 mg/l</b> elicited the 25% of mortality	
<i>Jenynsia lineata</i>	Gly. (IPA salt)	SST (96h.)	Mortality	Not observed lethal effect	Pérez & Miranda (unpublished)
	Eskoba III Max®			<b>12 mg/l</b> elicited the 20% of mortality	

Abbreviations, acronyms and notes: see Table 1

Table 4. Effects of glyphosate, different commercial formulations of glyphosate and POEA on fish.

Comparable outcomes with treatments of Roundup® and Glyphos® (other commercial formulation containing POEA) were reported for amphibians (Table 5). In laboratory tests, values of LC<sub>50</sub> varied from 2.6 mg/L of Glyphos® for tadpoles of the hylid *Scinax nasicus* (Lajmanovich et al., 2003) to 11.6 mg a.e./L of Roundup® for tadpoles of *Litoria moorei* (Mann & Bidwell, 1999). Middle concentrations (8 mg a.i./L) caused 100% of mortality in tadpoles of the toad *Rhinella arenarum* (Pérez & Miranda unpublished) exposed to Eskoba III Max® (Fig. 6), though LC values of 3.2 mg a.i./L were reported for this toad exposed to Roundup Ultra-Max (Lajmanovich et al., 2010). However, lower concentrations have shown significant effects in

mortality and growth. For instance, Chen et al. (2004) reported 100 % of mortality in tadpoles of *Rana pipiens* treated with 0.75 mg a.e./L of Vision® at pH of 7.5. Cauble & Wagner (2005) observed 50% of mortality for tadpoles of *Rana cascadae* treated with 1.94 mg a.i./L of Roundup® and an earlier metamorphosis time with 1 mg a.i. /L (Table 5). In addition, 2 mg a.i./L significantly reduce the survival and growth in three of five tadpoles exposed to Roundup® (Relyea, 2004). The same author reported in a mesocosms experiment a 100% of mortality for the *Rana sylvatica* and *Hyla versicolor* tadpoles and around 98 % of mortality for *Rana pipiens* and *Bufo americanus* tadpoles due to a direct herbicide effect (Relyea, 2005). Tadpoles seem to be more sensible to commercial formulations than juveniles and adults.

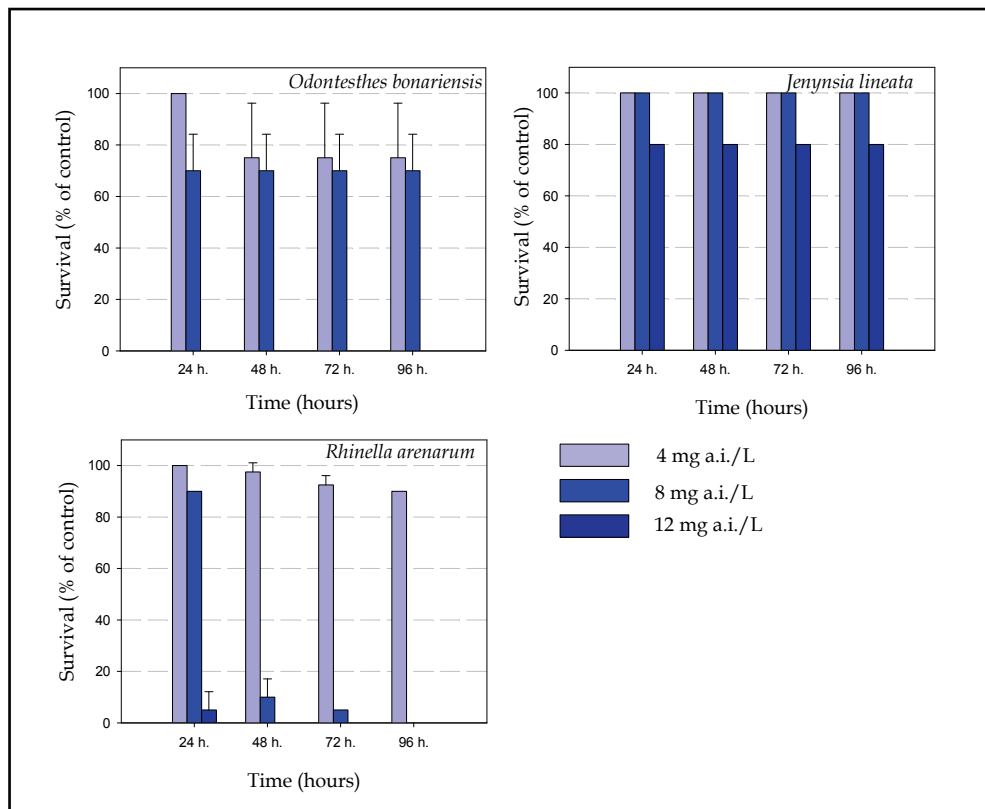


Fig. 3. Acute lethal effects of Eskoba III Max® on two fish species (*Odontesthes bonariensis* and *Jenynsia lineata*) and on tadpoles from *Rhinella arenarum*

POEA itself resulted more toxic than Roundup®, being this surfactant the more noxious component of several commercial formulations. Different authors concluded that the high mortality in fish and amphibian are actually due mainly to POEA surfactant and not to glyphosate itself (Folmar et al., 1979; Servizi et al., 1987; Perkins et al., 2000). In the fish *Pimephales promelas*, the relative contribution of glyphosate acid to the toxicity of Roundup® was about 30% (Folmar et al., 1979), while glyphosate (as IPA salt) was not toxic for 5 species of Australian frogs, and therefore without any contribution to Roundup® toxicity



(Mann & Bidwell, 1999). In fish, values of LC<sub>50</sub> obtained in POEA treatments varied from 1 to 13 mg a.i./L (Folmar et al., 1979; Servizi et al., 1987), being these values up to 4 fold more toxic than Roundup®. Besides, Perkins et al. (2000), found LC<sub>50</sub> values of 6.8 mg a.e./L for POEA treatments in African *Xenopus laevis* tadpoles, showing 1.8 fold higher toxicity than commercial formulation.

VERTEBRATES	ASSESSED CHEMICAL	STUDY TYPE	ASSESSED PARAMETER	EFFECTS CONCENTRATION (mg/L) ##	REFERENCES
<b>-Amphibians (frogs and toads)</b>					
<i>Lymnodynastes dorsalis</i>	Gly. (IPA salt)	SST (48 h.)	Morality	48h LC <sub>50</sub> = > 400	Mann & Bidwell, 1999
	Roundup®			48h LC <sub>50</sub> = 3.0	
	Roundup® Biactive			48h LC <sub>50</sub> = > 400	
	Touchdown®			48h LC <sub>50</sub> = 12.0	
<i>Heleioporus eyrei</i>	Gly. (IPA salt)	SST (48 h.)	Morality	48h LC <sub>50</sub> => 373	Mann & Bidwell, 1999
	Roundup®			48h LC <sub>50</sub> = 6.3	
	Roundup® Biactive			48h LC <sub>50</sub> => 427	
	Touchdown®			48h LC <sub>50</sub> = 16.1	
<i>Crinia insignifera</i>	Gly. (IPA salt)	SST (48 h.)	Morality	48h LC <sub>50</sub> = > 466	Mann & Bidwell, 1999
	Roundup®			48h LC <sub>50</sub> = 3.6	
	Roundup® Biactive			48h LC <sub>50</sub> = > 494	
	Touchdown®			48h LC <sub>50</sub> = 9.0	
<i>Xenopus laevis</i>	Rodeo®	SST (96 h.)	Morality	96h LC <sub>50</sub> = 5407	Perkins et al. 2000
	Roundup®			96h LC <sub>50</sub> = 9.4	
	POEA			96h LC <sub>50</sub> = 2.7	
<i>Scinax nasicus</i>	Glyphos®	SST (96 h.)	Mortality #	96h LC <sub>50</sub> = 2.6	Lajmanovich et al., 2003
<i>Rana pipiens</i>	Vision®	SST (8 d.)	Mortality #	0.75 mg/L elicited 100 % mortality	Chen et al., 2004
<i>Rana pipiens</i>				Not observed significant effects	
<i>Rana clamitans</i>				2 mg/L reduce survival & growth	Relyea, 2004
<i>Rana catesbeiana</i>	Roundup®	SST (16 d.)	Mortality & growth	2 mg/L reduce survival & growth	
<i>Bufo americanus</i>				2 mg/L reduce growth	
<i>Hyla versicolor</i>				Not observed significant effects	
<i>Rana sylvatica</i>				3.8 mg/L elicited 100% mortality	Relyea, 2005
<i>Rana pipiens</i>				3.8 mg/L elicited 98% mortality	
<i>Bufo americanus</i>	Roundup®	MES (15 d.)	Mortality & biomass #	3.8 mg/L elicited 98% mortality	
<i>Hyla versicolor</i>				3.8 mg/L elicited 100% mortality	
<i>Pseudacris crucifer</i>				Not observed significant effects	
<i>Rana cascadae</i>	Roundup®	SST (42 d.)	Mortality & metamorphosis#	1.94 mg/L elicited mortality and earlier metamorphosis times	Cauble & Wagner, 2005
<i>Rhinella arenarum</i>	Gly. (IPA salt)	SST (96h.)	Morality	Not observed significant effects	Pérez & Miranda (unpublished)
	Eskoba III Max®			8 mg/L elicited 100% mortality	

Abbreviations, acronyms and notes: see Table 1

Table 5. Effects of glyphosate, different commercial formulations of glyphosate and POEA on frogs and toads.

### 5. Conclusions

- Reviewing the available information on toxicity of glyphosate and its formulations on different groups of aquatic organisms, we have concluded that they are hazardous to the

aquatic environment. Several contributions reviewed here reported significant effects of the herbicide at concentrations lower than EEC (2.6 mg a.i./L). Herbicide could be very noxious in standing waters like ponds, or in irrigation canals and impounded waters, where EEC can be reached. In these scenarios, toxicity could be exacerbated by other stressors and water characteristics (e.g. high temperature and pH, low O<sub>2</sub> concentration, presence of clay colloids, water hardness and other chemicals). Besides, toxicity also has showed to depend on organism life stage.

- Overall, ecotoxicological sub-lethal endpoints based on behavioral traits (e.g., predator avoidance, feeding, and locomotion) and other endpoints (e.g. growth, reproduction and metabolism) seem to be more sensitive indicators of effects (i.e. reporting lower effective concentrations) and give more insights into patterns of toxicity than survivorship tests (i.e. lethality). In addition, in doses dependent effects studies, commonly results are expressed as LC<sub>50</sub> or EC<sub>50</sub>. However, it is not possible to predict, for instance, if the 10 % of mortality or reduction in growth (i.e at lower herbicide concentration) do not have significant effects on population and eventually in the community. On the other hand, studies focused in natural or assembled communities (e.g. microcosm and mesocosms experiments) have provided interesting and significant outcomes regarding direct and indirect herbicide effects that could not be reached in single species laboratory tests. Although these laboratory tests are an essential protocol to rapidly identify the direct impacts of pesticides on organisms, they prevent an assessment of effects on organisms embedded in their natural ecological contexts.

- Glyphosate itself (as acid or salt) is generally considered to be slightly or moderately toxic to aquatic organisms (i.e., LC<sub>50</sub> or EC<sub>50</sub> between >1 to < 100 mg/L). However, some algae and aquatic plants showed higher sensibility, being glyphosate very toxic (EC<sub>50</sub> between >0.1 to < 1 mg/L). Aquatic plants seem to be more sensitive to glyphosate than microalgae. The high toxicity of glyphosate in algae and aquatic plants is related with the mode of action of this compound (an herbicide) that interferes with plant metabolisms. On the other hand, much lower glyphosate toxicity was observed for other aquatic organisms (i.e. bacteria, protozoa, invertebrates, fish and amphibians). However, snails and worms seem to be exceptions; showing significant effects in growth, reproduction and metabolism at concentrations of < 1 mg/L of glyphosate.

- Commercial formulations and specially those containing the surfactant POEA, showed higher toxicity than the active ingredient itself for all the aquatic organisms studied. Roundup® showed to be up to 7 folds more noxious than glyphosate in algae and aquatic plants, up to 13 folds in protozoa, up to 42 folds in fish, up 70 folds in crustaceans, and up to 130 folds in frogs and toads. Algae and aquatic plants, showed significant effects with concentrations < 3 mg a.i./L. however, lower values were registered in studies of periphyton and micro plankton communities. Roundup® concentration of 10 µg a.i./L elicited changes in marine microbial community structure and 0.13 mg a.i./L of Rodeo® caused an increment in periphyton primary production. In addition, significant effects at concentrations relevant to environmental toxicity thresholds were also observed for other groups of aquatic organism. In protozoa, *Euglena gracilis* showed high sensibility, with 0.1 mg a.i. /L of Roundup® and Avans® eliciting significant sublethal effects. Different species of crustaceans showed lethal effects with values lower than 3 mg a.i./L of Roundup®. In Frogs and toads, relevant concentrations of glyphosate based products (< 2 mg a.i./L) elicited lethal and sublethal effects. Fish seems to be less sensitive to commercial

formulations, though some contributions showed significant sublethal effects in metabolism and enzyme activity at concentrations (< 2.5 mg a.i./L) of Roundup®

- The high toxicity observed for several commercial formulation of glyphosate was generally related with the content of POEA. In protozoa and invertebrates, POEA contributed with more than the 80% of Roundup® toxicity. Crustaceans showed values of EC<sub>50</sub> and LC<sub>50</sub> that ranged from 0.097 to 2 mg/L of POEA. In fish, glyphosate only contributed to the toxicity of Roundup® with around 30%, and values of EC<sub>50</sub> ranged from 1 to 13 mg/L of POEA. In frogs and toads POEA seems to be the most toxic compound in commercial formulations. Glyphosate alone (as IPA salt) was not toxic for 5 species of Australian frogs (LC<sub>50</sub> > 343 mg a.e./L) and therefore contributed little to Roundup® toxicity. In contrast, POEA could show lower contribution in algae and aquatic plants (as from about 46%).
- Stated the hazard of glyphosate and commercial formulations of glyphosate on aquatic environments and ecological implication of the effects reviewed here, we stressed the paucity of contributions studying the effects of glyphosate on several potential endangered aquatic species (e.g. hydroids, sponges, worms, flatworms, insects, and urodela species). We also emphasize the necessity of studies in natural communities or in assembled communities in order to evaluate direct and indirect effects upon different trophic levels.
- Finally we consider that glyphosate and commercial formulations of glyphosate could have particularly significant disruptive effects to waterbodies like ponds. Ponds have been widely recognized as very important freshwater habitats. These relative small and shallow still aquatic environments are very rich in genetic and taxonomic biodiversity; they are important refuges for amphibians and also for a bewildering variety of plants and animals, including many scarce and endangered species. In addition ponds are important places for insects hatching, fish larvae and juveniles refuges and net sites for wetland birds.

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Herbicides are much more than just weed killers. They may exhibit beneficial or adverse effects on other organisms. Given their toxicological, environmental but also agricultural relevance, herbicides are an interesting field of activity not only for scientists working in the field of agriculture. It seems that the investigation of herbicide-induced effects on weeds, crop plants, ecosystems, microorganisms, and higher organism requires a multidisciplinary approach. Some important aspects regarding the multisided impacts of herbicides on the living world are highlighted in this book. I am sure that the readers will find a lot of helpful information, even if they are only slightly interested in the topic.

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