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GLYPHOSATE: DISCOVERY, DEVELOPMENT, APPLICATIONS, AND PROPERTIES

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1.1 HISTORICAL PERSPECTIVE AND MODE OF ACTION

N-(phosphonomethyl)glycine (glyphosate) is a phosphonomethyl derivative of the amino acid glycine. Glyphosate is a white and odorless crystalline solid comprised of one basic amino function and three ionizable acidic sites (Fig. 1.1). Glyphosate was actually invented in 1950 by a Swiss chemist, Dr. Henri Martin, who worked for the small pharmaceutical company, Cilag (Franz et al. 1997). The product had no pharmaceutical application and was never reported in literature. In 1959, Cilag was acquired by Johnson and Johnson, which sold its research samples, including glyphosate, to Aldrich Chemical. Aldrich sold small amounts of the compound to several companies in the 1960s for undisclosed purposes, but no claims of biological activity were ever reported. In its Inorganic Division, Monsanto was developing compounds as potential water-softening agents and over 100 related aminomethylphosphonic acid (AMPA) analogs were synthesized. When these compounds were tested as herbicides by Dr. Phil Hamm, two showed some herbicidal activity on perennial weeds. However, the unit activity was too low to be a commercial herbicide.

Dr. Hamm enlisted the efforts of Monsanto chemist Dr. John Franz. He repeatedly told Dr. Franz that "he just wanted something five times as strong

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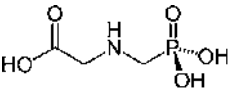


Figure 1.1. The structure of glyphosate.

… that's all." "He convinced me to take a shot at making analogs and derivatives," recalled Dr. Franz. "That didn't yield anything, and I was ready to drop the project. But then I began trying to figure out the peculiarities of those two compounds, and I wondered if they might metabolize differently in the plants than the others … I began to write out metabolites … you could write a list of about seven or eight … it involved completely new chemistry. Glyphosate was the third one I made" (Halter 2007). Glyphosate was first synthesized by Monsanto in May 1970 and was tested in the greenhouse in July of that year. The molecule advanced through the greenhouse screens and field testing system rapidly and was first introduced as Roundup® herbicide by Monsanto Company (St. Louis, MO) (Baird et al. 1971).

Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Amrhein et al. 1980), which is present in plants, fungi, and bacteria, but not in animals (Kishore and Shah 1988). The enzyme catalyzes the transfer of the enolpyruvyl moiety of phosphoenolpyruvate (PEP) to shikimate-3-phosphate (S3P). This is a key step in the synthesis of aromatic amino acids, and ultimately, hormones and other critical plant metabolites. The active site of the EPSPS enzyme in higher plants is very highly conserved (CaJacob et al. 2003). The mechanism of inhibition is also unique in that the binding site for glyphosate is reported to closely overlap with the binding site of PEP (Franz et al. 1997). A diagram of the shikimate pathway and glyphosate's inhibition site is shown in Figure 1.2. No other mode of action for glyphosate has been observed even when very high doses are applied to glyphosate-resistant (GR) soybean and canola (Nandula et al. 2007).

Glyphosate is currently labeled for use in over 130 countries, and current global volume is estimated to be approximately 600 kilotons annually (Research and Markets 2008). The current U.S. glyphosate label of Monsanto Agricultural Herbicides lists over 100 annual broad-leaved and grass species controlled. In addition, over 60 perennial weed species are also included on the label as of the writing of this chapter. It is the broad spectrum perennial weed control that makes glyphosate a very effective product. The ability of the product to translocate to growing meristematic tissues and inhibit an enzymatic process present in plants allows applicators to control underground meristems, corms, rhizomes, and other potential vegetative structures, which regenerate when only upper vegetative material is killed.

Because of its unique properties, glyphosate was initially utilized to control perennial weeds on ditch banks, in right of ways, and fallow fields. However, because it also killed crops, its uses in mainstream agriculture were limited until the use of minimum and no-till practices began to evolve. Spraying

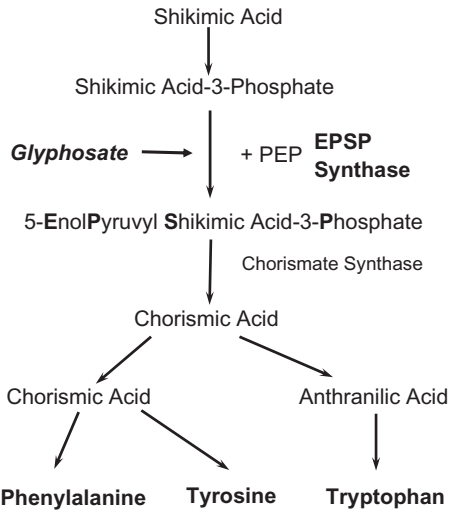


Figure 1.2. The site of inhibition of glyphosate from Dill (2005).

glyphosate to control weeds prior to planting allows growers to substitute chemical weed control with light-duty spray equipment for tillage. This practice saves fuel, preserves soil from erosion, and allows better water permeation into the soil (Dill 2005). Conservation tillage practices have continued to grow with the introduction of GR crops (Dill et al. 2008).

1.2 UPTAKE AND TRANSLOCATION OF GLYPHOSATE

The herbicidal efficacy of glyphosate is strictly dependent on the dose of glyphosate delivered to the symplastic or living portion of the plant. Since glyphosate was first announced (Baird et al. 1971) as a broad spectrum herbicide (and before the evolution of GR weeds), it could be said that all plants could be controlled given delivery of the appropriate dose of glyphosate. The delivery of this efficacious dose has continually been the topic of investigation now for almost 40 years with at least 40 individual weed species now studied in detail to determine the efficiency of uptake and the extent of translocation. The corollary science of pesticide application is an extensive area covering the physics of spray application and the reader is directed to a standard text (Monaco et al. 2002), while here we focus on uptake and translocation.

The first uptake efficiency and translocation studies of ^{14}C-glyphosate (Sprankle et al. 1973) characterized the principal features of glyphosate that we know today: phloem transport and consequent delivery to meristematic growing points in the roots and vegetation. The phloem movement of glyphosate intimately linked the efficiency of translocation to plant health and developmental stage, which are tied to environmental conditions. The early work is

well summarized in the book *The Herbicide Glyphosate* (Caseley and Coupland 1985). The discovery of the mode of action of glyphosate to be the inhibition of EPSPS (Steinrucken and Amrhein 1980) was largely due to the very rapid large accumulation of shikimic acid (Amrhein et al. 1980), which now routinely serves as a means to measure glyphosate toxicity (Singh and Shaner 1998).

Uptake and translocation studies are two different types of studies that are often combined as one to the detriment of both. Uptake studies should focus on the drop size and solute concentrations (and not really the total dose), whereas translocation studies require precise dose amounts so that distribution ratios can be calculated. There is a conundrum in uptake studies between volume and concentration when trying to deliver the desired dose. It is virtually impossible to deliver by hand application the droplets dictated by typical carrier volumes; the drops are just too small and too numerous. Consequently, the experimental dose is usually applied in a much smaller volume and/or much larger drop, dramatically distorting the concentration ratios of herbicide:surfactant: carrier volume ruining the lessons to be learned about the efficiency of spray solution penetration. Understanding the impact of spray solution composition on the efficiency of glyphosate penetrating the cuticle to the apoplast and the stepwise entry into the symplast where phloem transport can occur is critical to optimizing herbicide formulation. Normally, the amount of glyphosate "inside" the leaf or not removed by washing is considered the efficiency of uptake. Uptake is dependent on several interdependent factors: droplet size and droplet spread, cuticle composition and thickness, surfactant type and concentration, ionic strength and salt concentration, humidity, and, most importantly, glyphosate concentration. Because of the critical linkages between these factors, the most informative uptake studies are done with a sprayed application using a standard field nozzle and carrier (Feng et al. 2000; Prasad 1989, 1992). However, it is extremely difficult to deliver a precise dose due to the practical problems of leaf intercept of a spray application, and so the leaf intercept efficiency must be included. The interaction of drop size, surfactant, and herbicide concentration does impact the leaf surface cytology and can be correlated to efficiency of uptake (Feng et al. 1998, 2000). The cytotoxic damage caused by the excess surfactant/cuticle surface area provided by a large drop quickly "kills" the loading site for translocation and prematurely stops phloem loading. The exact correlation of drop size and concentration to penetration was determined by using a droplet generator (Prasad and Cadogan 1992). The herbicide concentration in very small droplets did overcome the drop-size factors, and the smaller droplets had minimal negative effect on epidermal cytology (Ryerse et al. 2004), thereby, avoiding the inhibition of transport caused by too much local cell damage (often seen in hand-applied large drops). The concept that small spray droplets do not actually dry but soak into the leaf was shown by coapplication with heavy water (deuterium oxide, D_2O), indicating that the surfactant forms channels to allow the herbicide to penetrate the cuticle as measured by the appearance of D_2O in the leaf (Feng et al. 1999). Spray applications on GR corn then allowed the

separation of local droplet-herbicide toxicity from droplet-surfactant injury related to drop size to show that large drops, while being retained less efficiently, were more efficient at loading glyphosate and allowing improved translocation. Consequently, studies that spray ^{14}C-glyphosate provide the best means to mimic field conditions and simultaneously understand the formulated droplet uptake characteristics (Feng and Chiu 2005; Feng et al. 2000, 2003b).

Translocation efficiency is dramatically affected by the self-limitation feature of glyphosate toxicity (vide infra) creating another paradox, optimizing translocation (improving with time) with increasing toxic effect (increasing with time). The negative effects on apical meristems with a small dose of glyphosate are readily accounted for by the observation that individual plant tissues have different sensitivities to glyphosate (Feng et al. 2003a). This toxicity affects the overall glyphosate efficiency and distribution pattern to sink tissues. Dewey (1981) noted that glyphosate easily loaded the phloem, moved from source to sink, and did not usually leave the symplastic assimilant flow. Gougler and Geiger (1981) used a sugar beet model system to demonstrate that glyphosate loads the phloem passively, and this result holds true as no significant active transport of glyphosate has ever been measured. They subsequently showed that reductions in photosynthesis resulted directly in limiting glyphosate translocation (Geiger et al. 1986) and further that glyphosate created a self-limitation of translocation due to its toxicity shutting down photosynthesis and sucrose metabolism (Geiger and Bestman 1990). These observations strongly suggest that the standard practice of overspraying a plant with cold glyphosate at a field rate and then spotting the ^{14}C-glyphosate on a particular leaf to measure translocation is a bad idea. First, the translocation from that source leaf will depend on "its" perception of sink strengths based on its location on the plant. Second, the self-limitation due to whole plant toxicity will prematurely limit translocation. Third, the unknown proportional mixing of cold and ^{14}C-glyphosate precludes learning about the concentration of glyphosate in a tissue. Because translocation studies are more concerned with how "much" glyphosate goes "where" from a source location, then one can simply apply a precise dose to a specific location. The faster the uptake, the better, because the first minute amounts of glyphosate delivered to sinks will begin to initiate the self-limitation, which ultimately stops translocation. Hence, a rapid delivery (but not locally cytotoxic) dose allows more glyphosate to be translocated and reveal the proportional sink strengths from that source location.

The use of GR plants compared with wild-type or a sensitive plant allows the separation of the effects of physiological barriers, like metabolic toxicity from physical barriers such as membranes, cell walls, and cuticles (Feng and Chiu 2005; Feng et al. 2003b). It is not always possible to have a GR plant for this comparison and so that situation can be created by using an ultralow dose of ^{14}C-glyphosate. That is, at some very low dose, the toxicity of glyphosate no longer impacts the uptake and delivery. This concept is particularly useful

when characterizing the mechanism of glyphosate resistance in horseweed (Feng et al. 2004). By comparing resistant and sensitive plants below the toxic effect level, the physiological impact of the resistance mechanism on glyphosate translocation and partitioning can be revealed. Studies with GR plants demonstrate restricted translocation in rigid ryegrass (Lorraine-Colwill et al. 2002; Powles and Preston 2006) and horseweed (Feng et al. 2004), but equal translocation in Palmer amaranth (Culpepper et al. 2006; Sammons et al. 2008). Equal translocation requires a modified hypothesis to explain symplastic translocation because apparently, there is no self-limitation of glyphosate delivery. Hetherington et al. (1999) showed increased translocation in GR corn, which is explained by the removal of toxic self-limitation to improve translocation efficiency. Removal of the source perception of toxicity requires a break in the symplastic phloem source–sink connection. The unabated translocation of glyphosate to a sensitive sink tissue would be a simple method of depleting the effective herbicide in the plant by isolating glyphosate in dying sink tissues, mimicking herbivory, and allowing the main plant to resume normal growth. Such a case is described by Patrick and Offler (1996) where an apoplastic step or intervention in phloem delivery insulates the sink from excessive solute concentration or osmotic changes. Studies with GR soybean demonstrate a clear example of self-limitation for apical meristem translocation, but with equal translocation to root tissue from a common source leaf, implicating sink apoplast unloading in soybean root tissue (Sammons et al. 2006). The species of plants using apoplastic unloading is not known and, if common, would change the general understanding we have of source–sink relationships. The facile ability of glyphosate to move from source to sink poses many opportunities to elucidate the regulation of symplastic and apoplastic movement of normal assimilants.

1.3 GLYPHOSATE'S FUNGICIDAL ACTIVITIES

The sensitivity of plant EPSPS enzymes to glyphosate accounts for its efficacy as an herbicide. However, glyphosate is generally recognized as having little to no fungicidal or bactericidal activities. In pure culture, growth of many fungi was inhibited by glyphosate, but only at extremely high concentrations (100 to more than 1000 mg g^{-1} for ED_{50}) (Franz et al. 1997). The results of our own *in vitro* screens confirmed that glyphosate has weak activity against many fungi (Table 1.1).

Most GR crops do not metabolize glyphosate and coupled with the use of glyphosate-insensitive CP4 EPSPS results in persistence of glyphosate in crops. Soybean is an exception and has shown slow metabolism of glyphosate to AMPA (Duke et al. 2003; Reddy et al. 2004). GR crops enable the evaluation of disease control effects of glyphosate in the absence of crop injury. We showed in 2005 that glyphosate applied to GR wheat at or below the field use

TABLE 1.1. Glyphosate Growth Inhibition (90% Effective Concentration [EC90]) of Important Agronomic Fungi as Measured by an *In Vitro* High-Throughput Screen

Fungi Genus	EC90 ($\mu g\,g^{-1}$ or ppm)
Septoria	<100
Pseudocercosporella	<100
Botrytis	<100
Phytophthora	1000
Rhizoctonia	1000
Fusarium	1000
Gaeumannomyces	1000
Puccinia (rust)	5000
Pyricularia	5000

rate of 0.84 kg a.e. ha^{-1} reduced the incidence of leaf and stripe rusts caused by *Puccinia triticina* and *Puccinia striiformis*, respectively (Feng et al. 2005). Laboratory studies showed that disease control was proportional to the spray dose and was correlated to systemic glyphosate concentrations in leaves. Wheat rusts were controlled by tissue glyphosate concentrations at less than 5 ppm, which is 1000 times less than the activity predicted by the *in vitro* screen (Table 1.1). We attributed this difference to the fact that *Puccinia* species are obligate pathogens that may not be amenable to *in vitro* screens. Stripe rust control by glyphosate was confirmed in the field under a natural heavy infestation. Leaf rust control by glyphosate has also been reported by Anderson and Kolmer (2005), and there are reports of activity on other diseases in cropping systems (Sanyal and Shrestha 2008).

Since our initial observation of disease control activities in GR wheat, our attention has shifted to *Phakopsora pachyrhizi*, an obligate pathogen that causes Asian soybean rust (ASR). We reported preliminary data on the activity of glyphosate against ASR in GR soybeans (Feng et al. 2005). Subsequent laboratory studies confirmed that leaf systemic glyphosate was responsible for controlling ASR, and efficacy in the field required application rates of glyphosate at 0.84–1.68 kg ha^{-1} (Feng et al. 2008). Additional laboratory studies using excised soybean trifoliates demonstrated rate-dependent activity of glyphosate against ASR at leaf concentrations ranging from 50 to 200 ppm. Analysis of leaf tissues showed that these concentrations may be reached within 24 h after spray application of glyphosate at 0.84–1.68 kg ha^{-1}.

Field studies conducted in the United States, Brazil, Argentina, and South Africa demonstrated significant reductions in ASR severity and yield loss from the application of glyphosate at rates between 0.84 and 2.5 kg ha^{-1}. These results have been corroborated by independent field studies from several

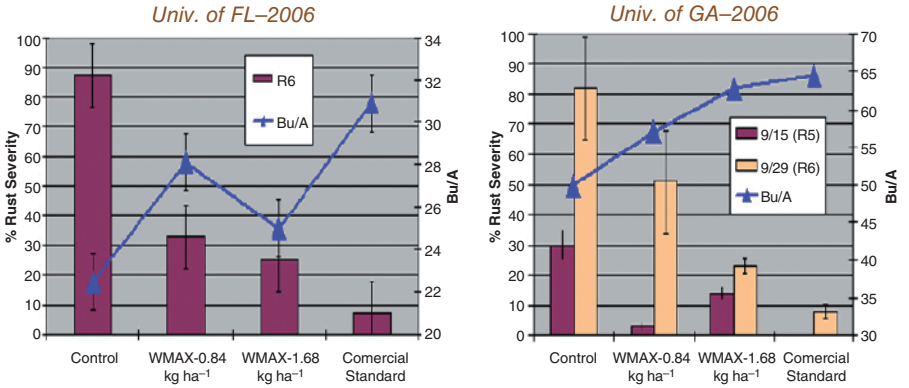


Figure 1.3. Results of field trials conducted by two universities on the effect of glyphosate on percentages of Asian soybean rust severity and yield (Bu/A) in soybeans. Glyphosate (Roundup WeatherMAX®) was applied at 0.84 or 1.68 kg a.e. ha^{-1} at R5 or R6 growth stages. The commercial fungicide standard was the labeled rate of pyraclostrobin. WMAX, Roundup WeatherMAX at indicated rates in kg a.e. ha^{-1}.

universities (R. Kemerait et al. pers. comm.; D. Wright et al. pers. comm.; Harmon et al. 2006). Figure 1.3 shows field results obtained from Universities of Florida and Georgia in 2006. The results showed dose-dependent decrease in ASR severity and preservation of yield from applications of glyphosate at 0.84–1.68 kg ha^{-1}. ASR control by glyphosate was less than that of a fungicide control.

We attributed glyphosate's activity to inhibition of fungal EPSPS based on observations that rust control was proportional to glyphosate tissue concentrations and not mediated via induction of pathogenesis-related genes (Feng et al. 2005). Infected plants treated with glyphosate show marked accumulation of shikimic acid, which is a well-established marker for the inhibition of plant EPSPS by glyphosate. Experiments were conducted to determine if shikimate accumulation might also serve as a marker for inhibition of fungal EPSPS. GR soybean leaves do not accumulate shikimate when treated with glyphosate because these plants are engineered with the glyphosate-insensitive CP4 EPSPS (Fig. 1.4). Shikimate levels also remained low when plants were infected with ASR, but without the glyphosate treatment, indicating a low basal level of shikimate in *P. pachyrhizi*. Significant increase in shikimate levels were observed only in infected leaves treated with glyphosate, suggesting that the source of the shikimate is from the fungi. There was an increase in shikimate levels with glyphosate applications from 4 to 10 days after inoculation, and this was coincident with the incubation period of *P. pachyrhizi* in soybeans and also with a reduction in disease severity. These results provided strong evidence that rust control activity of glyphosate is due to inhibition of fungal EPSPS.

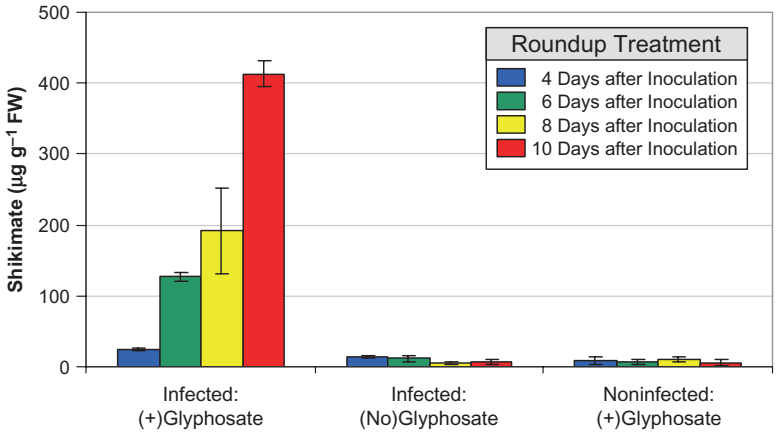


Figure 1.4. Shikimate accumulation in ASR-infected RR soybean leaves after glyphosate treatment. Leaf shikimate levels per gram fresh weight (FW) were measured 2 days after glyphosate treatment (0.84 kg ha^{-1}), as a function of glyphosate spray timing (4–10 days after inoculation) on infected plants with glyphosate, infected plants without glyphosate, and noninfected plants with glyphosate treatments. RR soybean plants are resistant to glyphosate and do not accumlate shikimate in response to glyphosate application.

More direct evidence of fungal EPSPS inhibition by glyphosate was obtained by cloning of *P. pachyrhizi* EPSPS. The expression of the *P. pachyrhizi EPSPS* gene complemented the EPSPS-deficient (aroA-) *Escherichia coli* strain thus confirming activity. The growth of the transformed *E. coli* strain was inhibited by glyphosate, demonstrating the sensitivity of *P. pachyrhizi* EPSPS to glyphosate. Enzyme kinetic analysis showed that the *P. pachyrhizi* EPSPS was more sensitive to glyphosate than that of *E. coli* and with a temperature optimum of <37°C. Additional laboratory studies demonstrated a lack of antifungal activity in glyphosate metabolites, which further support the conclusion that glyphosate's antifungal activity is due to direct action on fungal EPSPS.

Similar EPSPS enzymes are found across many classes of plant pathogenic fungi including the Oomycetes, Deuteromycetes, Ascomycetes, and Basidiomycetes. It is therefore reasonable to assume that glyphosate's antifungal activity should be evident in a broader range of fungi. We have shown that glyphosate can suppress disease symptoms and provide yield protection under both greenhouse and field conditions against a range of plant pathogenic fungi. Activity has been demonstrated against powdery mildew (*Microsphaera diffusa*) and *Cercospora* leaf spots (*Cercospora kikuchii* and *Cercospora soja*) in soybeans, against powdery mildew (*Erysiphe pisi*) in peas, and against downy mildew (*Peronospora destructor*) in onions. Our investigations are continuing to determine the potential benefits of disease suppression from the application of glyphosate in GR crops.

1.4 EFFECT OF GLYPHOSATE ON NONTARGET ORGANISMS

Glyphosate is generally no more than slightly toxic to higher organisms including mammals, birds, fish, aquatic invertebrates, and terrestrial invertebrates (such as earthworms and honeybees). The enzyme inhibited by glyphosate, EPSPS, is found only in plants, bacteria, and fungi. This specific mode of action contributes to the low toxicity observed for glyphosate for many taxonomic groups of nontarget organisms.

The environmental toxicology of glyphosate has been extensively reviewed. Regulatory reviews of glyphosate have been conducted by the United States Environmental Protection Agency (USEPA 1993), the World Health Organization (WHO 1994), the European Union (EC 2002), and other countries. An extensive compilation of regulatory studies and open literature studies, as well as an ecological risk assessment, is presented in Giesy et al. (2000). An assessment of risk from overwater application was reported by Solomon and Thompson (2003). A brief review of the ecological effects of glyphosate use in glyphosate tolerant crops is also available (Cerdeira and Duke 2006). The EPA ECOTOX database (http://cfpub.epa.gov/ecotox/) is also a source of regulatory and open literature ecotoxicological studies on glyphosate. Rather than present a comprehensive review of glyphosate effects on nontarget organisms, this section focuses on a few key points regarding ecological toxicology and risk assessment for glyphosate.

Glyphosate toxicity studies have been conducted with a number of different forms of glyphosate. When evaluating the results of glyphosate nontarget organism studies, it is important to note the form of glyphosate that has been tested. Glyphosate has carboxylic acid, phosphonic acid, and amine functionalities (Fig. 1.1). In the protonated acid form, glyphosate is a crystalline solid that is soluble in water at concentrations just over 1% at 25°C. A 1% solution prepared by dissolving crystalline glyphosate without buffering has a pH of 2 (Franz et al. 1997). The pH of glyphosate solutions increases with dilution. The acid form of glyphosate can be neutralized with dilute base to form salts, which are much more soluble in water. In its salt form, glyphosate is soluble at concentrations approaching 50%; these concentrated salt solutions have a pH between 4 and 5. In commercial end-use herbicide products, glyphosate is generally present in the salt form. Counterions used in glyphosate formulations include isopropylamine, potassium, and ammonium.

Commercial products typically also include a surfactant to facilitate the movement of the polar compound glyphosate through the waxy cuticle of plant foliage. While glyphosate and its commercial formulations are generally recognized to pose low toxicity to terrestrial organisms (such as birds, mammals, honeybees, and soil macroorganisms), some commercial formulations have been found to have greater toxicity to aquatic organisms than glyphosate (Folmar et al. 1979) due to the presence of surfactant in the formulation. Table 1.2 compares the toxicity of glyphosate as the acid, as the isopropylamine salt, and as the original Roundup agricultural formulation (MON 2139). Especially for fish, the salt form has less toxicity than the acid

TABLE 1.2. Relative Toxicity of Glyphosate Acid, Glyphosate Isopropylamine Salt, and the Original Glyphosate Formulation, Roundup (MON 2139)

		LC_{50}/EC_{50}[a] (mg a.e. L^{-1})		
Species	Exposure Duration	Glyphosate Acid	Glyphosate IPA salt	Original Roundup Formulation (MON 2139)[b]
Rainbow trout (*Oncorhynchus mykiss*)	96 h	71.4[b] ST	>460[c] PNT	1.3[d] MT
Bluegill (*Lepomis macrochirus*)	96 h	99.6[b] ST	>460[c] PNT	2.4[d] MT
Daphnia magna	48 h	128[b] PNT	428[c] PNT	3.0[d] MT
		LD_{50} (units as indicated)		
Rat	Single dose	>4275[e] mg a.e. $kg\,bw^{-1}$ PNT		1550[f] mg a.e. $kg\,bw^{-1}$ ST
Bobwhite (*Colinus virginianus*)	5 d	>4971[b] mg a.e. kg^{-1} diet ST	—	>1742[f] mg a.e. kg^{-1} diet ST
Honeybee (*Apis mellifera*)	Contact 48 h	>100 µg ae/bee[f] PNT		>31 µg a.e./bee[f] PNT
Earthworm (*Eisenia foetida*)	14 d		>2300 mg a.e. kg^{-1} soil[g] PNT	>1550 mg a.e. kg^{-1} soil[f] PNT

[a]For this comparison, the lowest end points from studies conducted with similar methodology (e.g., fish weight, water chemistry) were employed. EPA toxicity classification (USEPA 2008) is given under the endpoint value except for earthworms where a European toxicity classification is used (Canton et al. 1991). Units for formulation studies have been converted when necessary from mg formulation L^{-1} to mg a.e. L^{-1} for direct comparison of glyphosate concentrations of the acid and salt using the conversion factor 0.31.

[b]Regulatory study reported in USEPA (2008). These values are the values reported for the Analytical Bio-Chemistry Laboratories (ABC, Columbia, MO) studies in Giesy et al. (2000), but with a correction for 83% purity of the test substance.

[c]Values are reported in Giesy et al. (2000) as >1000 mg glyphosate IPA salt L^{-1}; however, review of the study reports indicates this concentration is expressed as the 62% aqueous salt solution rather than a.e. The correction has been made to a.e. using a conversion factor of 0.46.

[d]Folmar et al. (1979). LD_{50} values in this paper are expressed as mg a.e. L^{-1}.

[e]Giesy et al. (2000), with a correction for test substance purity of 85.5%.

[f]Giesy et al. (2000), with a conversion factor of 0.31 applied to convert from formulation units to a.e. units.

[g]Giesy et al. (2000). The LD_{50} value is >3750 mg a.e. kg^{-1} converted from the original study value of 5000 mg kg^{-1} as a 62% IPA salt solution using a salt to acid conversion factor of 0.75; however, since the original test substance was only 62% IPA salt, the original LD_{50} value of 5000 mg kg^{-1} has been corrected to glyphosate acid equivalent using the conversion factor 0.46.

PNT, practically nontoxic; ST, slightly toxic; MT, moderately toxic.

form, which in turn has significantly less toxicity than the original Roundup formulation.

It is also important to note that commercial herbicide products containing glyphosate can contain a number of different surfactants with varying degrees of aquatic toxicity. For example, there are a number of different formulations

TABLE 1.3. Comparative Toxicity of Three Glyphosate Formulations

	LC_{50} (mg formulation L^{-1})		
	Roundup Biactive®	Roundup Transorb®	Roundup Original®
Species	MON 77920	PCP[a]: 25344	PCP: 13644
Green frog (*Rana clamitans*)	>57.7	7.2	6.5

[a]Pest Control Product Registration Number (Canada).

with variations of the Roundup brand name, which exhibit varying degrees of aquatic toxicity (Table 1.3). When reporting results of glyphosate formulation testing, it is very important to provide the complete name of the product tested and any additional information that is available, such as the EPA registration number.

Because there are several forms of glyphosate that can be tested, it is critical that toxicity results clearly indicate whether the values are expressed as glyphosate acid equivalents (a.e.), glyphosate salt (often referred to as active ingredient), or as formulation units. It is also important to note that most concentrated glyphosate formulations have a density greater than 1; therefore, test substance should be measured on a weight basis for accurate conversion between forms based on weight percent units.

The toxicity of glyphosate formulations to amphibians has been a topic of recent investigation by a number of laboratories. Results from amphibian studies by Bidwell and Gorrie and Mann and Bidwell are summarized in Giesy et al. (2000). There have also been a number of more recent investigations regarding the acute toxicity of Roundup formulations to amphibians (e.g., Edginton et al. 2004; Howe et al. 2004; Relyea 2005a, 2005b, 2005c). Altogether, a total of 20 species of amphibians from three continents have been tested for acute toxicity to Roundup formulations. The lowest LC_{50} reported for any of these species for the most sensitive growth stage was 0.88 mg a.e. L^{-1} for *Xenopus laevis* (Edginton et al. 2004). Considering only regulatory studies, the lowest LC_{50} value for a fish species reported for a glyphosate formulation is 5.4 mg formulation L^{-1} (or 1.7 mg a.e. L^{-1}), which is less than two times greater than the lowest amphibian value. Since the United States and the European Union apply a 10- to 100-fold safety factor, respectively, between toxicity values and predicted exposure values, the risk assessments conducted using fish end points are also protective for amphibian species.

Results from monitoring studies can be used to put the reported toxicity values into perspective relative to exposure. Glyphosate concentrations in 51 water bodies in the midwestern United States were measured during three different runoff periods in 2002 (Scribner et al. 2003). The maximum concentration of glyphosate measured in these samples was 8.7 µg a.e. L^{-1} and the ninety-fifth centile concentration ranged from 0.45 to 1.5 µg a.e. L^{-1} for the

three sampling periods. A total of 30 sites in southern Ontario, Canada, representing rivers, small streams, and low-flow wetlands were sampled biweekly (April to December) during 2004 and 2005. The maximum concentration measured in these samples was 40.8 μg a.e. L^{-1}. In the wetlands with known amphibian habitat, the upper ninety-ninth centile confidence limit indicates that glyphosate concentrations would typically be below 21 μg a.e. L^{-1} (Struger et al. 2008). Both of these studies indicate that glyphosate concentrations in the environment are well below concentrations at which toxicity to aquatic animals has been observed in laboratory studies. Consistent with this margin of safety, the EPA recently determined that glyphosate poses no risk of direct effects to the aquatic stage of a threatened aquatic animal (California red-legged frog) (USEPA 2008).

One additional point to consider with respect to the risk assessment for glyphosate formulations is that the tallowamine surfactant often used in these formulations has been demonstrated to rapidly partition out of the water column (Wang et al. 2005). The Wang et al. study, which measured both the disappearance of MON 0818, the surfactant blend in the original Roundup formulation (MON 2139), from the water column and the reduction in toxicity to *Daphnia magna* over time, indicated that the half-life of the surfactant in two sediments was less than 1 day, and the decline in surfactant concentration was correlated with the reduction in toxicity. This rapid partitioning to sediment may also be expected for other surfactants containing long alkyl chains. A number of studies have been conducted that employ extended exposures (16–40 days) in laboratory tests with constant concentrations of glyphosate formulations. Exposures of this duration are not representative of exposures that would occur in the natural environment. Thus, the results of such studies should only be used as an indicator of future investigations to conduct under more realistic exposure scenarios.

The generally low toxicity of glyphosate to nontarget organisms, the rapid disappearance of surfactant from the water column, and the large margin of safety between concentrations of glyphosate in surface water and concentrations at which toxic effects to aquatic animals from glyphosate formulations have been observed, combine to indicate that glyphosate applications in accordance with the label do not pose an unreasonable risk of adverse effects to nontarget organisms.

1.5 PHYSICAL AND ENVIRONMENTAL PROPERTIES OF GLYPHOSATE

Due to its amphoteric nature, glyphosate is readily dissolved in dilute aqueous bases and strong aqueous acids to produce anionic and cationic salts, respectively. The free acid of glyphosate is modestly water soluble (1.16 g L^{-1} at 25°C), but when converted to monobasic salts, its solubility increases substantially. Due to its limited aqueous solubility, glyphosate is generally formulated as

concentrated water solutions of approximately 30–50% in the form of the more soluble monobasic salt (isopropylamine, sodium, potassium, trimethylsulfonium, or ammonium) in a number of commercial herbicidal products. Neither glyphosate acid nor the commercial salts are significantly soluble in common organic solvents. The lack of solubility of glyphosate in nonaqueous solvents has been attributed to the strong intermolecular hydrogen bonding in the molecule (Knuuttila and Knuuttila 1985). The physicochemical properties of glyphosate indicate a favorable environmental profile. For instance, the intermolecular hydrogen bonding results in low volatility of glyphosate (2.59×10^{-5} Pa at 25°C). Glyphosate's low volatility and its high density ($1.75\,g\,cm^{-3}$) suggest that it is unlikely to evaporate from treated surfaces and move through the air to injure nontarget sources or remain suspended in the air for a long time after application.

With the advent of glyphosate-tolerant crops and the widespread use of glyphosate products in so many different crops (Duke and Powles 2008), glyphosate has been the subject of numerous studies for potential to produce adverse effects. The environmental characteristics of glyphosate have been reviewed by many scientists from the industry (Franz et al. 1997), government regulatory agencies in several countries (USEPA 1993), scientific institutions (Giesy et al. 2000), and international organizations (WHO 1994). A summary of the physical, chemical, and environmental properties of glyphosate from these reviews is shown below.

Chemical decomposition does not contribute to the degradation of glyphosate in the environment because glyphosate is stable to hydrolytic degradations in sterile water in most environmentally relevant pH ranges. Glyphosate is also photolytically stable in sterile water and soil. However, photodegradation can occur in water under certain conditions. Studies using artificial light and solution containing calcium ions reveal slow photodegradation, while studies using natural or simulated sunlight and sterile water show no photodegradation (Franz et al. 1997). Similarly, under intense artificial lights, glyphosate in natural river water degrades via oxidative transformation induced by photochemical excitation of humic acids as reported for other pesticides (Aguer and Richard 1996). Although photodegradation of glyphosate in water can occur, it is not a major pathway of degradation of glyphosate in the environment.

In contrast, glyphosate is readily degraded by microorganisms in soil, nonsterile water, and water/sediment systems. In soil, indigenous microflora degrade glyphosate, under both aerobic and anaerobic conditions. The principle metabolite is AMPA. AMPA is further degraded by soil microflora, although at a slower rate than glyphosate. Studies demonstrate that in soil, up to 79–86% of glyphosate is biodegraded to carbon dioxide during a 6-month period (Franz et al. 1997). The results of over 93 field trials conducted in Europe, Canada, and the United States show that glyphosate dissipates with field half-lives in all cases of less than 1 year, and typically less than 38 days (Giesy et al. 2000). Laboratory and field studies also demonstrate that dissipa-

tion times are not affected by the rate of application and that glyphosate and AMPA do not accumulate following multiple applications, either during the same year or over tens of years (Giesy et al. 2000). Biodegradation is also the principle mechanism of degradation of glyphosate in environmental waters under both aerobic and anaerobic conditions. In all cases, the results demonstrate the biodegradation of glyphosate to AMPA and carbon dioxide, and the subsequent biodegradation of AMPA to carbon dioxide.

Glyphosate is only used as a postemergence herbicide, and the potential for root uptake of glyphosate from soils has been reported to be negligible. Lack of glyphosate soil activity is due to its rapid microbial degradation and strong soil-binding properties (Giesy et al. 2000). Glyphosate has been shown to bind tightly to most soils. In laboratory batch equilibrium studies, partition coefficient (K_{oc}) values ranged from 884 to 60,000 for seven soils. Studies have been conducted to investigate the uptake of radiolabeled glyphosate into rotational crops following soil applications to a primary crop. The maximum uptake into plants grown in soil treated with glyphosate was in all cases less than 1% of the total applied. These results demonstrate that glyphosate entry into plants via the root system as a result of applications of glyphosate to the soil is negligible.

1.6 GLYPHOSATE TOXICOLOGY AND APPLICATOR EXPOSURE

Glyphosate and glyphosate-based herbicides are backed by one of the most extensive worldwide human health and safety databases ever compiled for a pesticide product. Before any pesticide product can be registered, distributed, or sold, it is subjected to a rigorous battery of tests to determine that the product does not pose any unreasonable risks to consumers or the environment, when used according to label directions. Governmental regulatory agencies mandate these tests and have experts that review the submitted data for each pesticide. Glyphosate has been thoroughly reviewed and registered by the Canadian Pesticide Management Regulatory Agency (PMRA 1991), the USEPA (1993), the European Commission (EC 2002), and other regulatory agencies around the world. In addition, glyphosate has been reviewed by the WHO (1994), the Joint Meeting of the Food and Agricultural Organization (FAO) Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues (WHO/FAO 1987, 2004), and third party toxicology experts (Williams et al. 2000).

Comprehensive toxicological studies in laboratory animals have demonstrated that glyphosate has low oral, dermal, and inhalation toxicity and shows no evidence of carcinogenicity, mutagenicity, neurotoxicity, reproductive toxicity, or teratogenicity. In the absence of a carcinogenic potential in animals and the lack of genotoxicity in standard tests, the USEPA (1993) placed glyphosate in its most favorable cancer category, Group E, meaning that there is "evidence

of non-carcinogenicity for humans" and the WHO/FAO (2004) concluded that glyphosate was unlikely to pose a carcinogenic risk to humans.

Of almost equal importance to the toxicology data is human pesticide exposure potential. The term "pesticide exposure" may mean different things to different people. If someone had been in a farm field when pesticides were being applied, the person might feel that he or she had been exposed to pesticides. In terms of determining potential risk, however, there is general agreement that exposure should be based on the amount of pesticide that has penetrated into the body, the so-called internal dose (Chester and Hart 1986; Franklin et al. 1986).

Exposure related to the professional use of glyphosate-based formulations, through the monitoring of the single active ingredient, glyphosate, has been the subject of a number of studies. Biomonitoring and passive dosimetry, and exposure modeling are approaches that can be used to estimate applicator exposure to pesticides. Biomonitoring results represent systemic (internal) exposure from all possible routes, whereas the results obtained from passive dosimetry quantify external deposition. There is general agreement that biological measurements as obtained through biomonitoring provide the most relevant information for safety assessments (Chester and Hart 1986; Franklin et al. 1986). There have been six published glyphosate biomonitoring studies (Abdelghani 1995; Acquavella et al. 2004; Centre de Toxicologie du Quebec 1988; Cowell and Steinmetz 1990a, 1990b; Jauhiainen et al. 1991). The authors of each study quantified glyphosate in urine. Urine is an ideal medium for quantifying systemic dose because glyphosate is not metabolized by mammals and is excreted essentially unchanged in urine with a short half-life (Williams et al. 2000).

The most extensive biomonitoring study is the Farm Family Exposure Study (FFES), conducted by investigators at the University of Minnesota with guidance offered by an advisory committee of recognized international experts in exposure assessment (Acquavella et al. 2004). The study monitored farm families. Urine samples were collected the day before glyphosate was to be applied, the day of application, and for 3 days after application. The detection method was capable of detecting 1 part per billion (ppb) glyphosate. In the FFES, 48 farmers applied a Roundup branded herbicide and provided 24-h urine samples the day before, the day of, and for 3 days after the application. Approximately 50% of the applications were on more than 40 ha and application rates were at least 1 kg ha^{-1}. Overall, 40% of the farmers did not have detectable glyphosate in their urine on the day of application. Some farmers did have detectable glyphosate in their urine samples, and the urinary concentrations ranged from <1 to 233 ppb. The maximum systemic dose was estimated to be 0.004 mg kg^{-1}. This would suggest that it is very unlikely for an applicator to get a systemic glyphosate dose that would even approach any level of toxicological concern. For comparison, according to the USEPA (1993), the lowest no observed effect level (NOEL) from glyphosate toxicology studies is considered to be 175 mg kg^{-1} day^{-1}. Regulatory agencies estimate risk to pesticide applicators by using a ratio of the estimated exposure to a relevant NOEL.

This ratio is referred to as the margin of exposure (MOE). Typically, MOEs that are less than 100 will exceed a level of concern for worker risk. Based on estimates of systemic dose, a farmer who did 20 applications per year for 40 years would have a MOE of approximately 1.75 million fold.

In summary, numerous comprehensive toxicological studies in animals conducted over many years clearly demonstrate that there are no significant hazards associated with glyphosate exposure. Glyphosate does not cause cancer, birth defects, mutagenic effects, nervous system effects, or reproductive problems. The comprehensive biomonitoring study of Acquavella et al. (2004) showed that people who regularly work with glyphosate have very low actual internal exposure. Taken together, the results from exposure studies in humans and animal laboratory toxicity studies demonstrate that glyphosate in real-world use conditions would not be expected to pose a health risk to humans when used according to label directions (Williams et al. 2000).

1.7 COMMERCIAL PROCESS CHEMISTRY FOR PREPARING GLYPHOSATE

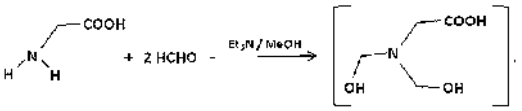
Many chemical routes for synthesizing glyphosate have been reported (Franz et al. 1997). Such a large number of routes is related to the fact that glyphosate is relatively stable in a variety of reaction environments (i.e., pH, temperature, oxidative, reductive, etc.), thus giving rise to a diversity of synthesis methods.

Although there are many routes reported, only a small fraction of these have yield and other characteristics that make them suitable for commercial operation. Currently, there are two dominant families of chemical pathways for commercial manufacturing of glyphosate: the "alkyl ester" pathways and the "iminodiacetic acid (IDA)" pathways. Each is discussed below.

1.7.1 Alkyl Ester Pathways

A significant number of Chinese manufacturers use a process based on an "alkyl ester" pathway. Although several variations of this pathway exist, commercially, the primary alkyl ester pathway is based on that developed and patented by the Alkaloida Chemical Works of Hungary (Brendel et al. 1984). The "Alkaloida" process uses glycine, dimethylphosphite (DMP), and paraformaldehyde as raw materials.

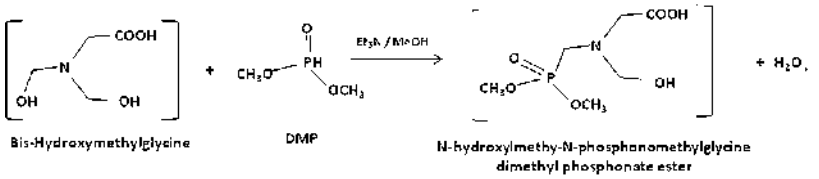
In the Alkaloida process, the reaction takes place in a nonaqueous medium, where glycine is first added to a mixture of triethylamine and paraformaldehyde (approximately two equivalents) in methanol. Under these conditions, a hydroxymethylglycine intermediate is formed:



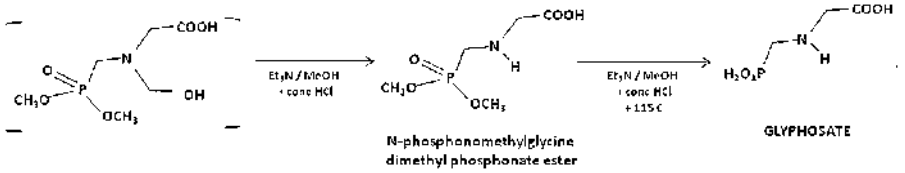
GLYCINE

Bis-Hydroxymethylglycine

DMP is then added to the reaction mixture, forming the following phosphonate ester:



Concentrated HCl is then added at room temperature, resulting in the removal of the hydroxymethyl group. Then subsequent heating of the solution results in further hydrolysis of the phosphonate ester to produce glyphosate:



The various intermediates are not isolated; thus, the reaction system is simple in that the reactions can be carried out in a "single pot." The final solution (containing glyphosate, methanol, etc.) is further processed to isolate glyphosate or a glyphosate solution suitable as a product.

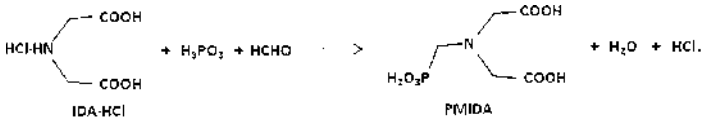
Some of the key advantages to this process (e.g., more stable and neutral pH, lower temperature operation) come from carrying out the reaction in an organic solvent instead of in an aqueous solution and the base choice (Et_3N). These preferred conditions give rise to favorable reaction conditions such that the overall yield of glyphosate is improved.

Process technology developments have led to the recovery and recycling of methanol and Et_3N to the process. Also, attention has been given to developing technologies to recover chloromethane generated during hydrolysis. This captured chloromethane can be sold or used in other processes (e.g., organosilicone production), improving the overall economics of the process.

There are variations of the above process, such as the use of diethyl phosphate (DEP) instead of DMP and other optimized solvents and reaction conditions. Process research continues on the alkyl ester pathways, as a significant amount of China's glyphosate production is based on these processes.

1.7.2 IDA Pathways

The other predominant family of pathways for the commercial production of glyphosate is based on IDA. In general, for these pathways, it is the hydrochloride salt of IDA (IDA·HCl) that participates in a phosphonomethylation reaction via a modified Mannich reaction to form the N-phosphonomethyliminodiacetic acid (PMIDA):



IDA·HCl PMIDA

One might envision/guess that performing the above phosphonomethylation reaction with glycine rather than IDA would directly generate glyphosate; however, phosphonomethylation of glycine via the Mannich reaction produces glyphosate only in low yield because glyphosate very readily undergoes an additional phosphonomethylation, forming bis-phosphonomethyl glycine. Thus, one can think of the second carboxymethyl group on IDA as a "protecting" group that prevents a second phosphonomethylation from occurring.

Often during the phosphonomethylation reaction to produce PMIDA, both HCl and phosphorous acid are conveniently supplied by feeding PCl_3 to an aqueous solution of IDA. PCl_3 reacts with water accordingly to generate phosphorous acid and hydrochloric acid *in situ*:

$$PCl_3 + 3H_2O \longrightarrow H_3PO_3 + 3HCl.$$

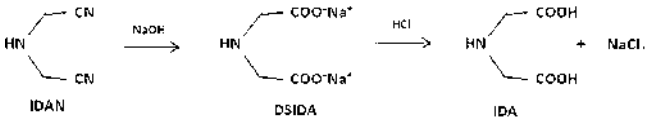
Once PMIDA is formed, it can be isolated, and the protecting group can be removed via oxidation to form glyphosate:

PMIDA GLYPHOSATE

This oxidation can be achieved by concentrated sulfuric acid, hydrogen peroxide, electrolysis, or oxygen/air over a catalyst.

The production of IDA is often part of the integrated glyphosate process. There are three primary approaches that glyphosate producers use to produce IDA, and they are summarized below.

1.7.2.1 IDA from Iminodiacetonitrile (IDAN) Caustic is added to IDAN to produce disodium iminodiacetate (DSIDA). Hydrochloric acid is then added to produce IDA:

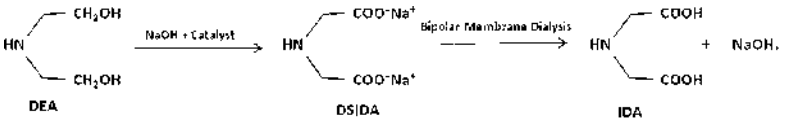


IDAN DSIDA IDA

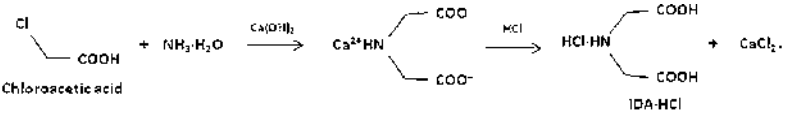
Since IDAN is produced from HCN, forming IDA via IDAN is favored in situations where inexpensive or by-product HCN is available.

1.7.2.2 IDA from Diethanol Amine (DEA) Another means of generating IDA is from DEA. DEA is converted to DSIDA by reacting with caustic

over a catalyst. As above, DSIDA can then be hydrolyzed to IDA or (as shown below) some producers use membrane dialysis to generate IDA and NaOH:



1.7.2.3 IDA from Chloroacetic Acid In this approach, chloroacetic acid is added to a solution of NH_3 and $Ca(OH)_2$. After the reaction, the solution is then neutralized with HCl to form the hydrochloride salt of IDA:



Of these various strategies for producing IDA, this chloroacetic acid method is the least efficient, as it generates significant quantities of strong acid ($CaCl_2$) waste, leading to lower yields than the IDAN or DEA routes.

1.8 GLYPHOSATE FORMULATION

This section will describe some of the properties of formulations of glyphosate and issues faced in the selection of formulation ingredients. This is meant as a general overview of the subject and not an exhaustive review of the subject area or exhaustive literature review. The formulations discussed will be those principally sold in the United States, not worldwide, although most of the formulations discussed are or have been sold in many countries.

Formulations containing glyphosate have been sold under the trade name of Roundup (Monsanto Company) for more than 30 years. As the original patents on the use of glyphosate as an herbicide and salts of glyphosate expired, other brands such as Touchdown® (Syngenta, Basel, Switzerland), GlyphoMAX® (Dow AgroSciences LLC, Indianapolis, IN), and Gly Star® (Albaugh, Inc., Ankeny, IA), to name only a few, have also come into the market. These commercial mixtures are water solutions of glyphosate salts with most containing a surfactant. Some dry, water-soluble granule or powder formulations have also been sold. Consulting the National Pesticide Information Retrieval System (NPIRS®) (http://ppis.ceris.purdue.edu/) Web site, more than 50 different registered products containing glyphosate are found.

In the design of a glyphosate product formulation, the selection of the type of salt and surfactant has been the principal ingredients studied. The formulation must be stable over the range of temperature extremes that the product will experience in the market place. The formulation must be easily diluted in

water and be sprayable without clogging the spray nozzles of application equipment. It must also perform in an efficacious manner as an herbicide. Additional requirements of the formulations are that they have the minimal toxicity to humans and the environment.

1.8.1 Glyphosate Acid and Salt Solubility

The solubility of glyphosate acid is 1.57% in water at 25°C (Vencill 2002). This solubility is too low to be useful for a soluble concentrate commercial product. While it may be possible to formulate the acid as a suspension concentrate, a liquid soluble concentrate product is typically preferred and can have fewer physical stability issues. Hence, the vast majority of commercial products sold to date have been salts of glyphosate. Glyphosate has three acid sites (or exchangeable protons) and one amine available for protonation (Fig. 1.1); thereby, several different types of salts of glyphosate are easily obtained. The simplest forms of salts are produced by reaction of a base with glyphosate acid. As these salts are formed, solubility of the salt can be expressed in terms of the amount of salt soluble, or % ai in solution. This makes the comparison of the amount of glyphosate anion in solution between different salts slightly difficult as the molecular weights of the cations are different. To make comparison easier, the amount of equivalent glyphosate acid dissolved in a salt solution has typically been referred to as "% a.e." The solubility in water of selected salts is shown in Table 1.4 for a variety of glyphosate salts prepared at a 1:1 molar ratio of cation to acid.

One of the first glyphosate formulations sold contained the isopropylamine (IPA) salt. Several other salts have been sold in commercial products since that time, including sodium, tetramethylsulfonium (TMS), potassium, ammonium, monoethanolamine, and dimethylamine salts. An acid salt

TABLE 1.4. Solubility in Water of Various Glyphosate Salts, 1:1 Mole Ratio of Glyphosate:Base (Vencill 2002)[a] and Unpublished Data

Cation	% ai w/w Soluble (20°C)	% a.e. w/w Soluble at 20°C (pH)
H+		1.16 (pH 2.5)[a]
Li+	19	18 (pH 4.5)
Na+	34	30 (pH 3.6)
K+	54	44 (pH 4.2)
TMS+	78.6 (pH 4.06)	54 (pH 4.06)
	50 (pH 4.2)	34 (pH 4.2)
IPA+	63	47 (pH 4.6)
NH_4+	39	35 (pH 4.3)

[a]See references.

NH_4, ammonium; H, hydrogen; IPA, isopropylamine; Li, lithium; K, potassium; Na, sodium; TMS, tetramethylsulfonium.

formulation where the nitrogen atom is protonated using sulfuric acid has also been sold.

Salt solubility is an important factor in preparing a soluble concentrate formulation of glyphosate. The solubility must be high enough such that when the formulation is exposed to extreme low temperatures, the salt will not crystallize and precipitate. Testing of formulations at low temperature expected in the market place is one of the typical hurdles for a formulation to overcome. It is important in these tests that a seed crystal of the salt be added to the formulation since a supersaturated solution can appear to be stable, without a stimulus to crystallize. The seed crystal will give this stimulus and help avoid a false reading.

Most salt formulations of glyphosate contain a "mono" salt of glyphosate or nominally 1 mol of neutralizing cation to 1 mol of glyphosate anion. A way to increase the solubility of a lesser soluble salt is to make a di-cation salt, making use of the second acid site on the glyphosate molecule. This has particularly been used with the ammonium salt as described by Sato et al. (1999). The product Touchdown IQ® (Syngenta) contains this salt. The sesquisodium salt of glyphosate, 1.5 mol of Na per mole of glyphosate, was sold as a water-soluble powder under the product name Polado® (Monsanto Company).

1.8.2 Density of Solutions

Formulations sold in agricultural markets typically describe the active ingredient loading based on the weight of active ingredient per gallon or per liter. The first glyphosate formulation sold as Roundup contained 3 lb of glyphosate a.e. or 4 lb of glyphosate IPA salt per gallon. In metric units, this loading is approximately 360 g L^{-1} of glyphosate a.e. or 480 g L^{-1} of glyphosate IPA salt. The amount of glyphosate on a weight percent basis in the formulation was 31% glyphosate as the isopropylamine salt or 41.6% IPA salt of glyphosate. It is a simple calculation to obtain the weight per volume for a formulation as shown below in Equations 1.1 and 1.2:

$$\text{Solution specific gravity} \times 1000 \times \%\,\text{w/w} = \text{g L}^{-1}\ \text{active ingredient}, \quad (1.1)$$

$$\text{Solution specific gravity} \times 8.3283 \times \%\,\text{w/w} = \text{lb gal}^{-1}\ \text{active ingredient}. \quad (1.2)$$

The specific gravity of a solution is defined as the density of a given solution divided by the density of water at a given temperature. The solution density of glyphosate salt solutions (and hence the active ingredient loading of a formulation) can be affected by the choice of glyphosate salt. Table 1.5 shows the specific gravity of several different solutions of salts of glyphosate. The weight percent of the equivalent amount of glyphosate acid in each solution present as a salt is shown for each salt. This value is abbreviated as % a.e. or percent glyphosate a.e.

TABLE 1.5. Specific Gravity for a Variety of 30% a.e. Glyphosate Salt Solutions and g L^{-1} Loading of Glyphosate (Wright 2003)

Cation	SG 30% a.e.	SG 30% ai
Potassium	1.25	1.20
Ammonium	1.18	1.16
Isopropylamine	1.16	1.11
Ethanolamine	1.24	1.17
Trimethylsulfonium	1.19	1.13

TABLE 1.6. A Partial List of Glyphosate Salts Sold in Commercial Products with a Representative Product Name

Salt Cation	Representative Trade Name	lb gal^{-1} a.e. Glyphosate	% a.e. w/w Glyphosate
Isopropylamine	Roundup® (Monsanto Company)	3	30.4
Tetramethylsulfonium	Touchdown® (Syngenta)	5	39.5
Diammonium	Touchdown IQ® (Syngenta)	3	28.3
Potassium	Roundup WeatherMAX® (Monsanto Company)	4.5	48.8
Dimethylamine	Durango® (Dow AgroSciences LLC)	4	39.7

While the weight per volume or loading of glyphosate possible in a solution of glyphosate salt is determined by the % soluble salt and density of the solution, it is practically limited by the solubility of the salt in water. While two formulations prepared with different salts may contain the same percent glyphosate by weight (% a.e.) the amount of glyphosate expressed in terms of weight per volume can be different. This is demonstrated by comparing the pound per gallon and % a.e. in Touchdown (Syngenta) and Durango® (Dow AgroSciences LLC) that have similar % a.e. concentrations, but the density of the TMS salt solution is much greater than the DMA salt. Some of the salts of glyphosate that have been sold in commercial products are shown in Table 1.6.

1.8.3 Surfactant Selection

The biological efficacy of glyphosate, perhaps more than any other herbicide, can be very dependent on the surfactant in the spray solution. Most of the glyphosate formulations on the market contain a surfactant. When considering

a surfactant to include in a formulation, there are two main items to consider: identification of a surfactant that boosts efficacy and identification of a surfactant that is compatible in the formulation. There is a legion of research that has been documented on various surfactants and how they affect the biological performance of glyphosate, much more than could be discussed in a book chapter. The purpose of this section will be to deal with some of the issues to be solved in the selection of a surfactant based on formulation criteria.

Identifying a surfactant that is soluble in concentrated salt solutions can be difficult as many types of surfactants are not soluble in salt solutions and particularly insoluble in glyphosate salt solutions. After the selection of the surfactant, determine if it is soluble in a given concentration of a glyphosate salt solution at room temperature. One measure of the compatibility of the surfactant in the formulation is to measure the cloud point of the solution. As explained by Lange (1999), the "turbidity arises from attractive micelle-micelle interactions. At a higher temperature, phase separation into a water-rich phase and a surfactant-rich phase generally occurs." This can occur at a maximum and a minimum temperature. This is an important consideration when designing a formulation that will experience a wide variety of climatic conditions. If the cloud point is lower than the maximum temperature the product will experience, the formulation may separate into layers that may not be easily reconstituted.

Under most textbook definitions of cloud point, it is described that the higher the ethylene oxide (EO) content of a surfactant, the more soluble it will be in water. This is due to the availability of more oxygen molecules with which water can hydrogen bond. However, in water solutions containing a high amount of salt, as with salt solutions of glyphosate, this is not the case. In fact, most nonionic surfactants such as alkylphenol or alcohol ethoxylates are not soluble to a great extent in solutions containing an appreciable amount of glyphosate salt. The one exception to this rule is alkyl polyglycoside (APG) surfactants (Hill et al. 1996). These nonionic surfactants are highly soluble in salt solutions in general and particularly in glyphosate salt solutions.

Many commercial glyphosate formulations contain the so-called cationic surfactants, or surfactants that can retain a positive charge under acidic conditions. Alkylamine ethoxylates are such surfactants. These surfactants can be compatible in glyphosate salts, but the compatibility is affected by the type of cation carried by the glyphosate salt (Lennon et al. 2006). The compatibility is also affected by the amount of ethoxylation on the alkylamine. With these surfactants, the cloud point does not follow the expected rule of the cloud point of water solutions being higher with higher amounts of EO on the amine. Table 1.7 shows cloud points of formulations containing 30% ae IPA glyphosate salt with ethoxylated cocoamine surfactants at increasing concentration and increasing EO. Note that the cloud point actually decreases with added EO rather than increases as one may find in pure water.

Other types of adjuvants can be used with the application of glyphosate formulations. These adjuvants can be a number of different materials such as

TABLE 1.7. Cloud Point Measurements of Cocoamine Ethoxylate Surfactants in 30% ai Isopropylamine (IPA) Glyphosate Solutions

EO (mol)	% w/w Surfactant	Cloud Point (°C)
5	10	>99
5	15	>99
5	20	>99
10	10	>99
10	15	88
10	20	76
15	10	68
15	15	54
15	20	45

EO, Ethylene oxide.

surfactants, antifoam agents, defoaming agents, drift control materials, and water-conditioning agents. Some of these materials can be included in a formulation without difficulty. Perhaps the most commonly added adjuvant to glyphosate applications as a tank mix ingredient is ammonium sulfate. Adding ammonium sulfate to formulations of glyphosate can be problematic in that you are adding more salt to an already high-salt-containing solution. Particularly in the agricultural formulations, it is desirable to maximize the amount of active ingredient provided in the formulation. Most glyphosate product labels recommend adding 1–2% ammonium sulfate to the spray solution. Adding the amount necessary to obtain this 1–2% concentration to the formulation would greatly reduce the amount of glyphosate in the formulation. Thus, in the U.S. market, few formulations have been sold that contain an appreciable amount of ammonium sulfate.

1.8.4 Dry Granular Formulations

Formulations of glyphosate can be made in the form of water-soluble solids. Both the sodium and ammonium salts have been sold in these types of formulations. The first dry formulation sold in the market was a water-soluble powder, Polado (http://ppis.ceris.purdue.edu/). This product was the sesquisodium salt or 1.5 mol of sodium per mole of glyphosate acid. The monosodium salt of glyphosate was sold as a water-soluble granule in Europe as Roundup Ultragran® (http://ppis.ceris.purdue.edu/). The ammonium salt of glyphosate has been more commercially successful. The ammonium salt as described by Kuchikata et al. (1996) is less hydroscopic than other salts, which offers advantages to the formulator in that it will require less water impermeable packaging. Dry ammonium glyphosate formulations have been sold as Roundup WSD (Monsanto Company) and other commercial labels particularly in South and Central America. A combination of glyphosate, diquat dibromide, and

surfactant (Crockett et al. 2006) is also sold as a water-soluble granule under the product name of QuikPro® (Monsanto Company) specifically for the industrial market.

1.8.5 Combination or Package Mix Formulation

Formulations containing more than one active ingredient are commonly referred to as "package mix" formulations. Several products have been sold that contain glyphosate and another herbicide. Typically, this is to place another type of herbicide that offers some benefit to the user such as residual activity or an herbicide with different selectivity. Products sold into the agriculture market have included Bronco® (Monsanto Company) (glyphosate plus alachlor), Landmaster® (Monsanto Company) (glyphosate plus 2,4-D), Fallowmaster® (Monsanto Company) (glyphosate plus dicamba), and Fieldmaster® (Monsanto Company) (acetochlor, atrazine, glyphosate). One benefit of these products is that they offer the convenience of having both active ingredients in the same formulation or container. This can be both a blessing and a curse in that the ratio of active ingredients in the formulation is fixed, which does not allow the user to adjust the ratio of active ingredients based on soil type or species of weeds present in a given field. When preparing a formulation with more than one active, it will typically reduce the concentration in the final formulation for each active ingredient over what could be provided with either active ingredient could have been formulated when provided in separate formulations. In any formulation containing two or more actives, one of the first tests to be conducted is to ensure that one active ingredient does not have a chemical reaction with another that would cause decomposition of one active ingredient. This is particularly true with glyphosate, which can act as a proton donor to aid in the hydrolysis of many actives that contain an ester moiety.

1.8.6 Lawn and Garden Formulations

Sales of glyphosate formulations in the lawn and garden or household consumer market have slightly different requirements when compared with the products sold to farmers in an agricultural market. One principal difference is that the formulations can contain lower concentrations. This is done principally for the convenience of the user. A significant portion of products sold are prediluted or ready-to-use (RTU) formulations. These formulations generally contain the active ingredient as well as surfactants and other additives to potentiate activity in a water solution diluted to a dose that is ready to be applied by the user. These formulations also typically come in a container that is also the applicator, such as a trigger actuated sprayer. Concentrate formulations are also sold to be diluted into pump sprayers. These concentrate formulations can contain as little as 6% and up to 50% glyphosate salt.

In dealing with the consumer expectations, most of the innovations with glyphosate formulations in recent history have been to develop products to provide fast developing symptoms, or yellowing and desiccation of weeds. Arnold et al. (1993) described that pelargonic acid (nominally a C_9 fatty acid or nonanoic acid) can be added to glyphosate formulations to achieve the fast developing symptoms. By controlling the pH of the solution to near neutral, the formulation will be homogeneous; at lower pH values, the fatty acid will separate from the formulation. Faster symptoms in a more concentrated formulation have been obtained by the combination of glyphosate, diquat, and surfactant as described by Crockett et al. (2006). This patent describes that the selection of surfactant is very important so that it allows the glyphosate to get into the plant and the translocation of the glyphosate to occur so that the diquat will not antagonize the biological performance of the systemic herbicide glyphosate. The amount of diquat in the product is only enough to provide yellowing and desiccation of the leaves of the treated weed.

Products that contain both glyphosate and a residual herbicide to keep weeds from germinating in the treated area have been sold. Those products include glyphosate + oxyfluorfen (Ortho®Season-Long®, Scott's Miracle-Gro Company, Marysville, OH), glyphosate + imazapyr (GroundClear®, Scott's Miracle-Gro Company), and glyphosate + imazapic (Roundup Extended Control®, Monsanto Company). Other specialized formulations are also sold such as glyphosate + triclopyr as Roundup Poison Ivy & Tough Brush Control® (Monsanto Company). This combination of actives was developed particularly for use on brushy weeds and vines as described by Wright et al. (2004).

1.9 CONCLUSION

This chapter was meant as an overview of more recent research on the use and environmental, toxicological, and physical aspects of the herbicide glyphosate. Obviously, this compound has been studied extensively over the last 30+ years, and after over three decades of use, glyphosate-based products continue as an important tool for weed control to be used by farmers across the globe. The compound continues to be the leading herbicide used in row crops, orchards, fallow lands, and pastures. Glyphosate's unique and favorable environmental and toxicological properties and its ability to control a broad spectrum of weed species will keep it a key weed management tool.

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