Effects of Transgenic Glyphosate-Resistant Crops on Water Quality

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Glyphosate (*N*-[phosphonomethyl] glycine) is a highly effective, non-selective herbicide. Herbicide-resistant crop (HRC) has been the most successful trait used in transgenic crops throughout the world. Transgenic glyphosate-resistant crops (GRCs) have been commercialized and grown extensively in the Western Hemisphere and, to a lesser extent, elsewhere. GRCs have generally become dominant in those countries where they have been approved for use, greatly increasing the utilization of glyphosate. Potential effects of glyphosate on ground and surface water are lower than the effects of the most herbicides that are replaced when GRCs are adopted. Perhaps the most positive indirect effect is that GRCs crops promote the adoption of reduced- or no-tillage agriculture, resulting in a significant reduction in soil erosion and water contamination. Glyphosate and its degradation product, aminomethylphosphonate (AMPA), residues are not usually detected in high levels in ground or surface water in areas where glyphosate is used extensively. There are some concerns about AMPA in water since it has higher mobility and persistence in the environment than glyphosate. However, neither glyphosate nor AMPA are considered to be significantly toxic. Of greater concern are the formulation ingredients, which can vary from country to country, from product to product, and even over time with the same product. There is some published evidence that formulation ingredients might adversely affect amphibians in some situations.

Key words: Agriculture, Ground Water, Nonpoint Source Pollution, Toxic Substances, Water Quality

Introduction

Herbicide resistance and insect resistance are the only two types of transgene-conveyed traits for crops that have so far had a marked effect on agriculture (Gutterson and Zhang, 2004). The term 'herbicide-resistant crop' (HRC) describes crops made resistant to herbicides by transgene technology. HRCs have been the subject of numerous previous reviews (Cerdeira and Duke, 2006; Cerdeira and Duke, 2007; Cerdeira *et al*., 2007b; Dekker and Duke, 1995; Duke, 1998; Duke, 2002; Duke, 2005; Duke and Cerdeira, 2005; Duke *et al*., 1991; Duke and Powles, 2008; Duke *et al*., 2002; Gressel, 2002; Hess and Duke, 2000; Warwick and Miki, 2004) and two books (Duke, 1996; McClean and Evans, 1995), and special issues of the journal *Pest Management Science* in 2005 and 2008. A review has covered agronomic and environmental aspects of HRCs (Schuette *et al*., 2004). Other reviewers have discussed the environmental impacts of all transgenic crops, with coverage of HRCs (Carpenter *et al*., 2002; Uzogara, 2000). Lutman *et al*., 2000 and Kuiper *et al*., 2000 published brief reviews of environmental consequences of growing HRCs. Other reviews have focused entirely on GRCs (Cerdeira and Duke, 2007; Cerdeira *et al*., 2007b)

The vast majority of HRCs used in agriculture are glyphosate-resistant crops (GRCs). So, in this review, we focus on the potential effects of GRCs on soil and water quality. Different formulations of glyphosate will not be discussed, as the actual composition of additives to these products, other than the active herbicide ingredients, are generally trade secrets and can vary between geographical regions and with time. The potential environmental impact of a technology is often geography and/or time dependent. Thus, extrapolation of the results and conclusions of studies to all situations is impossible. Generalizations from reported studies may not cover every situation. For a realistic assessment of risk, we will contrast certain risks of GRCs with the risks that the GRCs displace.

Glyphosate-resistant crops

Glyphosate (*N*-[phosphonomethyl] glycine) is a highly effective, non-selective herbicide. Prior to introduction of GRCs, glyphosate was used in non-crop situations, before planting the crop, or with specialized application equipment to avoid contact with the crop (Duke, 1988; Duke *et al*., 2003. ; Franz *et al*., 1997). It inhibits the shikimate pathway by inhibiting 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS). This results in reduced aromatic amino acids and deregulation of the pathway. The latter effect causes massive flow of carbon into the pathway, with accumulation of high levels of shikimic acid and its derivatives. Glyphosate is particularly effective because most plants metabolically degrade it very slowly or not at all, and it translocates well to metabolically active tissues such as meristems. Its relatively slow mode of action allows movement of the herbicide throughout the plant before symptoms occur. Glyphosate is only used as a post emergence herbicide, as it has little or no activity in soil. Glyphosate is an anion and is sold as a salt with different cations (e.g., isopropyl amine, trimethylsulfonium, diammonium).

Most GRCs are produced using the CP4 gene of *Agrobacterium* sp, found to encode a highly efficient, glyphosate-resistant EPSPS. Plants transformed with this gene are highly resistant (ca. 50X) to glyphosate Nandula *et al*., 2007. Glyphosate oxidoreductase (GOX), encoded by a gene from the microbe *Ochrobactrum anthropi* (strain LBAA), degrades glyphosate to glyoxylate, a ubiquitous and safe natural product, and aminomethylphosphonate (AMPA). This gene has been used along with the CP4 gene in GR canola. GR canola also as a resistance factor of about 50X Nandula *et al*., 2007. A multiple missense mutation in endogenous maize EPSPS produced by site-directed mutagenesis (GA21 gene) has been utilized to generate commercial glyphosate resistance in some varieties of maize (Lebrun *et al*., 1997).

To date, GR soybean, cotton, canola, sugarbeet, and maize are available to farmers of North America (Table 1). All varieties use the *CP4* EPSPS gene, except for the GA21 maize varieties. The GOX gene is also found in GR canola. The adoption rate of GR cotton and soybeans in North America has been high (ISB, 2008). This has been in large part because of the significantly reduced cost of excellent weed control obtained with the GRC/glyphosate package (Gianessi, 2005; Gianessi, 2008). Simplified and more flexible weed control also contributed to the rapid adoption. Approximately 62% of the canola acreage in the USA was planted in GR varieties in 2005 (Sankula, 2006,). Adoption of GR soybeans was more rapid in Argentina than in the U.S. (Monjardino *et al*., 2005; Penna and Lema, 2003). Initially, the economic advantage was not been as clear with GR maize, but after a lag phase adoption has increased rapidly to to approach the level of adoption of cotton.

Surface and groundwater quality

In a recent review, (Borggaard and Gimsing, 2008), concluded that the risk of ground and surface water pollution by glyphosate seems limited because of sorption onto variable-charge soil minerals (e.g. aluminum and iron oxides) and because of microbial degradation. Although sorption and degradation are affected by many factors that might be expected to affect glyphosate mobility in soils, glyphosate leaching seems mainly determined by soil structure and rainfall. Glyphosate in drainage water runs into surface waters but not necessarily to groundwater because it may be sorbed and degraded in deeper soil layers before reaching the groundwater. According to the World Health

Organization WHO, 2004 quidelines, under usual conditions, the presence of glyphosate and AMPA in drinking-water does not represent a hazard to human health. For this reason, the establishment of a guideline value in drinking water for glyphosate and AMPA is not deemed necessary.

An extensive review conducted by Vereecken, 2005, about the mobility and leaching of glyphosate concluded that in the USA and Europe there was a low occurrence of glyphosate in groundwater. An interesting finding from a study by Laitinen *et al*., 2007, suggested that plant translocation of glyphosate to roots should be included both in leaching assessments and pesticide fate models. After glyphosate fate was simulated with the PEARL 3.0 model, the observed and simulated glyphosate residues in soil after canopy applications did not correlate, highlighting the importance of the translocation process in glyphosate fate in soil. Their studies indicated that some soil glyphosate residues must originate from exudation from plant roots, and that the translocation process should be included both in leaching assessments and pesticide fate models.

Klier *et al*., 2008, studying glyphosate behavior based on the pesticide transport model LEACHP and the model PLANTX to simulate the pesticide uptake by plants implemented in the modular modeling system EXPERT-N, concluded that glyphosate transport measurements and the mathematical modeling results indicate that, due to the high sorption of glyphosate to the soil matrix and the high microbial capacities for glyphosate degradation, soil leaching risks can be considered to be low. On the other hand, Mamy *et al*., 2008, found that the main metabolite of glyphosate, AMPA, was more persistent than glyphosate and because of the detection of AMPA in the deep soil layer, the replacement of both trifluralin and metazachlor due to glyphosate resistant oilseed rape might not contribute to decreasing environmental contamination by herbicides. They also concluded that predictions of the pesticide root zone model (PRZM), underestimated the dissipation rate of glyphosate and the formation of AMPA in the field.

Scorza and Da Silva, 2007, using the PEARL model to establish a ranking considering the main pesticides and their potential to contaminate groundwater in Brazil, evaluated 4,374 agronomic prescriptions used in the Dourados river watershed and concluded that the most used pesticides on the watershed area were glyphosate followed by 2,4-D, fipronil, methamidophos, imazaquin, parathion-Me, trifluralin, and atrazine. Although glyphosate scored high in the amount used, their simulations revealed that the pesticides with the highest potential of groundwater contamination were bentazon, imazethapyr, fomesafen, 2,4-D, methamidophos, imazaquin, followed by the less used thiodicarb, and monocrotophos.

Long term studies conducted in Canada with the herbicides glyphosate, dicamba, 2,4-D, bromoxynil, methylchlorophenoxyacetic acid (MCPA), diclofop, and triallate showed no residues of glyphosate in groundwater Miller *et al*., 1995. Various studies have shown that glyphosate contaminates surface water less than several alternative herbicides (summarized by Carpenter *et al*., 2002). Once in surface water, it dissipates more rapidly than most other herbicides. In the intensely farmed maize-growing regions of the mid-western USA, surface waters have often been contaminated by herbicides, principally as a result of rainfall runoff occurring shortly after application of these to maize and other crops (Wauchope *et al*., 2002). A model was used to predict maize herbicide concentrations in the reservoirs as a function of herbicide properties comparing broadcast surface pre-plant atrazine and alachlor applications with glyphosate or glufosinate post-emergent herbicides with both GR and glufosinate-resistant maize (Wauchope *et al*., 2002). Because of greater soil sorptivity, glyphosate loads in runoff were generally one-fifth to one-tenth those of atrazine and alachlor, indicating that the replacement of pre-emergent maize herbicides with glyphosate would dramatically reduce herbicide concentrations in vulnerable watersheds. A more recent study by Shipitalo *et al*., 2008 found in a multi-year study of GR soybeans grown in no-tillage or tilled conditions, that glyphosate runoff in surface water was below drinking water standards, whereas levels of certain other herbicides used as a comparision were not always below maximum allowable levels. AMPA levels in runoff water were also low.

In a comprehensive survey of the U.S. Geological Service, USGS, 1998, more than 95% of all samples collected from streams and rivers contained at least one pesticide, compared to about 50% for ground water. Glyphosate was not among them. Although this study was done before the widespread adoption of GRCs, glyphosate was widely used as both a preplant and postharvest herbicide, as well as a harvest aid. Other studies also found no glyphosate in ground water in the United States where glyphosate is applied on no-tillage cropping systems (Kolpin *et al*., 1998) and in Brazil in various cropping systems (Cerdeira *et al*., 2003; Cerdeira *et al*., 2007a; Cerdeira *et al*., 2005; Lanchote *et al*., 2000; Paraiba *et al*., 2003). Similar results were found for surface waters (Clark *et al*., 1999).

Leaching of glyphosate and/or its metabolite AMPA was studied in a low-tillage field and a normal tillage field. A significant difference between the soil residual concencentrations of AMPA was seen, with the higher concentration found where low-tillage had been practiced and where glyphosate had been used several times in the years before sampling soil. Spatial and temporal variations in concentrations of glyphosate and AMPA have been observed in pre-and post-application 45-cm deep soil cores divided into 15-cm intervals (Meyer *et al*., 2005). Simonsen *et al*., 2008, studying the fate of glyphosate and its byproduct AMPA in soil, found that both compounds were better extracted from soil when phosphate was used as an extraction agent, compared with pure water indicating that the risk of leaching of aged glyphosate and AMPA residues from soil is greater in fertilized soil.

Degradation of pesticides in aquifers has been evaluated, and glyphosate was found to be degraded under both anaerobic and aerobic conditions, as opposed to some other herbicides such as MCPA and mecoprop (Albrechtsen *et al*., 2001). Certain pesticides were not degraded in water under aerobic or anaerobic conditions (dichlobenil, bentazon, isoproturon, and metsulfuron-methyl). This could be important when using glyphosate on transgenic crops, if the herbicide leached sufficiently to reach ground water, which is a more anaerobic environment. Half-lives of glyphosate vary from 60 h for ground water samples exposed to sunlight to 770 h for those stored under dark conditions (Mallat and Barceló, 1998).

Ground water contamination risks for a particular herbicide use should be evaluated in the context of the herbicides are replaced. As shown on Table 2. special attention should be given to atrazine, the most used herbicide under conventional crops considered. Atrazine was used in most acreage before GRC introduction. Atrazine is banned in Europe due to the water contamination potential. Wauchope, 1987 has shown that it has a high potential for groundwater contamination despite its moderate solubility, which explains the detection of the pesticide in concentrations that exceed the health advisory level in some wells in the United States located on irrigated lands (Belluck *et al*., 1991). According to Shipitalo *et al*., 2008, replacing atrazine and alachlor with glyphosate can reduce the occurrence of dissolved herbicide concentrations in runoff exceeding drinking water standards.

Glyphosate is considered to have a low risk for leaching Wauchope *et al*., 1992 and has a low GUS (Ground-water Ubiquity Score) index (Cerdeira *et al*., 2007b). The GUS index Gustafson, 1989 assesses the leachability of molecules and the possibility of finding these herbicides in groundwater. The index is based on two widely available herbicide properties: half-life in soil ($t_{1/2}^{soil}$) and partition coefficient between soil organic carbon and water (Koc). It can be calculated by the equation:

$$GUS=\log_{10}(t_{1/2})\times[4-\log_{10}(Koc)] \text{ (Table 2)}$$

Aquatic biota

Peterson and Hulting, 2004 compared the ecological risks of glyphosate used in GR wheat with those associated with 16 other herbicides used in spring wheat in the northern Great Plains of the USA.

A Tier 1 quantitative risk assessment method was used. They evaluated, among other things, acute risk to aquatic vertebrates, aquatic invertebrates, and aquatic plants, and also estimated groundwater exposure. They found less risk with glyphosate than with most other herbicides to aquatic plants and groundwater (Table 3).

As we mentioned earlier, glyphosate is less likely to pollute ground and surface waters than many of the herbicides that they replace. A life-cycle assessment technique used to compare conventional sugarbeet agricultural practices with risks that might be expected if GR sugarbeet were grown suggested that growing this GRC would be less harmful to the ecology of water for the herbicide-resistant crop than for the conventional crop (Bennett *et al*., 2004). These results suggest less impact of GRCs on aquatic vegetation than conventionally-grown crops.

Glyphosate was also evaluated for ecological risk assessment, and it was found not to bioaccumulate, biomagnify, or persist in an available form in the environment (Solomon and Thompson, 2003). This study also showed that the risk to aquatic organisms is negligible or small at application rates <4 kg/ha and only slightly greater at application rates of 8 kg/ha. Solomon *et al*., 2007; also found no significant effect on aquatic organisms of use of glyphosate as aerial spray in Colombia to erradicate coca plantations. Analyses of surface waters in five watersheds showed that, on most occasions, glyphosate was not present at measurable concentrations. Similarly, studies with surface water and sediment with glyphosate have also shown that adsorption to the bottom sediments, microbial degradation, the persistence of glyphosate in freshwater pond and effect on fishes used in the in situ bioassays posed no serious hazard (Tsui and Chu, 2008).

Conclusions

Glyphosate/GRC weed management offers significant environmental and other benefits over the technologies that it replaces Duke and Powles, 2008. We have provided an abbreviated survey of the potential impacts (risks and benefits) of GRCs on soil and water quality. Clearly, we and many of the authors that have written on this topic emphasize that risks and benefits of any GRC are very geography and time dependent. For example, increasing GR weeds in GRCs are changing how farmers use these crops, and in most cases reducing the environmental benefits of GRC systems. Glyphosate is more environmentally and toxicologically benign than many of the herbicides that it replaces. Its effects on soil and and water are relatively small. Soil erosion causes long term environmental damage. Being a broad spectrum, foliarly applied herbicide, with little or no activity in soil, glyphosate is highly compatible with reduced- or no-tillage agriculture and has contributed to the adoption of these practices in the Western Hemisphere. This contribution to environmental quality by GRCs is perhaps the most significant one. Numerous regulatory tests of glyphosate and glyphosate products, using rigorous protocols meeting international standards, as well as product post-marketing surveillance, have failed to reveal any effects that could help substantiate any claims of adverse health and environmental outcomes (Farmer *et al*., 2008). On the other hand, the degradation product of glyphosate, AMPA, has higher mobility and persistence in the environment. The environmental implications of this have not been well studied.

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Table 1. Transgenic GRCs that have been or are now available to farmers (de-regulated) in North America. (adapted from Duke and Cerdeira, 2005; and updated from the Information Systems for Biotechnology ISB, 2008

Crop	**Year made available**
Soybean	1996
Canola	1996
Cotton	1997
Maize	1998
Sugarbeet[1]	1990
Alfalfa[2]	2005
[1]Never grown by farmers, withdrawn in 2004, but re-introduced in 2008.	
[2]Re-regulated by court order in 2007.	

Table 2. Leaching potential of the main herbicides used on conventional main crops compared to glyphosate, according to indexes Ground-water Ubiquity Score (GUS) (Adapted from Inoue *et al.*, 2003).

Herbicides	**K_{oc} (ml/g)**	**$T_{1/2}$ (days)**	**GUS**	**Acreage (x1000)**	**LD_{50} (mg/kg)[1]**
Atrazine	165	60	L	42813	3090
Metolachlor	200	195	L	27295	1200-2780
Imazetapyr	22	75	L	25490	>5000
Pendimethalin	17200	44	NL	21558	1050
Trifluralin	7000	45	NL	21242	>5000
Dicamba	2	14	L	18237	757-1707
Acetochlor	55	20	L	14839	1426-2148
Cyanazine	190	14	IN	10772	182-332
Chorminuron	110	40	L	8882	4100
Glyphosate	24000	47	NL	-	>5600

NL= Does not leach, IN=Intermediate, L=Leaches easily, K_{oc}= Adsorption coefficient (mg/g^{-1}) $T_{1/2}$= Half-life LD_{50}= Lethal dose, [1]Lethal dose data from Extoxnet

Table 3. Predicted relative ecological risks of herbicide active ingredients based on modeling. (adapted from Peterson and Hulting, 2004)

Active Ingredient	**Application rate (g ai/ha)**	**Groundwater value (ppb)**	**RR[b]**	**Aerobic soil half-life (days)**
Glyphosate	840	0.0005	1	2
2,4-D	560	0.005	10	5.5
Bromoxynil	1,100	0.0004	0.8	2
Clodinafop	67	0.00003	0.06	1
Clopyralid	146	0.06	120	26
Dicamba	280	0.1	220	18
Fenoxaprop	90	0.000006	0.01	1
Flucarbazone	34	0.2	400	NA
MCPA	1,457	0.26	520	25
Metsulfuron	9	0.004	8	28
Thifensulfuron	22	0.0001	0.2	6
Tralkoxydim	280	0.001	2	5
Triallate	1,100	0.04	80	54
Triasulfuron	34	0.05	100	114
Tribenuron	16	0.00003	0.06	2
Trifluralin	1,100	0.009	18	169

[a]Abbreviations: RR, relative risk; NA, not available

[b]RR: Relative Risk compared with glyphosate, value in bold indicates greater risk relative to glyphosate