Environmental and health effects of the herbicide glyphosate

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BACKGROUND: The WHO reclassified the herbicide glyphosate as probably carcinogenic to humans, and concerns about potential side effects of the large-scale use of glyphosate have increased. We are interested in potential indirect effects of glyphosate on animal, human and plant health due to shifts in microbial community composition and antibiotic resistance in soil, plant surfaces and intestinal tracts.

OBJECTIVES: We review the scientific literature on glyphosate use, its toxicity to macro- and microorganisms, effects on microbial compositions, and potential indirect effects on plant, animal and human health. We hypothesize that glyphosate use has increased antibiotic resistance and propose study designs for testing this hypothesis.

DISCUSSION: Although the acute toxic effects of glyphosate on mammals are low, the chronic effects on human and animal health could be considerable due to accumulation in the environment. Intensive glyphosate use has led to the selection of glyphosate-resistant weeds and microorganisms. Shifts in microbial compositions due to selective pressure by glyphosate may have contributed to the proliferation of pathogens. Research on a link between glyphosate and antibiotic resistance is scarce. We hypothesize that the selection pressure for glyphosate resistance in bacteria could lead to shifts in microbiome composition and increases in antibiotic resistance.

CONCLUSION: We recommend interdisciplinary research on the associations between glyphosate use, distortions in microbial communities, expansion of antibiotic resistance and the emergence of animal, human and plant diseases. Independent research is needed to revisit the tolerance thresholds for glyphosate residues in food and animal feed taking all possible health risks into account.

Glyphosate use and residues

The herbicide glyphosate, N-(phosphonomethyl) glycine (Fig. 1), has brought several benefits to farmers since its introduction in 1973. First, large scale no-till farming, which is enabled by killing the previous crop and weeds by herbicide, often glyphosate, and then seeding the next crop in cultivated strips of land, has benefitted soil and environmental quality (Campbell et al. 1998; Locke et al. 2015). No-till crop production resulted in a reduction in soil erosion, conservation of soil moisture, an increase in soil organic matter and a reduction in fuel use (Robertson et al. 2014). Second, the development and use of glyphosate resistant crops has improved large-scale farming operations due to effective weed control (Duke, 2015). Third, glyphosate is also used to facilitate harvesting by terminating mature crops like potatoes and sunflowers. Glyphosate is also widely used in urban areas for weed control.

The total acreage treated with glyphosate has steadily increased, especially since the introduction of glyphosate-resistant soybean and canola in 1996 (Fig.2), cotton in 1997, and corn in 1998. The annual glyphosate application rates per acre of soybeans have also increased, partly due to the development of glyphosate resistant weeds (Fig. 3). In 2012, about 127,000 tons of glyphosate were used in the USA (Fig. 4) and 700,000 tons worldwide (Swanson et al. 2014; US Geological Survey 2012). Glyphosate use is widespread, both in industrialized and developing countries, especially on glyphosate resistant crops, which constitute 60-80% of all genetically-modified crops (Fig. 5). In the USA, glyphosate and AMPA are now pervasive in soils, surface water and groundwater (Battaglin et al. 2014).

Farm products contain widely varying amounts of glyphosate and its degradation product aminomethyl phosphonic acid, AMPA (Arregui et al. 2004; Bøhn et al. 2014; Cuhra 2015). The maximum residue limits also vary widely, ranging from 0.05 mg/kg in milk up to 500 mg/kg in various types of fodder (Codex Alimentarius 2013; Cuhra 2015; EPA 2013). To reduce glyphosate residues in plant products, a gene from *Ochrobactrum anthropi*, which encodes for the enzyme glyphosate oxidase (*GOX*), has been inserted into some glyphosate resistant crop cultivars, so that glyphosate is broken into AMPA and glyoxylate within the plants (Monsanto 2013). The residues of glyphosate and AMPA are taken up by animals and humans together with their food, and accumulate in various organs besides being excreted in urine. Glyphosate was detected in the urine of a high proportion of farm animals and humans (Krüger et al. 2013 and 2014a,b; Schrödl et al. 2014; Shehata et al. 2014).

Mode of action of glyphosate

Glyphosate is toxic to both monocotyledonous plants (like grasses) and dicotyledonous plants (most broad-leaf plants). Uptake and translocation of glyphosate in plants is enhanced by surfactants in the formulated product, such as polyoxyethylene amine (POEA) in Roundup®. Its herbicidal activity is associated with the inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), stopping the sixth step in the shikimate pathway that is required for the production of aromatic amino-acids and secondary compounds with defense functions in plants (Krüger et al. 2013; Schrödl et al. 2014). In addition, it chelates several micro-elements, in particular Mn, which is needed for the reduction of the flavin mononucleotide co-factor for EPSPS.

The shikimate pathway is not only present in plants but also in fungi and bacteria, rendering many taxa of microorganisms sensitive to glyphosate. This pathway is absent from animals, which is the basis for the lack of acute toxicity of glyphosate in animals. However, not all organisms with the shikimate pathway are equally sensitive to glyphosate. For example, *Agrobacterium tumefaciens* strain CP4 has a gene coding for a different version of EPSPS that is resistant to glyphosate inhibition. This gene was inserted initially in soybeans and later in other crops to provide glyphosate resistance (Padgette et al. 1995). Over time, naturally resistant plants have appeared in many fields that were treated repeatedly with glyphosate, with resistance mediated through a variety of mechanisms (Pollegioni et al. 2011; Schafer et al. 2014; Shaner et al. 2012). Similarly, bacterial and fungal strains with low sensitivity to glyphosate have been selected (Li et al. 2015; Liu et al. 2013; Priestman et al. 2005). These differences in sensitivity could affect the microbial composition of various habitats with glyphosate, including soil, plant surfaces and animal intestinal tracts.

Glyphosate effects on animals and humans

Although the acute toxicity to mammals is considered to be low (McComb et al. 2008), there is increasing interest in potential chronic effects of glyphosate and its degradation products as they accumulate in the environment (Battaglin et al. 2014; Mesnage et al. 2015; Séralini et al. 2014). For example, glyphosate and its break-down product AMPA increased the reactive oxygen species in human erythrocyte cultures, albeit at relatively high concentrations > 0.25 mM for 24 h (Kwiatkowska et al. 2014). However, chronic exposure to glyphosate (2 yr) at low doses (50 ng/L) in drinking water caused liver and kidney damage as well as various tumors in laboratory rats (Mesnage et al. 2015; Séralini et al. 2014). It has been hypothesized that the combination of glyphosate and “hard” water could have led to chronic kidney disease among rice farmers in Sri Lanka (Jayasumana et al. 2014). Moreover, increases in infertility and malformation among pigs that were correlated with glyphosate concentrations in various organs have raised concerns about glyphosate in the feed (Krűger et al. 2014a,b).

The World Health Organization recently warned about the potential negative health effects of glyphosate, classifying glyphosate as probably carcinogenic to humans (Guyton et al. 2015; IARC 2015). Subsequently, the Colombian Government decided to stop the large-scale aerial application of this herbicide to eliminate coca plants (http://www.nytimes.com/2015/05/15/world/americas/colombia-halts-us-backed-spraying-of-illegal-coca-crops.html?_r=0). Recently, medical scientists urged the US National Toxicology Program to reassess the toxicology of glyphosate and its formulations as well as interactions with other herbicides (Landrigan and Benbrook 2015). In the medical literature, AMPA is known as a glutamic acid receptor in the central nervous system (Catarzi et al. 2006), but little is known about the effects of excess AMPA on the functioning of the nervous system.

Glyphosate effects on microorganisms

Soil, rhizosphere and plant microbiomes.

Formulated glyphosate products are taken up by the foliage of plants and transported to the roots, from which they can exude into the rhizosphere (Yamada et al. 2009; Zobiole et al. 2010). Glyphosate is absorbed to clay and organic matter particles, slowing degradation by microorganisms (Banks et al. 2014). Surfactants, added in glyphosate formulations to enhance plant penetration, may slow down degradation even further. Because many microorganisms are sensitive to glyphosate, its application can affect the microbial composition and enzymatic activity in the rhizosphere and surrounding soil (Arango et al. 2014; Banks et al. 2014; Cherni et al. 2015; Druille et al. 2015; Schafer et al. 2014). For example, the plant growth promoting rhizobacteria *Burkholderia* and *Pseudomonas* spp., as well as arbuscular mycorrhizal fungi and nitrogen fixing *Rhizobium* spp., were negatively affected by glyphosate treatments (Druille et al. 2015; Schafer et al. 2014; Zobiole et al. 2010). However, the impact of glyphosate on soil microbial communities remains controversial (Wolmarans and Swart 2014). In studies comparing application of glyphosate with an untreated soil control, microbial communities seemed to “bounce back” from short-term treatment (Arango et al. 2014), with only minor or no effect on overall microbial structure, biomass, or activity (Shigueyoshi Nakatani et al. 2014), probably reflecting the great diversity and compensatory ability of microorganisms in soil. However, these studies have generally not employed DNA sequencing methods which would target subtle shifts in microbial composition or specific genera (Schafer et al. 2014).

Long-term negative effects of intensive farming practices, including herbicide use, on soil quality and functioning have been shown to be considerable (Squire et al. 2015). In addition, negative effects of glyphosate on the uptake of certain micronutrients like Mn, Zn and Fe and reduced resistance of plants to several root pathogens including *Fusarium* spp. have been shown convincingly (Fernandez et al. 2009; Johal and Huber 2009; Rosenbaum et al. 2014; Yamada et al. 2009). Moreover, *Fusarium* sp. appears relatively insensitive to glyphosate (Zobiole et al. 2010), potentially shifting the balance of *Fusarium* and antagonistic microorganisms such as *Pseudomonas fluorescens* in favor of the root pathogen (Kremer and Means 2009; Yamada et al. 2009; Zobiole et al. 2010). Many *Fusarium* species produce mycotoxins, heightening the concern about the general increase in *Fusarium* diseases.

Effects on microorganisms in animals.

Glyphosate-contaminated animal feed and water can affect intestinal microbial communities. For example, lactic acid producing bacteria generally are negatively affected by glyphosate (Clair et al. 2012; Krűger et al. 2013). These normally produce bacteriocins and suppress pathogenic bacteria like *Clostridium botulinum,* (Krűger et al. 2013; Rodloff and Krűger 2012), and botulism has increasingly been found in cows that had high concentrations of glyphosate in their feed and urine (Gerlach et al. 2014; Krűger et al. 2013 and 2014a). Similarly, *Bifidobacterium* and *Enterococcus* spp. in poultry were negatively affected by glyphosate at 0.08-0.15 mg/g, while pathogenic bacteria like *Salmonella* and *Clostridium* spp. were less sensitive (MIC=1.2-5 mg/g) to this herbicide (Shehata et al. 2013). The tested concentrations were high, but the glyphosate concentration in the poultry feed was also high: 0.19-0.4 mg/g (Shehata et al. 2014). Glyphosate in animal feed affects not only intestinal bacteria but also fungi. A negative correlation was found between glyphosate concentrations in urine and the density of Mucorales in the rumen of dairy cows in Germany (Schrödl et al. 2014). Although Mucorales were resistant to glyphosate *in vitro*, it is possible that this change in prevalence was due to a disturbance of the intestinal microbiota. Fortunately, addition of humic acids and charcoal to animal feed can bind some of the glyphosate and alleviate its negative effects on animal health (Gerlach et al. 2014; Shehata et al. 2014).

Resistance to glyphosate and antibiotics.

Some bacteria and fungi are highly resistant to glyphosate (Fei et al. 2013; Wolmarans and Swart 2014). For example, *Agrobacterium tumefaciens* strain CP4 was resistant to glyphosate before its widespread use. Since the intensive use of glyphosate, however, resistance has been found increasingly in many other bacteria, with resistance mediated through a variety of mechanisms (Fei et al 2013): (i) target site mutation like an amino acid substitution in the EPSP synthase in *Staphylococcus aureus* (Priestman et al. 2005), (ii) overexpression of a membrane efflux transporter, for example in *E. coli* and *Pseudomonas* (Staub et al. 2012), (iii) degradation of glyphosate, for example by cyanobacteria (Arunakumara et al. 2013), and (iv) scavenging of free radicals by mycothiol in Actinobacteria providing general stress resistance (Liu et al. 2013). It has been proposed that mechanisms conferring resistance to glyphosate in bacteria also mediate resistance to clinically-important antimicrobial agents (Kurenbach et al. 2015; Liu et al. 2013). For example, mycothiol provides resistance not only to glyphosate but also to a wide range of antibiotics, including penicillin G (Liu et al. 2013). Regulation of the AcrAB efflux pump was modified in *E. coli* exposed to a commercial formulation of glyphosate (1,240 μg/ml), and this was associated with resistance to 0.03-0.09 μg/ml Ciprofloxacin (Cip) and 5-10 μg/ml Kanamycin (Kan) (Kurenbach et al. 2015). Similarly, *Salmonella enterica* serovar Typhimurium was more resistant to Cip at 0.08 μg/ml and Kan at 8-40 μg/ml when exposed to glyphosate, but potential changes in efflux pump were not investigated (Kurenbach et al. 2015). Glyphosate-resistant Enterobacteriaceae isolates (n=75) selected on Roundup-amended plates (7,500 ppm glyphosate) from the gastrointestinal tract of cattle and pasture soil showed >80% cross-resistance against Tetracycline, Erythromycin, Polymixin B, Rifampicin, and Ampicillin, but not against the extended spectrum β-lactamase (ESBL) antibiotic cefotaxime (Jeong et al. unpublished data). However, positive relationships were found between field-induced glyphosate resistance and ESBL production in various other Enterobacteriaceae (http://www.ensser.org/fileadmin/files/Science_in_the_Eye_of_the_Storm/II_Monika_Kr%C3%BCger_-_Collateral_damages_of_the_herbicide__glyphosate__in_dairy.pdf). In agreement with this finding, unidentified bacteria (n=102) that were isolated from citrus roots on penicillin (20 ppm) amended agar had 62% cross-resistance to Roundup (7,500 ppm glyphosate), which is applied frequently in citrus groves, while penicillin is never used (van Bruggen et al., unpublished data). It remains to be seen if the overall architectures of ESBL antibiotics and AMPA are similar enough for specific cross-resistance to develop (Fig. 1).

Discussion and conclusion

Scientific literature about possible environmental and organismal side effects of glyphosate is increasing rapidly. Although the acute toxic effects of glyphosate on mammals are low, concerns have emerged about chronic effects on the health of humans and animals, with literature suggesting a linkage between increased glyphosate use and a wide variety of human diseases, including various forms of cancer (Swanson at al. 2014). Data on causality are, unquestionably, sparse. However, sufficient data are accumulating (Landrigan & Benbrook, 2015) that additional controlled experiments in animal models to assess possible chronic toxic effects would appear to be indicated.

Intensive glyphosate use has led to the selection of glyphosate resistant weeds and increased application rates. In response to glyphosate selection pressure, glyphosate resistance in microorganisms may rise, and shifts in microbial communities will take place in soil, plants, animal feeds and intestinal tracts, with proliferation of specific plant and animal pathogens and potential impacts on plant, animal, and human health (Berg et al. 2014; Hoffman et al. 2015; O'Doherty et al. 2014). Antibiotic resistance is widespread in soils and the intestinal tract of farm animals (CDC 2013; Marti et al. 2013; Udikovic-Kolic et al. 2014; Demanèche et al. 2008; Stine et al. 2007), and may well increase the risk of clinically-important antimicrobial resistance (Brolund et al, 2014; CDC 2013; Smith et al, 2005). In not all instances is it possible to directly relate rates of antimicrobial resistance to antimicrobial use (Marti et al. 2013; Udikovic-Kolic et al. 2014); in such settings, glyphosate may serve as a selective driver for the emergence of antimicrobial resistance, acting through common resistance mechanisms.

Considering that the microbiomes associated with soil and fresh plant products transfer to animal and human intestinal tracts (Berg et al. 2014), and the microbiomes of excrements return to soil and water, it is likely that there are microbial cycles that are characteristic for particular management systems (Semenov et al. 2010; van Bruggen et al. 2015). Management of weeds with multiple glyphosate applications could result in microbiomes that are relatively glyphosate- and antibiotic resistant. We hypothesize that the selection pressure for glyphosate resistance and the associated resistance to antibiotics in the soil microbiome results in transfer of antibiotic resistant bacteria from soil to plants, animals and humans through the food web. We suggest investigating this question by comparing the microbiomes and antibiotic resistance in food production and consumption systems where glyphosate is used or not and where manure with or without antibiotic resistance is used or not.

We expect that the problems associated with the large-scale and intensive use of glyphosate are much more encompassing than originally anticipated by the regulatory agencies. Glyphosate use may need to be restricted to prevent accumulation of glyphosate and AMPA in soil, water, feed and food. Residual concentrations need to be monitored more extensively, and results need to be published. Pending results from additional independent research, the tolerance levels may need to be lowered. Finally, stricter enforcement of tolerance levels and rejections of loads shipped internationally may be required. A global effort will be needed to collect these types of high-quality data across the range of settings in which glyphosate is currently used; only as such data become available will we be able to design and implement strategies to counter further escalation of the problems associated with glyphosate use.

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Figure legends

Fig. 1. Chemical structures of Glyphosate, its degradation product AMPA, and the antibiotics ampicillin and penicillin (http://www.chemspider.com/Chemical-Structure.6013.html).

Fig. 2. Percentages of agricultural areas under corn, cotton and soybean treated with total herbicides, total insecticides and glyphosate from 1990 to 2014.
Adapted from: http://sustainablepulse.com/wp-content/uploads/GMO-health.pdf and USDA NASS (2014).

Fig. 3. Glyphosate application rate (kg/ha/yr) on soybeans; standard error 0.078 kg/ha/yr.
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Fig. 4. Distribution of glyphosate use (0-160 kg/ha) in the USA (US Geological Survey 2012).
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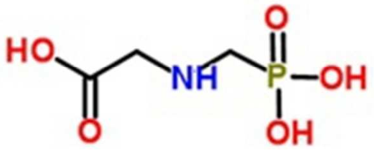
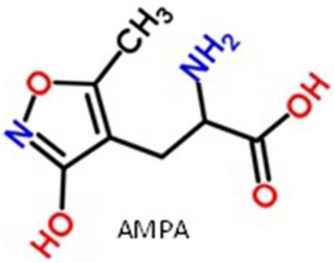
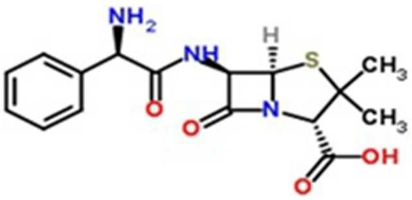
Fig. 5. World-wide production of genetically modified (GM) crops in 2010.
http://www.economist.com/blogs/dailychart/2011/02/adoption_genetically_modified_crops
The most commonly grown GM crops are Roundup®-ready soybeans, corn, and canola, >80% in 2009 down to 60% in 2013 (Duke and Powles 2009; James 2014).



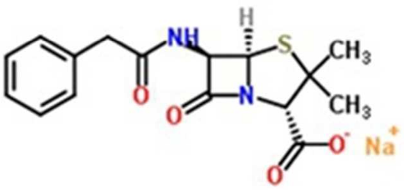
Glyphosate



AMPA



Ampicillin



Penicillin G

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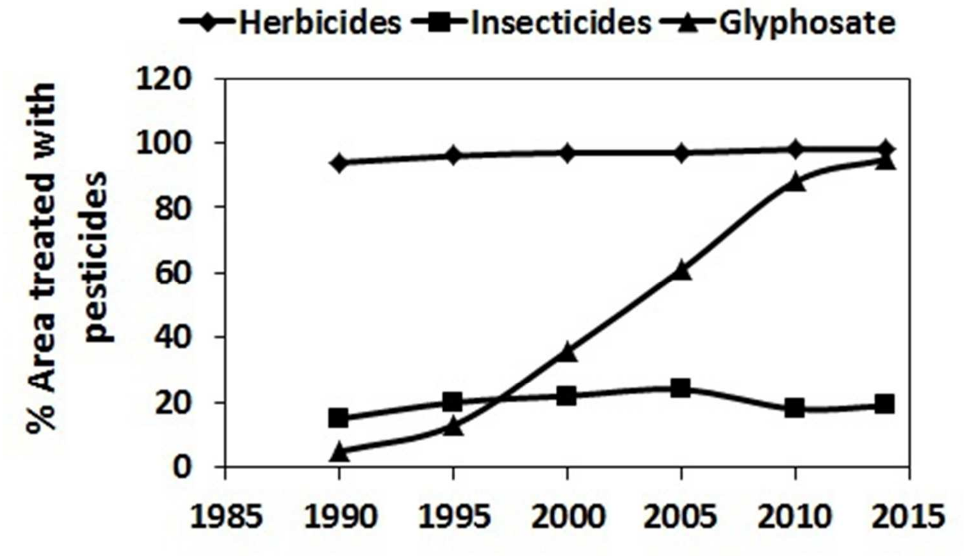

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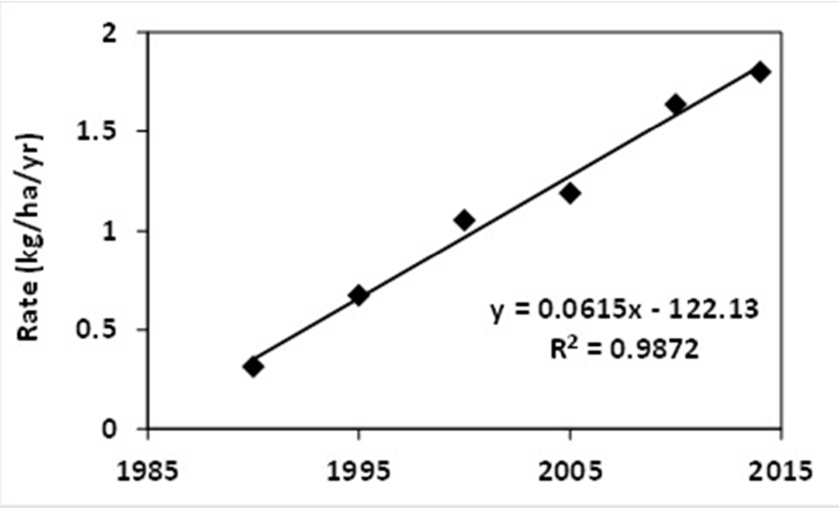
128x80mm (96 x 96 DPI)



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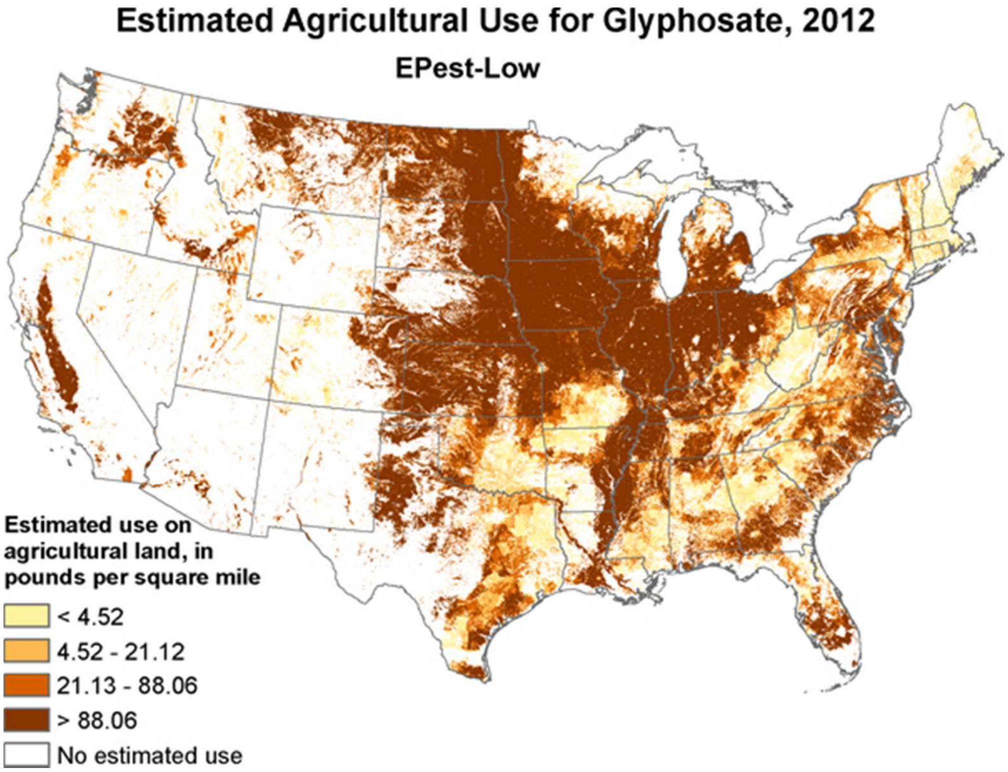

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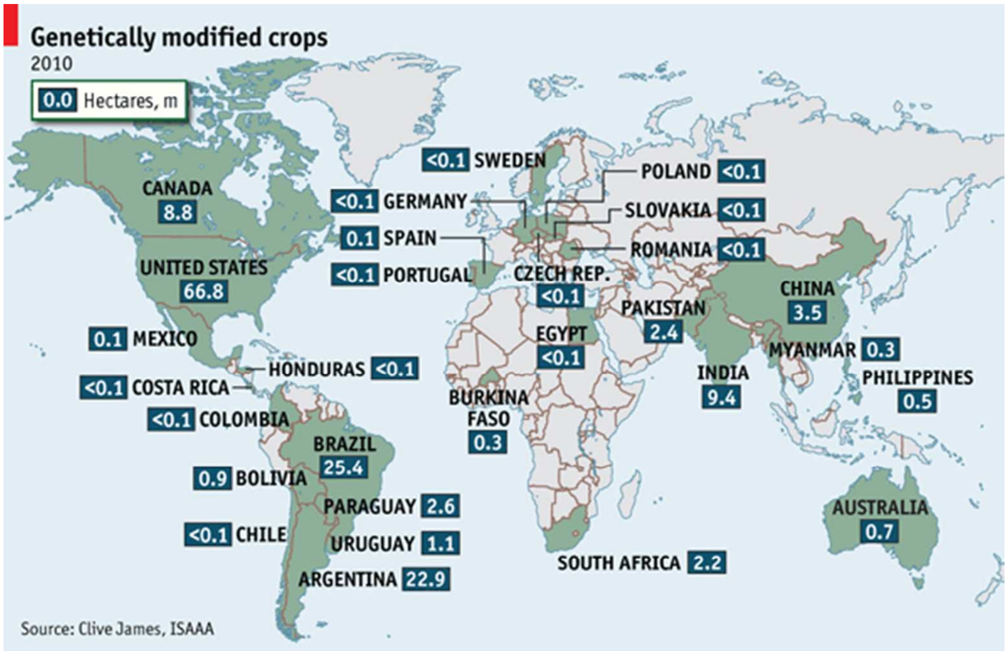

Genetically modified crops

2010

Source: Clive James, ISAAA

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209x135mm (72 x 72 DPI)