

Herbicide tolerance and GM crops

Why the world should be
Ready to Round Up glyphosate

**EXECUTIVE
SUMMARY
& REPORT**

June 2011



GREENPEACE

Contents

Herbicide tolerance and GM crops

This report examines the environmental and health implications of the widespread and intensive use of the herbicide glyphosate in association with GM (Roundup Ready) crops.

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Executive Summary

Glyphosate is the active ingredient in many herbicides sold throughout the world, including the well-known formulation, Roundup. Glyphosate-based herbicides are used widely for weed control because they are non-selective; glyphosate kills all vegetation.

Glyphosate has been promoted as 'safe'. However, mounting scientific evidence questions the safety of glyphosate and its most well known formulation, Roundup. **The evidence detailed in this report demonstrates that glyphosate-based products can have adverse impacts on human and animal health, and that a review of their safety for human and animal health is urgently needed.**

The widespread and increasingly intensive use of glyphosate in association with the use of GM (genetically modified, also called genetically engineered or GE) crops poses further risks to the environment and human health. GM crops specifically engineered to be tolerant to glyphosate are known as 'Roundup Ready' (RR). These RR varieties allow farmers to spray the herbicide over the top of the growing crop, killing virtually all weeds without affecting the crop. The use of glyphosate on GM RR crops such as soy, maize and cotton has increased dramatically in North and South America, where they are predominantly grown.

GM RR crops are marketed by the US agrochemical giant Monsanto, and are associated with its own formulation of glyphosate herbicide, Roundup. Monsanto's sales pitch to farmers promised, and still does, reduced labour and financial savings by simplifying and reducing the costs of weed control. The reality is turning out to be different, with increasing health, biodiversity and environmental concerns and the development of weed resistance.

Given the problems that are now evident, no new GM glyphosate-tolerant crops should be authorised. In broader terms, GM herbicide-tolerant crops have been developed for an industrial farming model. They are therefore intrinsically linked to unsustainable farming practices that damage the basic natural resources food production is based upon, and their cultivation should be banned.

Glyphosate is present in soils, waters and our food as a result of its widespread use with GM Roundup Ready crops.



Exposure to glyphosate

People, plants and animals can be exposed to glyphosate and Roundup in many ways. Farmers, bystanders and other operators can be exposed during its application, and neighbouring natural habitats by drift from the area where it is being applied. Aerial application is used on some crops, such as on the vast monoculture plantations of GM RR soya in the Americas, which greatly increases the chances of accidental exposure of neighbouring populations or habitats.

Exposure to glyphosate and Roundup also occurs via their residues, frequently found in food and the environment. The Maximum Residue Levels (MRLs) in food for glyphosate and its breakdown product were agreed by the UN-based Codex Alimentarius Commission in 2006, but appear to be related more to the type of agricultural practices characteristic of each food crop rather than to safety thresholds for human health.

In light of the new scientific evidence on the health and environmental impacts of glyphosate it is essential to re-evaluate MRLs in order to align them with updated safety assessments.

In the environment, glyphosate can be held in the soil by binding to particles but, depending on soil chemistry, can also leach into groundwater. Glyphosate can also wash directly into drains and surface waters and it has been detected in both. Glyphosate and its degradation product have been detected in studies of drainage surface waters in Canada, the US and Denmark. These findings have implications for surface water quality and drinking water quality. Given the evidence that glyphosate can cause harm to health and the environment, the leaching of glyphosate has also serious implications for aquatic life.

Glyphosate is present in soils, waters and our food as a result of its use as an herbicide. Therefore, rigorous assessment of the safety of glyphosate to plant, humans and animals is of great importance.

Human health problems related to glyphosate

Independent scientific studies are underscoring the call for an urgent reassessment of glyphosate and its related products. These studies associate exposure to glyphosate with a number of negative effects on human and animal health, including long term or chronic effects:

- **Birth defects in the Argentinean state of Chaco**, where GM soya and rice crops are heavily sprayed with glyphosate, increased nearly fourfold over the years 2000 to 2009. Similar defects were also found in a woman from Paraguay exposed to glyphosate-based herbicides during pregnancy. These defects were compatible with those induced in laboratory experiments at much lower concentrations than normal commercial glyphosate concentrations.
- **Glyphosate is a suspected endocrine disruptor**. This means it could disrupt production of vital reproductive hormones, such as progesterone and oestrogen. Published studies demonstrate various endocrine effects in animals and human cells associated with glyphosate.
- Studies of illness patterns in human populations (epidemiological studies) have linked glyphosate exposure to **non-Hodgkin's lymphoma** (a type of blood cancer) whilst laboratory studies have confirmed that glyphosate and/or its associated products exhibit characteristics typical of cancer causing agents (i.e. genotoxicity or mutagenicity) in animals and both human and animal. Together, these studies suggest that glyphosate may contribute to cancer. Evidence that glyphosate **may also affect the nervous system** and may even be implicated in Parkinson's disease.

Scientific evidence highlighting these health effects must be taken very seriously. An urgent reassessment of the health impacts of glyphosate and its related products must take place.

Executive Summary (cont.)

Glyphosate affects biodiversity

Glyphosate can impact on biodiversity in a number of different ways and can have short and long term, as well as direct and indirect negative effects. Evidence is accumulating that glyphosate can have a damaging impact on aquatic organisms as a result of its normal use in agriculture or forestry. Several studies have suggested that, under close-to-field conditions, glyphosate-based products, including Roundup, have a direct toxic effect on the adults and tadpoles of a range of amphibian species. Despite these findings, Monsanto still claims that Roundup has ‘*no adverse effect on aquatic animals*’ (Monsanto 2010a).

Many aquatic animals - from microscopic algae to fish and mussels - have been found to be affected by exposure to glyphosate and/or Roundup. The observed effects included: shorter life spans and reduced reproductive rates in rotifers (a type of freshwater invertebrate); changes in population structure in phyto- (or plant-) plankton; increased mortality in aquatic worms; and changes in liver cells in carp. A recent study found genotoxic effects in the red blood cells of European eels when exposed to Roundup for a short period. There is also a suggestion that glyphosate may affect the nervous system of aquatic animals in a manner similar to an organophosphate.

Glyphosate can also have a direct impact on non-target plants in the environments where it is used through spray drift or deliberate over spraying. This could lead to the loss of rare or endangered species or an overall reduction in diversity and numbers. Research carried out in the UK on the use of glyphosate on GM RR beet showed significant indirect effects of this form of weed control. These included reduced weed numbers in arable fields and reduced weed seed production both of which are potentially deleterious to species further up the food chain, including threatened bird species, if repeated over a number of years.

‘...If GM herbicide-tolerant beet were to be grown and managed as in the FSEs [UK Farm Scale Evaluations 2000- 2003] this would result in adverse effects on arable weed populations, as defined and assessed by criteria specified in Directive 2001/18/EC, compared with conventionally managed beet. The effects on arable weeds would be likely to result in adverse effects on organisms at higher trophic levels (e.g. farmland birds), compared with conventionally managed beet’
(ACRE 2004)

It is apparent that glyphosate and its formulated commercial products (e.g. Roundup) can be harmful to species at many stages along the food chain, including the aquatic food chain. Regulators must ensure that usage of herbicides is safe for wildlife when it is used for purposes it has been approved for. Therefore, the safety of glyphosate to biodiversity urgently needs to be re-assessed.

Glyphosate impacts on the soil-plant system

The impact of glyphosate on soil biodiversity and the soil-plant system is of concern because of the effects observed with GM RR crops. Glyphosate enters the soil by being directly sprayed on it, via the roots of plants that have been sprayed, or from dead vegetation. Importantly, glyphosate affects the rhizosphere – the region of the soil surrounding the roots that is essential to the health and nutrient uptake of the plant. Surprisingly, the approvals processes for glyphosate and its formulated products around the world, including the EU, currently do not require exhaustive testing of its soil impacts.

Studies of earthworms exposed to glyphosate showed reduced growth rate, reduced cocoon hatching and behaviour to avoid treated areas. Earthworms are vital to soil health so any adverse effect on them is likely to affect soil health.

Independent researchers are now publishing studies showing that glyphosate has an impact on key functions of the rhizosphere. These include:

- Reduction in the uptake of essential micronutrients by crops
- Reduction in nitrogen fixation, resulting in reduced yields
- Increased vulnerability to plant diseases

Such changes can have a direct impact on the health and performance of crops. Plant diseases - such as take-all in cereals, damping off, root rot and sudden death syndrome in soya - are encouraged by the changes in soil biology and chemistry that glyphosate induces. These impacts are of concern to farmers and environmentalists and need to be addressed urgently.

GM Roundup Ready crops include oilseed rape or canola (pictured), soya, maize and cotton. Such crops do not contribute to sustainable agriculture practices.



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Glyphosate and the plague of resistant weeds

When glyphosate first appeared in the mid 1990s, weed resistance to herbicides as a result of GM RR crops was rarely discussed, although the phenomenon of weed resistance to herbicides was well known. Now, 15 years later, weed resistance to glyphosate is one of the most well documented effects and is a major environmental concern of the cultivation of GM RR crops.

Since the introduction of RR crops, there has been a dramatic increase in the number of weed species exhibiting glyphosate resistance. Glyphosate resistance has now been confirmed in over 20 species, with over 100 resistant strains identified, primarily in the Americas. Many scientists attribute this increase to the over reliance on glyphosate to control weeds in fields of GM RR soya, maize and cotton.

'No-tillage corn and soybean production has been widely accepted in the mid-Atlantic region, favouring establishment of horseweed. Within 3 years of using only glyphosate for weed control in continuous glyphosate-resistant soybeans, glyphosate failed to control horseweed in some fields. Seedlings originating from seed of one population collected in Delaware were grown in the greenhouse and exhibited 8- to 13-fold glyphosate resistance compared with a susceptible population' (Van Gessel 2001)

Controlling glyphosate-resistant weeds in GM RR crops is now a major problem for farmers. Monsanto acknowledges this, and has published guidance on how to deal with the growing weed resistance problems in GM RR crops. Monsanto's recommended strategies include:

- the use of either stronger formulations of glyphosate or of mixtures of glyphosate and other herbicides, e.g. the notorious 2,4-D – one active ingredient of Agent Orange, the defoliant used by the US Army during the Vietnam; and
- producing GM seeds with several herbicide tolerant genes (gene stacking), which would allow other herbicides, in addition to glyphosate, to be sprayed over crops.

These strategies add to the amount of herbicides being used therefore increasing the overall toxic burden from GM RR crops and continue the industrial agriculture treadmill of herbicide usage and resistance. The development of more weeds with resistance to multiple herbicides seems probable. The widespread nature of weed resistance, and the additional herbicides required to control these weeds means that Monsanto's promise of cheaper and easier weed control with GM RR crops has not been delivered

The toxicological profiles for mixtures of herbicides are not clear. However, **it is clear that GM RR crops have brought about an escalation in the pesticides 'arms race' with an increasing toxic burden on the environment and people.**

Conclusion

Recent studies demonstrate that glyphosate-based herbicides, such as Roundup, can have harmful effects to human health and the environment. Exposure of humans to glyphosate has been linked to various health effects including reproductive effects, cancer and neurological effects. Glyphosate interacts with soil chemistry and biology, resulting in a variety of impacts including reduced plant nutrition and increased vulnerability to plant disease. Glyphosate may also leach into surface and groundwaters, where they may damage wildlife and possibly end up in drinking water. Glyphosate and Roundup are far from benign herbicides and a review of their safety for human and animal health and for the environment is urgently needed.

GM RR crops have greatly increased glyphosate usage, especially in the Americas where they are primarily grown. Given the new evidence of glyphosate toxicity, this is of great concern. The rise in glyphosate resistant weeds is associated with GM RR crops, and the escalation in the 'arms-race' against these resistant weeds fuels concerns that even more glyphosate will be used in the future with GM RR crops, in stronger formulations and possibly with additional herbicides. This facet of GM herbicide-tolerant crops should be enough to lead to a ban on their cultivation.

GM herbicide-tolerant crops, as epitomised by GM RR crops, are not part of sustainable agriculture practices. They are part of an industrial agriculture system that involves large-scale monocultures that depend on costly, polluting inputs such as herbicides. There is no doubt that there is an urgent need to find sustainable solutions to agriculture. As the recent UN/ World Bank global assessment of agriculture (IAASTD) recently stated, 'business as usual is no longer an option' (IAASTD 2009b). Sustainable solutions will not come from GM crops, and definitely not from GM herbicide-tolerant crops.

GM Roundup Ready crops have led to increasingly intensive use of glyphosate, as they allow farmers to spray the herbicide over growing crops.



Scientific evidence shows that glyphosate can have immediate and long-term, direct and indirect toxic effects on plants and animals, as well as indirect effects linked to the changes it causes in the ecosystem.

1] Introduction

Glyphosate - the active ingredient in many herbicides sold throughout the world - has always been promoted as 'safe'. But is it?

Mounting scientific evidence suggests that there can be adverse impacts on human and animal health, and the environment. The safety of glyphosate is in serious doubt.

The most well-known formulated herbicide based on glyphosate is 'Roundup', sold by the US-based agricultural biotechnology corporation Monsanto, the world's leading producer of glyphosate. Monsanto is also the leading producer of genetically modified (GM, also called genetically engineered, GE) seed, producing glyphosate-tolerant GM crops that are marketed as 'Roundup Ready' (RR). The glyphosate-tolerance of these crops subsequently leads to a widespread use of glyphosate-based products; thus, the close correlation between crop and herbicide is a major cause for concern.

This report examines the increasing evidence on the impacts of glyphosate-based products - and glyphosate's main breakdown product aminomethylphosphonic acid (AMPA) - on health, the environment, biodiversity and farmers. It also looks at the use of glyphosate or Roundup on GM RR crops: how its use in connection with these crops is resulting in widespread weed resistance; what that means for future herbicide usage; and the wider considerations about GM herbicide-tolerant (HT) crops.

This report comes at a time when the use of Roundup has increased dramatically around the world. At the same time there is a growing body of evidence indicating its harmful impacts.

1.1 Glyphosate: how does it work?

Glyphosate is a water-soluble, broad-spectrum, non-selective herbicide that is absorbed by the leaves and transported to all parts of the plant, including the roots. It is therefore capable of completely killing even deep-rooted plants, in contrast to other products - such as paraquat - which affect only the leafy part of the plant above ground. This property, combined with marketing campaigns promoting it as a 'safe' product, has made glyphosate a very popular herbicide.

A Monsanto employee discovered the herbicidal nature of the glyphosate molecule in 1970. Monsanto introduced the first commercial Roundup product (Monsanto 2005a), which uses glyphosate as the active ingredient, in 1974. It is claimed that Roundup is now used in 130 countries on 100 different crops (Monsanto 2005a).

The enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) is present in all plants, fungi and bacteria. Glyphosate chelates (or binds) manganese, making it unavailable to the EPSPS. Because manganese is essential for EPSPS to work (Johal & Huber 2009), inhibiting it in this way subsequently affects an essential biochemical pathway in plants, the shikimate pathway, leading to a shortage of vital molecules for building proteins and causing the plant's death.

Because EPSPS is not found in animals, it is assumed that glyphosate is relatively harmless to mammals, insects, fish and birds. However, independent research shows that this is not the case. In addition glyphosate breaks down in the natural environment to form aminomethylphosphonic acid (AMPA), which is very similar in chemical structure to glyphosate. There is evidence that AMPA can also have impacts on animal and human health, and the environment.

On its own, glyphosate is not very effective as a herbicide. Therefore, it is marketed in formulated products mixed with other chemicals known as adjuvants or surfactants. These chemicals enable the herbicide to stick to foliage and allow the glyphosate molecule to penetrate the cuticle on the leaves and enter cells and the plant's circulatory systems. Glyphosate is then transported to all parts of the plant, including the tips of the roots.

So, when examining the impact of this herbicide on health and the environment, it is important to take AMPA and the adjuvants or surfactants into account. The impacts of these chemicals - singularly and in combination - are explored in this report.

1.2 GM crops: a perfect match for Roundup

Roundup is Monsanto's top-selling range of herbicides, and all products contain glyphosate as the active ingredient. There are many different formulations of the product sold under the same brand name around the world - for instance Roundup PowerMax and Roundup Weathermax, or under other brand names such as QuikPro¹.

Monsanto's patent on glyphosate ran out in 2000. However, Monsanto had already secured markets for their glyphosate by introducing GM seeds - soya, maize, cotton and canola - that are specifically engineered to be glyphosate tolerant. All Roundup Ready seeds are GM, as there are no conventional methods to produce herbicide tolerance to Roundup. These GM seeds have been marketed from the mid 1990s onwards² as Roundup Ready (RR). Because Monsanto does not guarantee crop performance with non-Roundup-brand herbicides, farmers are encouraged to use only Monsanto's Roundup on the GM crops rather than other brands of glyphosate herbicide (Monsanto 2011).

GM RR varieties allow farmers to spray the herbicide over the growing crop, killing virtually all weeds without affecting the crop itself. Monsanto's sales pitch to farmers promised - and still does - simplified weed control and reducing the number of spray passes required, subsequently reducing the costs of weed control (Monsanto 2009a). However, the reality is turning out to be different with increasing health (Chapters 2 and 3), biodiversity (Chapter 5) and environmental (Chapters 4 and 6) concerns and the development of weed resistance (Chapter 7).

GM RR crops are primarily grown in the Americas. In 2009, more than 90% of the soya crop planted in the US was GM RR (National Agricultural Statistics Service 2009). GM RR maize and cotton were also widely grown. While GM RR soya also dominates the soya crop in Argentina and Paraguay, adoption of the technology elsewhere in the world has been met with less enthusiasm. In Brazil, take-up of GM RR soya has been much slower, with 40% of the soybean crop being non-GM in 2009/10 (The Crop Site 2010). In Europe, no GM RR crops have so far been approved for cultivation.

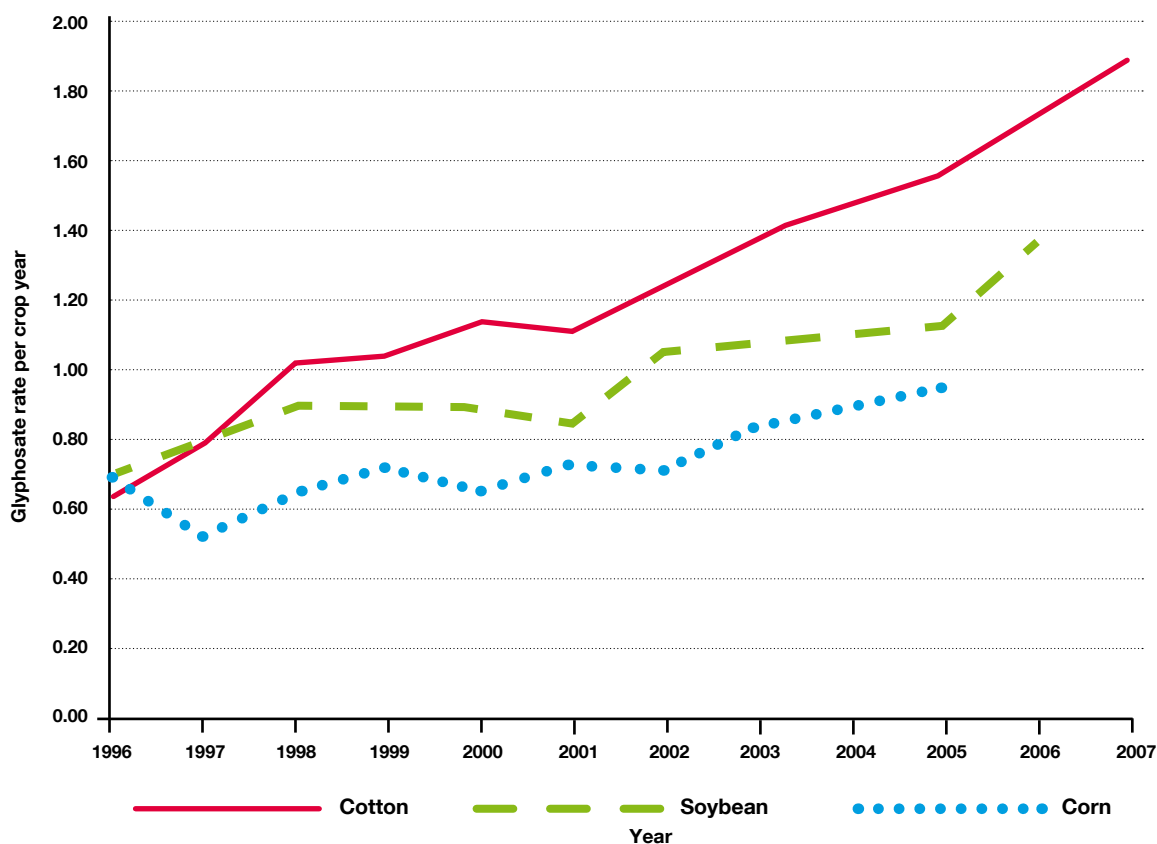


Fig. 1: Average glyphosate application rate per crop year for corn, soya and cotton in the US. Application rates of glyphosate-based herbicides have increased steadily since the introduction of glyphosate-tolerant RR cotton, soya and corn in the mid 1990s. Data are from US National Agricultural Statistic Service who define "rate per crop year" as the average one-time rate of application multiplied by the average number of applications. Redrawn from Benbrook (2009) with permission.

¹ <http://www.monsanto.com/products/Pages/agricultural-herbicides.aspx>

² http://www.cera-gmc.org/?action=gm_crop_database

Glyphosate is present in soils, waters and our food as a result of its widespread use with GM Roundup Ready crops.



Since the introduction of GM RR seeds, the amount of glyphosate used in countries where these crops are grown has increased dramatically. In a series of reports, Charles Benbrook analysed USDA data charting the rise of glyphosate usage in the US since the introduction of GM RR crops (Fig. 1; Benbrook 2001; 2004; 2009). He noted a 39% rise for maize (1996-2005); nearly 200% for cotton (1996-2007) and nearly 100% for soybean (1996-2006). Peer-reviewed literature also notes considerable increases of glyphosate associated with the introduction of GM RR crops in the US (e.g. Duke, 2005; Cerdeira & Duke 2006). Similar trends have followed the introductions of GM RR soya in Argentina (Binimelis et al. 2009) and Brazil (Lucas 2006). It is apparent that the introduction of GM RR seeds has been instrumental in the increased use of glyphosate - much of it as part of Monsanto's Roundup range - in recent years.

Since the introduction of GM Roundup ready (RR) seeds, the amount of glyphosate used in countries where these crops are grown has increased dramatically.

1.3 Other uses of glyphosate

The potential markets for glyphosate extend beyond GM RR crops to many types of arable crops and many types of land management (see, e.g. Monsanto 2005a).

Increasingly, Monsanto is marketing GM insect-resistant crop varieties that also include glyphosate-tolerance (RR) genes³. In addition, crops with the GM RR gene and tolerance to other herbicides such as dicamba are being developed by biotech companies to deal with glyphosate-resistant weeds (see Chapter 7) (Behrens et al. 2007; Service 2007; Stride 2010).

1.4 Summary

Glyphosate is the active ingredient in many herbicides. It is generally sold as formulations that include other ingredients in order to increase its effectiveness by allowing it to adhere to plant leaves. The most well-known of these formulations is the Roundup range of herbicides sold by Monsanto. Although first marketed in 1974, glyphosate use increased drastically following the introduction of GM RR crops in the mid 1990s. Monsanto maintains a high market share of glyphosate sales by selling Roundup as a package with its GM glyphosate-resistant seeds.

The dramatic increase in the use of glyphosate has serious implications for health and the environment. These implications are described in the following chapters.

Aerial spraying of RR crops such as soya increases exposure of people to glyphosate-based herbicides.



Since the early days of their commercialisation, glyphosate and Roundup have been marketed as 'safe' or benign. Yet increasingly, the scientific literature indicates that these products are far from being safe. Independent scientific studies are now providing details of Roundup's effects, especially its chronic effects on human health.

2] Glyphosate impacts on human health

Glossary of terms

Axon the long fibre of a neuron.

Congenital present at birth.

Dendrites threadlike extensions of the cytoplasm of a neuron.

Dopamine a chemical produced by the brain, which functions as a neurotransmitter.

Free radicals electrically-charged reactive atoms or molecules in cells, which can damage other molecules within cells.

Globus pallidus part of the nucleus of the brain.

Implantation the attachment of the embryo to the lining of the uterus.

Lymphocytes vertebrate white blood cells.

Necrosis premature death of cells.

Mitochondria cell organelles responsible for energy production.

Mitochondrial transmembrane potential - difference in voltage across a mitochondrial membrane.

Mitochondrial energy energy generated by mitochondria in cells.

Ossification the process of creating bone.

Oxidative stress various pathologic changes seen in living organisms in response to excessive levels of free radicals in the environment.

Plasma the fluid portion of the blood.

RNA transcription the synthesis of RNA from a DNA template.

Serotonin a chemical produced by the brain, which functions as a neurotransmitter.

Steroidogenic acute regulatory (StAR) protein protein that regulates steroid hormone synthesis.

Seminal tubules tubes that carry sperm from the testes.

Substantia nigra movement centre in the brain.

2.1 Chronic effects

2.1.1 Reproductive

Birth defects in the Argentinean state of Chaco, where GM soy and rice crops are heavily sprayed with glyphosate, increased nearly fourfold over the years 2000 to 2009, according to a report released by the Chaco state government in April 2010 (Otaño et al. 2010). Paganelli et al. 2010 also reported 'several cases of malformations together with repeated spontaneous abortions were detected in the village of Ituzaingo' Cordoba, which is surrounded by GMO-based agriculture'. On its own, this information does not implicate glyphosate, for other pesticides are also used on the soy and rice fields. However, taken together with laboratory studies and other epidemiological information (patterns of illness in the human population), it raises concerns that can no longer be ignored.

'...Several cases of malformations together with repeated spontaneous abortions were detected in the village of Ituzaingo' Cordoba, which is surrounded by GMO-based agriculture'.

(Paganelli et al. 2010)

In Paraguay, 52 women who were exposed to glyphosate-based herbicides during pregnancy delivered offspring with congenital (i.e. present at birth) malformations. These birth defects showed striking similarities to those induced by glyphosate in laboratory experiments (Paganelli et al. 2010). However, they cannot yet be linked directly to glyphosate exposure.

The congenital malformations included microcephaly (small head), anencephaly, and cranial malformations. Anencephaly occurs when the neural tube fails to close during pregnancy, resulting in the absence of the majority of the brain, skull and scalp.

Herbicide tolerance and GM crops

Why the world should be
Ready to Round Up glyphosate

In 2009, Argentinean researchers lead by Professor Carrasco showed that even weak concentrations (down to 0.02%) of a commercial glyphosate formulation caused disruption to the development of the craniofacial skeleton of tadpole embryos (Fig 2). Other effects included shortening of the trunk, reduced head size and eye defects. The authors concluded that their results were 'compatible with the malformations observed in the offspring of women chronically exposed to glyphosate-based herbicides during pregnancy' (Paganelli et al. 2010). Although this study has recently been criticised by the agrochemical industry (Saltmires et al. 2011), the study nonetheless raises concern regarding the impacts of glyphosate on reproduction.

Previous studies also indicate the potential of glyphosate to disrupt reproduction. One study showed that applying glyphosate and Roundup at dilutions far below those used in agriculture severely affected human embryonic and placental cells, producing mitochondrial damage and two types of cell death, necrosis and programmed cell death, within 24 hours. Cell deaths occurred at concentrations corresponding to the level of residues in food expected from Roundup-treated GM crops (Benachour & Séralini 2009). The authors concluded that, if this occurred in the body, it would result in impacts on fertility, as well as carbohydrate metabolism, immune system function and water balance.

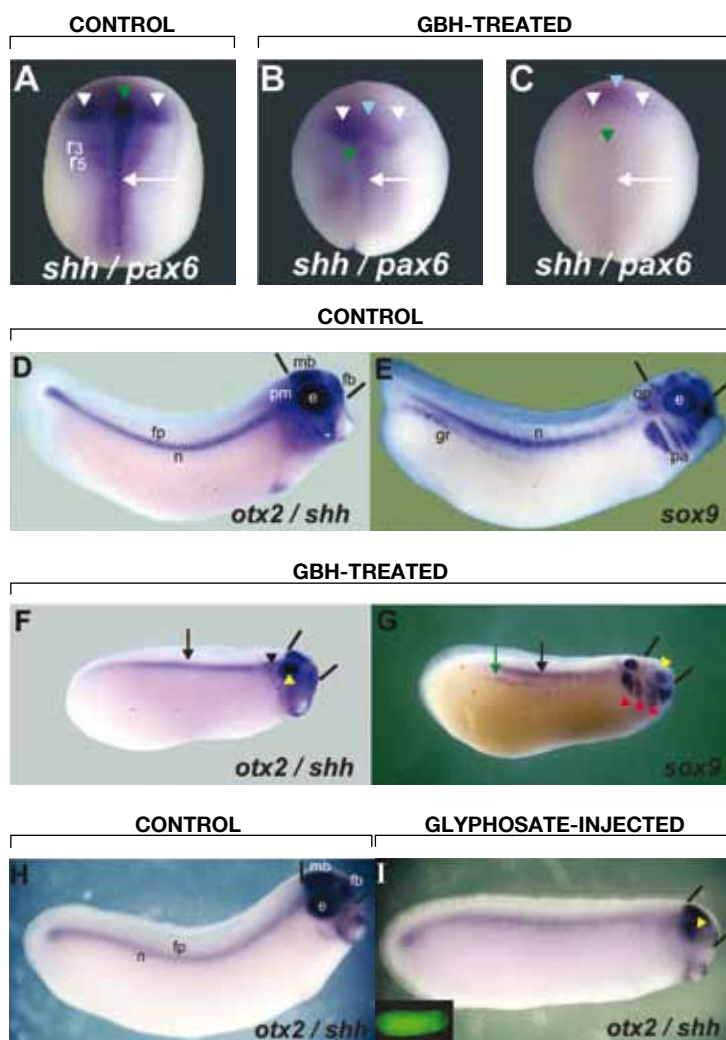


Fig 2: Glyphosate-based herbicides (GBH), and glyphosate itself, interfere with early development in both frog and chicken embryos (Paganelli et al. 2010). Clear differences in frog embryos are seen here, with malformations present in those exposed to a 1/5,000 (0.02%) dilution of GBH but note, the last panel are injected with glyphosate.

Reproduced with permission from American Chemical Society. For full details, see Paganelli et al. (2010)

'Applying glyphosate and Roundup at dilutions far below those used in agriculture severely affected human embryonic and placental cells, producing mitochondrial damage and two types of cell, death necrosis and programmed cell death, within 24 hours.'

(Benachour & Séralini 2009)

Other studies demonstrate glyphosate and/or Roundup's endocrine disrupting effects:

- **Roundup disrupted the production of the female reproductive hormone progesterone in mouse cells by disrupting expression of the steroidogenic acute regulatory (StAR) protein** (Walsh et al. 2000).
- **Glyphosate at dilutions 100 times lower than agricultural rates inhibited activity of the enzyme aromatase, which is responsible for synthesis of another female reproductive hormone oestrogen.** Roundup itself had an even greater effect. This effect occurred once the glyphosate and Roundup had entered the cells, but prior to entry Roundup had the opposite effect causing 40% increase in aromatase activity (Richard et al. 2005). The authors concluded this might explain premature births and miscarriages observed in female farmers using glyphosate (Savitz et al. 1997; Arbuckle et al. 2001).
- **Hokanson et al also demonstrated a synergistic effect of glyphosate with oestrogen, with implications for pregnancy-induced hypertension and foetal growth retardation.** (Hokanson et al. 2007)
- **In 2007, Benachour et al. demonstrated that low levels of glyphosate inhibit aromatase in human embryonic cells resulting in reduced oestrogen production, with adjuvants in Roundup increasing the effect.** (Benachour et al. 2007)

- **Roundup, but not glyphosate, inhibited the conversion of androgens to oestrogen.** However glyphosate was anti-androgenic at levels 40 times lower than residues permitted in soybeans (Gasnier et al. 2010).
- **Pre-pubertal exposure to the product Roundup Transorb delayed puberty, altered the structure of seminal tubules in the testes of male rats, and reduced testosterone production** (Romano et al. 2010).

The implications of these effects on reproduction and the developing foetus are profound, with work by Mose et al. 2008 confirming that **glyphosate does cross the placenta**.

Finally, high-dose experiments on rats resulted in decreased total implantations and viable foetuses, reduced litter size, reduced foetal weight and pup weight, and reduced ossification of the breastbone (US EPA 1993, 2006; IPCS 1994).

2.1.2 Cancer

The Chaco report (Otaño et al. 2010) mentions a significant increase in cancer and particularly child cancer including leukaemia, lymphoma and brain tumours. Once again, while these could be caused by a number of factors including other pesticides, there is support from epidemiology and laboratory studies to indicate that glyphosate might be contributing to these cancers.

A number of epidemiological studies have linked exposure to glyphosate to **non-Hodgkin's lymphoma** (Nordsstrom et al. 1998; Hardell & Eriksson 1999; Hardell et al. 2002; McDuffie et al. 2001; De Roos et al. 2003; Eriksson et al. 2008;) and **multiple myeloma** (De Roos et al. 2005). Three studies of people exposed to the aerial spraying of illegal crops in Columbia have found **DNA damage** amongst those who had experienced acute effects from the spray (Mueckay & Malondao 2003; Paz-y-Mino et al. 2007; Bolognesi et al. 1997).

A number of laboratory studies have shown glyphosate, Roundup and/or the metabolite of glyphosate, AMPA, to be **genotoxic or mutagenic** in human cells, including liver (Mañas et al. 2009a; Gasnier et al. 2010; Mañas et al. 2009b), and lymphocytes (Lioi et al. 1998a; Bolognesi et al. 1997; Vigfusson & Vyse 1980). Numerous other studies have demonstrated genotoxicity or mutagenicity in mouse, bovine, fish, caiman, tadpole, fruit fly, sea urchin, onion and bacterial cells (Rank et al. 1993; Kale et al. 1995; Bolognesi et al. 1997; Clements et al. 1997; Peluso et al. 1998; Lioi et al. 1998b; Kaya et al. 2000; Grisolia 2002; Siviková & Dianovský 2006; Bellé et al. 2007; Cavaş & Könen 2007; Cavalcante et al. 2008; Guilherme et al. 2009; Mañas et al. 2009b; Mañas et al. 2009a; Poletta et al. 2009).

Other ways in which glyphosate may be contributing to cancer include:

- **its ability to deregulate cell division**, a hallmark of tumour cells, demonstrated to occur in sea urchin embryos at concentrations up to 4,000 times lower than normal sprayed concentrations (Marc et al. 2002, 2003, 2004);
- **its inhibition of RNA transcription**, demonstrated in sea urchin embryos at concentrations 25 times lower than normal sprayed concentrations (Marc et al. 2005); and
- **its ability to cause oxidative stress**, demonstrated for glyphosate and/or Roundup in human lymphocytes (Lioi et al. 1998a; Pieniazek et al. 2004) and skin cells (Gehin et al. 2005; 2006), as well as in bovine lymphocytes (Lioi et al. 1998b), bullfrog tadpoles (Costa et al. 2008), pregnant rats and their foetuses (Beuret et al. 2005), rat liver cells (El-Shenawy 2009), mouse kidney cells and liver DNA (Bolognesi et al. 1997), and in rice leaves (Ahsan et al. 2008).

2.1.3 Neurological

Glyphosate may affect the nervous system and may even be implicated in neurodegenerative diseases such as **Parkinson's disease**. Both Roundup and glyphosate were found to inhibit growth of 'neurite-like structures' (axons or dendrites), at concentrations lower than those measured in plasma and tissue of farmers exposed to Roundup (Axelrad et al. 2003). Two other studies on rats have demonstrated that glyphosate depletes **serotonin and dopamine** (Anadón et al. 2008); and caused a loss of mitochondrial transmembrane potential in rat brain cells, especially in the *substantia nigra* region of the brain (Astiz et al. 2009). The brain is very dependent on mitochondrial energy to maintain normal physiology, and **loss of mitochondrial function** is associated with many human neurodegenerative disorders. Damage in the *substantia nigra* is implicated in Parkinson's disease. Additionally, the central nervous system - and particularly the *substantia nigra* - is highly sensitive to free radical damage, which results from **oxidative stress**. A number of studies reported earlier show that glyphosate and Roundup cause oxidative stress in various different cells, including brain cells.

These laboratory findings are reflected in an epidemiological study and one reported clinical case. In a study of children born to pesticide applicators in Minnesota in the US, 43% of the children reported to have ADD/ADHD (Attention Deficit Hyperactivity Disorder) had parents who were exposed to glyphosate-containing herbicides (Garry et al. 2002). A 54-year-old man developed skin lesions six hours after he accidentally sprayed himself with a glyphosate herbicide, and one month later developed a 'symmetrical Parkinsonian syndrome'. Two years later, magnetic resonance imaging revealed effects in the *globus pallidus* and *substantia nigra* regions of the brain associated with Parkinson's disease (Barbosa et al. 2001).

2.2 Acute effects

A number of deaths have resulted from intentional ingestion (suicide), preceded by metabolic acidosis, respiratory and kidney failure, cardiac arrest, seizures, and coma (IPCS 1994; Chang & Chang 2009).

The most commonly reported acute effects from occupational and bystander exposures to glyphosate-based herbicides are those of the skin, eyes, respiratory, gastrointestinal and cardiac systems. They include:

- irritation, swelling, tingling or burning of the skin, dermatitis, photo-contact dermatitis;
- conjunctivitis, painful eyes, corneal injury, burning eyes, blurred vision, double vision, swelling of the eye and lid;
- oral and nasal discomfort, unpleasant taste, tingling and irritation of throat, sore throat, swollen tongue;
- burning in chest, cough;
- nausea, vomiting, headache, fever, diarrhoea, shakes and chills, tiredness, lethargy; and
- rapid heartbeat, raised blood pressure, dizziness, light-headedness, tingling in hands and feet; aching arms (IPCS 1994; Goldstein et al. 2002; Bradberry et al. 2004).

One recent epidemiology study in the US reported that exposure to glyphosate herbicides was associated with both asthma and rhinitis ('runny nose') (Slager et al. 2009).

Significant exposure to glyphosate herbicides has occurred in Ecuador and Columbia as a result of the aerial spraying campaign to eradicate coca in Columbia and along its border with Ecuador since 1997. Symptoms reported there include many of those reported above and, in addition, red eyes, skin rashes and blisters, skin infections, abdominal pain, gastrointestinal infections, respiratory infections, difficulty in breathing, numbness, insomnia, depression, debilitation, weeping eyes (Gallardo 2001; Oldham & Massey 2002; Paz-y-Miño et al. 2007).

2.3 Summary

Chronic effects related to glyphosate and its derivative products can be classified in the following categories: **reproductive** (birth defects), **cancer**, **neurological** (even implicated in causing Parkinson's disease), and **acute effects** linked with the direct use of the product by farmers or exposure of bystanders.

There is concern that birth defects experienced by women in Argentina and Paraguay may result from their exposure to glyphosate used on GM soya and rice crops. Other studies have demonstrated glyphosate's potential to disrupt reproduction by its ability to cause mitochondria damage, necrosis and cell death in human embryonic and placental cells; and to cause endocrine disruption, including disruption of progesterone and oestrogen production, and delayed male puberty.

Epidemiological studies have linked exposure to glyphosate with non-Hodgkin's lymphoma and multiple myeloma, as well as DNA damage among people who had experienced acute symptoms from glyphosate exposure. These findings are supported by laboratory studies that demonstrate that glyphosate can cause genotoxicity, mutagenicity, oxidative stress and dysregulation of cell division. Potential chronic neurological effects include Parkinson's disease and ADD/ADHD, while acute exposure symptoms include a wide range of effects on skin, eyes, respiratory, gastrointestinal and cardiac systems.

These effects must be taken very seriously and an urgent reassessment of the health impacts of glyphosate and its related products must now take place.

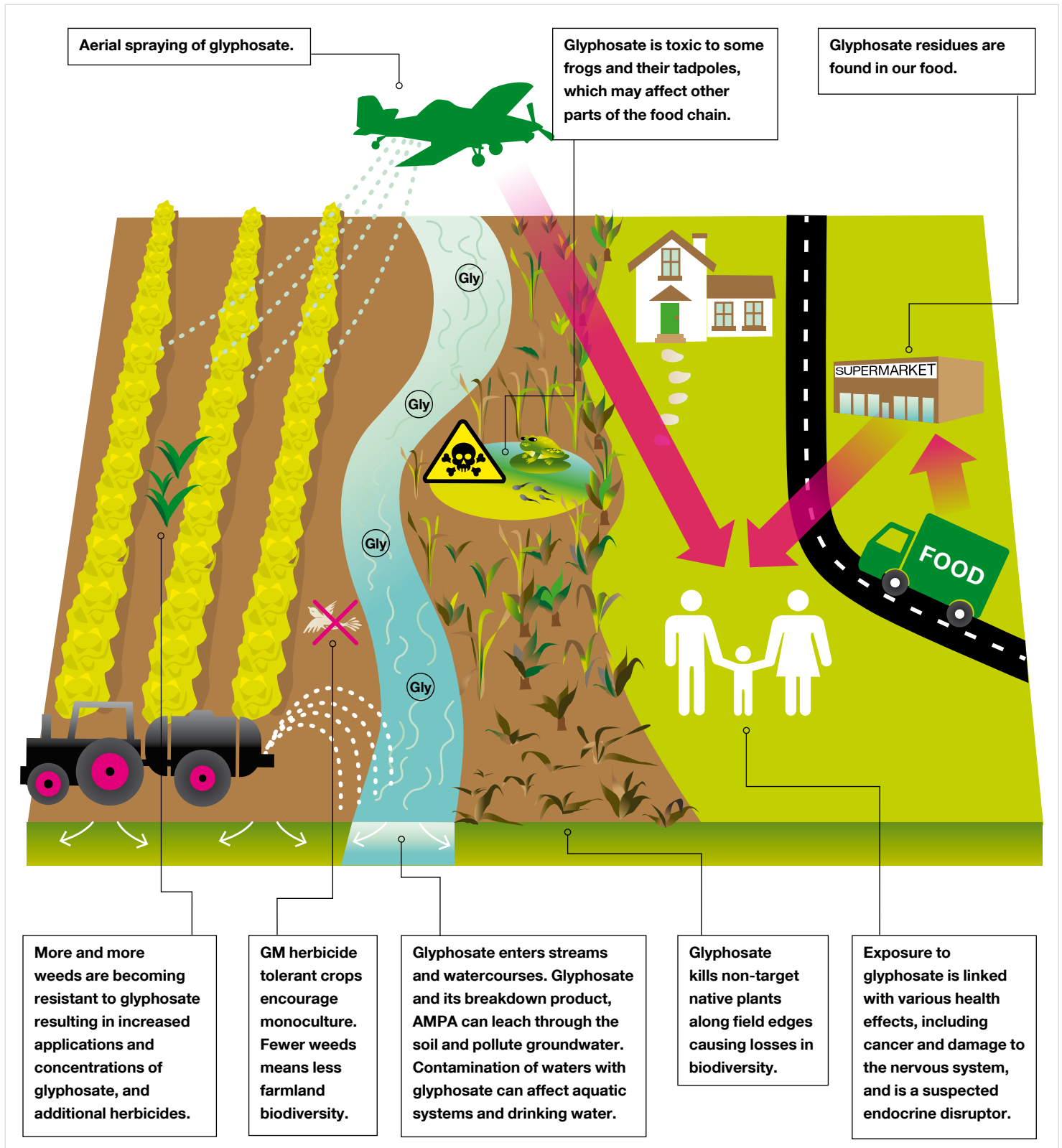


Fig 3: Environmental and human health effects of glyphosate

*An urgent
assessment of the
health impacts of
glyphosate and its
related products
must now take
place.*



3] Glyphosate residues in food

The use of Roundup on food and feed crops means that residues of glyphosate and other chemicals used in the various formulations will be found in our food (Fig. 3). However, data on the presence of glyphosate and its breakdown product aminoglyphosate acid (AMPA) in food, feed and animal products from glyphosate sprayed crops are sparse.

Maximum Residue Levels (MRLs) are the maximum permitted concentration of pesticide residue in a food or animal feed. MRLs are primarily trading standards, but they are also intended to ensure that pesticide residues do not pose a risk for consumers. The current MRLs for residues of glyphosate in food were agreed by the United Nations Food and Agriculture Organisation's Codex Alimentarius (or food code) in 2006 and are listed in Table 1. The MRLs are for the combined levels of both the herbicide and its main breakdown product, AMPA.

Glyphosate is frequently used to desiccate cereal and oilseed rape crops immediately prior to harvesting. This results in residues in crops and processed products. The MRLs in Table 1 are generally higher for crops where glyphosate is applied directly than when it is used for weed control prior to sowing. That is, MRLs are higher for crops where glyphosate is used as a desiccant to dry grain prior to harvest (e.g. wheat and barley) or for crops where GM RR crops are commercially grown (e.g. soya, maize, cotton, rapeseed), than those for crops (e.g. pea and bean) where glyphosate is not sprayed on the crop itself, but may be used for clearing a field before planting. Thus, MRLs appear to be based upon the levels likely to be found in a specific product as a result of expected usage of glyphosate, rather than on safety concerns.

Table 1: Maximum Residue Levels (MRLs) for glyphosate in foods⁴

Commodity	MRL mg/kg
Animal products	
Poultry meat	0.05
Meat (from mammals other than marine mammals)	0.05
Edible offal of poultry	0.5
Edible offal of pigs	0.5
Edible offal mammalian (except pigs)	5.0
Eggs	0.05
Crops	
Banana	0.05
Beans (dry)	2.0
Sugar cane	2.0
Peas (dry)	5.0
Maize	5.0
Sunflower seed	7.0
Sugar cane molasses	10
Soya bean (dry)	20
Wheat bran unprocessed	20
Rape seed	20
Cereal grains	30
Cotton seed	40
Sorghum straw and fodder. Dry	50
Oat straw and fodder. Dry	100
Maize fodder. Dry	150
Bean fodder. Dry	200
Wheat straw and fodder Dry	300
Barley straw and fodder. Dry	400
Hay or fodder (dry) of grasses	500
Alfalfa fodder	500
Pea hay or pea fodder (dry)	500

⁴ Source: UN FAO Codex Alimentarius. <http://www.codexalimentarius.net/pestres/data/pesticides/details.html?sessionid=7BA965F7D5BAA909CE2C7C2A6E3FAF97?d-16497-o=2&id=158&d-16497-s=3>

Sampling of foodstuffs often detects glyphosate and/or AMPA.

- In Denmark, sampling of cereals in successive years in the late 1990s found glyphosate and/or its degradation product AMPA in more than half of the cereal samples. The average concentration of glyphosate in 46 samples from the 1999 harvest was 0.11 mg/kg compared with 0.08 mg/kg in 49 samples for the 1998 harvest (Granby & Vahl 2001).
- In the UK, sampling of food for glyphosate residues has largely concentrated on cereals, including bread and flour. Glyphosate has been regularly detected, and usually below the current MRL (Pesticides Residues Committee 2010, 2009, 2008 and 2007a). In 2006, the UK's Pesticide Residues Committee monitoring found a sample of wheat flour containing 0.8 mg/kg above the Codex MRL of 0.5 mg/kg (Pesticide Residues Committee 2007b).
- Residues of glyphosate in tofu and soya pieces were reported in the UK in 2006. Six out of eight samples of tofu/soya pieces originating from Brazil contained glyphosate with the highest level recorded being 1.1 mg/kg (Pesticide Residues Committee 2007a).
- In an EU survey of pesticide residue frequency, glyphosate was found in 9.54% of samples in 2007 (EFSA 2009).

WHO/FAO 2005 reported on feeding trials in pigs that were given feed containing 40, 120 and 400 mg/kg of glyphosate and AMPA. At the highest level (400 mg/kg), glyphosate residues in the liver were 0.72 (1.4 including AMPA) mg/kg and in kidneys, 9.1 (11) mg/kg. These residues are comparable with the MRL for edible offal from pigs of 0.5 mg/kg (Table 1). Despite this, no animal products have been sampled in the EU in recent years (EFSA 2009) nor in the US (USFDA 2008), meaning that exposure of consumers has not been monitored in recent times.

The levels found in cereals and animal products are below the current MRLs but indicate that consumers of cereal-based products are regularly exposed to glyphosate and AMPA residues. Importantly, the MRLs seem more dependent on the levels likely to be found in a specific product rather than on whether a specific residue level is safe or not.



Fig 4: Food label displaying RR soya ingredients

Summary

Despite the extensive use of products that contain glyphosate, there are limited data on residues in food and feed, including animal products such as offal, consumed by people and animals. However, there are data showing that glyphosate and AMPA are found in food destined for people at levels below the current MRLs.

The MRLs do not appear to be based on whether a specific residue level is safe or not but more on the levels likely to be found in a specific product as a result of agricultural practice, e.g. the use of glyphosate as a dessicant. As recent scientific studies (see Chapter 2) question the human safety of glyphosate-based products, the basis upon which these MRLs are made is called into question.

Along with a rigorous review of the environmental and the health impacts of glyphosate, a revision of the existing MRLs is also needed. In light of the new scientific evidence on glyphosate impacts it is essential to re-evaluate MRLs in order to ensure that they remain in line with updated safety assessments.



In light of new scientific evidence on glyphosate impacts, it is essential to re-evaluate maximum residue levels in order to ensure that they remain in line with updated safety assessments.



*“...Glyphosate,
when applied in late
autumn, can leach
through the root
zone at unacceptable
concentrations in
loamy soils”*

(Kjær et al. 2003)

4] Glyphosate in water

“From soil and plant applications of glyphosate herbicide, it is expected that a small amount of the applied glyphosate may enter surface waters through runoff or attached to soil particles that wash off treated field”
(Monsanto 2003)

Glyphosate is highly soluble in water and therefore has the capacity to be mobile in aquatic systems. In fact, glyphosate is far more soluble (in the range 10 000-15 700 mg/l at 25°C) than other herbicides, such as atrazine (in the range 20-35 mg/l) and isoproturon (in the range 70-72 mg/l), which are already known to leach from the soil to contaminate surface waters. However, as will be discussed further in Chapter 6, it is glyphosate's capacity to bind tightly to soil particles that prevents it from being highly mobile. Binding can immobilise it in the soil provided that there are sufficient suitable sites. This varies depending on the soil type and composition. Studies have found that binding of glyphosate is greater in soils with lower pH (i.e. more acidic) (Gimsing et al. 2004) and that phosphates (Simonsen et al. 2008) can compete for binding sites. All of this adds to the complexity of glyphosate's movements in the soil, and predictions of its leachability.

A report by the World Health Organisation (WHO 2005) confirmed that glyphosate is found in surface waters at levels between 0.5 µg/l and 1 µg/l and its environmental breakdown product, AMPA, was present at levels around 6 µg/l⁵. The levels of glyphosate exceed the maximum allowed for pesticides in drinking water under EU law (see below) and would require water companies to undertake expensive filtration before the water could be supplied to the public.

Glyphosate has often been detected during monitoring of surface waters and groundwater. A comprehensive study of streams in the midwest US examined the presence of herbicides, including glyphosate and AMPA, at different stages in the crop-growing cycle (Battaglin et al., 2005). Glyphosate was detected during every season up to a maximum concentration of 8.7 µg/l. This is over 80 times the EU maximum permitted concentration of 0.1 µg/l in drinking water (European Union Council 1998) but substantially below the US drinking water maximum concentration of 700 µg/l (US EPA 2009). Such a massive difference in permitted concentrations is hard to justify, especially given the growing body of evidence on the harm glyphosate can cause to health and aquatic life. AMPA was also detected above 0.1 µg/l in more than half of the samples taken through the year, most frequently after crops had emerged from the soil. The maximum concentration of AMPA recorded in this study was 3.67 µg/l.

Further evidence that glyphosate can enter surface waters comes from monitoring in Alberta, Canada, where it was found in 8 out of 13 sites and in 22% of samples taken in wetland and streams, with a peak concentration of 6.07 µg/l (Humphries et al. 2005). In Denmark, a major government-sponsored study on the leaching of pesticides was undertaken between 1999 and 2009. The conclusions of an interim report were that 'glyphosate, when applied in late autumn, can leach through the root zone at unacceptable concentrations in loamy soils' (Kjær et al. 2003). Glyphosate was detected to the depth of the drainage system and not in groundwater (Kjær et al. 2003, 2005). The final report (Rosenbom et al. 2010) declared that glyphosate and AMPA exhibited 'pronounced leaching'. A peer-reviewed paper based on the study stated 'both glyphosate and AMPA can leach through structured soils, they thereby pose a potential risk to the aquatic environment'.

The Danish study monitored pesticides leaching from different soil types, crops/agronomy and climates. Loamy soils (with roughly equal amounts of sand, silt and clay) were found to be more prone to leaching of glyphosate and AMPA than coarse sandy soils, where matrixes of aluminium and iron provide the right conditions for sorption and degradation. On loamy soils, autumn application resulted in detectable concentrations of glyphosate and AMPA in the drainage water in the upper metre of soil, often at concentrations exceeding the EU's maximum concentration for drinking water. The maximum concentrations of glyphosate recorded in drainage water at the two most vulnerable sites were found in 2009 (31 µg/l and 4.7 µg/l respectively). Average concentrations of glyphosate in drainage water following the first drainage after application were well above 0.1 µg/l for some crops, for instance maize in 2005 (4.04 µg/l) and peas in 2001 (0.54 µg/l), both following the application RoundUp. Detection of glyphosate and AMPA was mainly confined to drainage water although it was detected at three sites below the drainage system. At one site in the wet August of 2008, glyphosate was frequently detected in groundwater, with a maximum concentration of 0.67 µg/l.

The Danish results show that, in certain soil types with low sorption capacity, glyphosate can easily find its way into surface waters at concentrations that well exceed the EU drinking water maximum of 0.1 µg/l. In more exceptional circumstances, i.e. after heavy rain, it can also find its way into groundwater.

Standards for protecting aquatic life from glyphosate have not been widely set. None are agreed in the US or the EU, for example. In Canada, an interim standard of 0.65 µg/l was agreed upon as long ago as 1989 (Canadian Council for Environment Ministers, 1999) and work is presently in progress to establish a new one. US researchers investigating small water bodies in areas where glyphosate-based herbicides were used found levels up to 328 µg/l, well above the level set in Canada to protect freshwater aquatic life (Battaglin et al. 2008).

The amount of glyphosate entering watercourses is dependent on the weather immediately following its application. Heavy rain on low sorption soils is most likely to result in glyphosate washing into drainage systems.

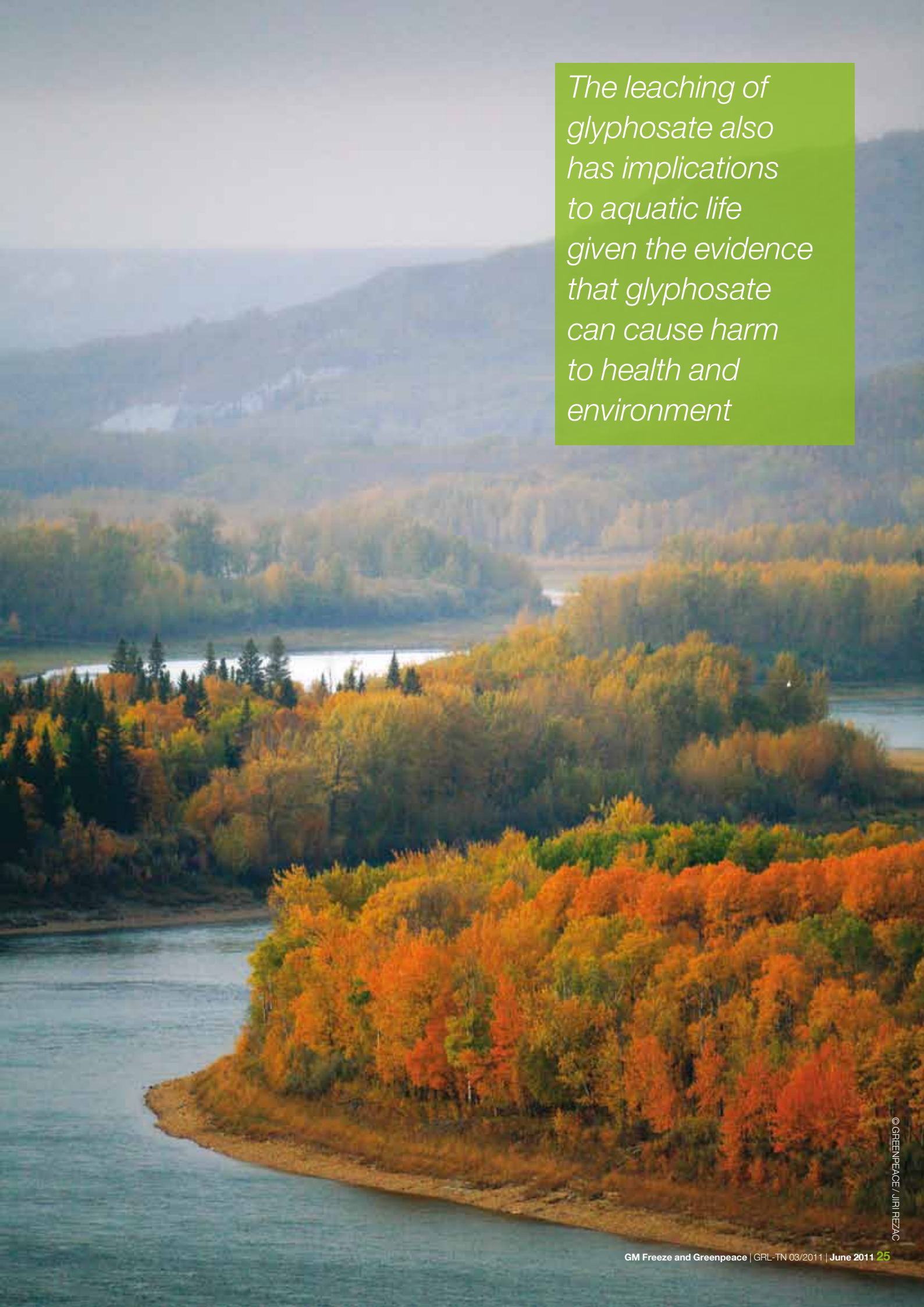
Glyphosate can enter surface waters from land-based spraying either by becoming attached to soil particles, by leaching or by spray drift at concentrations that, in the EU, would have to be removed before waters entered the public supply. For example, small catchment studies in Sweden (Keuger 2005), France (Delmas 2004) and Greece (Papadopoulou-Mourkidou 2004) have confirmed that glyphosate can leach into drainage systems and surface waters. Losses amount to a small percentage of the glyphosate applied in the catchment but can exceed levels permitted in drinking water.

The use of glyphosate on paved surfaces in urban settings can also result in glyphosate quickly entering drainage water - and hence, surface waters - immediately after rainfall. A study in France showed that glyphosate can enter watercourses more readily from urban areas via the sewerage system than in rural environments due to applications on roads and railways. High levels were linked to rainfall events (Botta et al. 2009). Glyphosate is banned from use on hard surfaces in Denmark and by half of Swedish municipalities (Kristoffersen 2008).

Conclusion

Glyphosate is mobile in the root zone in soils with weak sorption capacity. This results in the presence of glyphosate and its degradation product, AMPA, in drainage water and surface waters. Groundwater has been polluted in similar soils after spraying followed by heavy rainfall. Run-off from the weed treatment of paved areas can also contribute to levels of glyphosate in watercourses.

These finding have implications for surface water quality and drinking water quality. The leaching of glyphosate also has implications to aquatic life given the evidence that glyphosate can cause harm to health and the environment (see Chapters 2 and 5).



*The leaching of
glyphosate also
has implications
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to health and
environment*

5] Glyphosate impacts on biodiversity

Although promoted as a benign herbicide, accumulated scientific evidence detailed in this chapter shows that glyphosate, and its formulated commercial products such as Roundup, can affect biodiversity.

Glyphosate can impact on plants and animals via:

- direct toxic effects of exposure to the spray;
- chronic effects caused by long-term exposure in the ecosystem; and
- indirect effects due to changes in the ecosystem.

5.1 Direct toxic effects

Since Roundup was first introduced in the 1970s, Monsanto has consistently claimed that glyphosate and Roundup are not likely to harm animals. It argues that, because glyphosate destroys an enzyme in plants that is not present in animals, it will not affect them.

For example, Monsanto says 'glyphosate-containing products labelled for forestry use have shown no adverse effect on aquatic animals' (Monsanto 2010a) and that these products present 'extremely low toxicity to mammals, birds and fish' (Monsanto 2010b).

However, there is now a significant body of evidence from the peer-reviewed scientific literature showing that these claims can no longer be supported where Roundup formulations are applied. The toxicity of glyphosate is strongly increased by the adjuvants and surfactants that it is mixed with in order for it to adhere to foliage and penetrate into plant cells, allowing it to then be transported (or translocated) to all parts of the plant. Approvals of products are based on separate assessments of glyphosate and the adjuvants and surfactants⁶ but not the combined commercial product. At least 12 different adjuvants have been used in glyphosate-based formulations (Cox 2004). In most cases, the mixtures and ratios are commercially confidential.

5.1.1 Toxicity to amphibians

Declines in the numbers and the diversity of amphibian species across the world have been widely reported since the 1980s. It is estimated that one in three of species globally is threatened with extinction (Williams 2004). Causes such as habitat loss, habitat fragmentation, disease and environmental contamination have been put forward as contributing to this decline.

In the past, testing pesticides on amphibians, as part of the approval process, was rare. When it did occur, it was only over short time periods (Reylea 2005a). However, the global decline of amphibian numbers led researchers to focus on agro-chemicals as a potential cause of their decline. Glyphosate and Roundup formulations were selected for independent toxicity studies because of their widespread use.

The conclusions from several projects suggest that, under close-to-field-conditions, glyphosate-based products, including Roundup, have a direct toxic effect on the adults and tadpoles of a range of amphibian species:

- Roundup was found to have the potential to cause substantial mortality in many amphibian species in a controlled study of aquatic communities that included algae and tadpoles from five North American species of toads and frogs (Reylea 2005b).
- Three species of North American frog and toad tadpoles exposed to Roundup in artificial ponds exhibited very high mortality (96-100%) over three weeks, which the author suggests could lead to population decline in the wild (Reylea 2005c).
- Western chorus tadpoles exposed to the glyphosate product Roundup WeatherMax at 572 µg/l glyphosate acid equivalents (a.e.) resulted in 80% mortality, which the authors suggested resulted from a unique surfactant formulation. Exposure to WeatherMax or Roundup Original Max at 572 µg/l a.e. also lengthened the larval period for American toads (Williams & Semlitsch 2010).
- Frog tadpoles (*Rhinella arenarum*) exposed to concentrations used in commercial formulations showed decreases in the activities of AChE (acetylcholinesterase) (Lajmanovich et al. 2010).

The findings of these studies suggest that glyphosate-based products harm amphibians at concentrations which occur as a result of their normal use in agriculture or forestry. This group of animals includes many species that are predators of pests in and around agro-ecosystems and forest ecosystems. Losses of the order reported above in wild populations could have significant impacts upon pest populations and a long-term impact on crop yield and quality.

⁶ Adjuvants have been categorised as extenders, wetting agents, sticking agents and fogging agents designed to enhance the activity or other properties of a pesticide mixture. Surfactants are formulants for reducing surface tension, thereby increasing the emulsifying, spreading, dispersibility or wetting properties of liquids or solids.

5.1.2 Aquatic toxicity

In the last few years, independent research on the toxicity of glyphosate and its formations has found that they are biologically active in aquatic systems at concentrations that could arise from routine applications. Many different aquatic organisms have been affected.

- Rotifer (*Brachionus calyciflorus*) (microscopic aquatic animals) exposed to different concentrations of glyphosate had longer embryonic developmental time, longer durations of juvenile and reproductive periods, shorter average lifespan, a reduced net reproductive rate and reductions in the intrinsic population growth rates (Vera et al. 2010).
- The parasitic horsehair worm (*Chordodes nobilii*) showed a number of responses, including reduced infective capacity of larvae and 50% mortality in adults, when exposed to glyphosate concentrations lower than expected in freshwater environments, and lower than specified in the relevant legislation, (Achiorno et al. 2008).
- Phytoplankton and periphyton⁷ communities showed changes in the microbial population structures consistent with a direct toxicological effect of glyphosate (Pérez et al. 2007).
- A study of the combined effects of glyphosate with the trematode parasite (*Telogaster opisthorchis*) and the fish parasite (*Galaxias anomalous*) found that the glyphosate and the parasite acted synergistically on aquatic vertebrates at environmentally-relevant concentrations. Researchers suggested that glyphosate might increase the risk of disease in fish (Kelly et al. 2010).
- Disturbance in the marine microbial communities was caused by exposure to 1 µg/l Roundup concentration, a value typical of those reported in coastal waters during run-off events (Stachowski-Haberkorn et al. 2008).
- A freshwater mussel (*Lampsilis siliquoidea*) was found to be acutely sensitive to Roundup and its separate components. Researchers tested the specific active ingredient (technical-grade isopropylamine (IPA) salt of glyphosate), the surfactant (MON 0818) and the commercial product itself. MON 0818 was found to be the most toxic, but juvenile *Lampsilis siliquoidea* were found to be acutely sensitive to all three (Bringoff et al. 2007).
- Carp (*Cyprinus carpio*) exhibited changes to the internal appearance of liver cells and changes to mitochondria at Roundup concentrations between 2 and 40 times lower than used in practice (Szarek et al. 2000).

It is clear that glyphosate can be toxic to many aquatic organisms if it enters watercourses (see Chapter 4).

5.2 Does glyphosate affect the nervous system?

Acetylcholinesterase (ACh) is an enzyme that breaks down acetylcholine, which transmits nervous impulses between nerves. Organophosphate and carbamate insecticides inhibit ACh formation, resulting in nervous impulses being maintained. Eventually, insects die - as do mammals and birds if they are exposed to high enough levels of these pesticides. Sub-acute levels are known to cause changes in birds and mammals in their temperature regulation, food consumption and reproduction (Grue et al. 1997).

Monsanto (1982) and some studies (e.g. Rom & Markowitz 2007) found that glyphosate has no ACh-inhibiting activity, despite the presence of phosphate in its molecule, meaning it is not classed as an organophosphate chemical. However, other studies report that glyphosate does suppress ACh activity in the brain, but not in muscles, of species examined (Lajmaonovich et al. 2010; Gluszcak et al. 2006; Gluszcak et al. 2007). The implications of these changes arising from exposure to glyphosate have yet to be fully investigated and this needs to be carried out within a review of glyphosate herbicides.

5.3 Impacts on non-target plants

As expected from its broad-spectrum use, glyphosate also impacts non-target wild plants in field margins or in water bodies. However, there are considerable cascading effects on farmland biodiversity. Glyphosate, in its formulated products such as Roundup, is a highly effective herbicide on all types of vegetation until weed resistance develops (see Chapter 7), and therefore has the capacity to cause major changes to the agro-ecosystem. These impacts include the loss of botanical diversity in the agro-ecosystem, including the loss of rare species growing in arable fields. In Iowa, US, glyphosate is classed as 'high risk' for off-target plants growing in soya and maize fields (i.e. those of botanical interest but not major weeds in the crops) (Iowa University State Extension 2003). Accidental drift from glyphosate in approved use has also been found to impact on rare plants in Australia (Matarczyk et al. 2002). Monsanto runs an 'endangered species initiative'⁸ in the US, which specifies particular areas of land with sensitive species present. However, this does not automatically preclude the use of glyphosate sprayed from the air in the area outside the buffer zone surrounding the site.

⁷ Periphyton is the complex of algae and micro-organisms attached to underwater surfaces.

⁸ <http://www.monsanto.com/ourcommitments/Pages/glyphosate-endangered-species-initiative.aspx>

Impacts of GM herbicide-tolerant crops on weed abundance and the biodiversity food chain were studied during the Farm Scale Evaluations (FSE) in the UK between 2000 and 2003 (Heard et al. 2003a; Heard et al. 2003b; Roy et al. 2003). The only GM RR crop trialled was beet. The other GM herbicide-tolerant crops (oilseed rape and maize) were tolerant to a different herbicide, glufosinate ammonium. Equivalent data are not available for any GM RR crops elsewhere in the world.

The conclusions were clear:

'Based on the evidence provided by the FSE results published in October 2003, if [GM herbicide-tolerant RR] beet were to be grown and managed as in the FSEs this would result in adverse effects on arable weed populations, as defined and assessed by criteria specified in Directive 2001/18/EC, compared with conventionally managed beet. The effects on arable weeds would be likely to result in adverse effects on organisms at higher trophic levels (e.g. farmland birds), compared with conventionally managed beet'
(ACRE, 2004)



Fig 5: Use of glyphosate-based herbicides on RR crops affects biodiversity. The use of glyphosate on RR beet reduces the numbers of weeds which form the base of the food chain needed to support farmland birds, such as the skylark (ACRE 2004).

The FSE research showed how weed seed production (seed rain) was reduced by the use of glyphosate on GM RR beet and warned that 'relatively small differences could eventually sum to produce a large effect if they were sustained over several crop rotations, say for 10 or more years' (Heard et al. 2003a).

Data on GM RR maize and oilseed rape were not included in the FSE reports, as only glufosinate ammonium-tolerant GM varieties of these crops were trialled. In the trials, glufosinate ammonium-tolerant GM oil seed rape demonstrated similar impacts on biodiversity to GM RR beet but the herbicide-tolerant GM maize apparently showed less adverse effects on weed abundances than the non-GM variety, but the trial was invalidated by the use of a herbicide, atrazine, that was subsequently banned. Retrospective analysis suggested that the removal of the atrazine from the analysis very much reduced any effects on weed abundance (Perry et al. 2004).

5.4 Summary

There is a growing body of scientific evidence that glyphosate is harmful to species at many stages along the food chain, including the aquatic food chain. Scientific evidence shows that glyphosate (and its formulated commercial products such as Roundup) can have immediate and long-term, direct and indirect toxic effects on plants and animals, as well as indirect effects linked to the changes it causes in the ecosystem.

This new evidence of glyphosate toxicity, together with the increase in glyphosate usage associated with GM RR crops (see Chapter 1) is now of great concern. It is time that regulators examined the new evidence of harm in aquatic ecosystems that is now emerging from independent research on toxicity and mobility in soil and aquatic systems.

6] Glyphosate impacts on the soil-plant system

Glyphosate enters the soil by being directly sprayed on it, via the roots of plants that have been sprayed, or from dead vegetation. Glyphosate is soluble in water and can be washed into the soil by rainfall or irrigation. In some soils, it can bind tightly to soil particles. This means it cannot be washed deeper into soil and is less likely to be degraded by soil microbes. In other soil types, it remains mobile in soil water and can be leached into drains and decomposed. Tightly-bound glyphosate can be displaced by other chemicals, such as phosphate, meaning it can become present in soil water again.

The interactions between the chemical, physical and biological components of the soil and glyphosate are complex (Kremer & Means 2009; Zablowicz & Reddy 2004). Given this complexity, the requirements for the risk assessment of glyphosate by regulators around the world are surprisingly limited. For instance, in the EU, applicants are only required to provide data on the persistence of glyphosate in the soil and the impact on earthworms and other functional groups of soil organisms. Detailed examination of the impact of glyphosate on the make-up and activity of soil microbial species including pathogens is not required (EU Commission, Directorate General for Health – DG SANCO 2002).

Glyphosate binds so tightly to soil particles that it is rendered inactive and hence unavailable to organisms. It is therefore claimed that glyphosate has limited biological availability in the soil (Monsanto 2005b; Geisy et al. 2000). However, several important interactions between glyphosate and soil microbes have been identified that impact on the function of plants (Kremer & Means 2009). These interactions are detailed in Fig. 6.

When glyphosate is available in the soil, it affects microbial communities leading to:

- Reduction in mineral uptake by crops;
- Increased microbial biomass and activity;
- Proliferation of phytopathogens in crops;
- Reduction in nitrogen fixation and nodulation, leading to increased demands for nitrogen fertiliser.

6.1 Availability of glyphosate in the soil

The impact that glyphosate will have on the soil ecosystem is largely dependent on whether it is bound to soil particles or unbound and free. Glyphosate molecules bind with particles present in the soil that have a high binding capacity - such as aluminium hydroxide and ferric oxides, minerals or organic matter - and subsequently become inert (Shushkova et al. 2009). The extent to which this happens varies from soil to soil, depending on its composition and on the presence of other chemicals and mineral nutrients. When glyphosate is not bound (or only loosely bound) to soil particles, it is available for microbes to break down and utilise as a source of energy and nutrients. It is when this happens that glyphosate starts to impact on the environment.

Soil chemistry can also play an important part in how much or how little glyphosate binds to the soil. Phosphate competes with glyphosate molecules for soil-binding sites. In experiments, soils to which a phosphate solution has been applied are found to have raised levels of glyphosate and AMPA in solution, thus making it mobile in the soil (Simonsen et al. 2008) and available for microbes to break down or leach through the soil.

The rhizosphere is the thin layer of soil immediately surrounding plant roots, an area that is extremely important for the uptake of nutrients into the plant. It is markedly different from the bulk soil (Chin-hua & Palada 2006; see Fig 6). Glyphosate appears to interfere with the biological and chemical processes in this important rhizosphere, unintentionally affecting plant growth and nutrition.

6.2 Activity and abundance of soil microbes

Molecules of glyphosate and its breakdown product AMPA, which are not bound to soil particles, are available for soil and rhizosphere microbes (also called micro-organisms) to utilise as a source of nutrients and energy, thus leading to increased microbial biomass and activity (Haney et al. 2000; Wardle & Parkinson 1990). Glyphosate can therefore affect the structure of the microbial community, increasing the abundance of some microbes and decreasing others.

The existence of the EPSPS enzyme in microbes and fungi means they can be affected by the presence of free glyphosate in the soil, and changes in microbial populations occur in the rhizosphere of GM RR crops depending on how susceptible they are to glyphosate. Some groups increase, such as manganese-oxidising bacteria, and some decrease, such as pseudomonads that act against fungal pathogens. Thus, important roles for microbes such as growth promotion and biological control can be disrupted (Kuklinsky-Sobral et al. 2005).

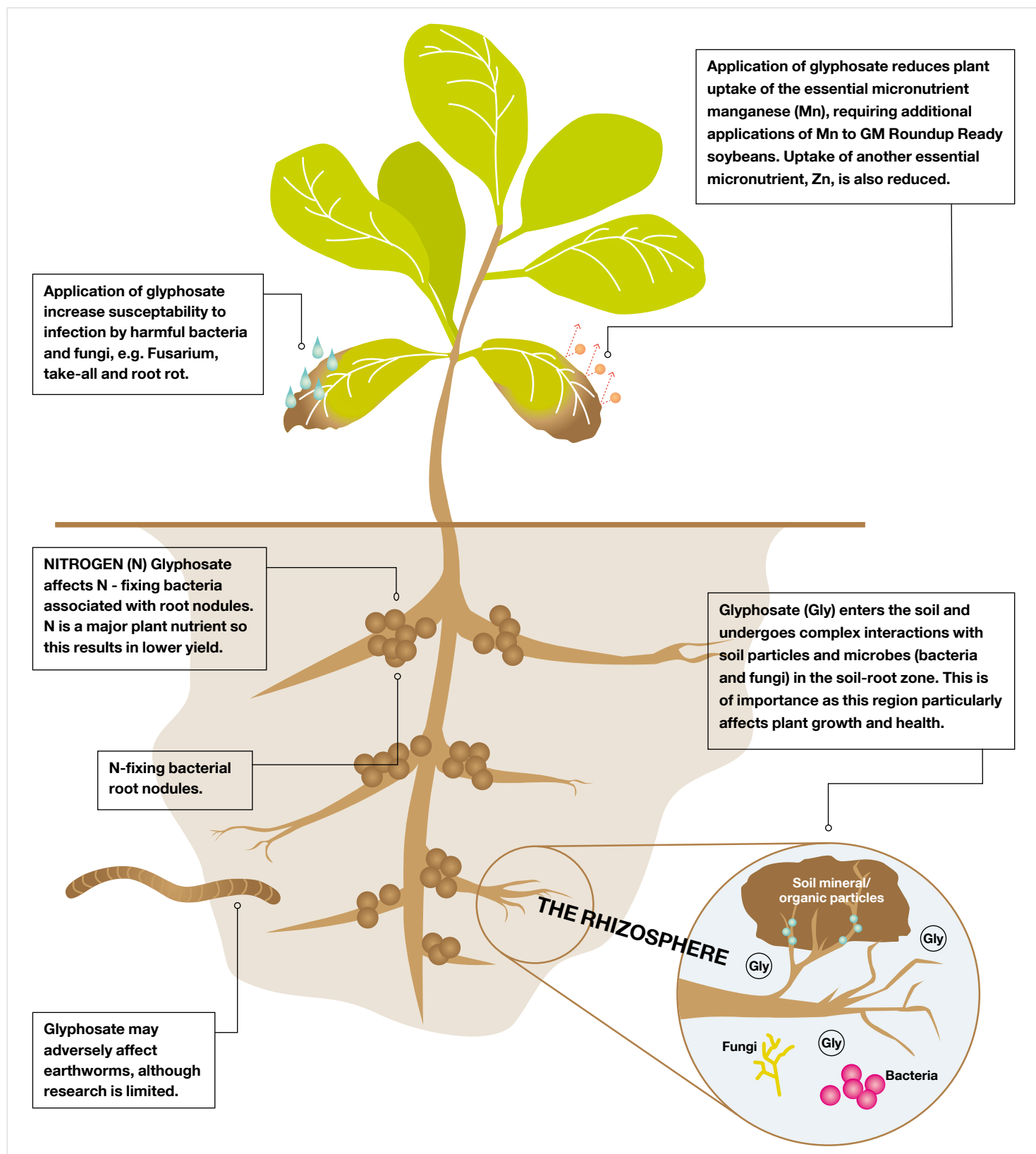


Fig 6: Plant-soil interactions of glyphosate.

A recent study on Monsanto's new variety of GM RR soya (RR2) found that glyphosate negatively impacted 'the complex interactions of microbial groups, biochemical activity and root growth that can have subsequent detrimental effects on plant growth and productivity' (Zobile et al. 2011b). The plant roots had higher numbers of *Fusarium* species and reduced numbers of manganese-reducing bacteria.

These imbalances in the soil and rhizosphere microbial community can affect soil and plant health. They may reduce the availability of plant nutrients, or cause increased vulnerability to disease.

6.3 Reduced nutrient uptake by plants

Research is beginning to uncover the complex relationship between glyphosate use and plant nutrients, such as manganese and zinc. Manganese is vital to plants, enabling photosynthesis, enzyme function, nitrate assimilation and the formation of vitamins (Ducic & Polle 2005). Zinc is vital in the structure and function of enzymes (Broadly et al. 2007).

Glyphosate has been shown to interact with micronutrients, such as manganese, in the rhizosphere and appears to reduce their availability to plants. These interactions possibly happen because glyphosate affects micro-organisms in the rhizosphere (see Chapter 5). Laboratory experiments with sunflowers show that after glyphosate application there was 'an impairment of the manganese-nutritional status, which was still detectable after a waiting time of up to 21 days' (Tesfamariam et al. 2009). This confirmed previous field observations and research suggesting that glyphosate affected microbes associated with the mobilisation of rhizosphere manganese. This then influences the mobility of manganese in the rhizosphere and hence reduces the amount that is available to the plant even when the element is plentiful in the soil (Kremer & Means 2009). Applications of manganese to GM RR soya are necessary to counteract this reduction in manganese uptake compared with the conventional varieties (Gordon 2007).

Studies on GM RR soya confirm these findings and suggest that other nutrients may also be affected by a reduced availability to the plant. Research in Brazil showed that the application of glyphosate 'interfered [with the] mineral nutrition of soybean and the total contents of [nitrogen, manganese, copper, zinc and iron]' (Serra et al. 2011). Recent research on Monsanto's GM RR soya 2 has shown that it also suffers from reduced macro-and micro-nutrient as a result of glyphosate applications (Zobiole et al. 2011a).

6.3.1 Nitrogen fixation impaired

It is known that nitrogen fixation and uptake is affected in GM RR soya. Conventional agriculture normally relies upon the addition of a nitrogen fertiliser to the soil. However, some crops - such as legumes (peas, beans etc.) - are able to utilise atmospheric nitrogen. They do this by forming symbiotic relationships with 'nitrogen-fixing' bacteria in the soil. The bacteria form characteristic nodules around the roots of the plants. There is evidence that the functioning of these nodules in GM soya plants is affected by the presence of glyphosate.

One study found that 'nodulation was always lower on GR [GM glyphosate-resistant] soybean with or without glyphosate compared with conventional varieties with non-glyphosate or no herbicide' (Kremer & Means 2009). The authors suggested that 'glyphosate and perhaps genetic modification in the GR plant may affect the numerous processes involved with nitrogen fixation symbiosis', which processes include nitrogenase activity and leghaemoglobin (an oxygen-carrying protein found in plants) content. This effect could have a significant impact on the long-term sustainability of the crop, which relies on fixation to provide 40% to 70% of the nitrates required by the crop (Kremer & Means 2009). In the longer term, this will lead to an increased dependency on the external addition of nitrates (Bohm et al. 2009), along with the all the environmental concerns associated with the use of nitrate fertilisers (Tillman 1999).

It is clear that GM RR crops, especially RR soya, suffer from a range of nutrient deficiencies induced by the application of glyphosate.

6.3.2. Glyphosate toxicity to earthworms

Independent research on the impact of glyphosate in earthworms is limited. However, there is evidence that repeated use can damage this vitally important group of species. Growth rates of one earthworm species were reduced in culture chambers for 100 days by a range of glyphosate concentrations (Springett & Gray 1992). Similar findings were found in a different species (Yasmin & D'Souza, 2007). Another study found a significant change in the hatching of earthworm cocoons exposed to glyphosate and that the earthworms actively avoided glyphosate-treated soil in the laboratory (Casabé et al. 2007). The conclusion was that the study 'showed deleterious effects of [glyphosate] ... formulations when applied at the nominal concentrations recommended for soya crops'.

The repeated use of glyphosate on GM crops and in general weed control across a wide range of agro and forest ecosystems could possibly have long-term impacts on earthworm populations. Given the vital importance of earthworms in improving soils and in the food chain, there is an urgent need to assess the impact of long-term exposure.

6.4 Increased vulnerability to plant diseases

'The synergistic activity of glyphosate weed control in predisposing plants to infectious organisms has been observed for many diseases ... and the extensive use of glyphosate in agriculture is a significant factor in the increased severity or 're-emergence' of diseases once considered efficiently managed'
(Johal & Huber 2009)

The impact of glyphosate on the ability of plants to fight off disease has now been extensively studied. Pathogenic (or harmful) fungal diseases have been found to worsen in the presence of glyphosate in 13 crops, including soybeans, wheat, cotton, sugar beet and canola. Diseases include take-all in cereals, damping off, root rot and sudden death syndrome in soybeans (Johal & Huber 2009).

Several plant diseases caused by *Fusarium* species (pathogenic fungi) are increased by glyphosate. The presence of the herbicide in the rhizosphere enables these fungi to become the dominant group (Kremer & Means 2009). Soil-dwelling *Fusarium* species cause a wide range of diseases in crop plants, ornamentals and grasses (Nelson et al. 1983). Therefore, any increase arising from the use of glyphosate could be significant in a wide range of different land uses, including GM RR crops.

Research on the impact of glyphosate on microbes inhabiting soil outside the rhizosphere is limited (Kremer & Means 2009). Other research found that numbers of the soil-borne pathogen (or disease) *Pythium ultimum* were significantly greater in root exudates from bean plants treated with glyphosate than those that were not (Kuklinsky-Sobral et al. 2005). Other *Pythium* species were not stimulated in this way.

The toxicity of glyphosate to soil microbes is highly variable and is thought to be dependent on the sensitivity of the microbe's EPSPS enzyme to glyphosate. For instance, five species of the ubiquitous family of soil bacteria *Pseudomonas* were found not to be sensitive to glyphosate, while the activity of one commonly-found member of the family, *Pseudomonas fluorescens*, was inhibited by glyphosate (Schultz, Krüper & Amrhein 1985). *Pseudomonas* species are thought to be important in the breakdown of glyphosate in the soil (Gimsing et al. 2004). Other microbes use glyphosate and AMPA as a source of phosphorus.

There are a number of possible explanations why GM RR soya treated with glyphosate should suffer from increased *Fusarium* activity compared with crops not sprayed with glyphosate (Powell & Swanton 2008):

- Stimulation of pathogenic fungi by glyphosate, either via the roots or by direct application to the soil.
- Glyphosate may inhibit the production of antagonistic chemicals produced by the plant to protect itself (for example, Phytoalexins are produced by plants to protect them from harmful microbes).
- Glyphosate may inhibit the production of protective plant tissues such as lignin.
- Increased levels of pathogen inoculum on weeds if control in the GM RR crop is delayed.
- Enhanced stimulation of pathogens compared with microbes that are antagonistic to pathogens.

6.5 Case studies of glyphosate impacts on soil-plant system

6.5.1 Root rot in soybeans

The roots of soybean plants growing near giant ragweed plants (*Ambrosia trifida*) that had been treated with glyphosate in Indiana, US, were found to have black lesions on 90 to 95% of their roots, caused by the pathogenic fungus *Corynespora cassiicola*. By comparison, lesions were found on only 5-10% from plants growing next to non-treated ragweed. Yield losses from plants affected by glyphosate sprayed weeds were four times those of other soybean plants (Huber et al. 2005).

6.5.2 Citrus variegated chlorosis (CVC)

The symptoms of this disease are a yellowing of the leaves of citrus plants, similar to that caused by manganese and zinc deficiencies. Weed control in citrus plantations in Brazil involved the use of glyphosate. The method developed by the International Plant Nutrition Institute in Brazil was to stop using glyphosate for weed control around the trees and replace it with a mulch of grass cut from between the rows (Yamada & Castro 2005). This controlled weeds and restored manganese and zinc to sufficient levels in the soil. The removal of glyphosate also reduced the incidence of crown rot (*Phytophthora* species).

6.5.3 Take-all in cereals

The take-all fungus, *Gaeumannomyces graminis* (ascomycota), is a major root-rot pathogen of cereals and grasses. Glyphosate is used extensively to clear weeds before cereals are sown, and it has been recognised for many years that take-all infection is increased following the use of the herbicide (Johal & Huber 2009). Take-all is also increased following applications of glyphosate associated with the cultivation of GM RR soya the preceding year in comparison with the use of other herbicides. It is thought that this increase in take all severity is thought to be associated with Mn-deficiency induced by glyphosate (Johal & Huber 2009).

6.6 Summary

When sprayed, glyphosate enters the soil either directly or indirectly. In the soil, the inter-relationships between glyphosate, AMPA, soil minerals and microbes are complex.

It is not known exactly how glyphosate influences soil processes, or soil health. However, **it is now clear that presence of glyphosate in the rhizosphere can affect the uptake of essential micronutrients from the soil, the fixing of nitrogen by root nodules and increase plant's vulnerability to diseases.**

7] Weed resistance and glyphosate - the failure of GM Roundup Ready technology

As with GM crops in general, GM herbicide-tolerant crops are primarily grown in North America, Brazil and Argentina (ISAAA 2011; see Chapter 1). Farmers who adopted these GM crops did so because the seeds were advertised (e.g. Monsanto 2009a) as allowing better and easier weed control that would make crop management much simpler, cheaper and less time intensive (see Chapter 1). Consequently, GM RR soya, maize and cotton have gained significant market shares in the Americas.

When they first appeared in the mid 1990s, weed resistance to herbicides as a result of GM RR crops was rarely discussed, although the phenomenon of weed resistance to herbicides was well known. Now, 15 years later, weed resistance to glyphosate is one of the most well documented effects and a 'major environmental concern' of the cultivation of GM RR crops (IAASTD 2009a).

7.1 A false dawn

For the first few years following their introduction in 1996, GM RR crops did indeed perform as the adverts had promised. Roundup was widely, and possibly indiscriminately, used on GM RR crops, as evidenced by the increase in glyphosate usage (see Chapter 1). Farmers soon became dependent on glyphosate as their main means of chemical weed control in fields of GM RR soya, maize and cotton. They were unaware - or possibly unconcerned - that weed resistance could occur on their farms. However, it was only a very short time before the first glyphosate resistant weed - horseweed (*Conyza canadensis*) - was confirmed in areas of soybean cultivation in 2000 (Zelaya et al. 2004).

US scientists have suggested that the rapid spread of glyphosate resistance in horseweed can be traced to the use of zero tillage in GM RR crops. Zero tillage is where the soil is not ploughed or tilled. It used as a soil and carbon conservation tool by both sustainable and industrial agricultural systems. In industrial agricultural systems, it is often associated with GM herbicide-tolerant crops where weed control is achieved by the use of herbicides.

'No-tillage corn (Zea mays L.) and soybean (Glycine max (L.) Merr.) production has been widely accepted in the mid-Atlantic region, favouring establishment of horseweed (Conyza canadensis (L.) Cronq). Within 3 years of using only glyphosate for weed control in continuous glyphosate-resistant soybeans, glyphosate failed to control horseweed in some fields. Seedlings originating from seed of one population collected in Delaware were grown in the greenhouse and exhibited 8 to 13-fold glyphosate resistance compared with a susceptible population.'
(Van Gessel 2001)

A survey of 1,200 farmers across six US States in 2005/06 found that only 3 out of 10 farmers thought that glyphosate-resistant weeds were a serious issue and a 'substantial number of farmers underestimated the potential for glyphosate resistant weed (GR) populations to evolve in an agro-ecosystem dominated by glyphosate as the weed control tactic' (Johnson et al. 2009). Other studies have confirmed that farmers do not start to manage for weed resistance until they have appeared in their fields (Beckie 2006):

'Part of the reason growers do not manage proactively is because most know other options are still available and expect companies to continue to provide new technology. Unfortunately, companies are not being as successful in discovering selective herbicides with new modes of action as they have been in the past.'

(Green 2008)

In Argentina, the first glyphosate resistant weed to be recorded was Johnsongrass (*Sorghum halepense*) in 2002 (Binimelis et al. 2009). However, early warnings were not heeded, and farmers who raised concerns in the Las Lijatas (northern Argentina) area were reassured.

'Little attention was paid to the uncontrolled clumps [of glyphosate-resistant Johnsongrass] and the farmer complained that no effective action was taken because they were misled by technical advisors who stated that the situation was not problematic. This year, upset by the increasing severity of the resistance problem, he decided to speak up and talk to the local press to raise awareness and speed up proper action.'

(Valverde & Gressel 2006)

This lack of attention to the early signs of weed resistance may have been important in deepening the problems of weed resistance in Argentina. Johnsongrass is ‘one of the world’s worst weeds’ (Bryson 2000) and hence there is real concern about its sudden resurgence in GM RR soya.

‘...The evolution of glyphosate-resistance in S. halepense [Johnsongrass] is a major threat to glyphosate-resistant soybean productivity in northern fields of Argentina.’
(Vila Aiud et al. 2008)

A glyphosate-resistant biotype (variety or strain) of Johnsongrass has also been found in Arkansas in 2005, once again in areas of GM RR soya cultivation (International Survey of Herbicide Resistant Weeds 2010).

The first reported case was confirmed in Rigid Ryegrass (*Lolium rigidia*) in Victoria, Australia in 1997 (Powles 1998). Since then the number of species and strains with glyphosate resistance has increased dramatically (Fig. 7). From zero in 1996, glyphosate resistance has

now been confirmed in over 20 species and over 100 resistant strains have been identified (Table 2). Although these weeds occur in several continents, they primarily occur in the US and the Americas (Fig. 7), where GM RR crops are primarily grown (see Chapter 1). Weed scientists link this rapid growth to the expansion of GM RR crops and there is widespread concern about the agricultural and economic impacts this has and will be causing.’

‘Because glyphosate is the herbicide most often used in no-till and minimum-till systems, GR [glyphosate resistant] volunteer crop plants and glyphosate-resistant or tolerant weeds will jeopardise the sustainability of those systems.’
(Mallory-Smith & Zapotila 2008)

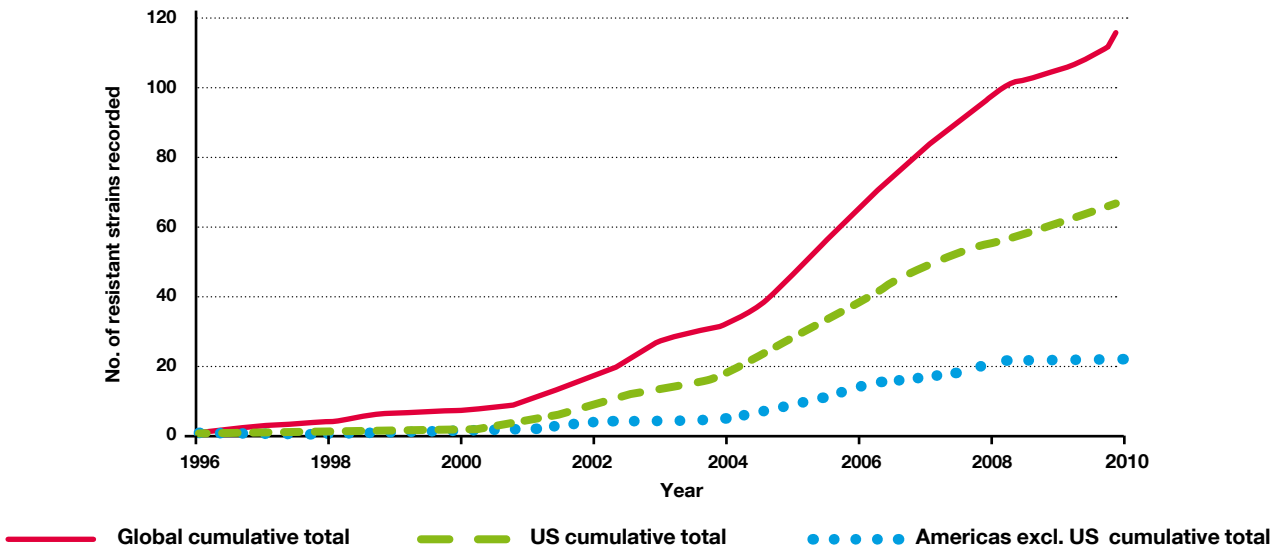


Fig 7: Increase in the occurrence of glyphosate resistant weeds over the past 15 years in the US, the Americas and globally. GM Roundup Ready (RR) crops (resistant to glyphosate) were first introduced in the mid 1990s and grown principally in the US and elsewhere in the Americas (South America and Canada). The occurrence of glyphosate-resistant weed strains follows the pattern of GM RR cultivation with the highest frequency occurring in the Americas, notably the US, and increasing in the years since the introduction of GM RR crops. Based on data from the International Survey of herbicide Resistant Weeds www.weedscience.org.

Table 2: Infestation levels of glyphosate-resistant weed species around the world⁹

Glyphosate-resistant weed	Country	Number of locations where resistance confirmed¹⁰	Estimated maximum total area infested (all locations) (ha)	Crops affected	Year glyphosate resistance first detected
Palmer amaranth <i>Amaranthus palmeri</i>	US	11	2 030 000	Maize, cotton and soya	2005
Common water hemp <i>Amaranthus rudis</i>	US	7	413 000	Maize and soya	2005
Common ragweed <i>Ambrosia artemisiifolia</i>	US	7	4 960	Soya	2004
Giant ragweed <i>Ambrosia trifida</i>	US, Canada	12	9 780	Soya, cotton and maize	2004
Australian Fingergrass <i>Chloris truncata</i>	Australia	1	4.05	Cropland	2010
Hairy Fleabane <i>Conyza bonariensis</i>	US, Colombia, Brazil, Spain, South Africa, Israel, Australia	9	8 620	Roadsides, fruit and orchards, maize, wheat, soya	2003
Sumatran fleabain <i>Conza sumatrensis</i>	Spain	1	20.2	Orchards	2009
Horseweed/Marestail <i>Conyza canadensis</i>	US, Brazil, Spain, China, Czech Rep	23	3 340 000	Soya, maize, cotton, rice, fruit, orchards, road side, railway, nursery	2000
Sourgrass <i>Digitaria insularis</i>	Paraguay, Brazil	5	81 400	Soya, orchards	2006
Junglerice <i>Echinochloa colona</i>	Australia	2	607	Cropland	2007
Goosegrass <i>Eleusine indica</i>	Colombia, Malaysia	3	208	Cropland, coffee, cotton	1997
Wild poinsettia <i>Euphorbia heterophylla</i>	Brazil	1	202	Soya	2006
Italian ryegrass <i>Lolium multiflorum</i>	Chile, Brazil, US, Spain, Argentina	11	8 680	Cropland, orchards, fruit, cotton, soya, barley, wheat	2001
Rigid ryegrass <i>Lolium rigidia</i>	Italy, Spain, France, South Africa, Australia, US	14	10 400	Orchard and vineyards, asparagus, cereals, wheat, almonds, cropland, sorghum	1996
Perennial ryegrass <i>Lolium perennes</i>	Argentina	1	20.2	Barley, cropland, soya, wheat	2008
Ragweed Parthenium <i>Parthenium hysterophorus</i>	Colombia	1	40.4	Fruit	2004
Kochia <i>Kochia scoparia</i>	US	2	4 050	Maize, cotton, cropland and soya	2007
Buckhorn Plantain <i>Plantago lanceolata</i>	South Africa	1	20.2	Orchards and vineyards	2003
Johnsongrass <i>Sorghum halepense</i>	US, Argentina	4	40 500	Soya	2005
Liverseedgrass <i>Urochloa panicoides</i>	Australia	1	20.2	Sorghum, wheat	2008
Blue grass <i>Poa annua</i>	US	1	4.04	Turf	2010
Total global area glyphosate resistant weeds (2010)			5 960 000		

⁹ Data from the International Survey of Herbicide Resistant Weeds (www.weedscience.org). It does not include glyphosate resistant volunteer crops, which are also common in RR crops.

¹⁰ Represents the number of locations where the resistant biotypes are found. The number of infestations at each location range from one to 100 000.

The confirmation of glyphosate resistance in more species in the near future seems highly probable. Twenty-one other weeds in Argentina have been listed as ‘just barely controlled by glyphosate’ and ‘might be the next to upgrade to full resistance by another evolutionary step’ (Valverde & Gressel 2006). These include field bindweed (*Convolvulus arvensis*), curled dock (*Rumex crispus*) and morning glory (*Ipomoea purpurea*).

It is very clear that weed resistance to glyphosate is an increasing problem across the globe and most prominently where GM RR crops are widely grown. The ramifications of this increased weed resistance are that extreme measures are recommended by Monsanto to prolong the effectiveness of GM RR crops.

*‘...More worrisome are glyphosate-resistant populations of far more economically damaging weed species [than *Lolium*].’*

(Powles 2008)

7.2 Monsanto’s Reaction to reports of resistant weeds

Monsanto is highly aware of the growing resistance problem in GM RR crops but appears reluctant to take on liability for the extra costs currently being borne by farmers.

‘Growers must be aware of and proactively manage for glyphosate-resistant weeds in planning their weed control program. When a weed is known to be resistant to glyphosate, then a resistant population of that weed is by definition no longer controlled with labelled rates of glyphosate. Roundup agricultural herbicide warranties will not cover the failure to control glyphosate-resistant weed populations.’

(Monsanto, undated)

Monsanto has published guidance on how to deal with the growing weed resistance problems in GM RR crops (Monsanto 2011) and has already begun developing prevention strategies based on the use of combinations of herbicides and timing of applications.

- The first strategy is **the use of either stronger formulations of glyphosate¹¹ or of tank mixtures of glyphosate and other herbicides**. For instance, 2,4-D – one of the active ingredients of Agent Orange, the defoliant used by the US Army during the Vietnam War – is recommended for burning down weeds prior to sowing marestail (Monsanto 2008).
- The second strategy is **to produce seeds with several herbicide tolerant genes (gene stacking) by crossing different GM herbicide-tolerant varieties** so that different herbicides can be applied to the growing crop in rotation or in tank mixes. This will ensure that weeds that are resistant to glyphosate will be killed by other herbicides (see, for example, Monsanto 2009). For instance, Monsanto has recently announced an agreement with the chemical and biotechnology company BASF to develop crops stacked with glyphosate and dicamba tolerant genes (Monsanto 2010c; see also Behrens et al. 2007; Service 2007). Less is known about the toxicity of dicamba but there are concerns it may be toxic to aquatic organisms¹².
- The third strategy is **to use herbicides that remain active in the soil (residual herbicides or residuals)**, which kill seedling weeds as soon as they germinate.

It is clear from these strategies that weed resistance is a serious problem for the continued efficiency of GM RR crops, and tackling the problem requires extreme measures. Currently, no cost estimates are available for the increased amount of farmer expenditure on weed control as a result of weed resistance, but they are likely to be considerable. These extreme measures may have environmental and possibly health implications.

‘Most of the documented cases of evolved GR [glyphosate resistant] weeds in the past six years have been in GR crops.’

(Duke and Powles 2008)

¹¹ For example, ‘Roundup Weather MAX’. See <http://www.monsanto.com/products/Pages/roundup-weathermax-herbicide.aspx>

¹² Pesticide Action Network. See http://www.pesticideinfo.org/Detail_Chemical.jsp?Rec_Id=PC32871

Farmers are having to resort to stronger formulations of glyphosate or herbicide mixtures to cope with weeds resistant to glyphosate. This has resulted in an escalation of the pesticides 'arms race', with an increasing toxic burden on the environment.



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7.3 False solutions create more problems

The strategy of applying tank mixes pre and post-emergence and residual herbicides will add to the amounts being used and to the overall toxic burden on the environment from GM RR crops.

This strategy could also create new problems if herbicides are overused, leading to the evolution of weeds with resistance to several herbicides. The problem of multiple weed resistance is already well established in the US soya and corn belts. Several weeds that have developed glyphosate resistance are also resistant to other herbicides. In soya crops one strain of common waterhemp (*Amaranthus rudis*) already has resistance to sulphonylureas (e.g. thifensulfuron-methyl), triazines (e.g. atrazine) and glyphosate. Another has resistance to sulphonylureas (e.g. cloransulam-methyl), triazines (e.g. atrazine) and diphenyl ethers (e.g. Lactofen). Twelve other strains have double-resistance. In all there are already seven biotypes in soya crops with multiple resistances that include a resistance to glyphosate. In maize, there are three multi-resistant biotypes including glyphosate. Many of these problem weeds are common to both crops (International Survey of Herbicide Resistant Weeds 2010).

Options for using tank mixes of herbicides or soil residuals to cope with weed resistance are already limited in both crops by the numerous (over 20) weed species that already have resistance to one or more active ingredients.

The future strategy for dealing with glyphosate-resistant weeds involves the use of a wide range of active ingredients in mixtures or singly with a cocktail of adjuvants used in each formulated product. The toxicology of such mixtures is unclear, as approval processes tend to focus on the individual products rather than any additive and/or synergistic effects. Nor can the development of more weeds with multiple resistances in the future be ruled out. Sustainable solutions will not come from the continual adherence to the crop monocultures reliant on chemical weed control that GM RR crops epitomise.

7.4 Conclusion

The rapid evolution of weeds that are resistant to glyphosate is a result of farmers becoming over-reliant on one herbicide for weed control. This is particularly associated with GM RR crops. Now that resistance to glyphosate is widespread in weeds within GM RR soy, maize and cotton crops, farmers have to resort to using mixtures of herbicides.

Thus, the promise of reduced herbicide use and cheaper and easier weed controls has not been delivered. However, it is clear that GM RR crops have brought about an escalation in the pesticides 'arms race', with an increasing toxic burden on the environment involving significant uncertainty about the overall safety of glyphosate for people and biodiversity.

8] Conclusions

There are many problems with GM crops, fundamentally their tendency to produce unexpected and unpredictable effects. This is related to the process by which they are created, the forcible insertion of gene(s) into a plant genome. No GM crops should be cultivated in the environment, nor enter the food chain.

In this report, the focus is on one GM trait, that of herbicide resistance - in particular, it looks at the close association between Roundup Ready crops and the herbicide glyphosate. The evidence presented in the report demonstrates that glyphosate-based herbicides, such as Roundup, can have harmful effects on human health and the environment.

Exposure of humans to glyphosate has been linked to various health effects, including reproductive effects, cancer and neurological effects. Glyphosate interacts with soil chemistry and biology, resulting in a variety of impacts including reduced plant nutrition and increase vulnerability to plant diseases. Glyphosate may also leach into surface and groundwater, where it may damage wildlife and possibly end up in drinking water.

Thus, glyphosate and Roundup are far from benign herbicides. A review of their safety for human and animal health and for the environment is urgently needed.

GM RR crops have greatly increased glyphosate usage, especially in the Americas where they are primarily grown. Given the new evidence of glyphosate toxicity, this is of great concern. The rise in glyphosate-resistant weeds is associated with GM RR crops, and the escalation in

the 'arms-race' against these resistant weeds fuels concerns that even more glyphosate, in stronger formulations and possibly with additional herbicides, will be used on GM RR crops in the future. Similar problems would be highly likely to arise if other GM herbicide-resistant crops are widely cultivated and farmers become dependent on a single herbicide, e.g. GM crops resistant to glufosinate ammonium, marketed as 'Liberty Link'. This facet of GM herbicide-tolerant crops should be enough to lead to a ban on their cultivation.

GM herbicide-tolerant crops, as epitomised by GM RR crops, are not part of a sustainable agriculture system. As with all GM crops they have been developed for, and are economically profitable within, an industrial agriculture system that involves large-scale monocultures that depend on costly, polluting inputs such as herbicides, synthetic fertilisers and fossil fuels.

In contrast, ecological farming both relies on and protects nature by taking advantage of nature's goods and services, such as biodiversity, nutrient cycling, soil regeneration and natural enemies of pests, and by integrating these natural goods into agro-ecological systems.

The widespread and increasingly intensive use of glyphosate in association with the use of GM RR crops, poses risks to the environment and human health. Given the problems that are now evident, no new GM glyphosate-tolerant crops should be authorised. As part of broader considerations of the way forward for agriculture, no GM herbicide-tolerant crop can form part of a sustainable agriculture model, and the cultivation of all such crops should be banned.

GM herbicide-tolerant crops, as epitomised by GM RR crops, are not part of a sustainable agriculture system...In contrast, modern agro-ecological farming practices both relies on and protects nature by taking advantage of nature's goods and services.

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No GM herbicide-tolerant crop can form part of a sustainable agriculture model, and the cultivation of all such crops should be banned

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