



**Friends of
the Earth
Europe**

for the people | for the planet | for the future



The environmental impacts of glyphosate

Friends of the Earth Europe, June, 2013

Table of Contents

Introduction	3
Uses of glyphosate	3
Impacts on biodiversity	4
Glyphosate in the soil	5
Effects on wild life in agricultural areas	6
Glyphosate in Water	7
Summary of data on glyphosate in surface waters	8
Effects on amphibians	9
Effects on aquatic and marine organisms	10
Endocrine disruption	12
Conclusions and demands	12
References	14

Prepared by Friends of the Earth Europe | June 2013

This briefing has been produced with the financial assistance of the European Commission funded Development Fields project. The contents of this briefing are the sole responsibility of Friends of the Earth Europe and can under no circumstances be regarded as reflecting the position of the European Commission.

The environmental impacts of glyphosate

Friends of the Earth Europe, June 2013

Introduction

Glyphosate is the world's best-selling chemical herbicide. Glyphosate-containing herbicides, such as Monsanto's Roundup, are the most widely used herbicides in Europe, applied in farming, forestry, parks, public spaces and gardens. Glyphosate-containing herbicides are also crucial to the production of genetically modified herbicide resistant crops. In recent years a number of scientific studies have raised concerns about glyphosate's safety and there have been calls for glyphosate-containing herbicides to be banned. New research by Friends of the Earth has detected glyphosate residues in the urine of 44 percent of people tested, from 18 different European countries.

Uses of glyphosate

Glyphosate is a systemic, broad-spectrum herbicide. This means that it moves throughout the plant, and kills any plant not genetically modified to resist it. Glyphosate's chemical name is N-(phosphonomethyl)glycine and its main effect is to block an enzyme that plants need to make amino acids and proteinsⁱ. When the enzyme is blocked, plants die within a few days. Glyphosate is never used on its own as a herbicide, it is always combined with other chemical ingredients. For example, a class of chemical called 'surfactants' are added to increase penetration of glyphosate into plant cells.

Glyphosate-containing herbicides cannot be used to control weeds in a growing crop, unless the crop has been genetically modified to resist glyphosate. This is because the herbicide would kill the crop as well as the weeds. But glyphosate is still heavily used in the production of non-GM crops, and it has approval in Europe for a wide range of uses. It is used on cereals, oilseed rape, maize and sunflowersⁱⁱ, as well as for weed control in vineyards, olive groves and fruit orchardsⁱⁱⁱ. Glyphosate is approved for use on grass pastures, forestry and in sensitive habitats. It is approved for clearing railway lines and in some countries it is even approved for use in rivers and lakes. Glyphosate is also widely approved for use in parks, public spaces, streets and gardens. In short, glyphosate may be used almost anywhere, whether in the countryside or in towns and cities.

No genetically modified (GM) herbicide resistant crops have yet been authorised for commercial production in the European Union, but there are currently 14 GM glyphosate resistant crops awaiting approval for cultivation. 10 of these are for GM maize varieties, as well as cotton, sugar beet and soybeans. Monsanto claims that if these GM crops are approved, there will be a reduction in pesticide use^{iv}. But based on what has happened in the United States since the introduction of GM crops, it has been predicted that the introduction of GM glyphosate resistant sugar beet, maize and soybean in the European Union could lead to an 800% increase in glyphosate use by 2025, with overall herbicide use going up by 72% compared to current levels^v.

Impacts on biodiversity

Glyphosate-containing herbicides are used to control plants considered to be weeds, or to clear vegetation. But other plants, animals, invertebrates (e.g. insects) and micro-organisms may also be exposed to glyphosate-containing herbicides:

- when glyphosate is being sprayed, for example insects flying through;
- from eating treated crops, or by eating prey that has been feeding on treated crops;
- from herbicide spray that has been blown by wind onto field margins, or into wild habitats next to a treated area;
- from glyphosate applied to rural or urban areas that has been washed by the rain into groundwater, streams, rivers and coastal waters;
- from glyphosate spray that has fallen onto the soil, moved through plants to their roots, or been incorporated into the soil when a treated plant dies.

These 'non-target' organisms may experience direct toxic effects from the herbicide, or be indirectly affected by changes to ecosystems or food resources. Direct and indirect impacts may be caused by glyphosate, by the other ingredients in glyphosate-containing herbicides, or by the combined action of the different chemicals.

In 2002, when the EU granted approval to glyphosate, the assessment of its effects on organisms and ecosystems was limited to laboratory-based toxicity studies, using high doses and a small number of species^{vi}. This approach has been criticised because there was very little consideration of the ecological aspects of toxic effects, such as the consequences for other species^{vii}. In addition, the species used in the studies were often chosen because they could be easily cultured in laboratories, not because they were especially relevant to

agricultural ecosystems^{viii}. In 2012, after a series of expert workshops and consultations, the European Food Safety Authority recommended that much more extensive modelling was needed to work out the effects of pesticides on species and ecosystems, and that “*to protect biodiversity, impacts need to be assessed at least at the level of the watershed/landscape*”^{ix}. This type of assessment has not yet been carried out for glyphosate.

If GM glyphosate resistant crops are introduced in the EU, this will allow glyphosate to be used throughout the growing season, extending exposure of both the number of non-target species, and stages of their life cycles. Other herbicides could also be used to combat the rapid development of glyphosate-resistant weeds, which could lead to cocktails of different pesticides being applied to fields, as is starting to happen in the United States^x.

Glyphosate in the soil

Glyphosate-containing herbicides may contaminate soils in and around treated areas. Once in soil, the relationship between glyphosate and soil ecosystems is complex, and varies from soil to soil. Glyphosate is soluble in water^{xi} but it also binds onto soil particles under certain conditions^{xii}, particularly in clays. So it may quickly wash out of sandy soils, or last for more than in a year in soils with a high clay content^{xiii}. Even when bound to soil particles, it may dissolve back into soil water later on, for example in the presence of phosphates^{xiv}.

Glyphosate can also form complexes with metal ions^{xv}, potentially affecting the availability of nutrients in the soil.

Glyphosate may be used as a source of energy and nutrients by some soil micro-organisms, increasing their numbers. At the same time, it may be toxic to other species^{xvi xvii}, so reducing their populations. Some fungal species that cause plant diseases have been found to increase in soils treated with glyphosate^{xviii}. In contrast, populations of micro-organisms that suppress disease-causing fungi have been found to decrease in soils treated with glyphosate^{xix}. So the presence of glyphosate in the soil could change the balance of bacteria and fungi, in turn altering soil ecosystem functions and plant health.

Glyphosate has been shown to interfere with the uptake of essential minerals in agricultural crops^{xx}. Despite its widespread use in forestry, there are few studies of glyphosate’s effect on forest soils, although it has been found to persist in the upper organic layers of forest soils

for 360 days (at 16-18% of initial levels)^{xxi} indicating the potential for long term effects. It is also plausible that application of glyphosate to natural ecosystems could replicate the disruption of nitrogen fixation^{xxii} observed in glyphosate-resistant soya crops.

In laboratory studies, Argentinean researchers found that glyphosate-containing herbicides could also be toxic to earthworms, causing damage to cells and DNA at levels “close to the applied environmental concentrations”^{xxiii}. In similar studies, earthworms were found to avoid glyphosate-treated soils^{xxiv}, the growth rates of some earthworm species were reduced by the application of glyphosate-containing herbicides^{xxv xxvi}, and the hatching of cocoons was delayed^{xxvii}.

Effects on wild life in agricultural areas

Glyphosate's mode of action means that any plant sprayed with it will be injured or killed. Glyphosate is considered to be a high risk herbicide for non-target plants^{xxviii}. On farmland, continued application of glyphosate-containing herbicides can significantly affect the numbers and diversity of plant species around field edges. Studies examining the effect of glyphosate spray drift^{xxix xxx} found effects on the growth and on the species composition of wild plant communities exposed to glyphosate-containing herbicides at levels between 1% and 25% of normal agricultural rates.

Common weeds can be important food sources for insect, bird and animal species in agricultural areas. Weeds provide food and nectar sources for insects, which in turn feed birds. Weed seeds can also be vital winter foods for many declining bird species, such as corn bunting and skylark^{xxxi}. Farm Scale Evaluations (FSE) of GM crops in the UK between 1999 and 2003, examined the number of weeds and their seed production in non-GM intensively-managed sugar beet fields, compared with those in GM glyphosate resistant sugar beet crops^{xxxii}. The results showed a significant loss of weeds and weed seeds in the GM glyphosate resistant sugar beet, compared to the conventional crop. The UK government's scientific advisory committee spelled out the significance of the results, stating that *‘if [GM glyphosate resistant] beet were to be grown and managed as in the FSEs this would result in adverse effects on arable weed populations [which] would be likely to result in adverse effects on organisms at higher trophic levels (e.g. farmland birds), compared with conventionally managed beet.*^{xxxiii}

A follow-up modelling project concluded that the effects of GM glyphosate resistant crops could affect different species, depending on their feeding and life cycle requirements. The authors noted that, in the results of their model, *“Skylarks showed very little response to the introduction of GMHT rape. By contrast, the consequences of introducing GMHT sugar beet were extremely severe, with a rapid decline, and extinction of the skylark within 20 years. This contrasts with the cirl bunting, which showed little response to the introduction of GMHT beet, but severe consequences arose as a result of the use of GMHT rape”*^{xxxiv}.

Similarly, the decline of Monarch butterfly populations in North America since the mid-1990s has been linked (in part) to the use of glyphosate-containing herbicides on GM maize and soya crops. However, this is not due to direct toxicity of the herbicide to the butterflies. Monarch caterpillars are very dependent on one species of plant, the common milkweed, as their primary food source. Monsanto’s guidance for farmers specifically mentions that its glyphosate-containing herbicide Roundup WeatherMAX *“will provide suppression and/or control of....milkweed, quackgrass, etc”*^{xxxv} (emphasis added). Common milkweed plants have been lost at very high rates from fields of glyphosate-resistant crops^{xxxvi}, and it is estimated that common milkweed has been largely eliminated from 100 million hectares of US cropland following the introduction of glyphosate-resistant crops^{xxxvii}. While not directly toxic to the butterflies, the use of glyphosate interrupts the caterpillar stage of their lifecycle^{xxxviii}.

Glyphosate in Water

Pesticides may be washed by rain into the water in ditches, river and streams (called surface waters). They may also be washed down through soil and rock layers into underground water sources, such as aquifers (called groundwater). Groundwater is often used as the main source of drinking water supplies, although surface waters may feed into artificial reservoirs. Monsanto has stated in the past that glyphosate is not a major problem in water because it *“sometimes is detected in surface waters, but historically, glyphosate has not been included among herbicides that cause concern in water supplies”*^{xxxix}. The reason given for this, is that *“(b)ecause glyphosate binds tightly to most soils, it has a low potential to move through soil to contaminate groundwater”*^{xl}.

But long-term research in Denmark found that glyphosate could be washed down through some soil types by rain, into field drains and on to rivers and streams. Glyphosate levels reached 31µg/litre and 4.7µg/litre in drainage water at the two most vulnerable sites^{xii}. Urban runoff is also a source of glyphosate into streams and rivers, and as a result its use on paved surfaces is banned in Denmark, and half of Sweden's urban areas^{xiii}. Discharges through the sewerage system after rainstorms can be a source of pollution into rivers and streams. Monitoring of Copenhagen's sewage and storm water overflows^{xiii} found that glyphosate was always present.

In fact, glyphosate residues have been detected in surface waters across the European Union. The European Glyphosate Environmental Information Sources (EGEIS) summarised surface water monitoring data from 1993-2009 for thirteen European countries^{xiv}. Over 50,000 samples were included, and glyphosate was found in 29% of these samples. Residues of glyphosate's breakdown product (AMPA) were found in 50% of samples. These findings are supported by other monitoring projects, some of which found glyphosate in virtually all samples tested (see table.)

Summary of data on glyphosate in surface waters

Country	Date	Occurrence of glyphosate residues and concentrations recorded	Source
Several	2005(published)	0.5-1.0µg/l	WHO ^{xiv}
US	2002	36% of samples, up to 8.7µg/l	Battaglin et al ^{xv}
Canada	2002	22% of samples, up to 6.07µg/l	Humphries et al ^{xvii}
France	1999-2009	99% of samples, up to 86µg/l	Villeneuve et al ^{xviii}
US	2004-2008	Most rivers 100%, up to 430µg/l after a storm	Coupe et al ^{xix}
Germany	1998	Found in two rivers in the Ruhr, up to 0.59µg/l	Skart et al ⁱ
Hungary	2010-11	Found in 2010 only, up to 0.1µg/l	Mortl et al ⁱⁱ
Norway	1995-99	Up to 1µg/l	Ludvigsen et al ⁱⁱⁱ

Glyphosate contamination of surface waters is of significance for wildlife, but residues have also been detected at low levels in groundwater, which is used for drinking water. EGEIS summarised groundwater monitoring from over 8900 European locations between 1993 and 2009, and found a low percentage contaminated with glyphosate (1.3%), with 270 (0.7%) samples above the maximum permitted in drinking water (0.1µg/litre)^{liii}. Monitoring of small boreholes in four Danish counties found glyphosate present in 8.8% of the wells analysed, with 3.4% exceeding the drinking water maximum. In France, glyphosate accounted for 2.9% of all samples exceeding the drinking water limit in samples of raw water destined for public supply (2000-2002). Results of monitoring in Catalonia in north east Spain between 2007 and 2010 found the glyphosate in 41% of 140 groundwater samples, with a maximum of 2.5µg/litre and an average of 0.2µg/litre^{liv}.

Glyphosate is being detected in surface and groundwater wherever it is used. In 2007, despite its previous statement that glyphosate was not of concern for water supplies, Monsanto commissioned research from the UK's Water Research Centre (WRc) on the "*Removal of Glyphosate by Water Treatment*"^{liv}, setting out treatment options for ensuring drinking water complied with the EU's maximum permitted concentration for glyphosate.. The costs of such water treatment will have to be borne by the water companies.

If GM glyphosate resistant crops are approved for use in the EU, glyphosate contamination of surface and groundwater is likely to become even more widespread. In areas of the USA where GM glyphosate resistant crops are grown, glyphosate in river waters has been measured at levels up to 430µg/litre; glyphosate has been detected in the air and rain during the crop growing season^{lvi}, and in water from spring snow-melt^{lvii}.

Effects on amphibians

In recent years there has been growing concern about the world-wide decline in numbers and diversity of amphibian species^{lviii}. Amphibians are particularly vulnerable to pesticide exposure because they can absorb water-borne chemicals through their skin, as well as by eating contaminated food resources^{lix}. Glyphosate has been investigated as a possible cause of amphibian declines^{lx}, and a number of studies have found worrying results about the effect of exposure to glyphosate-containing herbicides on the growth and development of amphibians.

In laboratory experiments, frog embryos exposed to dilutions of glyphosate-containing herbicides showed facial and cranial malformations, as well as shortening of the body, smaller heads and defective eyes^{lxi}. Similarly, exposure to a glyphosate-containing herbicide reduced the snout-vent length of adult frogs^{lxii}. Exposure to glyphosate-containing herbicides has been found to extend the larval period of American toads^{lxiii}, and caused changes to the activity of a key enzyme involved in the nervous system in the tadpoles of the frog *Rhinella arenarum*^{lxiv}. In one study, exposure caused changes to the shape of tadpoles, including deepening of their tails. The scientist conducting the experiment noted that the changes were similar to those caused by the presence of predators^{lxv}.

Laboratory and controlled environmental experiments have found that glyphosate also causes increased mortality in growing tadpoles^{lxvi}, with one trial on North American tadpoles in artificial ponds finding mortality rates as high as 96-100% when glyphosate was applied at the manufacturers recommended rate^{lxvii}. A study^{lxviii} examining the effects of a glyphosate-containing herbicide on 13 species of frogs, toads, newts and salamanders found the toxicity varied between groups, with frogs and toads being more sensitive than salamanders. The authors commented that glyphosate-containing herbicides have the potential '*to cause substantial amphibian mortality at environmentally expected concentrations.*'

Glyphosate was given EU-wide approval in 2002, but the evidence from these studies show that its use could cause serious impacts on already threatened amphibian species. As one of the researchers has commented, "*our understanding of the possible effects of glyphosate based herbicides on amphibians has moved from a position of knowing very little and assuming no harm, to a position of more precise understanding of which concentrations and conditions pose a serious risk.*"^{lxix} Despite this, neither the US^{lxx} or EU^{lxxi} regulatory systems require direct testing of the impact of pesticide formulations on amphibians.

Effects on aquatic and marine organisms

There have also been investigations into the impacts of glyphosate-containing herbicides on organisms living in river, streams and coastal waters. Micro-organisms are vital to marine and freshwater ecosystems, because they form the basis of food chains. In laboratory experiments, the growth and species composition of microbial populations from marine

waters was disturbed at levels of glyphosate typical of those caused by run-off from the land^{lxxii}. Similar effects were found on microbial populations from freshwater systems^{lxxiii}. Another study found that photosynthesis in freshwater cyanobacteria was inhibited by glyphosate-containing herbicides^{lxxiv}, while tiny aquatic animals called Rotifers were found to have reduced life expectancy and reproductive rates, longer development times and lower overall populations^{lxxv}.

Toxic impacts have also been observed higher up marine and aquatic food chains. Freshwater mussels have been found to be acutely sensitive to pure glyphosate, surfactant ingredients and to the glyphosate-containing herbicide Roundup^{lxxvi}. Freshwater carp showed changes to liver cells and mitochondria (parts of all cells) after exposure to Roundup herbicide at levels 20 and 40 times lower than would be expected from normal agricultural practice^{lxxvii}. A study on the European eel concluded that “*environmentally relevant concentrations of Roundup can pose a health risk for fish populations*”^{lxxviii} and found that the herbicide damaged the DNA of the exposed fish.

Other effects were observed on interactions between fish and their parasites. In one study the parasitic horsehair worm showed reduced infective ability and increased adult mortality following exposure to very low concentrations of glyphosate^{lxxix}. A separate study examined the relationship between a glyphosate-containing herbicide and trematode flatworm parasites of fish. The authors concluded that interactions between the two could mean that “*at environmentally relevant concentrations...glyphosate might increase the risk of disease in fish*”^{lxxx}.

There is also evidence that glyphosate affects the activity of the enzyme acetylcholinesterase, which is vital for the operation of the nervous system. If acetylcholinesterase is not working properly, nerve impulses are not switched off, causing serious health problems and even death^{lxxxi}. Glyphosate has been found to suppress the activity of the enzyme in brown mussels^{lxxxii} and fish^{lxxxiii}, ^{lxxxiv}, ^{lxxxv}, ^{lxxxvi}. The consequences of the effects observed in these experiments have not been fully investigated.

Endocrine disruption

Laboratory studies have found evidence that glyphosate and Roundup formulations may be linked to endocrine disruption in animals and human cell lines^{lxxxvii lxxxviii lxxxix xc xci}, with effects occurring at concentrations below those used in agriculture. One study in Argentina found that very low doses of glyphosate-containing herbicides (as low as 0.02% of the concentration used in agricultural sprays) caused skeletal changes in tadpoles, along with other developmental effects such as shortened bodies, reduced head size and eye defects^{xcii}. Whether or not such effects could be occurring in wildlife after field application of glyphosate has not yet been established. Glyphosate is not currently included on lists of confirmed endocrine disrupting chemicals^{xciii xciv}.

Conclusions and demands

New research from Friends of the Earth has shown that people from all over Europe – in EU and non EU countries – have glyphosate residues in their urine. The evidence suggests that a significant proportion of the population could have glyphosate in their bodies – and it is not clear where it is coming from. Despite the fact that glyphosate is the world's best-selling chemical herbicide and glyphosate-containing herbicides are the most widely-used herbicides in Europe, very little testing is done for glyphosate residues in food, feed, or water. Tests for glyphosate in the body do not take place at all.

Friends of the Earth wants to know:

- Why do people have glyphosate in their urine? Where does it come from?
- Why haven't public authorities done any testing on glyphosate residues in humans?
- Why is food, animal feeds (such as imported soy) and drinking water so rarely tested for glyphosate?
- What are the health impacts of glyphosate in our bodies? Is it guaranteed that glyphosate residues are completely excreted? If not, what happens to the remaining residues?
- Why haven't there been any long-term health studies on on-going glyphosate uptake in humans?
- Why have the maximum residue levels (MRLs) for glyphosate in food and feed been steadily increased?
- Who is profiting from increasing glyphosate use?

- Why are authorities considering applications to grow glyphosate-resistant genetically modified crops in Europe?

Given the uncertainty about how glyphosate is entering people and the need to minimise exposure to glyphosate, Friends of the Earth demands that:

- The EU and national governments must immediately start a monitoring programme for glyphosate in food and feed, including imported animal feed crops such as GM soy. Levels of glyphosate (and its breakdown product AMPA) in the environment should also be monitored, covering aquatic systems and soil. These monitoring programmes should be comprehensive and the results should be made available to the public without delay.
- National governments must introduce a glyphosate reduction programme and desiccation (spraying crops shortly before the harvest) should be banned without delay. All other uses for glyphosate should be evaluated by 2015, existing maximum residue limits (MRLs) should be re-evaluated, and there must be no further increases in the MRLs.
- No glyphosate resistant genetically modified crops should be authorized in the EU.
- All food processors and retailers should minimise their customer's exposure to glyphosate residues by specifying glyphosate-free products from their suppliers. They should extend their internal pesticides monitoring programme and include glyphosate in their regular testing.

References

- ⁱ Hoagland RE & Duke SE (1982). Biochemical effects of glyphosate. In: *Biochemical Responses Induced by Herbicides*; Moreland DE, St. John JB & Hess FD (Eds.) ACS Symposium Series 181 pp. 175-205. American Chemical Society, Washington DC, USA.
- ⁱⁱ Monsanto International and Monsanto Europe (2010) *The agronomic benefits of glyphosate in Europe- benefits of glyphosate per market use REVIEW* p 1-82
- ⁱⁱⁱ Monsanto International and Monsanto Europe (2010) *ibid*
- ^{iv} Monsanto International and Monsanto Europe (2010) *ibid*
- ^v Benbrook CM (2012) *Glyphosate tolerant crops in the EU: a forecast of impacts on herbicide use*. Greenpeace International
- ^{vi} European Commission (2002) *Review report for the active substance glyphosate*
Document reference: Glyphosate 6511/VI/99-final
- ^{vii} Galic N, Schmolke A, Forbes V, Baveco H & van den Brink PJ (2012) The role of ecological models in linking ecological risk assessment to ecosystem services in agroecosystems. *Science of the Total Environment* Vol 115 pp 93-100
- ^{viii} Galic N, Schmolke A, Forbes V, Baveco H & van den Brink PJ (2012) *ibid*
- ^{ix} Nienstedt KM *et al* (2012) Development of a framework based on an ecosystem services approach for deriving specific protection goals for environmental risk assessment of pesticides *Science of the Total Environment* Vol 415 pp 31-38
- ^x GM Freeze and Pesticides Action Network UK, 2012. GM Herbicide Tolerant Crops –Less Equals More
- ^{xi} University of Hertfordshire, The Pesticide Properties Database.
<http://sitem.herts.ac.uk/aeru/footprint/index2.htm>
- ^{xii} Shushkova T, Ermakova I & Leontievsky A. 2009. Glyphosate bioavailability in the soil. *Biodegradation* 21: 403-410.
- ^{xiii} Bergström L, Börjesson E & Stenström J (2011) Laboratory and Lysimeter Studies of Glyphosate and Aminomethylphosphonic Acid in a Sand and a Clay Soil *Journal of Environmental Quality* Vol 40 pp 98–108
- ^{xiv} Simonsen L, Fomsgard IS, Svensmark B & Splid NH. 2008. Fate and availability of glyphosate and AMPA in agricultural soil. *Journal of Environmental Science and Health Part B* 43: 365-375.
- ^{xv} Eker, S., Ozturk, L., Yazici, A., Erenoglu, B., Römheld, V., Cakmak, I., 2006. Foliarapplied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus* L.) plants. *Plant and Soil* 284: 101-109
- ^{xvi} Haney RL, Senseman SA, Hons FM & Zuberer DA. 2009. Effect of glyphosate on soil microbial activity and biomass. *Weed Science* 49: 89-93
- ^{xvii} Wardle DA & Parkinson D. 1999. Effects of three herbicides on soil microbial biomass and activity. *Plant Science* 122: 21-28.
- ^{xviii} Zobiolo LHS, Kremer R, Oliveira RS, Constantin J. 2011b. Glyphosate affects microorganisms in rhizospheres of glyphosate-resistant soybeans *Journal of Applied Microbiology*. 110: 118-127.

- ^{xix} Kuklinsky-Sobral J, Araujo WL, Mendes R, Pizzirani-Kleiner AA & Azevedo JL. 2005. Isolation and characterization of endophytic bacteria from soybean (*Glycine max*) grown in soil treated with glyphosate herbicide. *Plant and Soil* 273: 91-99.
- ^{xx} Zobiole LHS, Kremer RJ, Oliveira RS, Constantin J. 2011a. Glyphosate affects chlorophyll, nodulation and nutrient accumulation of “second generation” glyphosate-resistant soybean (*Glycine max* L.) *Pesticide Biochemistry and Physiology* 99: 53-60.
- ^{xxi} Feng J.C. and Thompson D.G., 1990. Fate of glyphosate in a Canadian forest watershed. 2. Persistence in foliage and soils. *Journal of Agriculture, Food and Chemistry* 38: 1118-1125.
- ^{xxii} Kremer RJ & Means NE. 2009. Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. *European Journal of Agronomy* 31: 153-161
- ^{xxiii} Piola L., Fuchs J., Oneto M.L., Basack S., Kesten E., and Casabé N., 2013. Comparative toxicity of two glyphosate-based formulations to *Eisenia andrei* under laboratory conditions. *Chemosphere* S0045-6535: 01537-8. doi: 10.1016/j.chemosphere.2012.12.036.
- ^{xxiv} Casabé N, Piola L, Fuchs J, Oneto ML, Pamparato L, Basack S, Giménez R, Massaro R, Papa JC & Kesten E. 2007: Ecotoxicological assessment of the effects of glyphosate and chlorpyrifos in an Argentine soya field. *Journal of Soils and Sediments*, 7: 232-239.
- ^{xxv} Springett AJ & Gray RAJ. 1992. Effect of repeated low doses of biocides on the earthworm *Aporrectodea caliginosa* in laboratory culture. *Soil Biology and Biochemistry* 24: 1739-1744.
- ^{xxvi} Yasmin S. and D’Souza D., 2007. “Effect of pesticides on the reproductive output of *Eisenia fetida*,” *Bulletin of Environmental Contamination and Toxicology*, 79: 529–532
- ^{xxvii} Casabé N, Piola L, Fuchs J, Oneto ML, Pamparato L, Basack S, Giménez R, Massaro R, Papa JC & Kesten E. 2007: Ecotoxicological assessment of the effects of glyphosate and chlorpyrifos in an Argentine soya field. *Journal of Soils and Sediments*, 7: 232-239.
- ^{xxviii} Iowa University State Extension. 2003. Protecting Iowa’s rare and endangered plants. <http://www.extension.iastate.edu/Publications/PM1506.pdf>
- ^{xxix} Perry NH, Chaney A & Wilcox A (1996) The effect of herbicide and fertiliser application on herbaceous field margin communities *Aspects of Applied Biology* Vol 44 pp 339-344
- ^{xxx} Damgaard C, Strandberg B, Matthiassen SK & Kudsk P (2011) The combined effect of nitrogen and glyphosate on the competitive growth, survival and establishment of *Festuca ovina* and *Agrostis capillaris*. *Agriculture Ecosystems and Environment* Vol 142 pp 374–381
- ^{xxxi} Voříšek P., Jiguet F., Van Strien A., Škorpilová J., Klvaňová and Gregory R.D., 2010. European trends in farmland birds. *BOU Proceedings – Lowland Farmland Birds III*. <http://www.bou.org.uk/bouproc-net/lfb3/vorisek-et-al.pdf>
- ^{xxxii} Heard MS, Hawes C, Champion GT, Clark SJ, Firbank LG, Haughton AJ, Parish AM, Perry JN, Rothery P, Scott RJ, Skellern MP, Squire GR & Hill MO. 2003a. Weeds in fields with contrasting conventional and genetically modified herbicide-tolerant crop – I. Effects on abundance and diversity. *Philosophical Transactions of The Royal Society London B* 358: 1819-1832.

- ^{xxxiii} ACRE. 2004. *Advice on the implications of the farm-scale evaluations of genetically modified herbicide tolerant crops*.
http://webarchive.nationalarchives.gov.uk/20080727101330/http://www.defra.gov.uk/environment/acre/advice/pdf/acre_advice44.pdf
- ^{xxxiv} DEFRA, 2003 *Modelling the effects of farmland food webs of herbicide and insecticide management in the agricultural ecosystem* DEFRA EPG 1/5/188
http://webarchive.nationalarchives.gov.uk/20081023141438/http://www.defra.gov.uk/environment/gm/research/pdf/epg_1-5-188.pdf
- ^{xxxv} Monsanto, 2011. Genuity® Roundup Ready 2 Yield® and Roundup Ready® Soybeans 2011 Technology Use Guide <http://www.monsanto.com/SiteCollectionDocuments/weed-management-documents/GENRR2Y-RR-soybeans.pdf>
- ^{xxxvi} Hartzler, R.G. (2010) Reduction in common milkweed (*Asclepias syriaca*) occurrence in Iowa cropland from 1999 to 2009. *Crop Protection*, 29, 1542–1544.
- ^{xxxvii} Monarch Watch, 2008, Roundup Ready Crops and Resistant Weeds. Blog Thursday, January 17th, 2008 <http://monarchwatch.org/blog/2008/01/roundup-ready-crops-and-resistant-weeds/>
- ^{xxxviii} Pleasants J.N. and Oberhauser K.S., 2012. Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population. *Insect Conservation and Diversity* doi: 10.1111/j.1752-4598.2012.00196.x
- Brower, L.P., Taylor, O.R., Williams, E.H., Slayback, D.A., Zubieta R.R., and Rez I.M.R., 2011. Decline of monarch butterflies overwintering in Mexico: is the migratory phenomenon at risk? *Insect Conservation and Diversity* doi: 10.1111/j.1752-4598.2011.00142.x
- ^{xxxix} Monsanto, 2003 Backgrounder Glyphosate and water quality.
http://www.monsanto.com/products/Documents/glyphosate-background-materials/gly_water_bkg.pdf
- ^{xl} Ibid
- ^{xli} Rosenbom AE, Brusch W, Juhler RK, Ernstsens V, Gudmundsson L, Kjær J, Plauborg F, Grant R, Nyegaard P & Olsen P. 2010. The Danish Pesticide Leaching Assessment Programme Monitoring results May 1999–June 2009. *Geological Survey of Denmark and Greenland*, Ministry of Climate and Energy and Faculty of Agricultural Sciences.
- ^{xlii} Kristoffersen P, Rask AM, Grundy AC, Franzen I, Kempenaar C, Raisio J, Schroeder H, Spijker J, Verschwele A & Zarina L. 2008. A review of pesticide policies and regulations for urban amenity areas in seven European countries. *Weed Research* 48(3) published on-line. <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-3180.2008.00619.x/pdf>
- ^{xliii} Birch H., Mikkelsen P.S., Jenson J.K and Lützhøft, 2011. Micropollutants in stormwater runoff and combined sewer overflow in the Copenhagen area, Denmark. *Water Science and Technology* 64:485-493.
- ^{xliv} Horth H., 2010. EGEIS, Monitoring results for surface and groundwater.
<http://www.egeis.org/documents/11%20Detection%20in%20SW%20and%20GW%20draft%20v3.pdf>
- ^{xlv} WHO. 2005. Glyphosate and AMPA in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality

- ^{xlvi} Battaglin WA, Kolpin DW, Scribner EA, Kuivila KM & Sandstrom MW. 2005. Glyphosate, other herbicides, and transformation products In *Midwestern Streams, 2002. Journal of the American Water Resources Association* 41: 323-332.
- ^{xlvii} Humphries D, Brytus G & Anderson AM. 2005. *Glyphosate residues In Alberta's atmospheric deposition, soils and surface waters*. Water Research Users Group Alberta Environment.
- ^{xlviii} Villeneuve A., Larroudé S & Humbert JF., 2011. Herbicide contamination of freshwater ecosystems: impact on microbial communities. In: *Pesticides – Formulations, Effects, Fate*. Stoytcheva M. (Ed.) pp. 285-312, InTech, <http://www.intechopen.com/articles/show/title/herbicide-contamination-offreshwater-ecosystems-impact-on-microbial-communities>
- ^{xlivx} Coupe RH, Kalkhoff SK, Capel PD and Gregoire C, 2011. "Fate and transport of glyphosate and aminomethylphosphonic acid in surface waters of agricultural basin". *Pesticide Management Science*, 67, doi: 10.1002/ps.2212
- ⁱ Skark C., Zullei-Seibert N., Schottler U. & Schlett C. (1998) The occurrence of glyphosate in surface water. *International Journal of Environmental Analytical Chemistry* 70 : 93-104
- ⁱⁱ Mörtl M., Németh G., Juracsek J., Darvas B., Kamp L, Rubio F.and Székács A.,2013. Determination of glyphosate residues in Hungarian water samples by immunoassay *Microchemical Journal* 107 :143–151
- ⁱⁱⁱ Ludvigsen G.H.and Lode O., 2001. Results from the agricultural and environmental monitoring program of pesticides in Norway 1995 – 1999. *Fresenius Environmental Bulletin*, 10, 470-474.
- ^{liii} Horth H.,2010. Op cit
- ^{liv} Sanchis J *et al* ,2012. Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry *Analytical and Bioanalytical Chemistry* 402 :2335-2345
- ^{lv} Water Research Centre, 2007 Removal of glyphosate by water treatment. WRC ref: UC7374 Available at <http://www.roundup.nl/userfiles/WRC-report-UC7374-July-2007-Removal-of-glyphosate-and-AMPA-by-water-treatment.pdf>
- ^{lvi} Chang F-C, Simcik MF and Capel P., 2011. Occurrence and fate of the herbicide glyphosate and its degradate Aminomethylphosphonic acid in the atmosphere *Environmental Toxicology and Chemistry* 30 : 548–555
- ^{lvii} Battaglin W.A., Rice K.C., Focazio M.J., Salmons S. and Barry R.X., 2009. The occurrence of glyphosate, atrazine, and other pesticides in vernal pools and adjacent streams in Washington, DC, Maryland, Iowa, and Wyoming, 2005–2006. *Environmental Monitoring and Assessment* 155, 281-307.
- ^{lviii} Williams N. 2004. Fears grow for amphibians. *Current Biology* 14: 986-987.
- ^{lix} Khan M.Z. and nelson., 2005. Adverse Effects of some Selected Agrochemicals and Pharmaceuticals in Aquatic Environment with reference to Amphibians and Fish journal of Basic and Applied Sciences 1: at <http://www.jbaas.com/HTML/Previous%20Issues/Volume%20No.%201%20No.%201/Heading/H-5.html>

- ^{lx} Relyea RA. 2005c. The lethal impact of roundup on aquatic and terrestrial amphibians. *Ecological Applications* 15: 1118–1124.
- ^{lxi} Paganelli A, Gnazzo V, Acosta H, López SL & Carrasco AE. (2010) Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signalling. *Chemical Research in Toxicology* Vol 23 pp 1586-95
- ^{lxii} Paetoe L.J., Daniel M.J., Cue R.I., Pauli B.D., and Marcogliese D.J. (2012) Effects of herbicides and the chytrid fungus *Batrachochytrium dendrobatidis* on the health of post-metamorphic northern leopard frogs (*Lithobates pipiens*). *Ecotoxicology and Environmental Safety* Vol 80 pp 372-80.
- ^{lxiii} Williams BK & Semlitsch RD. (2010). Larval Responses of Three Midwestern Anurans to Chronic, Low-Dose Exposures of Four Herbicides. *Archives of Environmental Contamination and Toxicology* Vol 58 pp 819-827.
- ^{lxiv} Lajmaonovich RC, Attademo AM, Peltzer PM, Junges CM & Cabagna MC. (2011). Toxicity of four herbicide formulations with glyphosate on *Rhinella arenarum* (Anura: Bufonidae) tadpoles: B-esterases and glutathione S-transferase Inhibitors. *Archives of Environmental Contamination and Toxicology* Vol 60 pp 681-689
- ^{lxv} Reylea R.A. (2012) New effects of Roundup on amphibians: Predators reduce herbicide mortality; herbicides induce antipredator morphology. *Ecological Applications* Vol 22 pp 634–647
- ^{lxvi} Relyea RA. 2005b. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecological Applications*, 15: 618–627.
- ^{lxvii} Relyea RA. 2005c. The lethal impact of roundup on aquatic and terrestrial amphibians. *Ecological Applications* 15: 1118–1124.
- ^{lxviii} Reylea R.A and Jones D.K. (2009). The toxicity of Roundup Original MaxH to 13 species of larval amphibians. *Environmental Toxicology and Chemistry* Vol 29 pp 2004-2009.
- ^{lxix} Relyea, R. A. (2011). Amphibians are not ready for Roundup®. Pages 267-300 in J. Elliott, C. Bishop, and C. Morrissey, eds. *Wildlife Ecotoxicology—Forensic Approaches*. Springer.
- ^{lxx} Reylea R.A. (2011) Op cit.
- ^{lxxi} European Commission, (2011) COMMISSION REGULATION (EU) No 544/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for active substances
- ^{lxxii} Stachowski-Haberkorn S, Becker B, Marie D, Haberkorn H, Coroller L & De la Broise D. (2008) Impact of Roundup on the marine microbial community, as shown by an in situ microcosm experiment. *Aquatic Toxicity* Vol 89 pp 232-241.
- ^{lxxiii} Pérez GL *et al* (2007) Effects of the herbicide Roundup on freshwater microbial communities: a mesocosm study. *Ecological Applications* Vol 17 pp 2310-22
- ^{lxxiv} Vera M.S. *et al* (2012). Direct and indirect effects of the glyphosate formulation Glifosato Atanor® on freshwater microbial communities. *Ecotoxicology* Vol 21 pp 1805-16.
- ^{lxxv} Vera M.S. *et al* (2012). *ibid*
- ^{lxxvi} Bringolf RB, Cope WG, Mosher S, Barnhart MC & Shea D. (2007). Acute and chronic toxicity of glyphosate compounds to glochidia and juveniles of *Lampsilis siliquoidea* (Unionidae). *Environmental Toxicology and Chemistry* 26: 2094-2100.

- ^{lxxvii} Szarek J, Siwicki A, Andrzejewska A, Terech-Majewska E & Banaszkiwicz T. (2000). Effects of the herbicide Roundup on the ultrastructural pattern of hepatocytes in carp (*Cyprinus carpio*). *Marine Environmental Research* 50: 263-266.
- ^{lxxviii} Guilherme S, Gaivao I, Santos MA & Pacheco M. 2009. Tissue specific DNA damage in the European eel (*Anguilla anguilla*) following a short-term exposure to a glyphosate-based herbicide. *Toxicological Letters* 189S:S212:Z15.
- ^{lxxix} Achiorno CL, Villalobos C & Ferrari L. (2008). Toxicity of the herbicide glyphosate to *Chordodes nobilii* (Gordiida, Nematomorpha). *Chemosphere* 71: 1816-22.
- ^{lxxx} Kelly DW, Poulin R, Tompkins DM & Townsend CR. (2010). Synergistic effects of glyphosate formulation and parasite infection on fish malformations and survival. *Journal of Applied Ecology* 47: 498–504.
- ^{lxxxi} Grue CE, Gibert PL & Seeley ME. 1997. Neurophysiological and behavioral changes in nontarget wildlife exposed to organophosphate and carbamate pesticides: thermoregulation, food consumption and reproduction. *American Zoologist* 37: 369-388.
- ^{lxxxii} Sandrini J.Z., Rola R.C., Lopes F.M., Buffon H.F., Freitas H.F. Freita M.M., Martins C.D., and da Rosa C.E. (2013). Effects of glyphosate on cholinesterase activity of the mussel *Perna perna* and the fish *Danio rerio* and *Jenynsia multidentata*: In vitro studies. *Aquatic Toxicology* 130-131: 171-173. doi: 10.1016/j.aquatox.2013.01.006.
- ^{lxxxiii} Menéndez-Helman R.J., Ferreyroa G.V., dos Santos Alfonso M. and Salibrán A., (2012). Glyphosate as an acetylcholinesterase inhibitor in *Cnesterodon decemmaculatus*. *Bulletin of Environmental Contamination and Toxicology*, 88; 6-9.
- ^{lxxxiv} Cattaneo R., Clasen B., Loro V.L., de Menezes C.C., Pretto A., Baldisserotto B, Santi A., and de Avila L.A., 2011. Toxicological responses of *Cyprinus carpio* exposed to a commercial formulation containing glyphosate. *Bulletin of Environmental Contamination and Toxicology* 87:597-602.
- ^{lxxxv} Gluszcak L, Dos Santos Miron D, Crestani M, Da Fonseca MB, De Araújo Pedron F, Duarte MF & Vieira VLP. 2006. Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in piava (*Leporinus obtusidens*). *Ecotoxicology and Environmental Safety* 65: 237-241.
- ^{lxxxvi} Gluszcak L, Dos Santos Miron D, Moraes BS, Simões RR, Schetinger MRC, Morsch VM & LoroVL. 2007. Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (*Rhamdia quelen*) *Comparative Biochemistry and Physiology Part C* 146: 519-524.
- ^{lxxxvii} Walsh, L.P., McCormick, C., Martin, C., Stocco, D.M., 2000. Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. *Environ. Health Perspect.* 108, 769–776
- ^{lxxxviii} Romano, M.A., Romano, R.M., Santos, L.D., Wisniewski, P., Campos, D.A., de Souza, P.B., Viau, P., Bernardi, M.M., Nunes, M.T., de Oliveira, C.A., 2012. Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression. *Arch. Toxicol.* 86 (4), 663–673.
- ^{lxxxix} Romano, R.M., Romano, M.A., Bernardi, M.M., Furtado, P.V., Oliveira, C.A., 2010. Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology. *Archive of Toxicology* 84:309-317

^{xc} Hokanson R, Fudge R, Chowdhary R & Busbee D. 2007. Alteration of estrogen-regulated gene expression in human cells induced by the agricultural and horticultural herbicide glyphosate. *Human and Experimental Toxicology* 26: 747-52.

^{xc}_i Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M.C., Seralini, G.E., 2009. Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology* 262, 184–191

^{xc}_{ii} Paganelli A, Gnazzo V, Acosta H, López SL & Carrasco AE. 2010. Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signalling. *Chemical Research in Toxicology* 23: 1586-95.

^{xc}_{iii} PAN Pesticides Database viewed 24/4/2013

http://www.pesticideinfo.org/Detail_Chemical.jsp?Rec_Id=PC39197

^{xc}_{iv} University of Hertfordshire Pesticide properties Database

<http://sitem.herts.ac.uk/aeru/footprint/en/index.htm>