Glyphosate

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Background

Glyphosate, commonly known by its original trade name Roundup™ (manufactured by Monsanto), is the world's most widely used herbicide. Glyphosate-based herbicides are manufactured by many companies in many countries.

The herbicidal action of glyphosate is primarily due to its capacity to block the production of essential amino acids in plants and some microorganisms through a pathway called "shikimate", which is present only in plants. Thus, it was sold as 'safe' for animals and humans.

Glyphosate is sprayed on numerous crops and plantations, including about 80% of genetically modified (GM) crops (canola, corn, cotton, soybean, sugar beet); with relatively high levels permitted as residues in food and animal feed. It is used as a pre-harvest desiccant, and because it is a systemic herbicide it cannot be completely removed from food by washing, peeling or processing. It is widely used in home gardens and public places including roadsides, and semi-

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natural and natural habitats. Human exposure is widespread and constantly recurring. Residues are widespread in foods, particularly those containing cereals (from pre-harvest use) or GM corn or soy-derived products. It has been detected in drinking water, wine and beer, and even in non-food products derived from GM cotton. The extent of human exposure is reflected in the widespread presence of glyphosate in human urine wherever it has been tested, principally in Europe. It has also been found in urine and breast milk in the USA.

Very aggressive public relations and marketing by its developer, Monsanto, has resulted in the widespread belief that glyphosate is 'safe'. For example, Monsanto claimed that glyphosate is 'biodegradable' and that it 'left the soil clean'. However, in 2009, France's Supreme Court upheld judgements by two previous courts that these claims were false (Anon 2009). Registration processes continue to allow the use of the herbicide without raising concerns about safety even as new data identifying adverse effects emerges.

However, the 2015 classification by the International Agency for Research on Cancer (IARC) of glyphosate as a probable human carcinogen is resulting in massive widespread concern about its continued use, especially preharvest and in public places. Additionally, independent scientific studies and widespread poisonings in Latin America (resulting from aerial application) have begun to reveal numerous acute and chronic effects of glyphosate-based herbicides.

As a result, national bans and restrictions, and voluntary action by local authorities and retailers to curb use are rising dramatically. Sri Lanka was the first country to ban it completely, although that ban may be partially relaxed. The European Union has extended approval for glyphosate for only 18 months instead of the usual 15 years, has banned the use of the surfactant POEA in formulations, and proposed minimised preharvest use and use in public places. The European Food Safety Authority stated that there are so many data gaps for POEA that establishing acceptable exposure limits is impossible. Italy has also banned the preharvest use of glyphosate, its use in public places and those frequented by children and the elderly, and non-agricultural use on soils with high sand content to reduce the potential for contamination of groundwater.

Huge production capacity for glyphosate in China has resulted in the world being oversupplied with the herbicide. Total global production capacity is more than twice the global demand, putting pressure on the industry to decrease prices and disperse GM Roundup Ready crops.

Highly Hazardous Pesticide

IARC's classification of glyphosate as a probable human carcinogen means that it now meets the criteria for a Highly Hazardous Pesticide as defined by PAN (PAN International 2016b) and by FAO/WHO Joint Meeting on Pesticide Management as implemented by FAO in Mozambique (Come et al 2013).

Poisonings

Glyphosate herbicides have been frequently used in self-poisonings and many deaths have occurred, especially in Asia, from as little as 3/4 of a cup of formulated product.

There have also been many cases of unintentional poisonings amongst users and bystanders, the former often experiencing severe chemical burns and respiratory problems.

Widespread poisonings have occurred in Latin America as a result of aerial spraying of GM soybean crops, and of coca crops in Colombia—effects being recorded as far as 10 km away from the supposed spray zone. The coca spraying (instigated by a US government-funded programme to eliminate cocaine production in Colombia) was reported to have also resulted in widespread animal deaths.

Doctors in Argentina report vomiting, diarrhoea, respiratory problems and skin rashes in association with aerial spraying of glyphosate on GM crops. Other acute symptoms of poisoning commonly reported from unintentional exposure include abdominal pain, gastrointestinal infections, itchy or burning skin, skin infections (particularly prevalent in children), blisters, burning or weeping eyes, blurred vision, conjunctivitis, headaches, fever, rapid heartbeat, palpitations, raised blood pressure, dizziness, chest pains, numbness, insomnia, depression, debilitation, difficulty in breathing, respiratory infections, dry cough, sore throat, and unpleasant taste in the mouth. Less common effects reported include balance disorder, reduced cognitive capacity, seizures, impaired vision, smell, hearing and taste, drop in blood pressure, twitches and tics, muscle paralysis, peripheral neuropathy, loss of gross and fine motor skills, excessive sweating, and severe fatigue.

Acute toxicity

Glyphosate has a low toxicity rating (WHO Table 5) despite the substantial evidence of adverse health effects. Surfactants added to formulated glyphosate products may be more toxic: the surfactant POEA present in many formulations is about 5 times more toxic than the glyphosate itself. There are a number of other chemicals added to glyphosate formulations or contaminating them; some are known to be harmful, but many are regarded as trade secrets and it is unknown which might be contributing to the health effects.

Long-term toxicity

Glyphosate-based herbicides can interfere with numerous mammalian organs and biochemical pathways, including inhibition of numerous enzymes, metabolic disturbances and oxidative stress leading to excessive membrane lipid peroxidation, and cell and tissue damage. Genotoxicity and endocrine disruption also lead to chronic health and developmental effects.

Glyphosate has long been known to have antimicrobial properties, and was patented by Monsanto as an antimicrobial in 2010, with claims to be active against a very wide range of organisms. Recent studies show it can cause imbalances in the normal gastrointestinal microbiome, increasing vulnerability to pathogenic bacteria and influencing the response to antibiotics and intestinal functioning, in humans and animals.

Scientists have also found harmful effects on human cells at levels of glyphosate too low to have a herbicidal effect, some at levels similar to those found in food. These effects are amplified by the adjuvants in the Roundup formulation, which assist penetration of the cells by glyphosate. Several researchers have reported that glyphosate appears to accumulate in human cells.

Glyphosate at low concentrations damages liver, kidney and skin cells; in the latter, it causes aging and potentially cancer. Its ability to penetrate skin increases 5-fold when skin is damaged.

Doctors in Argentina have reported a dramatic upsurge in long-term effects in areas where genetically modified soy crops are aerial-sprayed with glyphosate. They include cancer, infertility, pregnancy problems, birth defects, and respiratory diseases.

Kidney

Kidney and liver are the main target organs for glyphosate, and a wide range of adverse effects are reported from laboratory studies, including cell damage and death, DNA damage and tumours. Glyphosate is implicated in an epidemic of 'chronic kidney disease of unknown cause' (CKDu) amongst farmers in Sri Lanka, Andhra Pradesh (India), and Central America, in part because of the herbicide's ability to chelate nephrotoxic metals.

Cancer, genotoxicity

The IARC monograph on glyphosate, published in 2015, concludes that "there is limited evidence in humans for carcinogenicity of glyphosate" and "there is sufficient evidence in experimental animals for the carcinogenicity of glyphosate". Besides evidence from carcinogenicity studies in rats and mice, the IARC considered as a rationale "two key characteristics of known human carcinogens" and concluded that there is strong evidence that exposure to glyphosate or glyphosate-based formulations is genotoxic and can induce oxidative stress. The latter mechanism was also ascribed to aminomethylphosphonic acid (AMPA), the major metabolite of glyphosate. As a result the IARC classified glyphosate as probably carcinogenic to humans (Group 2A).

In the same year, the European Food Safety Authority (EFSA) insisted on its evaluation that glyphosate is neither carcinogenic nor genotoxic, thereby joining similar assessments made earlier by the International Programme on Chemical Safety (IPCS) and the United States Environmental Protection Agency (US EPA). This occurs in spite of substantial laboratory and some epidemiological evidence that continues to accumulate and points to the opposite conclusion.

The evaluation of glyphosate by the European Chemicals Agency (ECHA) is still ongoing. Final results are expected by end of 2017 when the extension of the current approval for glyphosate in the European union also expires.

Studies have demonstrated that glyphosate and/or Roundup cause genetic damage in human lymphocytes and liver cells; bovine lymphocytes; mouse bone marrow, liver, and kidney cells; fish gill cells and erythrocytes; caiman erythrocytes; tadpoles; sea urchin embryos; fruit flies; root-tip cells of onions; and in *Salmonella* bacteria. Other studies have shown that it causes oxidative

stress, cell-cycle dysfunction, and disruption to RNA transcription, all of which can contribute to carcinogenicity.

Several epidemiological studies have linked exposure to glyphosate with non-Hodgkin's lymphoma, hairy cell leukaemia, multiple myeloma, and DNA damage.

Glyphosate and Roundup caused DNA damage in human buccal cells, and was clastogenic in mouse bone marrow cells, adding to a number of previous studies showing it to be genotoxic.

Endocrine disruption

A number of studies have demonstrated that both glyphosate and the Roundup formulation disrupt oestrogen, androgen, and other steroidogenic pathways, and cause the growth of human breast cancer cells.

One study summarises these effects occurring at doses substantially lower than those used in agriculture, or permitted as residues: at 0.5 mg/kg (40 times lower than levels permitted in soybeans in the US) they were anti-androgenic; at 2 mg/kg they were anti-oestrogenic; at 1 mg/kg they disrupted the enzyme aromatase; at 5 mg/kg they damaged DNA, and at 10 mg/kg they were cytotoxic. These effects can result in adverse effects in sexual and other cell differentiation, bone metabolism, liver metabolism, reproduction, development and behaviour, and hormone-dependent diseases such as breast and prostate cancer (Gasnier et al 2009).

In vivo experiments in rats show that low levels of glyphosate-based herbicides disrupt the production of testosterone, oestradiol and other steroid hormones, down-regulate the expression of oestrogen progesterone receptors, induce the aromatase activity and protein levels in the testis and cause abnormal sperm morphology.

The implications of the endocrine-disrupting effects can be profound and far-reaching, involving a range of developmental impacts including sexual and other cell differentiation, bone metabolism, liver metabolism, lipid metabolism, reproduction, pregnancy, growth, brain and organ development, cognition, behaviour, and endocrine-related diseases such as breast, testicular and prostate cancer, neurodegenerative and metabolic disorders (diabetes, obesity).

Reproductive and developmental

Exposure to glyphosate-based herbicides, even at very low doses, may result in reproductive problems including miscarriages, pre-term deliveries, low birth weights, and birth defects. Laboratory studies have shown that very low levels of glyphosate, Roundup, POEA, and the metabolite AMPA all kill human umbilical, embryonic and placental cells. Roundup can kill testicular cells, reduce sperm numbers, increase abnormal sperm, retard skeletal development, and cause deformities in amphibian embryos.

Monsanto has known since the 1980s, and the German government since 1998, that glyphosate causes birth defects. After analysing the industry data reported in the German authorities 1998 draft assessment report, independent scientists concluded: "a substantial body of evidence demonstrates that glyphosate and Roundup cause teratogenic effects and other toxic effects on reproduction", including heart, kidney, skeletal, lung and cranial problems (Antoniou et al 2012).

More recent studies show malformation in the heads of frogs that are similar to birth defects amongst people exposed to aerial spraying of Roundup over GM soy crops in Latin America.

Neurological

Glyphosate is assumed by regulators to have no neurological effects—the US EPA did not require neurotoxicity studies to be carried out for the registration of Roundup. However, a number of studies have shown that glyphosate can adversely affect nerve cells and affect neuronal development. There is emerging evidence that glyphosate can affect areas of the brain associated with Parkinson's disease, particularly the dopaminergic neurons. Epidemiological and case studies link glyphosate exposure with parkinsonian, Attention-Deficit/ Hyperactivity Disorder (ADHD) and autism.

Immune

Several studies indicate that glyphosate formulations may interfere with the immune system resulting in adverse respiratory effects including asthma, rheumatoid arthritis, and autoimmune skin and mucous membrane effects.

Environmental effects

Glyphosate has direct eco-toxicological effects and indirect effects. The later result from the unprecedented elimination of flora termed weeds. Direct and indirect effects have cascading impacts on the food chain and on biodiversity. Ecosystem functions of insects, such as natural pest control and pollination services, are jeopardised by the almost complete elimination of weeds because these plants are essential to most beneficial species. This may lead to huge difficulties in returning to ecologically sound agricultural systems. In aquatic ecosystems, the direct eco-toxicological effects of glyphosate of greatest concern are those that occur at a subtle level, which can result in significant disruption of the ecosystem.

Aquatic effects

Glyphosate is water soluble, and is increasingly found in the environment at levels that have caused significant effects on species that underpin the entire aquatic food chain. Glyphosate and/or Roundup can alter the composition of natural aquatic communities, potentially tipping the ecological balance and giving rise to harmful algal blooms. It can have profound impacts on microorganisms, plankton, algae and amphibia at low concentrations: one study showed a 70% reduction in tadpole species and a 40% increase in algae. Insects, crustaceans, molluscs, reptiles, and fish can also be affected, with vulnerability within each group varying dramatically between species. Effects include reproductive abnormalities, developmental abnormalities and malformations, DNA damage, immune effects, oxidative stress, modified enzyme activity, decreased capacity to cope with stress and maintain homeostasis, altered behaviour, and impaired olfaction that can threaten their survival. Amphibians are particularly vulnerable. Roundup is generally more toxic than glyphosate, especially to fish.

Terrestrial effects

Soil & plant health

As with the aquatic environment, it is the subtle effects causing disruption of the ecosystem that are of greatest concern, particularly effects on the agroecosystem. Glyphosate is toxic to some but not all soil microorganisms, altering microbial community dynamics in ways that are harmful to plants and to ecological balance. It increases microorganisms capable of metabolising the chemical. It can reduce some beneficial organisms such as saprophytic fungi that decompose dead plant material and are important for soil fertility. Numerous studies have shown that glyphosate stimulates the growth of a number of fungal pathogens that cause diseases in many crops. The upsurge in use of glyphosate in no-till agriculture has brought about a resurgence of some diseases. Glyphosate binds micronutrients in the soil and causes micronutrient deficiencies in plants that increase their susceptibility to disease, decrease their vigour, and produce micronutrient-deficient food crops. It can reduce the plant's production of lignin and phenolic compounds, which are also important for disease resistance. It can reduce nitrogen fixation in legumes such as soybean.

Glyphosate can cause metabolic and compositional changes, including altering the nutritional composition of foods, for example the protein and fatty acid content of soybeans. It can cause iron deficiency in soybeans, which is a concern for human health as human iron deficiency is widespread.

Earthworms and beneficial insects

Glyphosate has adverse effects on some earthworms; and a number of beneficial insects useful in biological control, particularly predatory mites, carabid beetles, ladybugs, and green lacewings. It can also adversely affect other insects that play an important part in ecological balance such as springtails, wood louse, and field spiders. Glyphosate, at levels commonly found in agricultural settings, impairs honeybees' cognitive capacities affecting their navigation with potential long-term negative consequences for colony foraging success

Birds and other animals

Glyphosate use may result in significant population losses of a number of terrestrial species through habitat and food supply destruction. There have been reports of numerous deaths of livestock and domestic animals as a result of the aerial spraying of glyphosate in Colombia.

Environmental fate

Glyphosate is a widespread environmental contaminant found in soils and sediments, a wide range of surface water bodies, groundwater and the marine environment.

Soils

The European Food Safety Authority (EFSA) describes glyphosate persistence in soil as being low to very high, and that of AMPA as

being moderate to very high, with a half-life varying from less than a week to more than a year and a half, depending on the extent of soil binding and microbial breakdown (glyphosate is broken down by microbial degradation). Residues have been found up to 3 years after application in cold climates. It is less persistent in warmer climates, with a half-life between 4 and 180 days. It is bound onto soil particles, and this was once thought to mean that glyphosate is not biologically active within soil, nor will it leach to groundwater. However, it is now known that it can easily become unbound again, be taken up by plants or leach out, indicating a greater risk of groundwater contamination.

Phosphate fertilisers reduce binding of glyphosate to soil particles, and so increase the amount of unbound glyphosate remaining in the soil, which is available for root uptake, microbial metabolism, and leaching into groundwater. The risk of leaching is greater in fertilised soils. Conversely, the presence of glyphosate in some soils can reduce retention and availability of phosphate reducing soil fertility.

Water

Glyphosate is soluble in water, and slowly dissipates from water into sediment or suspended particles. Although it does break down by photolysis and microbial degradation, it can be persistent for some time in the aquatic environment, with a half-life of up to nearly 5 months, and still be present in the sediment of a pond after 1 year.

Residues of glyphosate have been found in a wide range of ditches, drains, streams, rivers, ponds, lakes and wetlands in many countries including Argentina, Canada, China, throughout Europe, Norway, USA, and the UK; in wastewater in France and Canada, landfill leachate in the UK. Urban use on road and rail sides is contributing significantly to this contamination, with residues being found in sewage sludge and wastewater treatment plants. Contamination of 'vernal pools'—pools that are shallow and disappear in dry weather—are a concern for amphibia, for which these water sources are critical.

Residues have also been found in groundwater in Canada, Austria, Belgium, Denmark, Germany, Ireland, Spain, Sweden, Switzerland, Netherlands, UK, Sri Lanka, and USA. They have been detected in the marine environment off the Atlantic Coast of France; and in marine sediment in New Zealand, believed to have come largely from the spraying of urban roadside vegetation.

Bioaccumulation

EFSA gives a bioconcentration factor (BCF) of 1.2 (+ 0.61). However, bioaccumulation of glyphosate may be greater than predicted). The BCF for glyphosate is increased in the presence of POEA in the aquatic environment. This may be because POEA, which is known to enhance glyphosate transport into plant cells, also facilitates increased permeability in animal cells. A BCF for glyphosate varying between 1.4 and 5.9 was found in freshwater blackworm. Bioaccumulation has also been demonstrated in land snails, fish, aquatic plants. There are also suggestions of bioaccumulation in some human cell studies.

Atmospheric transport and deposition

Glyphosate is of low volatility, and residues in the air have been found in particulate matter, suggesting that airborne transport is via particles with deposition being largely in dust rather than vapour. It has been found in the rain in Belgium, Canada, France, and USA.

Resistance

Weed resistance to glyphosate was first recorded in 1996, in Australia; it is now recorded in 35 species of weeds and in 27 countries, most notably the USA.

Most of this resistance has been caused by the repeated use of glyphosate in GM crops, no-till agriculture, and amenity use. Some has resulted from a gradual evolution of exposed weed species, and some from gene flow from GM crops to weed relatives. The latter has been observed with sugar beet in France, canola in Canada, creeping bentgrass in USA, and also with corn and soybean. Now even Monsanto is recommending the use of other herbicides in addition to glyphosate in Roundup Ready crops (crops genetically modified to be tolerant of Roundup), to slow the onset of resistance in weeds.

So widespread is the resistance now that Dow has developed a GM corn resistant to both 2, 4-D and glyphosate, and Monsanto to develop a soybean resistant to both dicamba and glyphosate.

Climate Change effects

A number of glyphosate's adverse effects can be expected to increase with climate change: higher

temperatures enhance glyphosate's reduction of chlorophyll and carotenoids in freshwater green algae, increase toxicity to fish, and increase susceptibility to *Fusarium* head scab in cereals.

One study has shown that increased levels of carbon dioxide can result in increased tolerance of some grasses to glyphosate, indicating that as climate change progresses, grasses may become less susceptible to the herbicide.

Alternatives

There are numerous design, mechanical and cultivational practices, as well as some non-chemical herbicides based on plant extracts that can be used instead of glyphosate herbicides, depending on the weed species and the situation. Care must first be taken to determine whether the plant regarded as a weed is in fact really a problem to production, or should be regarded as a non-crop plant with beneficial uses or ecosystem services.

Chemical Profile

Identification

Common name

Glyphosate

Common trade name

Roundup

Chemical names and form

N-(phosphonomethyl)glycine

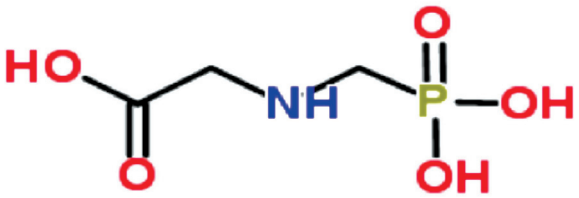
Glyphosate is a weak organic acid that consists of a glycine moiety (part of a molecule) and a phosphonomethyl moiety.

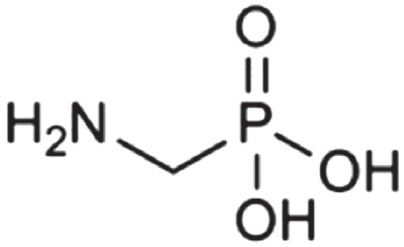
Technical grade glyphosate is a colourless, odourless crystalline powder, formulated as water-soluble concentrates and granules.

Most formulations contain the isopropylamine ammonium salt of glyphosate (glyphosate-isopropyl ammonium).

Molecular formula and structure

$C_3H_8NO_5P$





aminomethylphosphonic acid

Chemical group

Phosphinic acid

Other related chemicals

Glyphosate, diammonium salt
Glyphosate, dimethylammonium salt (glyphosate dimethylamine)
Glyphosate, ethanolamine salt
Glyphosate, monoammonium salt (glyphosate sel d'ammonium)
Glyphosate, potassium salt
Glyphosate, sesquisodium (or sodium) salt
Glyphosate, trimethylsulfonium salt (glyphosate-trimesium)

CAS numbers

Glyphosate	1071-83-6
Isopropylamine salt	38641-94-0
Monoamine salt	114370-14-8
Diammonium salt	69254-40-6
Sesquisodium salt	70393-85-0
Glyphosate-trimesium	81591-81-3
(Aminomethyl)phosphonic acid	1066-51-9

Trade names

Because glyphosate is so widely used and is off-patent, there are now many generic formulations and a very large number of trade names.

In many cases glyphosate formulations can be identified by G360, G450, G510, or G580, preceded by a trader's name. The number indicates the concentration of glyphosate in the formulation, i.e. G360 has 360 g/L of glyphosate.

In some cases, only the term 'Herbicide' is used, preceded by a variety of names such as Farmers Own, Growers, Harvest, etc.

Others make a play on the original product 'Roundup' by including 'up' in the name (Bright Up, Conto-Up, Dry-Up, Farm Up, Foldup, Ken-Up, Kleenup, Klin-Up, Move-Up, Set-Up, Sunup, Take-Up, Touch Up, Wes-Up, Zap Up); or the opposite, 'down' (Touchdown, Turndown); or 'round' (Myround, Roundsate, Seround, Weed Round).

Some names are variations of the word glyphosate (Glifocafe, Glifolag, Glifosate, Glifosato, Glifosol, Glyfo, Glyfosaat, Glyfosat, Glymax, Glyfoglex, Glofonex, Glyphogan, Glyphos, Glyphosat, Glyphotis); use the last syllable of glyphosate (Ancosate, Coopersate, Envisate, Farmfosate, Gofosate, Herbisate, Ken-phosate, Masate, Megasate, Narscosate, Pilarsate, Sulfosate, Sulfosato, Sunphosate, Supresate, Tec-forsate, Vefosate); or use the chemical constituent glycine (Glyacid, Glycel, Glycin).

Many other trade names bear no distinguishable relationship to Roundup or glyphosate. Some of these attempt to present a benign image (Aglow, Credit, Ecomax, Estelar, Esteem, Glyphos Bio Granny's Herbicide, Green fire, Lotus, Rainbow, Spirit, S-Star, Touchdown, Vision); but many more do just the opposite (Ammo, Armada, Arrow, Assassin, Avenger, Challenge, Clampdown, Decimate, E-Kill, Fire, Frontier, Harass, Hatchet, Knockout, Monster, Mustang, Panzer, Pounce, Punch, Q-Weapon, Raider, Rambo, Rival, Rodeo, Salute, Samurai, Scud, Sentry, Shoot, Siren, Slash, Smash, Squadron, Stampede, Sting, Swing, Terminator, Thunder, Tomahawk, Trounce, Turbo, Typhoon, Victorious, Wallop, Wipe-out).

Others just try to indicate the product kills weeds (Weedact, Weed All, Weedcut, Weed-go, Weedex, Weed Hoe, Weedkiller, Weedo, Weed Round, Weego).

Many more trade names are in local languages.

Some formulations combine glyphosate with other herbicides such as aminopyralid (Broadnet), 2,4-D (Bimasta, Campaign, Evo, Hat-trick, Kontraktor, Landmaster), dicamba (Fallowmaster), diquat (A-13692B), imazapyr (Tackle, Imasate), MCPA (Fusta, Rapid, Rextor, Panton), metsulfuron-methyl (Fusion), picloram (Fusta), pyrithiobac sodium (Staple Plus, a pre-plant herbicide for glyphosate-resistant soybeans), simazine (Ricochet), terbuthylazine (Folar, Terminate), and triclopyr (Glytron). The formulation Tag G2, registered in New Zealand, contains glyphosate, amitrole, oxyfluorfen, and terbuthylazine.

'Inert' ingredients and contaminants

Glyphosate formulations may contain a number of so-called 'inert' ingredients, most of which are not publicly known as, in many countries, the law does not require that they be revealed. Some information is available about formulations sold in the US, and the following list of 'inerts', provided by Cox (2004), can found in products in many other countries. The list is not exhaustive; it is indicative only. In addition, adjuvants may be added to glyphosate formulations prior to use, to improve their efficacy against weeds by enhancing penetration of glyphosate into the target plant. However, many of these may also increase the toxicity of glyphosate to other species; for example organosilicone surfactants, described as the most potent of adjuvants, and commonly added to glyphosate formulations, is now linked to decline in honeybees in the US (Mullin et al 2016).

POEA (*polyoxyethylene alkylamine; POE-tallowamine*)

- eye irritant, toxic to aquatic organisms
- penetrates cell membranes, disrupting their structure and function

This derivative of tallow, a fat rendered from cattle and sheep fat, is the most well known 'inert' contained in the original Roundup formulation, and many others. Registration data in New Zealand showed Roundup contained 18% POEA (Watts 1994). Other formulations may contain much higher levels, even as high as 60-80%, as in the formulation Genamin (Mesnage et al 2013a).

Propylene glycol

- genetic damage, reduced fertility, and anaemia in laboratory tests

Glycerine

- genetic damage in human cells and laboratory animals
- reduced fertility in laboratory animals

Sodium sulfite
- genetic damage in human cells and laboratory animals

Sodium benzoate
- genetic damage in human cells and laboratory animals
- developmental problems and reduced newborn survival in laboratory animals

Sorbic acid
- severe skin irritant
- genetic damage in laboratory tests

Sodium salt of o-phenylphenol
- skin irritant
- genetic damage and cancer in laboratory animals

Light aromatic petroleum distillate
- reduced fertility and growth of newborns in laboratory animals

Methyl p-hydroxybenzoate
- genetic damage in laboratory animals

3-iodo-2-propynyl butyl carbamate
- thyroid damage and decreased growth in laboratory animals

5-chloro-2-methyl 3(2H)-isothiazolone
- genetic damage and allergic reactions in laboratory tests

Other constituents of surfactants recommended for use with Monsanto's Rodeo formulation include:
- polyol fatty acid esters
- polyoxyethyl polyol fatty acid esters
- paraffin base petroleum oil
- propionic acid
- alkylpolyoxyethylene ether
- octylphenoxypolyethoxyethanol – skin and eye irritant
- n-butanol
- compounded silicone
- nonylphenoxypolyethoxyethanol – also used as a spermicide
- silicone antifoam compound
- isopropanol
- polydimethylsiloxane
  (Diamond & Durkin 1997)

Impurities found in technical grade glyphosate include *N*-nitroso-*N*-phosphonomethyl-glycine (also called *N*-Nitrosoglyphosate) (US EPA 1993). *N*-nitroso compounds are "genotoxic, carcinogenic to animals, and may play a role in human cancer development" (Hebels et al 2009). EFSA (2015b) also identifies formaldehyde as a low-level contaminant. Formaldehyde is a known human carcinogen (NTP 2014).

Registration data for Roundup in New Zealand showed the presence of 1% sulphuric acid and trace amounts of phosphoric acid (Watts 1994).

POEA is contaminated with 1,4-dioxane, reported at levels of 0.03% by the US EPA in 1991. This substance causes liver and nasal cancer in laboratory rodents (NTP 2005; Kano et al 2009) and is "reasonably anticipated to be a human carcinogen" (NTP 2005).

POEA

There are numerous studies showing that POEA is toxic; and often more toxic than glyphosate itself – the latter has been demonstrated in rats (acute toxic – Williams et al 2000), fish (oxidative stress, developmental toxicity – Williams et al 2000; Navarro & Martinez 2014); freshwater mussel (acute toxicity – Bringolf et al 2007); and microalgae and aquatic protozoa (acute toxicity – Tsui & Chu 2003).

In a comparative evaluation on aquatic species, Martin et al (2003) found that generally the toxicities are in the order of POEA > Roundup > glyphosate acid > salt of glyphosate. Servizi et al (1987) demonstrated that the acute toxicity of POEA to fish was more than 30 times that of glyphosate itself.

In studies using hepatic (HepG2), embryonic (HEK293) and placental (JEG3) cell lines to compare 10 formulations of glyphosate,[1] the most toxic formulations were those that contained POEA. POEA induced necrosis and disrupted the structure and function of cell membranes, with negative dose-dependent effects on cellular respiration and membrane integrity between 1 and 3 mg/L (Mesnage et al 2013a). It potentiates the effect of glyphosate, facilitating its penetration of cell membranes and bioaccumulation in cells (Richards et al 2005). The BCF for glyphosate is increased in the presence of POEA in the aquatic environment (Annett et al 2014). See Environmental Fate section for more on bioaccumulation of glyphosate.

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[1] Bayer GC, Clinic EV, Genamin T200, Glyphogan, Roundup Grand Travaux, Roundup Grand Travaux Plus, Roundup Ultra, Roundup Bioforce, Roundup 3plus, Topglypho.

In the late 1990s, the German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV) called upon Member States in the EU not to accept glyphosate products containing POEA because of its cytotoxicity; Germany finally removed them from their market in 2014 (Farrer & Falck 2014), and the EU banned them in 2016 (EC 2016). In 2015, EFSA, in response to a request to assess the risk of POEA as a co-formulant with glyphosate, had stated that it was unable to asses the risk to human and animal heath because of lack of or inadequate data on: dermal absorption, genotoxicity, long-term toxicity, carcinogenicity, reproductive / developmental toxicity, endocrine disrupting potential (particularly thyroid), residues in plants and livestock, fate and behaviour in the environment, and eco-toxicological properties. It therefore could not establish Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL), and Acute Reference Dose (ARfD). It did, however, note the higher toxicity of POEA compared with glyphosate on all endpoints; and that the data available "show that it should be classified as harmful if swallowed and possibly harmful if inhaled, causing severe eye irritation, skin irritation and skin sensitisation" (EFSA 2015c). EFSA (2015c) also noted that the formulation submitted to it for reapproval of glyphosate did not contain POEA.

It is not generally known which formulations of glyphosate contain POEA, but its use is believed to be widespread. For example, the New Zealand Environmental Protection Agency (NZ EPA) has current approvals for 91 formulations of glyphosate of which 69 contain POEA. It refuses to name them "because the composition of the formulations is commercial-in-confidence information" (NZ Parliament 2016). This situation is likely to be typical of many countries. The decree that banned the use of glyphosate formulations containing POEA in Italy named 55 formulations, including well known names such as Roundup, Rodeo, and Touchdown, and brands from Cheminova, Syngenta, Nufarm, Dow AgroSciences, and Arysta, as well as Monsanto and some Italian companies (MdS 2016).

Other inerts and adjuvants

TN-20, a surfactant common in glyphosate formulations, caused cell death and mitochondrial damage in rat cells, which disrupts the integrity of the cellular barrier to glyphosate and promotes its toxicity (Kim et al 2013).

The adjuvant Impacto (alkyl aryl polyglycol ether or alkylphenol ethoxylate), which is usually combined with glyphosate formulations in Argentina, increased cytotoxicity and oxidative stress of the glyphosate formulation Atanar in the human cell line HEp-2 (Coalova et al 2014).

	LC_{50} (mg/L) 24 hr	**NOEC (mg/L) 24 hr**
Atanor	376.4	300
Atanor + Impacto	4.7	2.4

Defarge et al (2016) found that 6 glyphosate formulations and 5 of their co-formulants decreased aromatase activity in human placental cell at concentrations much lower than for glyphosate alone.

Martini et al (2016) demonstrated that adjuvants other than POEA inhibited proliferation and differentiation of mammalian 3T3-L1 fibroblasts to adipocyctes.

It is clear, then, that exposure to a glyphosate-based herbicide entails exposure to a wide range of other chemicals as well as the glyphosate, about which little information is available and the full health effects of which have not been established. Some, such as POEA, are known to be more acutely toxic than the glyphosate itself. Others are clearly capable of causing serious chronic effects.

However, it should not be forgotten that glyphosate by itself is still toxic, causing a wider range of effects on humans and the environment.

Metabolites

The main metabolite of glyphosate is (aminomethyl)phosphonic acid (AMPA), with some glyoxylic acid (which further breaks down to carbon dioxide). A minor pathway has been identified in isolated soil bacteria, in which glyphosate breaks down to sarcosine and then to glycine (Annett et al 2014).

N-acetyl-glyphosate (also called *N*-acetyl-*N*-(phosphonomethyl)glycine) is a metabolite formed when glyphosate is applied to genetically modified 'Optimum Gat' soybean (FR 2008). It is assumed by the US EPA (2008) to be "toxicologically equivalent to glyphosate".

N-acetyl-glyphosate is in turn metabolised to *N*-acetyl (aminomethyl) phosphonic acid (*N*-acetyl- AMPA) - which is considered by the US EPA to be of low toxicity and "of limited concern" (FR 2008).

EFSA identified a gap for toxicological data on the metabolites N-acetylglyphosate and N-acetyl-AMPA, which are included for residue definition in GM glyphosate-tolerant plants.

Mode of action in weeds

The commonly accepted explanation of glyphosate's mode of action is as follows: glyphosate inhibits the enzyme 5-enolpyruvyl-shikimate 3-phosphate synthase, which is essential for the formation of aromatic amino acids (phenylalanine, tyrosine, tryptophan) in plants, by what is commonly referred to as the shikimic pathway. Without amino acids the plants cannot make protein; growth ceases, followed by cellular disruption and death. The shikimic pathway is not found in the animal kingdom, hence glyphosate was once thought to be "relatively non-toxic to mammals" (Anadón et al 2009).

However, the mechanisms leading to plant death may also be related to secondary or indirect effects of glyphosate and AMPA, which is phytotoxic, on plant physiology (Gomes et al 2014). Glyphosate forms complexes with some metal ions (though chelation), making them unavailable to plants and other organisms and thereby disrupting vital metabolic processes including enzyme activities. For example, it forms a complex with cobalt, an activator of some enzymes in higher plants (Ganson & Jenson 1998). Glyphosate also chelates copper, zinc, magnesium, calcium, cadmium, lead, manganese, nickel, and iron. Calcium and magnesium have the weakest chelating tendencies and copper the strongest of those tested (Lundager Madsen et al 1978; Glass 1984; Motekaitis & Martell 1985; Undabeytia et al 2002). Glyphosate was originally patented as a chelator by Stauffer Chemical Co in 1964, and used as a descaling agent to remove calcium and other mineral deposits from pipes (Jayasumana et al 2014).

A third mode of action that contributes to glyphosate's effects on plants is its impact on microbial communities. After glyphosate is absorbed through the foliage, it is translocated within the plant, down to the roots and released into the rhizosphere (soil surrounding the roots) (Kremer & Means 2009), where it disrupts the soil and root microbial community. As much as 80% of glyphosate absorbed after foliar application is translocated to the shoot apex and root tips (Cakmak et al 2009). Glyphosate's herbicidal action is now suggested to be in part due to, on the one hand, stimulation of soil-born pathogens which colonise the roots of the plants, and on the other hand the reliance of many plant defences on the shikimic acid pathway—so that the combination of increased pathogens and increased susceptibility to them is an important element in the death of the plant (Johal & Huber 2009). As far back as 1984 Johal & Rahe demonstrated that the death of bean plants treated with glyphosate resulted from parasitisation by fungal root rot pathogens in the growth medium (refer to section on Plant diseases for more on this).

Glyphosate can also reduce plants' ability to photosynthesise (Mateos-Naranjo & Perez-Martin 2013) by significantly reducing chlorophyll content (Kitchen et al 1981; Pline et al 1999; Eker et al 2006), and by impairing carbon metabolism through interference with sugar metabolism and translocation. It also causes oxidative stress (Gomes et al 2014).

Uses

Glyphosate is the world's most heavily used pesticide. Estimated global usage was 8.6 million tonnes from 1974 to 2014, with use rising 15-fold since GM Roundup Ready (RR) crops were introduced in 1996. Since the introduction of RR crops, glyphosate has been sprayed more intensely, i.e. more applications per hectare of a crop in a year with higher application rates. But global non-agricultural uses have also risen 5-fold since the introduction of GM crops (Benbrook 2016).

Glyphosate is a broad spectrum (non-selective), systemic, post-emergence herbicide used to control annual and perennial plants including grasses, sedges, broadleaf weeds and woody plants. It is used for crops, orchards, glasshouses, plantations, vineyards, pastures, lawns, parks, golf courses, forestry, roadsides, railway tracks, industrial areas, and home gardening; for root sucker control; and for weed control in aquatic areas.

It is also used for pre-harvest desiccation of cotton, cereals, peas, beans, and other crops, resulting in elevated residues in food and animal fodder from these crops. Germany has prohibited pre-harvest desiccation since May 2014 (Benbrook 2016). In the process known as hormesis, glyphosate at sublethal levels can actually stimulate plant growth and yield in older plants (Belz & Duke 2014). The sodium salt (e.g. Quotamaster) is used as a growth regulator on sugar cane—to hasten ripening, enhance sugar content, and promote earlier harvesting—and on peanuts.

Glyphosate has also been used to destroy drug crops grown in Colombia. Beginning in 2000, the USA funded the Colombian government to aerial spray crops of coca and opium—in 2006 alone 171,613 hectares were sprayed (Leahy 2007).

Weak solutions of the Roundup formulation are used to devitalise some plant material before importation into Australia and New Zealand to reduce biosecurity risks by preventing propagation of the plant material. For example, the New Zealand biosecurity authority required that the stems of cut flowers and foliage are immersed to within 50 mm of the flower in a 0.5% solution of Roundup for 20 minutes—this reputedly prevents propagation but allows about a week of shelf life (MPI 2014).

Glyphosate is patented as a synergist for mycoherbicides (natural fungi) used for biological control of weeds, as it enhances the virulence of the fungi (Johal & Huber 2009).

Glyphosate is applied by a wide range of methods, including aerial spraying, backpack sprayers, ground broadcast sprayers of various types, shielded and hooded sprayers, wiper applications, sponge bars, injection systems, and controlled droplet applicators.

The main drivers for global glyphosate use in recent years have been no-till farming, biofuels production and, especially, the development of plants genetically modified to be tolerant of glyphosate (CCM International 2009). The growing of GM corn, cotton, and soybean in the USA is credited with the 8-fold increase in glyphosate use between 1995 and 2005 (Johnson et al 2009).

Genetically modified (GM) crops

The first glyphosate-tolerant (Roundup Ready) crop was soybean, introduced in the United States in 1996 (Dill et al 2008); and soybean remains the crop with the highest use of glyphosate in USA at nearly double the amount used of the next largest crop, corn (Benbrook 2016). Roundup Ready maize and cotton varieties were also approved in the US in 1996, alfalfa in 2005 and sugar beet in 2008, but lawsuits delayed the release of the last 2 until 2011 and 2012 respectively. Globally, soybean still leads, but maize is second, followed by canola and cotton at a distant 4th (Benbrook 2016), grown mainly in USA, Canada, Argentina, Brazil, and Paraguay (Villar & Freese 2008).

By 2013, global GM crop acreage was 175.2 m ha, confined to only 27 countries (19 developing and 8 industrial), with the US alone accounting for more than a third of all GM plantings, at 68.4 m ha – that's 84.4% of all GM crops in industrial countries, and half of that country's cultivable land. Herbicide tolerant crops, the vast majority of which are glyphosate-tolerant, occupy 100 m ha (Ho 2014). However, figures from the biotech industry body International Service for the Acquisition of Bio-Tech Applications (ISAAA 2016a), shows global GM crop acreage peaking in 2014 and decreasing in 2015.
By 2009, about 95% of Argentina's annual crop of soybean was Roundup Ready; 200 million litres of glyphosate were applied to it every year, mainly by aerial spraying. This monocultural soybean was being grown on 42 million acres, accounting for nearly 50% of all farmland in Argentina (Trigona 2009; Valente 2009).

Bolivia also grows GM soybean on about 50% of its cropland; Paraguay grows about 15 m ha of GM soybean, maize and cotton (ISAAA 2016b); and Australia grows glyphosate-tolerant cotton and canola (ISAAA 2008). In 2015, almost 100% of the (270,000 hectare) Australian cotton crop was glyphosate-tolerant (Brookes 2016).

GM crops occupy 11.45% of cultivable land globally, compared with non-GM crops, which occupy 88.55% and are gown in about 196 countries. A number of countries have imposed explicit bans on GMOs, including 8 EU countries, Kenya and Russia (Ho 2014), and in 2012 Peru imposed a 10-year ban. Herbicide-tolerant crops have been banned in India (Ho 2013, 2014).

Increasing resistance of weeds to glyphosate (see section on Resistance) resulting from its severe overuse, has caused companies to look elsewhere for other herbicides to compliment glyphosate. Dow Agrosciences has developed a corn variety, Enlist Duo, which is tolerant of both glyphosate and 2,4-D. It was approved in the US in 2014. Registration was revoked in 2015 after a legal challenge (Towers et al 2015), but it was expected to be available again in 2016 (Dow Agrosciences 2015).

Meanwhile, Monsanto has developed a soybean seed tolerant of both glyphosate and dicamba, and has signed an agreement with DuPont for the supply of dicamba to combat glyphosate-resistant weeds in Roundup Ready soybean crops (Monsanto 2016).

Figures published in 2016 indicate that, over time, adopters of glyphosate-tolerant technology in soybean production used increasingly more herbicides compared to non-adopters, with the emergence of glyphosate resistance in weeds.

On average, adopters of glyphosate-tolerant soybeans used 28% (0.30 kg/ha) more herbicide than non-adopters, over the period 1998 to 2011 (Perry et al 2016).

Manufacturers

Glyphosate was first synthesised in Switzerland in 1950 by a Swiss chemist, Dr Henry Martin, working for a pharmaceutical company; the molecule was on-sold through a series of companies and tested for possible uses, until herbicidal action was identified by Dr John Franz, a scientist working with Monsanto Company of St Louis, Missouri, USA in 1970 (Benbrook 2016), and developed into the herbicide Roundup in 1974 (Monsanto undated).

Global production capacity is currently estimated to be around 1 million tonnes; actual production is 800,000 tonnes, split roughly 50/50 between Monsanto and China (PR Newswire 2016). Other producers include Nufarm, Syngenta, Dow AgroSciences and DuPont. In 2011 China's capacity alone was 835,900 tonnes, more than enough to meet global demand, and so there is huge over supply capacity. Hence there is significant pressure to decrease prices and to disperse Roundup Ready GM crops (Szekacs & Davas 2011).

Regulatory status

Glyphosate was first registered in the USA in 1974; it is now registered worldwide and is the most commonly used herbicide, especially on GM crops.

International regulatory action

None taken to date.

National regulatory action

Following the classification by the International Agency for Research on Cancer (IARC) of glyphosate as a probable human carcinogen, there has been a huge upsurge in regulatory and voluntary action against glyphosate.

In 2016, the European Union, due to renew its approval of glyphosate for another 15 years, amidst exceptional controversy extended the approval for only 18 months, banned the use of the surfactant POEA, and required minimised use in public places and minimise pre-harvest use (EC 2016). In the Standing Committee for Plants, Animals, Food and Feed (PAFF Committee), France, Germany, Greece, Portugal, Austria, Luxembourg and Italy all abstained in the vote to reapprove it, and Malta voted against it (Bos 2016). Malta has subsequently announced that it will be the first EU country to completely ban glyphosate (Pohlman 2016).

In May 2015, Bermuda suspended for 6 months the import of all glyphosate products, effective immediately, and pending further research. In February 2016, the ban was relaxed for ready-to-use products but not for concentrated forms. The government then instituted a monitoring process for residues in air, groundwater, soils, pond sediments, and GM foodstuffs in supermarkets (Government of Bermuda 2016; undated: Bernews 2016).

On May 27th 2015, Sri Lanka banned the import and use of glyphosate because of concerns about the high rate of kidney disease amongst farmers (Karunanayake 2015). However, it appears that the ban may be partially relaxed to allow use in tea plantations (Lankaweb 2016).

In September 2013, El Salvador's National Assembly approved the amendment of a law that would ban glyphosate, amongst 52 other pesticides. The Committee on the Environment and Climate Change in Congress set a deadline of 2 years for its phase out. However, the ban was not actioned, and in May 2016, members of the ruling party again took up the issue of the proposed banning of the 53 pesticides because of concerns about effects on agriculture (CentralAmericaData.com 2013, 2016).

Restrictions

In August 2016, Italy banned the use of glyphosate in public places and those frequented by children and the elderly. The banned areas include parks, gardens and courtyards, the edges of roads and railways, urban areas, sports fields and recreational areas, playgrounds and green areas within schools, and areas adjacent to health facilities. Italy has also banned pre-harvest use of glyphosate and non-agricultural use on soils composed of 80% or more of sand to reduce the potential for contamination of groundwater (MdS 2016).

In May 2015, France placed restrictions on sales of glyphosate to the public (it is no longer in self-service aisles but under the counter. The French Minister for Health has said that France will ban glyphosate regardless of the EU decision, although it is unclear when. All non-agricultural pesticide use will cease by 2020. There are

already over 400 pesticide-free towns in France, a further 400 with restrictions on pesticide use in public places (PAN UK 2016).

Netherlands has banned non-commercial use of glyphosate, to come into effect later this year (PAN UK 2016).

Germany's state ministers have drafted a resolution for "the supply to and use (of glyphosate) by private persons to be banned for precautionary reasons". Additionally, Hamburg has stopped the use of glyphosate (PAN UK 2016).

In 2010, a regional court in Argentina banned the spraying of pesticides, including glyphosate, near populated areas of Santa Fe province (Robinson 2010).

Voluntary action

Numerous local authorities around the world have taken action to stop their own use of glyphosate – for example in the UK (Brighton, Hammersmith & Fulham, Bristol, Glastonbury, Erewash, Aberdeen, Edinburgh, Frome, Shetland Islands); in New Zealand (Christchurch); in USA (Boulder, Colorado, Minneapolis, Thousand Oaks, California); in Spain (Barcelona); and Australia (Town of Bassendean) (PAN UK 2016; ACE 2016; Henry 2016).

Also in the US:

- Many Connecticut towns have adopted bans or restrictions on pesticide use on lawns and gardens.
- All public lands are managed organically in Greenbelt, Maryland; Marblehead, Massachusetts; Brandford, Connecticut
- Taos Country, New Mexico is considering a ban; Reno, Nevada is undertaking a pesticide-free pilot programme.

Many of the municipalities in Belgium will be pesticide-free by 2017, with no spraying of glyphosate or any other pesticide in public areas; and 42 communes in Luxembourg have banned the use of pesticides (PAN UK 2016).

In Canada, 8 out of 10 Canadian Provinces has in place some form of restriction on the use of non-essential cosmetic pesticides including glyphosate. It cannot be used in public areas or by the public out of doors.

Numerous retailers have also stopped supplying glyphosate to members of the public. Two Swiss retailers, Coop and Migros, have stopped selling glyphosate products. The German REWE retail group has stopped selling glyphosate products (PAN UK 2016).

In Canada, Grain Millers Inc will no longer buy oats that have been pre-harvest treated with glyphosate, and it requires all suppliers to sign an affidavit to this effect (Cross 2016).

In 2001, a court in Colombia ordered the government to stop aerial spraying of Roundup on illegal coca plantations on the border of Columbia and Ecuador (Antoniou et al 2010). Although it later re-commenced spraying, following the IARC decision Colombia again decided to stop the aerial spraying of glyphosate (PAN UK 2016).

A court order stopped aerial spraying of Roundup and other pesticides on Bedouin farmers' crops in the Negev region of Israel, between 2002 and 2004, after a coalition of Arab human rights groups and Israeli scientists reported high death rates of livestock, and a high incidence of miscarriages and disease amongst the people (Antoniou et al 2010).

High level calls for bans or restrictions

The President of the Portuguese Medical Association called for a global ban of glyphosate in 2016 (Sustainable Pulse 2016).

In 2015, the Brazilian public prosecutor requested the country's National Health Surveillance Agency (ANVISA) to perform an urgent toxicological re-evaluation of glyphosate with the expectation of a domestic ban (GM Watch 2015).

In May 2009, the Environmental Lawyers Association of Argentina filed a lawsuit in Argentina's Supreme Court for a ban on glyphosate, citing a study on glyphosate by Professor Carrasco's team (reported in the section on Reproductive toxicology). Argentina's defence ministry has banned the planting of glyphosate-tolerant soybean on lands it rents to farmers. Early in 2009 a court order banned crop spraying of soybean fields near Ituzaingó Anexo suburb of the central Argentinean city of Córdoba after multiple health complaints. The ban now applies to all fields within 1,000 metres of residential areas in the province of Córdoba (Misculin 2009; Trigona 2009).

In 2012, a network of 160 physicians, health workers and researchers demanded a ban

on aerial spraying of pesticides in Argentina, based on increases in cancer and a range of other illnesses since the introduction of glyphosate-tolerant GM soybeans. The illnesses affect development, reproduction, and skin; and immune, respiratory, neurological, and endocrine systems (Sirinathsinghji 2012). Then in 2015, a union of 30,000 doctors in Latin America demanded that glyphosate be banned (Vibes 2015).

Assessment of Health Impacts

The toxicity database for glyphosate is considered by the US EPA (2006) to be "complete and without data gaps". However, the US EPA did not require developmental neurotoxicity studies; neither did it require studies of its impact on hormones, or studies of inhalation toxicity.

Regulatory toxicological studies conducted to support the authorisation of glyphosate follow a standardised study design with a wide, but still limited range of endpoints, mainly focussed on the oral route of exposure. In addition they are focussed on the active ingredient, i.e. glyphosate itself, while people are typically exposed to glyphosate-based formulations. These limitations can contribute to overlooking important aspects of toxicity and underestimating risks and hazards. Specialised, non-standard in vivo studies as well as in vitro studies using specific cell lines or other test systems can offer insights about mechanisms and possible toxicological differences between formulations and the active ingredient (i.e. glyphosate). Such studies are significantly undervalued, if not ignored, during the regulatory process. Both types of studies – regulatory and specialised ones – are reported here, because a holistic approach should equally take into account these different types of studies to ensure a proper, holistic assessment of risks and hazards.

Absorption, distribution, metabolism and elimination

According to EFSA (2015a), about 20% of ingested glyphosate is absorbed; according to US EPA (2006), the figure is 30-36%. The absorbed portion is widely distributed with highest concentrations occurring in bone, kidneys and liver. It is eliminated via urine predominantly within 48 hr (EFSA 2015), with <1% remaining in the body (US EPA 2006). Unabsorbed glyphosate is excreted in faeces, mostly unchanged. Glyphosate is poorly metabolised in animals (<0.5%), to AMPA, according to the US EPA (1993). However, more recently, Anadón et al (2009) found 6.49% metabolism.

This pattern of toxicokinetics and metabolism is independent of sex, dose level, or repeated administration. In lactating goats and laying hens, some metabolisation of glyphosate in the liver into AMPA was observed (EFSA 2015).

Absorption through the skin is said to be "low" (US EPA 1993), less than 3% (EC 2002). But glyphosate penetration of damaged skin is about 5-fold greater than that of healthy skin according to Heu et al (2012b).

Small amounts of glyphosate can be absorbed through the skin from contaminated clothing: one study showed that absorption from cotton fabric was 0.74%, half of that absorbed from an aqueous solution (1.42%) in the same study (Webster et al 1996).

Poor absorption and rapid elimination of glyphosate are the reasons usually given for the assumption that normal exposure (i.e. not intentional self-poisoning) to glyphosate is unlikely to result in systemic effects (e.g. Williams et al 2000, an often-cited review). However, recent independent work has shown that both glyphosate and AMPA were eliminated slowly from plasma and, although bioavailability was only 23.21%, it is likely that glyphosate is distributed throughout the body by the blood's circulation and there may be considerable diffusion of it into tissues to exert systemic effects (Anadón et al 2009). See section on residues in food for information on residues in chickens and cows, which indicate that there can be widespread distribution in the body.

In metabolic studies of farm and laboratory animals, no significant degradation was observed for either glyphosate or AMPA in pig, cattle and chicken liver, fat or muscle before 24-26 months, 13 months for eggs and 16 for milk (EFSA 2015). Myers et al (2016) concluded that metabolism studies strongly point to bioaccumulation in the kidney and liver.

Nothing is known about absorption, distribution, metabolism and excretion after exposure via inhalation, which is a remarkable data gap.

Acute toxicity

The International Programme on Chemical Safety (IPCS) regards glyphosate as having very low acute toxicity to laboratory animals

(IPCS 1994). However, many formulations are significantly more toxic than glyphosate itself because of the toxicity of additional ingredients such as the commonly used surfactant, POEA.

US EPA (2006) toxicity categories for glyphosate:

- oral = category IV
- inhalation = category: none
- dermal = category IV
- eye irritation = category III
- skin irritation = category IV

The World Health Organisation Recommended Classification by Acute Hazard for glyphosate (WHO 2005):

- Class 5.

Lethal doses

The lethal dose, LD_{50}, is the dose that kills 50% of test animals.

1. Glyphosate (EFSA 2015)
   - Oral LD_{50} rat = >2,000 mg/kg
   - Dermal LD_{50} rat = >2,000 mg/kg
   - Inhalation LC_{50} rat = >5 mg/L (EC 2002)

2. Roundup (Williams et al 2000)
   - Oral LD_{50} rat = >5,000 mg/kg
   - Dermal LD_{50} = >5,000 mg/kg
   - Inhalation LC_{50} rat = 3.18 mg/L

3. POEA (Williams et al 2000)
   - Oral LD_{50} rat = 1,200 mg/kg
   - Dermal LD_{50} = >1,260 mg/kg

4. Isopropylamine (IPCS 1994)
   - Oral LD_{50} rat = 820 mg/kg

5. AMPA (EFSA 2015)
   - Oral LD_{50} rat & mice = >5,000 mg/kg
   - Dermal LD_{50} rat = > 2,000 mg/kg bw/day

Acute sublethal effects

Acute effects of glyphosate observed in laboratory studies included breathing difficulties, ataxia, and convulsions. Roundup has caused cardiac depression, mainly due to the surfactant POEA (IPCS 1994).

Skin and eye irritation

EFSA (2015) categorised glyphosate acid, but not its salts, as an eye irritant category 1.

FAO (2000) described glyphosate as causing moderate to severe eye irritancy in rabbits. US EPA (2008) described it as a mild skin irritant but not a skin sensitiser.

POEA is severely irritating to the skin and corrosive to the eyes in rabbits (Williams et al 2000).

Sub-chronic toxicity

No & Lowest Observed Adverse Effect Levels

The No Observed Adverse Effect Level (NOAEL) is the lowest dose of the chemical given to a test animal at which no harmful effects are observed, and the Lowest Observed Adverse Effect Level (LOAEL) is the lowest dose of the chemical at which a harmful effect is observed.

The sub-chronic NOAELs and LOAELs are:

- 90-day oral NOAEL glyphosate (EFSA 2015):
  rat = 414 mg/kg bw/day
  mouse = 500 mg/kg bw/day
  dog = 300 mg/kg bw/day

- 90-day dermal NOAEL glyphosate (EFSA 2015)
  rat = 414 mg/kg bw/day
  mouse = 500 mg/kg bw/day
  dog = 300 mg/kg bw/day

- 90-day oral NOAEL AMPA (EFSA 2015)
  rat = 400 mg/kg bw/day
  dog = 263 mg/kg bw/day

- 90-day oral toxicity of POEA (rat)
  NOAEL = 36 mg/kg/day, based on decreased body weight and intestinal irritation (Williams et al 2000)

- 21/28-day dermal (rabbit)
  NOAEL = 1,000 mg/kg/day
  LOAEL = 5,000 mg/kg/day, based on slight erythema and oedema in both sexes, and decreased food consumption by females

Target organs in the different species were the gastrointestinal tract (irritation with diarrhoea or at least loose stool; in rats also distension, increased organ weight and mucosal atrophy of the caecum), the bladder (cystitis), and the parotid salivary glands (histological findings). In addition, effects on body weight gain, food

consumption and efficiency, or on red blood cell parameters were observed (EFSA 2015).

Systemic effects

Trials in which laboratory animals were subjected to varying doses of glyphosate caused the following symptoms:

- at all dose levels—increased serum glucose; increased blood potassium and phosphorus levels;
- at high doses only—increased blood urea, nitrogen, and serum alkaline phosphatase; red nasal discharge; pancreatic lesions; growth retardation; salivary gland lesions; diarrhoea; changes in the relative weights of kidney, liver, thymus, heart and testes; inflammation of the gastric lining; increased bile acids; dermal exposure resulted in very slight erythema and oedema, decreased food consumption, and decreased serum dehydrogenase. The salivary gland lesions indicate that glyphosate may be weakly mimicking adrenalin (US EPA 1993; IPCS 1994; FAO 2000).
- Roundup, at 500 mg/kg bw, caused significant reductions of RBC, hematocrit, and hemoglobin, together with a significant increase of mean cell volume (MCV) in both sexes of mice (Jasper et al 2012)

A number of studies have shown adverse effects of glyphosate, and/or formulations, on mammalian enzymes:

- Glyphosate inhibited acetylcholinesterase (AChE) in human serum, a hallmark of organophosphate toxicity (El Demerdash et al 2001), and in rat brain, kidney, liver and plasma (Larsen et al 2016).
- Maternal exposure to glyphosate caused functional abnormalities in the specific activity of three enzymes found inside cells—isocitrate dehydrogenase, malic dehydrogenase, and glucose-6-phosphate dehydrogenase (G6PD)—in liver, heart, and brain of pregnant rats and their foetuses (Daruich et al 2001). All enzymes are involved in the generation of NADPH (nicotinamide adenine dinucleotide phosphate), which has many essential roles in metabolism.

Chronic toxicity

Studies show that glyphosate-based herbicides can interfere with numerous mammalian organs and biochemical pathways, including inhibition of numerous enzymes, metabolic disturbances and oxidative stress leading to excessive membrane lipid peroxidation, and cell and tissue damage. Genotoxicity and endocrine disruption also lead to chronic health and developmental effects.

EFSA (2015a) chronic NOAEL for glyphosate:

- rat, 2 yr = 100 mg/kg bw/day
- mouse, 18mth/2 yr = 150 mg/kg bw/day

Williams et al (2000) provided a chronic NOAEL for AMPA in rats of >2.8 mg/kg/day.

General effects

Laboratory trials have shown decreased body weight gain, increased incidence of cataract and lens abnormalities, increased liver weight, and degeneration of the liver and kidney at high doses (US EPA 1993), an increase in salivary glands weight, alterations in clinical chemistry parameters (e.g., increase in AP activity and lower urine pH), histological salivary gland changes mainly of the parotis, cataracts and stomach mucosa irritation or caecum distension were observed but not consistently in all studies (EFSA 2015).

Ho & Ching (2003) asserted that glyphosate has the potential to disrupt many important enzyme systems that utilise phosphoenol pyruvate, including energy metabolism and the synthesis of key membrane lipids required in nerve cells. Glyphosate acts in plants by preventing the binding of phosphoenol pyruvate to the active site of the enzyme 5-enolpyruvoyl-shikimate-3-phosphate synthetase (EPSPS) and, although this enzyme is specific to plants, phosphoenol pyruvate is a core metabolite in all organisms.

Glyphosate adversely affects a number, but not all, of enzymes in the cytochrome P450 (CYP) 'superfamily' (McLaughlin et al 2008). These enzymes are found in all domains of life (plants, animals, bacteria, viruses, etc), and are involved in the oxidation, peroxidation and reduction of both endogenous and exogenous compounds in a variety of metabolic and biosynthetic processes. They are commonly located in cell mitochondria and microsomes, and play a major role in the liver. In humans, glyphosate disrupts the CYP aromatase, which converts testosterone to oestrogen, in liver and placental cells (Gasnier et al 2009); and CYP19 in human placental cells (Richards et al 2005). It disrupts CYPs in rat liver (Hietanen et al 1983); in mouse testicular cells (Walsh et al 2000), and CYP 71B1 in plant cells (Lamb et al 1998).

Glyphosate, at low doses (15-70 μM), kills human skin cells by inducing mitochondrial membrane potential disruption leading to oxidative stress. Deregulation of the cell death mechanism or an improper removal of damaged cells can lead to cancerous lesions, and the authors warned that their study "confirms the potential public health risk" of glyphosate (Heu et al 2012a). Other studies have also showed that glyphosate induces the death of human skin cells through oxidative stress (Ellie-Caille et al 2009; Gehin et al 2005, 2006). At low, but not high, concentrations it causes damage to skin similar to that of the aging process, i.e. thickening of the skin and induction of subcellular cytoskeleton structures (Heu et al 2012b).

Gastrointestinal and microbial

Glyphosate has been known to have negative effects on microorganisms in the soil since at least 1998 (Carlisle & Trevors 1988), but it is only in recent years that the influence of glyphosate on microorganisms in the human digestive system (microbiome) has become a focus of attention. Glyphosate was patented by Monsanto as an antimicrobial in 2010, with claims to be active against all human and animal protozoa, a wide range of bacterial pathogens, and all organisms that contain the enzyme EPSPS (Williams 2010). A number of recent studies show that glyphosate can cause imbalances in the normal microbiome, known as dysbiosis, increasing vulnerability to pathogenic bacteria, as well as influencing the response to antibiotics and intestinal functioning, in humans and animals.

In an *in vitro* study on the effects of Roundup Max on poultry gut microorganisms, the highly pathogenic bacteria *Salmonella entritidis, Salmonella gallinarum, Salmonella typhimurium, Clostridium perfringens* and *Clostridium botulinum* were found to be highly resistant to glyphosate; but most of beneficial bacteria – e.g. *Enterococcus faecalis, Enterococcus faecium, Bacillus badius, Bifidobacterium adolescentis* and *Lactobacillus* spp. – were moderately to highly susceptible to it. This indicates poultry feed containing residues of glyphosate may be a predisposing factor in increased risk of pathogens in poultry and subsequently food-borne illness in humans (Shehata et al 2013; 2014b).

The bacteria *Clostridium botulinum* can cause severe diseases in humans and other animals through the production of the neurotoxin botulinum, including the food-borne disease botulism. The normal intestinal microflora is critical in keeping this pathogen in check. Lactic acid-producing bacteria such as lactobacilli, lactococci and enterococci, all generate bacteriocines that are effective against *Clostridium* and other pathogens. Enterococcal species were isolated from cattle, horses, and algae and their impact on *C. botulinum* studied with and without Roundup Max (460 gm/L glyphosate) and glyphosate alone (N-(Phosphonomethyle)glycine). Glyphosate at 0.1 mg/mL inhibited growth of *E. faecalis* but did not substantially inhibit growth or neurotoxin production of *C. botulinum* Type B; and Roundup was more toxic. The authors of the study proposed this as a predisposing factor in the increase in *C. botulinum* associated diseases in cattle in Germany over the last 10-15 years (Krüger et al 2013a). In support of this, Monsanto's 2010 patent of glyphosate as an antimicrobial acknowledges that it is active against all enteroccocal species (Abraham 2010).

Exposure to Roundup altered the response of the pathogenic bacteria *Escherichia coli* and *Salmonella enterica* serovar Typhimurium to a number of antibiotics from different classes. It increased the concentration of kanamycin and ciprofloxacin necessary to achieve a lethal dose (except for kanamycin against *E. coli*), and decreased it for chloramphenicol and tetracycline. Even though the levels needed to cause these changes were above maximum residue levels, they were lower than rates of application specified on product labels, and the results of the study have raised concerns about the effectiveness of prescribed antibiotic doses in the presence of glyphosate and consequently the development of antibiotic resistance (Kurenbach et al 2015).

In an *in vitro* study on rats, glyphosate impaired small intestinal motility, at concentrations that are reported to be present in human blood (0.003 – 0.014 g/L). Repetitive exposures over a short time frame can increase the effect. The authors suggest that the repetitive presence of glyphosate in low doses in intestine cells might play a crucial role in recurrent intestinal dysmotility[2] (Chlopecka et al 2014).

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2 Bayer GC, Clinic EV, Genamin T200, Glyphogan, Roundup Grand Travaux, Roundup Grand Travaux Plus, Roundup Ultra, Roundup Bioforce, Roundup 3plus, Topglypho.

In an *in vitro* study on sheep stomach, glyphosate isopropylamine significantly inhibited the activity of the enzyme carbonic anhydrase CA-II, much more than did chlorpyrifos, cypermethrin and lambda cyhalomethrin. Carbonic anhydrases (CA) are widely distributed zinc metalloenzymes that play crucial roles in the cells, tissues and organs of all living organisms through their roles in a number of metabolic processes, including acid-base regulation, respiration and transportation of carbon dioxide, homeostasis, bone resorption, calcification, and electrolyte secretion. CA-II is abundant in the gastrointestinal system and is important for gastric acid secretion and hence digestion (Kilinç et al 2015).

Samsel and Seneff (2013) have hypothesised that glyphosate residues in food may be linked to increasing coeliac disease in North America and Europe, because of glyphosate's adverse effect on the balance between beneficial and pathogenic gut biota, its ability to chelate metals, and its inhibition of some cytochrome P450 enzymes.

Kidney and liver

Kidney and liver are the main target organs for glyphosate with studies showing disruption of gene expression, alterations of enzyme levels, interference in mitochondrial metabolism, oxidative damage and, in the case of the kidney, tumours. Additionally, metabolic studies of farm and laboratory animals found residue levels of glyphosate and AMPA 10-100 times higher in kidney, and to a lesser degree often higher in liver tissue than in other tissues (EFSA 2015).

Kidney

A wide range of adverse effects is reported from laboratory studies, including cell damage and death, DNA damage and tumours.

In humans, ingestion of Roundup results in nephrotoxicity: patients who die from glyphosate ingestion usually have developed acute kidney injury (Wunnapuk et al 2014).

Glyphosate is implicated in what is termed an epidemic of chronic kidney disease of unknown cause (CKDu; also called Sri Lankan Agricultural Nephropathy – SAN) amongst farmers in Sri Lanka, Andhra Pradesh (India), and Central America. It is characterised by tubular interstitial nephritis associated with mononuclear cell infiltration, glomerular sclerosis, tubular atrophy, and tubular proteinurea. A strong association has been observed between CKDu and the consumption of hard water, but the mechanism remains unexplained. Glyphosate is thought to be the missing link, as it is widely used where CKDu is prevalent, is toxic to kidney cells and a chelator of metals, some of which are also nephrotoxic, e.g. arsenic and cadmium (Jayasumana et al 2014, 2015a). In a small case-control study, creatinine-adjusted values of urinary heavy metals and glyphosate were significantly higher in people with SAN than in controls (Jayasumana et al 2015b).

According to GE Free Cymru (2015), recently released EPA Memos from the early 1980s suggest significant damage to the kidneys of the rats in the 3-generational study: the incidence of tubular dilation in the kidney was higher in every treated group of rats when compared to controls. Tubular dilation and nephrosis was also accompanied by interstitial fibrosis in all test groups and in some of the lumens the researchers found amorphous material and cellular debris. Less than a third of the control rats showed signs of tubular dilation.

This is supported by the following results reported by EFSA (2015) for 2-generation studies on reproduction in rats, which noted the following kidney effects:

- F0 generation – cysts, dilated pelvis
- F1 generation – hydronephrotic kidneys, dilated pelvis.
- F1 pups – A low incidence of kidney hydro-nephrosis and dilated pelvis occurred without dose-response relation.
- F2 pups – dose-related incidence of hydronephrosis and dilated pelvis in kidney.

Roundup killed human embryonic kidney cells at levels 200-fold lower than those recommended for agricultural use. The LC_{50} was 57.2 mg/L (Mesnage et al 2013b).

In a study of the long-term toxicity of both GM Roundup-tolerant maize and Roundup, very significant chronic kidney deficiencies were found for all treatments; 76% of altered parameters were kidney-related (Séralini et al 2014).

Glyphosate also causes oxidative stress and genotoxicity in kidney cells, renal tumours, and several studies indicate birth defects involving kidneys – see relevant sections below.

A number of other effects on the kidney are reported by EFSA (2015):

- kidney papillary necrosis
- reduced mineralisation of renal pelvis and papillary epithelium with a concomitant increase in cortical and/or medullary mineral deposition
- bilateral hyaline degeneration and vacuolation of the cortical tubules in the kidneys (dogs)
- lesions (dogs)
- coarse surface
- cortical cysts
- renal tubular vacuolization (heifer)

US EPA 2006 reports interstitial nephritis, proximal tubule epithelial basophilia and hypertrophy; and US EPA (undated) states that kidney problems are a potential consequence of long-term exposure to glyphosate in drinking water above the maximum allowable concentration limit of 0.6 mg/L.

Liver

Numerous studies show that glyphosate alters liver enzyme activity, disrupts mitochondrial oxidative phosphorylation, damages liver cells, damages DNA, and can cause tumours. Some of these effects (enzymes, cell damage) occur at low levels of exposure. Some effects are stronger with formulations than technical glyphosate.

Enzyme activity:

- At levels 3-20 times lower than the US oral reference dose of 2 mg/kg/day, glyphosate in drinking water caused altered liver enzyme activity, in particular increase levels of reduced glutathione and glutathione peroxidase activity in the absence of tissue damage (Larsen et al 2012).
- Glyphosate (at 500 mg/L for 4 days and then 300 mg/L/day) decreased the hepatic level of cytochrome P450 and monooxygenase activities in rats (Heitanen et al 1983).
- Glyphosate also reduces activity of cytochrome P450 CYP 71B1 in plant cells (Lamb et al 1998).
- Roundup GII caused increased glutathione peroxidase activity and total glutathione, and lowered hepatic cytochrome P450 in rat liver cells, indicating that it may affect the metabolism of other xenobiotic substances (Larsen et al 2014).
- Roundup at 50 mg/kg caused significant increases in the levels of ALT, AST and gamma-glutamyl transferase (*y*-GT) in mice (Jasper et al 2012).
- Sublethal doses of Roundup modified liver enzymatic activity (inhibited monoxygenases) in rats (Hietanen et al 1983).

Oxidative phosphorylation:

- Glyphosate disrupted liver mitochondrial oxidative phosphorylation in rats (Olorunsogo et al 1979).
- Roundup, but not glyphosate, also depressed oxidative phosphorylation in rat liver cells (Peixoto 2005).

Cell damage:

- Glyphosate increased permeability of liver mitochondrial membranes to protons and calcium ions (Olorunsogo 1990).
- Roundup caused lipid peroxidation in male mice liver cells, an indicator of damage through oxidative stress (Jasper et al 2012).
- Roundup, at low concentrations of 1-10 mM,[3] damaged rat liver cells, including the mitochondrial membranes and nuclei (Malatesta et al 2008).
- Higher, sublethal, doses of Roundup depressed mitochondrial respiratory activity in rat liver cells (Peixoto 2005).
- Benedetti et al (2004) showed that brief exposure to a Brazilian formulation of glyphosate caused liver damage in rats (fibrosis and leakage of liver enzymes AST and ALP) and they regarded this to be indicative of irreversible damage to liver cells.
- Four Roundup formulations caused cell death in human liver cell lines, at concentrations described as “far below those used in agriculture”, and also below those legally allowed as residues in GM food. Whereas legal levels can be 200 mg/L, this study was carried out at dose levels of 40-96 mg/L. Effects were stronger for the Roundup formulations than for glyphosate alone (Gasnier et al 2010).

3 mM means millimolar concentration. 1M = 1 mole/litre; it is the per unit volume available to the species. nM = nanomolar; μM = micromolar.

Other effects on the liver reported by EFSA (2015) include:

- alterations in some clinical chemistry parameters in the mouse with the latter findings pointing to liver toxicity;
- at the highest dose levels increase in liver weight (rats);
- discolouration, mononuclear cell infiltrations, congestion; diffuse acute inflammation in the liver with pigment deposits (rats);
- acute diffuse inflammation with pigment deposits; macrovesicular vacuolation in the liver, correlated with changes in the blood biochemical parameters (i.e. urea, protein, albumin and bilirubin levels as well as liver enzyme activities) (dogs);
- fatty liver, chronic hepatitis in goats fatally poisoned;
- liver congestion, scarred liver capsule (cows);
- statistically significant increase in rabbit foetuses with liver haematoma and irregular shaped livers, at 500mg/kg/day.

Glyphosate also causes genotoxicity (see later section).

Carcinogenicity

Recently, 3 comprehensive documents have been published concerning the assessment of carcinogenicity of glyphosate and glyphosate-based formulations. One of these documents – the monograph of the International Agency for Research on Cancer (IARC) concludes that "there is limited evidence in humans for carcinogenicity of glyphosate" and "there is sufficient evidence in experimental animals for the carcinogenicity of glyphosate" (IARC 2015). Besides evidence from carcinogenicity studies in rats and mice the IARC considered as a rationale, "two key characteristics of known human carcinogens" and concluded there is strong evidence that exposure to glyphosate or glyphosate-based formulations is genotoxic and can induce oxidative stress. The latter mechanism was also ascribed to AMPA. As a result, the IARC classified glyphosate as probably carcinogenic to humans (Group 2A, according to the IARC classification).

The other 2 documents are the Renewal Assessment Report (RAR) including its Addendum submitted to the European Food Safety Authority, EFSA (RMS Germany 2015a,b) and the draft CLH Report submitted to the European Chemicals Agency, ECHA (BAuA 2016). Both were drafted by the German Federal Institute for Risk Assessment (BfR). They describe a number of carcinogenicity studies in rats and mice conducted by industry, in addition to those assessed by the IARC, which were not evaluated by the IARC because it did not have access to the original reports. According to Clausing (2015), PAN Germany (2016) and Portier et al (2016), the data presented in the RAR and the CLH-Report confirms the conclusion drawn by the IARC. Nevertheless the conclusion drawn by the BfR (and later confirmed by the EFSA) was opposite to that of the IARC and essentially the same as by IPCS (1994) and EPA (1993), i.e. that glyphosate is not carcinogenic. The reason for these differences will be discussed in more detail below. Yet another report was published by the FAO/WHO Joint Meeting on Pesticide Residues (FAO & WHO 2016), wherein the same conclusion was drawn as in the RAR and the CLH Report. But because the presentation of data was very abbreviated (less than one page of the report was dedicated to carcinogenicity) it is not further considered here.

Glyphosate was originally classified by the US EPA as a 'Group C', 'possible human carcinogen', on the basis of an increased incidence of renal tumours in mice. However, after "independent review of the slides, the classification was changed to D on the basis of a lack of statistical significance and uncertainty as to a treatment-related effect". D classification means "not classifiable as to human carcinogenicity" (HSDB 2006). Then, in 1993, the US EPA declared glyphosate as Group E 'evidence of non-carcinogenicity in humans' on the basis of 3 studies on rats and mice. All of these studies showed a variety of carcinogenic effects, but they were considered by the US EPA to be not caused by the glyphosate.

Animal studies

In an Addendum to the RAR (RMS Germany 2015b) the BfR re-assessed its own evaluation of 5 mouse and 2 rat carcinogenicity studies reported between 1981 and 2009 and identified a total of 11 statistically significant increases for 6 different tumour types distributed over 7 studies. This concerned haemangiosarcoma, malignant lymphoma and renal tumours in male mice, pancreatic carcinoma and liver adenoma in male rats and pancreatic C-cell adenoma in female rats. After acknowledging these statistically significant increases, the BfR dismissed them based on a "weight of evidence approach". This dismissal was heavily criticised

(Clausing 2015; PAN Germany 2016; Portier et al 2016), because the BfR's rejections were associated with neglecting important regulatory guidance (OECD 2002, 2012) or interpreting it in a strongly biased or even false way. This relates to the use of statistical methods and historical controls, an alleged virus infection in one study, and the wrong contention that carcinogenic effects of glyphosate were not reproducible and a high-dose effect only. The contentions made by the BfR (and repeated by the EFSA) were subsequently rebutted point by point. For details see Clausing (2015), PAN Germany (2016) and Portier et al (2016).

In addition, the BfR and the EFSA failed to apply a holistic approach and instead discussed carcinogenicity studies in animals, epidemiological research and mechanistic evidence separately. Of particular concern is the BfR's refusal to acknowledge the toxicological significance of an increase in malignant lymphoma observed in male mice of 3 different mouse studies, where the top dose of 2 of the studies was close to or even below the dose of 1,000 mg/kg body weight (considered as a "limit dose" by the BfR, though a questionable limitation from a guideline perspective). The finding of malignant lymphoma was clearly supported by historical control data (HCD) in one study (Kumar 2001), while HCD did not contradict the result of the second study (Sugimoto 1997), and no valid HCD were available for the third one (Wood et al 2009). Two more mouse carcinogenicity studies were available which did not show a significant increase in malignant lymphoma. Although not spelled out, these studies were obviously used by the BfR to claim lack of reproducibility. However, the BfR ignored the fact that with regard to malignant lymphoma one of these studies was invalid and the other one equivocal. For a more extended discussion see PAN Germany (2016).

Because malignant lymphoma in male mice provided the most compelling evidence for glyphosate's carcinogenicity, details of the five mouse studies are provided below.

Dietary admixture was the route of glyphosate administration in all 5 studies, and doses (mg/kg bw) were calculated based on records of body weight and food consumption. The numbers of animals with malignant lymphoma (incidences) are listed, followed by the number of animals per group in parentheses.

Knezevich and Hogan (1983)

Doses: 0 – 157 – 814 – 4.841 mg/kg bw
Incidences: cannot be given – see below
Remarks: according to the CLH Report (BAuA 2016) malignant lymphoma was not mentioned as a separate entity in the study report. The study results are difficult to compare with other studies, when equivocal terminology is used.

Atkinson et al (1993)

Doses: 0 – 165 – 838 – 4.348 mg/kg bw
Incidences: 4 – 2 – 1 – 6 (50)
Remarks: Allegedly no statistically significant differences were observed, but according to a footnote in the CLH Report (BAuA 2016) the histopathological assessment was incomplete, i.e. only lymph nodes with macroscopic changes were assessed microscopically concerning malignant lymphoma. This makes the study invalid with regard to this tumour type.

Sugimoto (1997)

Doses: 100 – 300 – 1.000 mg/kg bw
Incidences: 2 – 2 – 0 – 6 (50)
Remarks: Statistically significant increase (p=0.0085) with two-sided Cochran Armitage trend-test. High dose incidence (12%) above arithmetic mean (6.33%), but below range 19.23% of HCD (458 male mice, 12 studies 1993-1998) according to the CLH Report (BAuA 2016). OECD Guidance No. 116 (OECD 2012) discourages the use of arithmetic means and recommends medians (and interquartile ranges), because of the possible overemphasis on "rogue" outliers. Therefore, the HCD values presented in the CLH Report (BAuA 2016) are of limited value and do not invalidate the statistically significant finding of the study.

Kumar (2001)

Doses: 15 – 151 – 1.460 mg/kg bw
Incidences: 10 – 15 – 16 – 19 (50)
Remarks: Statistically significant with two-sided Z-test (p=0.002), one-sided Fisher's exact test (p=0.038) and one-sided Cochran Armitage trend-test (p=0.033). The high dose incidence (38%) was even above the HCD-range (30%, 250 male mice, 5 studies between 1996-1999) (cf. BAuA 2016). The study was allegedly excluded by the US EPA from evaluation, because of a viral infection, but the actual basis of EPA's decision is not known (BAuA 2016, p. 72) and no evidence of a viral infection was available to the European authorities.

Wood et al (2009)

Doses: 71 – 234 – 810 mg/kg bw
Incidences: 0 – 1 – 2 – 5 (51)
Remarks: Statistically significant with two-sided Cochran Armitage trend-test (p=0.037), two-sided Z-test (p=0.022), one-sided Chi-Square-test (p=0.034) and one-sided Fisher's exact test (p=0.028).

Note: According to OECD Guidance 116 (OECD 2012, p.133) the use of one-sided tests is legitimate and even more appropriate for the assessment of carcinogenicity. Furthermore, while not all statistically significant differences are biologically relevant, this applies also vice versa, i.e. biological relevance does not always need to be accompanied by statistical significance (OECD 2012, p.116).

In summary, glyphosate reproducibly elicited malignant lymphoma in male mice of three independent studies, in 2 of them with a clear dose-response-relationship. HCD were supporting this finding (one study), not contradicting this finding (one study) or were not available (one study). In addition, a significant increase of other tumours types was observed in one or more studies conducted in rats and mice.

Human evidence

A report released by Argentina's Chaco state government in April 2010 (Robinson 2010) refers to a significant increase in cancer in La Leonesa, an agricultural town, including a 3-fold increase in childhood cancers, particularly leukaemia, lymphoma, and brain tumours, coinciding with the dramatic increase in transgenic crops such as soy which are heavily sprayed with glyphosate. These effects could be caused by a number of factors including other pesticides, but there is support from epidemiology and laboratory studies to indicate that glyphosate might be contributing to these cancers.

A significant number of epidemiological studies have suggested possible links between exposure to glyphosate herbicides and several kinds of cancer, most commonly affecting the lymphatic system, i.e. non-Hodgkin's lymphoma (NHL), including hairy cell leukaemia (a rare type of NHL affecting circulating B-cell lymphocytes), and multiple myeloma. A total of 5 cohort studies and 14 case-control studies were reviewed by both the IARC (2015) and the BfR (RMS Germany 2015). Both institutions come to the same conclusion, i.e. that there is "limited evidence" in humans for the carcinogenicity of glyphosate, but they use this conclusion for opposite ends. The IARC considers the observed association between glyphosate use and NHL as supportive of the sufficient evidence in experimental animals (with main effects on the lymphatic system), while the BfR adopts – according to its own words – "a more cautious view since no consistent positive association is observed" (RMS Germany 2015b, p.90).

To strengthen its argument the BfR classified all case-control studies as "non-reliable", because glyphosate exposure or confounding factors (e.g. smoking status, earlier diseases) were allegedly not taken into consideration. However, according to Portier et al (2016), in most cases this was contrary to what was actually described in these publications. In addition, the BfR explained that "the most powerful study showed no effect". With this it referred to the Agricultural Health Study (AHS) a large cohort study conducted in the U.S. which was analysed from different perspectives (De Roos et al 2005; Engel et al 2005; Flower et al. 2004; Andreotti et al 2009). However, the AHS was criticised, because the median follow-up time of 6.7 years was too short to account properly for cancer latency and comprised only 92 NHL cases as compared to 650 cases in a pooled case-control study (Portier et al 2016; De Roos et al 2003).

Finally, the BfR pointed out that in epidemiological studies a differentiation between the effects of glyphosate and co-formulants is not possible. While BfR and EFSA are denying existing evidence for the induction of malignant lymphoma by glyphosate in mouse carcinogenicity studies, they imply the involvement of co-formulants in the association between glyphosate-based herbicides and NHL in humans.

Of the 46 analyses of an association between glyphosate and NHL (and other tumours of the lymphatic system) performed in the 14 case-control studies mentioned above, 35 yielded an odds ratio larger than 1. Eight out of these 35 analyses were statistically significant, i.e. the lower 95%-confidence limit of the odds ratio was larger than 1 for an association between glyphosate and NHL.

The following publications identified a significantly increased risk for tumours of the lymphatic system:

- McDuffie et al (2001) conducted a study across a large region of Canada with data collected between 1991 and 1994. They found an elevated risk of NHL associated with glyphosate use more frequent than 2 days/year (odds ratio 2.12).
- Hardell et al (2002) pooled 2 earlier studies conducted in Sweden with data collected between 1987 and 1992 (Nordström et al 1998 and Hardell & Erikson 1999). They calculated an odds ratio of 3.04, for increased risk of NHL associated with exposure to glyphosate.

- De Roos et al (2003) pooled data from three 1980 USA population-based case-control studies of NHL in Nebraska, Iowa and Minnesota, and Kansas. Their logistical regression analysis showed an association between exposure to glyphosate and NHL in men (odds ratio 2.1).
- A further study, carried out in Sweden between 1999 and 2002, "considerably strengthened" the association between NHL and exposure to glyphosate (Eriksson et al 2008). Other tumours of the lymphatic system (B-cell lymphoma, T-cell lymphoma, etc. were considered as well. Statistically significant odds ratios were calculated for different scenarios, ranging from 2.02 to 5.36.

It should be noted that all epidemiological studies published in peer-reviewed journals so far were conducted in the US, Canada or Europe, i.e. countries where a reasonable use of personal protective equipment (PPE) can be assumed for pesticide users and where – for Europe – no glyphosate-resistant varieties are planted, resulting in a more moderate use of glyphosate. As an example, in Germany during recent years, approximately 5,000 tonnes of glyphosate (active ingredient) were sprayed annually on about 4.8 million hectares (40% of Germany's arable land), resulting in less than 1 kg/ha (Dickeduisberg et al 2012). In contrast, areas growing glyphosate-resistant soy in Argentina or Brazil apply up to 10 kg/ha (Avila-Vazquez et al 2015, Benbrook 2016). In addition, PPE use is low in countries like Brazil and Argentina, e.g. only by 23% of rural workers in a study from Brazil (Ferreira Filho 2013). Therefore, thorough epidemiological studies in these high-exposure areas are urgently needed.

Mechanistic evidence

There is increasing toxicological evidence that glyphosate, Roundup, and the metabolite AMPA all have the potential to cause cancer through mechanisms such as genotoxicity, oxidative stress, and interference with hormonal functions.

The IARC assessed that there "is strong evidence that glyphosate can operate through two key characteristics of known human carcinogens" (IARC 2015, p.78). These were genotoxicity and oxidative stress.

Because genotoxicity is a concern in its own right, a separate section (below) has been devoted to this mechanism.

Oxidative stress

Oxidative stress is caused by an imbalance between the production of 'reactive oxygen species' (ROS) and an organism's capacity to detoxify them and/or repair the resulting damage. Damage to DNA and other subcellular structures can occur when this capacity to detoxify or repair is saturated. Hence, oxidative stress may be a causative factor in cancer as well as neurodegenerative and other diseases. Oxidative stress can be assessed by measuring lipid peroxidation (ROS-generation) using thiobarbituric acid-reactive substances (TBARS), by determining the activity of specific enzymes (e.g. superoxide dismutase, catalase, glutathione-S-transferase) or by other, less frequently used methods.

In human cells, oxidative stress was shown after treatment with glyphosate by Gehin et al (2005, 2006) and Elie-Caille et al (2010) in HaCat cells, a keratinocyte cell line, by Mladinic et al (2009b) in primary lymphocyte cultures and by Kwiatkowska et al (2014) in erythrocytes. In the latter study, significant effects were seen for glyphosate as well as for AMPA at concentrations as low as 0.25 mM. After incubation with glyphosate-based formulations, a significant increase of ROS was shown in HaCat cells (Gehin et al 2005, 2006), in Hep2 cells (Coalova et al 2014) and in Hep2G cells (Chaufan et al 2014).

Studies in laboratory rodents revealed a glyphosate-associated ROS-increase in blood plasma, liver and kidney of rats after repeated intraperitoneal injection of 10 mg/kg body weight (Astiz et al 2009a), and oxidative DNA damage in liver and kidney of CD-1 mice after a single intraperitoneal injection of 300 mg/kg body weight (Bolognesi et al 1997). Administration of glyphosate-based formulations to Swiss albino mice caused oxidative stress in liver and/or kidney at a single intraperitoneal injection dose of 50 mg/kg (Çavuşoğlu et al 2011) and after daily oral administration of 50 or 500 mg/kg for 15 days (Jasper et al 2012). Beuret et al (2005) gave a glyphosate-based formulation via drinking water containing 1% glyphosate to pregnant rats during the entire gestation. At the end of pregnancy, a significant ROS-increase was detected in the livers of dams as well as foetuses.

In an *in vitro* study, hippocampal slices from 15 day-old rats exhibited oxidative stress after 30 minutes of incubation with a 0.01% "Roundup Original" solution, corresponding to a glyphosate concentration of 36 mg/L (Cattani et al 2014).

The authors hypothesised that oxidative stress was resulting from the activation of NMDA receptors and voltage-dependent calcium channels. Martini et al (2016) described a ROS-increase in 3T3-L1 fibroblasts (a cell line derived from mouse adipose tissue) after exposure to a glyphosate-based formulation.

A large number of publications consistently presented evidence that glyphosate-based formulations can cause oxidative stress in fish including at environmentally relevant concentrations (de Menezes et al 2011; Guilherme et al 2010, 2012a; Lushchak et al 2009; Modesto & Martinez 2010a, 2010b; Murussi et al 2016; Ortiz-Ordeñez et al 2011; Samanta et al 2014; Sinhorin et al 2014). Navarro & Martinez (2014) showed that POEA, a surfactant used in some of the glyphosate-based formulations, can cause oxidative stress in fish at concentrations as low as 150 μg/L. However, it can be excluded that the effects seen after exposure to glyphosate-based formulations is solely caused by surfactants, because when comparing the effects of glyphosate and a glyphosate-based formulation in brown trout at water concentrations of 0, 0.01, 0.5 and 10 mg/L using global transcriptomic profiling. Webster & Santos (2015) provided evidence that glyphosate (active ingredient) as well as a formulation was inducing transcriptional changes indicative of oxidative stress. For more information on these studies, and studies showing oxidative stress in tadpoles, refer to the section on Aquatic toxicity.

Taken together, a plethora of papers demonstrated the induction of oxidative stress by glyphosate as well as glyphosate-based formulations in *in vitro* and *in vivo* tests with mammals and fish. For amphibians this effect was investigated with glyphosate-based formulations only. In spite of all this, the BfR refused to recognise the importance of this evidence. After denying the well-documented induction of malignant lymphoma by glyphosate in male mice (see above), the BfR reasoned that "from the sole observation of oxidative stress and the existence of a plausible mechanism for induction of oxidative stress through uncoupling of mitochondrial oxidative phosphorylation alone, genotoxic or carcinogenic activity in humans cannot be deduced for glyphosate and glyphosate-based formulations (RMS Germany 2015b p. iv).

Genotoxicity / mutagenicity

A pesticide is genotoxic if it causes damage to a gene that could result in cell death, or result in a change in the structure or function of the gene. The damage can be mutagenic (heritable) or non-mutagenic. Mutagenic means causing a change in the genetic structure, usually through base-pair substitution (change in amino acid sequence), deletion, or addition of gene fragments, or some other mechanism which include disruptions or breaks in chromosomes that result in the gain, loss, or rearrangements of chromosomal segments (clastogenicity). It also includes "sister chromatid exchanges", interchanges and re-attachments of strands in the chromosome during DNA replication, and induction (increase) in the frequency of micro-nuclei (small fragments formed when chromo-somes break). Besides causing inheritable dam-age (germ cell mutagenicity) one of the main health implications of genotoxicity in somatic cells is the induction of cancer.

The US EPA (2006) reported that glyphosate was non-mutagenic in the bacteria *Salmonella typhimurium*, Chinese hamster ovary cells, and rat bone marrow. FAO (2000) also reported it to be non-mutagenic in human lymphocytes (white blood cells) and mouse bone marrow. Both these determinations are based on test results reported by Monsanto (FAO 2000). Likewise, in the recent assessments performed on behalf of the EFSA (RMS Germany 2015b) and the ECHA (BAuA 2016), the BfR using a weight of evidence approach came to the overall conclusion "that glyphosate does not induce mutations *in vivo* and that no hazard classification for mutagenicity is warranted" (RMS Germany 2015b, p.49). Similar to the US EPA and the FAO, the BfR relied heavily on the regulatory studies submitted by industry, i.e. the "Glyphosate Task Force", a consortium of 25 glyphosate-producing corporations. On the other hand, the BfR cautioned that "for the different glyphosate-based formulations, no firm conclusions can be drawn with regard to a need for classification" and it "strongly recommends further genotoxicity studies in compliance with OECD test guidelines in general and for the representative formulation" (RMS Germany 2015b, p. iv). One would assume that there is a reason for this.

In general, a huge discrepancy exists between industry studies and results published in the scientific literature. Only 2 of the 36 industry studies reported in the RAR exhibited genotoxic effects (RMS Germany 2015a). On the other hand, 62 of the 87 tests published in the peer-reviewed scientific literature demonstrated genotoxic effects of glyphosate or glyphosate-based formulations. While some of the publications from the scientific literature had

methodological deficiencies, the vast majority of them were dismissed by the BfR for minor reasons or not even mentioned. The IARC, in contrast, made a thorough review of these publications and came to the conclusion that "(t)here is strong evidence that exposure to glyphosate or glyphosate-based formulations is genotoxic based on studies in humans *in vitro* and studies in experimental animals" (IARC 2015, p. 78).

While the BfR was quick to dismiss numerous scientific publications for minor or formal reasons, it ignored the fact that glyphosate has antimicrobial properties and therefore should not have been tested in microbial test systems. Glyphosate has been patented as a broad spectrum antibiotic (US patent number 7771736) and as an "antimicrobial agent" (US patent number 20040077608 A1). According to Luijten et al (2016), the Ames test is not suitable for testing antibiotics. The 36 industry studies mentioned above contain 16 Ames tests. In addition, for instance, Table 22 of the CLH-Report (BAuA 2016, p. 47/48) lists two negative assays in mouse lymphoma cells (reported in 1991 and 1996), but remains mute about 4 earlier mouse lymphoma assays commissioned by the Stauffer Chemical Company showing gene mutations (Majeska & Matheson 1982a,b and 1985a) and chromosomal aberrations (Majeska & Matheson 1985b) after exposure to glyphosate (cf. Hardell & Eriksson 1999).

The CLH Report points out that epidemiological data for genotoxicity of glyphosate is available, but cautions: "It must be taken into account that the study participants had been always exposed to plant protection products containing glyphosate but never to the active substance itself" (BAuA 2016, p. 57) This is commonplace and applies to almost all epidemiological data for pesticides. Nevertheless this information is particularly valuable, because these are human data. In the sense of a weight of evidence approach these findings should be evaluated together with the results of *in vitro* tests for mutagenicity, clastogenicity or DNA damage/ repair with glyphosate acid in mammalian cells as summarised in Table 22 of the CLH Report (BAuA 2016, p. 47/48). Of the 18 tests listed in this table, 7 were performed with cells of animal origin, 11 with cells of human origin. It is remarkable that 6 of the 7 tests performed with cells of animal origin were negative. In contrast the majority (i.e. 7 of the 11 tests) with cells of human origin were positive.

Below, a number of scientific publications about the genotoxic effects of glyphosate are summarized. Most compelling are the studies that show genotoxicity in human cells:

- Both glyphosate and Roundup caused DNA damage in human buccal (mouth) epithelial cells with short-term (20 mins) exposure to concentrations corresponding to a 450-fold dilution of concentrations normally used in agriculture, prompting the authors of the study to warn that inhalation may cause DNA damage in mouth and respiratory tissues of exposed individuals. Even a 1,350-fold dilution of a spraying solution of Roundup caused acute and genotoxic effects on human cells in this study (Koller et al 2012).
- Chromosomal damage was shown at 580 μg/mL of technical grade glyphosate under the condition of metabolic activation using the micronucleus formation as an endpoint (Mladinic et al 2009a).
- Glyphosate isopropylamine was tested at 0.7, 7 and 700 μM concentrations in a comet assay with human lymphocytes. A dose-dependent, statistically significant increase in glyphosate-exposed cells was observed for all concentrations (Alvarez-Moya et al 2014).
- After incubation of human lymphocytes with technical grade glyphosate, DNA damage was shown at concentrations of 3.5 μg/mL or higher in comet assays with and without metabolic activation (Mladinic et al 2009b).
- Glyphosate caused DNA damage in human liver cells at concentrations of 3 to 7.5 mM, but not in human lymphocytes at 0.2 to 6 mM (Mañas et al 2009a).
- Roundup caused dose-dependent DNA damage in human liver cells, with 50% DNA strand breaks at 5 mg/kg, described by the authors as "residual levels corresponding to 120 nM of glyphosate" (Gasnier et al 2009).
- Glyphosate was genotoxic in normal human cells at concentrations of 4 to 6.5 mM and in human cancer cells (fibrosarcoma) at 4.75 to 5.75 mM (Monroy et al 2005).
- Glyphosate caused a dose-dependent increase in chromosomal aberrations and an increase in sister chromatid exchange in human lymphocytes (Lioi et al 1998a).
- Glyphosate and Roundup caused dose-dependent increases in sister chromatid exchange in human lymphocytes; Roundup had a greater effect (Bolognesi et al 1997).
- Roundup at high concentrations caused an increase in sister chromatid exchange in human lymphocytes (Vigfusson & Vyse 1980).

- In the first data to be published on the potential genotoxicity of the metabolite AMPA, Mañas et al (2009b) have shown that it is clearly genotoxic, causing DNA damage in human liver cells at concentrations of 2.5 to 7.5 mM. It also caused chromosomal damage in human lymphocytes at 1.8 mM.

A variety of tests on other mammalian cells have further demonstrated the genotoxic ability of glyphosate, Roundup and AMPA:

- Glyphosate was clastogenic in mouse bone marrow cells, causing chromosomal aberrations and induction of micronuclei. Cytotoxicity was demonstrated by a significant decrease in mitotic index (Prasad et al 2009).
- Glyphosate caused the induction of micronuclei at high doses, possibly through oxidative stress, in mouse bone marrow (Mañas et al 2009a).
- In the study by Alvarez-Moya et al (2014) mentioned above, aimed at the comparison of the effects of glyphosate isopropylamine in the comet assay in 3 different test systems (human lymphocytes *in vitro,* erythrocytes in the fish species *Oreochromis niloticus, in vitro* and *in vivo*, and staminal nuclei of the plant *Tradescantia*, clone 4430, also *in vitro* and *in vivo*), using the same concentrations in all 3 systems, significant genetic damage was observed in all cell types and organisms tested. The results indicated that glyphosate is genotoxic in the cells and organisms studied at concentrations of 0.7-7 μM.
- Roundup caused the induction of micronuclei in mouse bone marrow. Both glyphosate and Roundup caused DNA strand breaks in mouse liver and kidney cells (Bolognesi et al 1997).
- Roundup, but not glyphosate, caused dose-dependent formation of DNA adducts in mouse liver and kidney cells (Peluso et al 1998).
- Glyphosate caused chromosomal aberrations and sister chromatid exchange in bovine lymphocytes (Lioi et al 1998b).
- Glyphosate caused sister chromatid exchange in bovine lymphocytes at concentrations of 56 to 1120 uM (Siviková & Dianovskỳ 2006).
- In a hamster ovary cell line (CHO K1), AMPA induced micronucleus formation both with (at 0.05 μg/mL and higher) and without metabolic activation (at 0.01 μg/mL and higher) while glyphosate itself caused an increase in micronuclei only after metabolic activation (at 10 μg/mL and higher) (Roustan et al 2014).
- AMPA caused the induction of micronuclei in mice (Mañas et al 2009b).

Roundup has also caused DNA damage and/or micronucleus induction in the broad-snouted caiman *Caiman latirostris* (Poletta et al 2009), bullfrog tadpoles *Rana catesbeiana* (Clements et al 1997), sea urchin embryos (Bellé et al 2007), neotropical fish *Prochilodus lineatus* (Cavalcante et al 2008; Moreno et al 2014), *Tilapia rendalli* (Grisolia 2002), goldfish *Carassius auratus* (Cavaş & Könen 2007), guppy fish *Poecilia reticulata* (de Souza Filho et al 2013), European eel *Anguilla anguilla* (Guilherme et al 2009, 2010, 2012b; Marques et al 2014), the pepper cory *Corydoras paleatus* (de Castilhos Gishi & Cestari 2012), the freshwater fish spotted snakehead *Channa punctatus* (Nwami et al 2013), and the tropical fish tambaqui (*Colossoma macropomum*) (Braz-Mota et al 2015). Refer to section on Ecotoxicity – Aquatic for further details.

Developmental exposure to glyphosate caused mutations in fruit flies (*Drosophila melanogaster*) (Kaya et al 2000). Roundup and Pondmaster "induced a very high frequency of lethals [sex-linked, recessive lethal mutations] in larval spermatocytes and in spermatogonia" of fruit flies (Kale et al 1995). And Roundup, but not glyphosate, caused chromosomal damage in root-tips cells of onions (*Allium cepa*) (Rank et al 1993).

Epidemiology

Four epidemiological studies on genotoxic effects of glyphosate-based formulations were available.

- In 2007, Paz-y-Miño et al found a significantly higher degree of DNA damage amongst 24 people exposed to aerial spraying of Roundup-Ultra (43.9% glyphosate + POEA + Cosmo-Flux 411 F) in northern Ecuador than in 21 non-exposed controls. The exposed group lived < 3 km from the Ecuador-Colombia border area where the aerial spraying occurred continuously during 3 days between December 2000 and March 2001, sporadic aerial spraying continuing for 3 weeks following the continuous spraying. The individuals had all presented symptoms of toxicity after several exposures (see section on 'Poisonings'). Half of the group had received spraying directly over their houses and half were living within 200 m to 3 km from

the sprayed areas. The application rate was 23.4 L/ha of Roundup-Ultra (equivalent to 10.3 L/ha of glyphosate), more than 20 times the US maximum recommended application rate.

- In a study carried out in the border region of Ecuador and Colombia, 22 women who had shown symptoms of poisoning, were found to have damaged DNA. All of the women studied showed the genetic damage, in about 36% of cells tested; and the level of damage was 500 times greater than in women living in the Amazon region 80 km away (ANON 2003; 2003b; Mueckay & Maldonado 2003).
- Bolognesi et al (2009) found a relationship between exposure to aerial glyphosate spraying in Colombia and DNA damage, although the authors dismissed it as small, transient and "not biologically relevant". The frequency of binucleated cells with micronuclei (BNMN) was compared before, 2 days after, and 4 months after aerial application. The study involved 137 women and their spouses from 5 areas of Colombia: Santa Marta where organic coffee is grown with no exposure to glyphosate or other pesticides; Boyaca where unidentified pesticides were used; Putumayo and Narino where glyphosate was aerial sprayed for coca eradication; and Valle del Cauca where it is aerial sprayed for sugar cane "maturation", i.e. to increase sugar content. The base line testing showed that the BNMN levels in people from areas where pesticides are used were 2.5 times higher than those from the organic area. There was significant increase in BNMN in all exposed areas 2 days after aerial spraying, especially amongst those who reported direct contact with the spray. In Putumayo, that level increased again at 4 months after spraying, but it decreased in Narino. There was a slight decrease in Valle del Cauca but it was not statistically significant. Despite the authors attempts to dismiss the results because they were not consistent, this study provides further evidence that exposure to glyphosate may cause DNA damage.
- As a follow-up to the 2007 study referred to above, Paz-y-Miño et al (2011) investigated 92 individuals from 10 communities in the northern border of Ecuador where aerial spraying ceased 2 years ago and compared them with 90 healthy individuals from several other provinces. No differences between the 2 groups were found (results summarized by IARC 2015), thus indirectly confirming the findings of the study published in 2007.

In summary, the available evidence lead the IARC to conclude that there is strong evidence glyphosate is genotoxic. The publications reviewed here illustrate how the IARC came to this conclusion.

Endocrine disruption

The US EPA Endocrine Disruptor Screening Program (US EPA 2015) reported that "glyphosate demonstrates no convincing evidence of potential interaction with the oestrogen, androgen or thyroid pathways in mammals or wildlife". This conclusion was drawn from a battery of Tier-1 tests, composed of *in vitro* and short-term *in vivo* tests, on glyphosate alone. No further long-term studies were carried out, and not all endocrine relevant endpoints were examined in each assay (for example oestrogen receptor (ER) binding or ER activation each was measured just in one assay). Furthermore, US EPA did not take into consideration any of the findings from studies that tested the formulations of glyphosate-based herbicides, which is what people and the environment are exposed to.

However, a number of studies since then have demonstrated that both glyphosate and the Roundup formulation do disrupt oestrogen, androgen, and other steroidogenic pathways. These studies indicate that glyphosate has the potential to elicit endocrine-disrupting effects in cell lines, and the effects in comparison to glyphosate are either significant or more pronounced in studies where Roundup (glyphosate and its adjuvants) is used.

Cell line or in vitro studies

Walsh et al (2000) demonstrated that Roundup, but not glyphosate, significantly inhibited the production of the hormone progesterone in mouse cells, by disrupting the expression of the steroidogenic acute regulatory (StAR) protein. The authors concluded that, as the StAR protein is also indispensable for steroidogenesis in the adrenal glands, a disruption in StAR protein expression may potentially affect carbohydrate metabolism, immune system function, and water balance, as well as fertility. It may have an impact on reproduction in humans, other mammals, birds, and amphibians.

In 2000, Lin & Garry found that both Roundup and glyphosate caused the proliferation of MCF-7 human breast cancer cells, but not via an oestrogenic mechanism.

In 2005, a research team from Caen University in France (Richard et al 2005) demonstrated that glyphosate and Roundup, at non-toxic concentrations, affected the enzyme aromatase, which is responsible for the synthesis of oestrogen. They found that glyphosate, at dilutions 100 times lower than agricultural rates, inhibited aromatase activity, interacted with the active site of the enzyme, and decreased aromatase mRNA levels. The effects were greater with the Roundup formulation than glyphosate alone. However, there appears to be a differential effect on aromatase: although both glyphosate and Roundup reduced aromatase activity once inside the cells (microsomal aromatase), glyphosate had no effect on aromatase at the cellular level, whereas Roundup caused up to 50% inhibition. The authors concluded that glyphosate has endocrine-disrupting effects in mammals, and that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation in cells, and that these effects could explain premature births and miscarriages observed in epidemiological studies involving women farmers using glyphosate (see Reproduction section for details of these).

In 2007, Hokanson et al demonstrated that a commercial glyphosate formulation ("a 15% home use preparation") dysregulated 680 out of 1,550 genes in MCF-7 human breast cancer cells. They also identified a synergistic effect with oestrogen (17B-estradiol). Oestrogen-regulated gene expression is a major factor in the regulation of a number of physiological functions, and the genes affected in this study have implications for tumour formation and growth, immune function, hypertension, pre-eclampsia (pregnancy-induced hypertension), and foetal growth retardation.

Also in 2007, Benachour et al demonstrated glyphosate-dependent endocrine disruption via aromatase inhibition in human embryonic and placental, and equine testis cells, at non-toxic levels of glyphosate. As little as 0.01% Roundup provoked a significant reduction of 19% of oestrogen production in exposed cells. The authors linked the effects to glyphosate itself, with a synergistic effect provoked by the adjuvants in the formulation. The embryonic cells, which are the most sensitive cells, showed evidence of either bioaccumulation or time-delayed effect, suggesting a cumulative impact of very low doses of glyphosate approximating the Acceptable Daily Intake (0.3 mg/kg). The authors expressed concern that significant levels of glyphosate are likely to reach the placenta and embryo in exposed pregnant women, given that little protective equipment is usually worn when this herbicide is applied. A study by Mose et al (2008) confirmed that glyphosate does cross the placenta: they found 15% of glyphosate in maternal circulation crossed to foetal circulation, although this figure could be higher as 32% of the glyphosate was unaccounted for after the experiment.

Using human liver HepG2 cells, Gasnier et al (2009), found that 4 different formulations of Roundup (but not glyphosate) inhibited aromatase activity with a non-linear biphasic effect on the aromatase mRNA levels: the greatest effect occurring at medium doses. Aromatase transcription and activity were disrupted from 10 mg/L. The authors also measured cytotoxicity with 3 assays, genotoxicity (comet assay), anti-oestrogenic (on ERα and ERβ) and anti-androgenic effects (on AR) using gene reporter tests. All parameters were disrupted at doses lower than agricultural rates, with all formulations, within 24h. These effects were more dependent on the formulation than on the glyphosate concentration. Endocrine disruption occurred from 0.5 mg/L[4] on the androgen receptor in MDA-MB453-kb2 cells for the most active formulation (R400). From 2 mg/L the transcriptional activities on both ERs were also inhibited. Cytotoxic effects started at 10 mg/L and DNA damage at 5 mg/L. Glyphosate alone had no anti-oestrogenic effect but "was clearly anti-androgenic at sub-agricultural and non-cytotoxic dilutions.

Similarly, Defarge el al (2016) measured the effects of 6 glyphosate-based formulations, 5 of their co-formulants (at concentrations below NOEC) and glyphosate alone on aromatase activity in human placental cell lines JEG-3. They found that glyphosate formulations and co-formulants decreased aromatase activity at concentrations much lower (up to 800 times) than the agricultural dilutions. Glyphosate alone also decreased aromatase activity but at 1/3 concentration of the agricultural dilution. Thus ingredients in glyphosate-based formulations appear to have a synergistic effect on aromatase.

An oestrogenic potential of glyphosate alone was also observed in human hormone-dependent breast cancer T47D cells, where exposure to environmentally relevant levels of glyphosate

4 800 times lower than the level permitted in some US animal feed, and 40 times lower than levels permitted in soybeans (FR 2000).

induced the activation of oestrogen response element (ERE) transcription activity by 5- to 13-fold, as well as ERα and β gene expression and resulted in proliferative growth of the breast cancer cells (Thongprakaisang et al 2013). This activation was inhibited by the addition of an oestrogen antagonist revealing an ER-mediated action.

Low, nontoxic doses of Roundup Bioforce (1 mg/L) and glyphosate (0.366 mg/L), similar to the levels found in the urine of those occupationally exposed (0.233 mg/L glyphosate), decreased testosterone levels by 35% in rat testes. Endocrine disruption in testicular cells can result in adverse effects, including epigenetic ones, on reproduction, including decreased sperm count and increased abnormal sperm (Clair et al 2012a). The study also found that glyphosate, at doses of 1-10 mg/L, increased aromatase mRNA in testicular cells, the enzyme that converts androgens to oestrogens.

In vivo studies

Effects of Roundup on testosterone and oestradiol (Romano et al 2010), as well as on corticosterone (Romano et al 2012) production have also been reported *in vivo* in rats, at doses below or equal to the NOAEL (50 mg/kg), following *in utero* and post-natal exposures. These developmental toxicity studies are discussed in detail in the next section.

In 2016, Varayoud et al ran a uterotrophic assay in which adult ovariectomised rats were injected subcutaneously for 3 days with a glyphosate-based herbicide (662 mg/mL of glyphosate potassium salt) at 0.5, 5, or 50 mg GBH/kg/day. Although the uterine weight and epithelial proliferation did not change, GBH affected the expression of oestrogen-sensitive genes. ERα protein expression was down-regulated at all doses, and expression of progesterone receptor (PR) mRNA was diminished. The expression of ERβ and PR was up-regulated in glandular epithelial cells, an effect that was also observed following exposure to 17β-estradiol.

In an 130-days *in vivo* study in rats, acute (0.5%) exposure to Roundup during an 8-day period increased the aromatase activity and aromatase protein levels in the testis at 68, 87 and 122 days after treatment (Cassault-Meyer et al 2014). Abnormal sperm morphology was also observed, suggesting the potential of Roundup to interfere with sex steroid hormones *in vivo.*

The implications of the endocrine-disrupting effects reported above can be profound and far-reaching, involving a range of developmental impacts including sexual and other cell differentiation, bone metabolism, liver metabolism, reproduction, pregnancy, development, behaviour, and hormone-related diseases such as breast and prostate cancer (Gasnier et al 2009).

Reproductive and developmental effects

Laboratory studies

Mesnage at al (2015c) reviewed all regulatory and independent studies on glyphosate and reported that some regulatory studies have detected reproductive and developmental toxicity below the NOAEL but dismissed the findings for reasons that are not in line with the principles of endocrinology. Among the reasons were the lack of dose-response, inconsistency of responses between genders and the necessity of biochemical disturbances correlated with organ lesions. A revision of such studies, following the principles of endocrinology, i.e. exposure starting already during prenatal life, is urgent.

A report by a group of internationally acclaimed independent scientists (Antoniou et al 2011) found that:

- Industry (including Monsanto) has known from its own studies since the 1980s that glyphosate causes malformations in experimental animals at high doses.
- Industry has known since 1993 that these effects also occur at lower and mid doses.
- The German government has known since at least 1998 that glyphosate causes malformations.
- The EU Commission's expert scientific review panel knew in 1999 that glyphosate causes malformations.
- The EU Commission has known since 2002 that glyphosate causes malformations, yet approved it anyway.

Antoniou et al (2012) concluded: "a substantial body of evidence demonstrates that glyphosate and Roundup cause teratogenic effects and other toxic effects on reproduction". They analysed industry data on glyphosate as reported in the German authorities 1998 draft assessment report, and found the following birth defects reported: increased heart malformations and abnormalities; absent kidneys; extra, distorted and rudimentary ribs; absent postcaval lobe of the lungs; reduced ossification of cranial centres and sacro-caudal vertebrae; undefined

skeletal malformations; and embryonic deaths. The German authorities had dismissed the birth defects on grounds such as a nonlinear dose-response (which is not in accord with current scientific understanding), and that some of the effects only happened at doses toxic to the mother (this situation is not unusual with industry studies, which use low numbers of animals and hence have to use high doses to find a statistically significant effect, in the process obscuring effects that may occur at low or medium frequency). As a result of the dismissal of these effects, the ADI set by the German regulators, of 0.3 mg/kg is 3 times higher than it should be based on these studies alone, according to Antoniou et al, who concluded that if more recent peer-reviewed studies were taken into account, the ADI would be 12 times lower, at 0.025 mg/kg/bw.

US EPA (undated) states that reproductive problems are a potential consequence of long-term exposure to glyphosate in drinking water above the maximum allowable concentration limit of 0.7 mg/L.

EFSA (2015b) relevant maternal NOAEL (glyphosate):
- rat = 300 mg/kg bw/day
- mouse = 50 mg/kg bw/day

(AMPA):
- rat = 150mg/kg bw/day

EFSA (2105b) relevant developmental NOAEL (glyphosate):
- rat = 300 mg/kg bw/day
- mouse = 50 mg/kg bw/day

(AMPA):
- rat = 400 mg/kg bw/day

Reported effects suffered by rats (at high doses only) include reduced maternal body weight; decreased total implantations and number of viable foetuses; increased number of early resorptions; reduced litter size; reduced foetal and pup weight; and reduced ossification of the breastbone (US EPA 1993, 2006; IPCS 1994).

Williams et al (2000) provided developmental NOAELs in rats of 400 mg/kg/day for AMPA (based on maternal and foetal body weight effects) and 15 mg/kg/day for POEA (based on decrease in food consumption and mild clinical signs); hence, both POEA and AMPA are considerably more developmentally toxic than glyphosate.

Despite the high NOAELs and LOAELs reported for regulatory purposes, independent laboratory studies have shown that glyphosate and Roundup formulations can cause adverse reproductive and developmental effects at much lower dose levels that are relevant to normal human exposure.

The endocrine-disrupting actions reported in the preceding section can cause such effects. Additionally, Richard et al (2005) showed that glyphosate killed a large portion of human placental cells after 18 hours, at concentrations (0.1%) lower than those recommended for use in agriculture. Roundup was more toxic than its active ingredient, but POEA potentiated the effect of glyphosate and facilitated its penetration of cell membranes. Similarly, Young et al (2015) found that Roundup was more cytotoxic to human placental cell lines than glyphosate alone at concentrations comparable to drinking water guidelines.

Oxidative stress and necrotic cell death could also trigger reproductive toxicity. Exposure of pubertal rat testis to Roundup caused oxidative stress and depleted antioxidant defences leading to Sertoli cell death (de Liz Oliveira Cavalli et al 2013). This may impact spermatogenesis and male fertility. The authors attribute cell death to an increase in Ca2+ uptake. Glyphosate alone, at doses between 1/50 and 1/250 of LD_{50}, also induced testicular oxidative stress in rats following 5 weeks exposure (Astiz et al 2009b), and decreased plasma testosterone levels.

Low subclinical doses of glyphosate, "indicative of involuntary exposure to residual agrochemicals", (10 mg/kg bw) caused oxidative stress in testicular tissue and decreased antioxidant defence systems resulting in modified hormonal parameters involved in reproductive function, including decreases in testosterone, follicle stimulating hormone and luteinizing hormone levels in the plasma of treated rats. The effects were worse with simultaneous exposure to glyphosate, dimethoate and zineb (Astiz et al 2009b).

Clair et al (2012a) also found that exposure to Roundup Bioforce and glyphosate "at agricultural levels" kills testicular Sertoli cells.

In 2009, Benachour & Séralini demonstrated that glyphosate, Roundup, POEA, and the metabolite AMPA all cause cell death in human umbilical, embryonic and placental cells, at dilutions far below those used in agriculture. The relative toxicities were as follows: POEA > Roundup > AMPA > glyphosate. Glyphosate alone acted rapidly at concentrations up to 1,000

times lower than recommended agricultural use (Ho & Cherry 2009). Benachour & Séralini (2009) concluded "this work clearly confirms that the adjuvants in Roundup formulations are not inert". The study also demonstrated synergistic toxicity between glyphosate, POEA and AMPA. The cell deaths occurred at concentrations of Roundup corresponding to low levels of residues in food: "the proprietary mixtures available on the market could cause cell damage and even [cell] death around residual levels to be expected, especially in food and feed derived from R[oundup] formulation-treated crops".

The cell deaths together with the endocrine disrupting effects reviewed in the previous section could result in pregnancy problems leading to abnormal foetal development, low birth weights, or miscarriages (Benachour et al 2007; Gammon 2009).

As reported in the previous section, glyphosate and some glyphosate-based formulations (e.g. Roundup) alter aromatase activity and oestrogen production. Low levels of oestrogens in the foetal brain at the time of sex differentiation for the male can result in reduced fertility at puberty or adulthood. Dallegrave et al (2007) found that exposure of rats to Roundup during pregnancy and lactation did result in adverse effects on male reproduction during puberty and adulthood: a decrease in sperm number per epididymis tail and in daily sperm production during adulthood, an increase in the percentage of abnormal sperms and a dose-related decrease in serum testosterone level at puberty, and degeneration of spermatids during both periods. In females the only adverse effect recorded was that of delayed vaginal canal opening, which is an important marker of pubertal onset. All the doses of Roundup tested were considerably higher than those people are likely to be exposed to; however, the effects occurred at even the lowest dose levels used.

A study on semen characteristics in rabbits showed that glyphosate can cause a significant adverse effect on libido, ejaculate volume, and sperm concentration, with increased abnormal or dead sperm (Yousef 1995).

Dallegrave et al (2003) found that a Roundup formulation used in Brazil induced developmental retardation of the foetal skeleton in rats, especially incomplete skull ossification and enlarged fontanel. The rats were exposed to high doses, ranging from 500 to 1,000 mg/kg glyphosate, but the teratogenic effects occurred at all dose levels in a positive dose–response pattern, with the rate of defect at the lowest dose level being double that of the controls.

Neonatal exposure of rats to 2 mg/kg of Roundup Full II for 7 days resulted in changes in the uterine morphology in female pups and altered the expression of proteins involved in uterine organogenetic differentiation (Guerrero Schimpf et al 2016). Therefore, Roundup exposure disrupts the postnatal uterine development at the neonatal and prepubertal period, at much lower concentrations than the NOAEL.

Romano et al (2010) described the formulation Roundup Transorb as "a potent endocrine disruptor *in vivo*" when male rats were exposed during the puberty period. They found that it significantly altered the progression of puberty, reduced testosterone production and altered seminiferous tubules. Gestational maternal exposure to Roundup Transorb (NOAEL 50 mg/kg) caused behavioural changes in mating, early offset of puberty in male offspring, as well as increases in testosterone and estradiol concentrations, mRNA expression and protein content in the pituitary gland, and the serum concentration of luteinizing hormone, sperm production and reserves (Romano et al 2012).

Rats treated with sublethal doses of Roundup Max during pregnancy had decreased implantation rates, increased resorption of foetuses, and the surviving offspring had decreased skeletal calcification (Gerislioglu et al 2010).

In 2009, Argentinean researchers led by Argentinean scientist, Professor Carrasco of the University of Buenos Aires Medical School, demonstrated significant consistent and systematic malformations in amphibian embryos resulting from very low dose exposure to glyphosate, and warned that comparable effects can happen in humans (Paganelli et al 2010). In the first part of the study amphibian embryos were immersed in a solution of Roundup Classic, containing 48% w/v of a glyphosate salt, diluted to 1/5000 (equivalent to 430 µM of glyphosate). The embryos suffered head deformities. In the second part, the embryos were injected with glyphosate alone at 8 and 12 µM per injected cell): the impact was even more severe, demonstrating that it is the active ingredient, not the adjuvants that are the problem. The glyphosate caused marked alterations in cephalic and neural crest development and shortening of the anterior-posterior axis in tadpole embryos, resulting in deformities in the cranial cartilages at the tadpole stage. Other effects included shortening of the trunk, reduced

head size, eye defects, genetic alterations in the central nervous system, increased death of cells that help form the skull, deformed cartilage, eye defects, and undeveloped kidneys. Carrasco also stated that the glyphosate was not breaking down in the cells, but was accumulating. The authors concluded 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). The malformations occurred at the equivalent of 2.03 mg/kg glyphosate; residues have been found in soybeans up to 17 mg/kg (Antoniou et al 2010). The findings lend weight to claims that abnormally high levels of cancer, birth defects, neonatal mortality, lupus, kidney disease, and skin and respiratory problems in populations near Argentina's soybean fields may be linked to the aerial spraying of Roundup (Valente 2009; Trigona 2009; Ho 2009; Antoniou et al 2010; Paganelli et al 2010).

Animal evidence

In Denmark, a pig farmer took 38 live borne but malformed one-day-old piglets into the laboratory because of extraordinary high percentages of malformations in piglets. Abnormalities included: ear atrophy, spinal and cranial deformations, leg atrophy; one eye not developed and one enlarged eye; piglets without trunk, with elephant tongue, and female piglet with testes; one malformed piglet had a swollen belly, and fore gut and hind gut were not connected. The Danish pig farmer reported to the Danish media that he switched to non-GM and glyphosate-free feed and instantly observed positive changes in the health of the sow herd[5]. Krüger et al (2014b) analysed tissues such as the lungs, liver, kidney, brain, muscles, gut wall and heart of the piglets, and found that all contained glyphosate at the following levels:

	Minimum (*µ*g/mL)	Maximum (*µ*g/mL)
lung	0.15	80
liver	0	29.25
kidney	0.1	38
muscles	4.4	6.4
brain	0.4	19.7
intestine	0.7	7.7
heart	0.4	80

A number of studies on sea urchins, oysters, aquatic snails, and fish also described endocrine-disrupting effects: these are reported in the Environmental impacts section.

Human evidence

Birth defects in the Argentinean state of Chaco, where GM soy and rice crops are heavily sprayed with glyphosate, increased nearly 4-fold over the years 2000 to 2009, according to a report released by the Chaco state government (Robinson 2010). Paganelli et al (2010) also reported that "several cases of malformations together with repeated spontaneous abortions were detected in the village of Ituzaingo´ [Cordoba], which is surrounded by GMO-based agriculture". Similar birth defects were also experienced by 52 women in Paraguay, who were exposed during pregnancy to herbicides (Paganelli et al 2010).

These birth defects, specifically neural tube defects and craniofacial malformations, showed striking similarities to those induced by glyphosate in laboratory experiments (Paganelli et al 2010). The congenital malformations in Paraguay included microcephaly (small head), anencephaly, and cranial malformations (Benítez Leite et al 2009). Anencephaly occurs when the neural tube fails to close during pregnancy resulting in the absence of the majority of the brain, skull and scalp.

Media items report that, in April 2012, Argentinean tobacco farmers filed a lawsuit against Monsanto and tobacco companies that had asked them to use Monsanto's products, which they knew or should have known cause birth defects. The majority of the small family-owned farms use Roundup. Birth defects and other effects cited in the complaint include cerebral palsy, Down syndrome, psychomotor retardation, missing fingers, blindness, epilepsy, spina bifida, intellectual disabilities, metabolic disorders, and congenital heart defects (Filip 2012).

A Canadian epidemiological study, the Ontario Farm Family Health Study, which involved 1,898 couples and 3,984 pregnancies, found an association between exposure to glyphosate-based herbicides, and spontaneous abortions (miscarriages) and pre-term deliveries. Pre-conception exposure of the father to glyphosate was associated with increased risk of pre-term delivery (odds ratio 2.4) and to a lesser extent of spontaneous abortion (odds ratio 1.5) (Savitz et al 1997). Use of glyphosate by the mother in the 3 months prior to conception was associated with an increased risk of late (12th to 19th

5 Full story: http://www.gmwatch.org/index.php/articles/gm-reports/13882

weeks) abortions (odds ratio 1.7). However, the risk was 3 times higher for older women (>34 years) than for women of the same age who had not been exposed to glyphosate (odds ratio 3.2) (Arbuckle et al 2001).

A positive association was found between decreased fecundity, as measured by time to pregnancy, and exposure of both spouses to a variety of pesticides including glyphosate (Curtis et al 1999), but a recent study in Colombia in which exposure to glyphosate was poorly characterised provided confused results and no clear link (Sanin et al 2009).

A systematic review of current studies on glyphosate and birth outcomes (and 2 studies examining time-to-pregnancy) in humans found ten studies testing associations on glyphosate. An excess of Attention Deficit Hyperactivity Disorder was found in children born to glyphosate appliers in one study, with no other significant associations described. The authors noted that current epidemiological evidence does not support public health concerns around birth outcomes, however, the authors also noted that the current literature is limited and methodological limitations, particularly a lack of direct measurements of glyphosate exposure, make it clear that negative findings are not definitive evidence that glyphosate brings no risk for human development and reproduction (de Araujo et al 2016).

Nervous system

A number of studies have shown that glyphosate can adversely affect neuronal development and nerve cells, including those in the brain's dopaminergic system. There are indications it may have a role in Parkinson's disease, and in autism and Attention Deficit Hyperactivity Disorder (ADD/ADHD) in children.

Laboratory studies

US EPA (2006) stated that they did not require neurotoxicity or developmental neurotoxicity studies, and found no evidence of neurotoxicity.

However, a study designed to determine if chronic exposure to low doses of the organophosphate diazinon rendered nerve cells more sensitive to other chemicals subsequently did show that Roundup especially, but also glyphosate, can affect nerve cells. Both inhibited growth of 'neurite-like structures' (axons or dendrites) even without pre-exposure to diazinon, but higher concentrations were required to cause this effect. Concentrations as low as 10 μM for glyphosate alone, and 0.5 nM for Roundup, inhibited growth of neurite-like structures in nerve cells pre-exposed to diazinon (Axelrad et al 2003). The authors reported that a "recent study of glyphosate (formulated as Roundup) exposure in farmers showed a maximum adult plasma/tissue concentration of 17 nM". Thus the level of internal exposure for farmers using Roundup herbicide may be sufficient to affect nerve cells, especially if those farmers have previously been exposed to organophosphate insecticides like diazinon.

Similarly it has been reported (Coullery et al 2016) that the exposure of neuronal cells to glyphosate (4mg/mL, 23mM) induces a delay in neuronal development and differentiation. The study did not show evidence of any lethal effect in cultured neurons, nonetheless the total axonal length and the number of branches were irreversibly reduced by the treatment with the herbicide.

Hernández-Plata et al (2015) recently showed that repeated intraperitoneal injections of glyphosate (50 to 150 mg/kg bw) caused decreased exploratory behaviour and spontaneous locomotor activity in rats immediately after each injection, showing an acute effect. The effect was also apparent 2 days after the last injection but not after 16 days suggesting that these alterations occur while a critical concentration of glyphosate is present in the system. However, the study also found that the D1 dopamine receptor binding was reduced in the nucleus accumbens in the brain and that acute glyphosate administration immediately decreases extracellular levels of basal striatal dopamine. These results suggest that glyphosate affects the dopaminergic system.

Anadón et al (2008) identified the possible role of glyphosate in neurodegenerative diseases, especially Parkinson's disease. Glyphosate produced a significant dose-dependent depletion of serotonin and dopamine, also increasing the metabolites of these 2 neurotransmitters in male rats.

This was followed by a study in 2009 (Astiz et al 2009a) that lent further weight to a role in Parkinson's disease. Low doses (10 mg/kg, intraperitoneal injection) of glyphosate, by itself and in combination with zineb and dimethoate, caused a loss of mitochondrial transmembrane potential in rat brain cells, especially in the substantia nigra region of the brain. 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 are highly sensitive to free radical damage which results from oxidative stress – and a number of studies reported earlier show that glyphosate and Roundup cause oxidative stress in various cells, including brain cells.

Gui et al (2012) discovered that chronic exposure of neuronal differentiated PC12 cells to glyphosate induced both apoptotic and autophagic cell death. Apoptosis is an energy-dependent, natural, genetically controlled process regulated by the Bcl-2 family proteins. Autophagy is a programmed cell death pathways but unlike apoptosis, its involvement in the pathogenesis of Parkinson's disease has been investigated only recently. Beclin-1, a Bcl-2-interacting protein, has been identified as the protein potentially regulating the 2 pathways. This provides a novel link between use of glyphosate and Parkinson's disease, and lends further support to the epidemiological findings and case reports that indicate glyphosate exposure may be causing Parkinson's.

A fourth study has found that glyphosate products (in this case Touchdown) can cause degeneration of the dopaminergic neurons, a hallmark of Parkinson's disease (Negga et al 2012). This study was carried out on roundworms, confirming the previous studies on rats.

Cattani et al (2014) demonstrated that 0.01%. Roundup (0.036 g/L glyphosate) induced 45Ca2+uptake and decreased cell viability in hippocampal slices from immature rats. The authors emphasised that the concentration of Roundup used in agriculture ranges from 1% to 2%. Exposure to Roundup affects the glutamate metabolism in the hippocampus from immature rats. The results showed that Roundup leads to glutamatergic excitotoxicity, oxidative damage and energy deficit in hippocampal cells from immature rats, which culminate in neural cell death.

Nervous system impacts have also been reported in fish (see section on Aquatic toxicity).

Human evidence

A study of children born to pesticide applicators in Minnesota, USA, found a significant correlation between exposure to glyphosate-based herbicides and neurodevelopment effects, and in particular ADD/ADHD. Forty three percent of the children who had parent-reported ADD/ADHD had parental exposure to glyphosate-containing herbicides (Garry et al 2002).

Accidental exposure, both dermal and inhalation, to a glyphosate herbicide has been linked to a case of parkinsonism. A 54-year-old man developed skin lesions 6 hours after he accidentally sprayed himself, then one month later developed a "symmetrical parkinsonian syndrome". Magnetic resonance imaging revealed effects, 2 years later, in the globus pallidus and substantia nigra regions of the brain, which are associated with Parkinson's disease (Barbosa et al 2001).

Using a proven GIS method to calculate the population exposure to various pesticides, researchers have recently correlated data about Parkinson's disease incidence, land use and pesticide usage in Nebraska. The analysis suggests that Parkinson's disease incidence is significantly associated with the exposure to several pesticides, including glyphosate. Individuals older than 75 years living in rural areas with low socio-economic level presented the highest rate of Parkinson's disease. The authors point out that the toxicity of a pesticide could be enhanced when administered with other pesticides (Wan & Lin 2016).

Nevison (2014) investigated whether the trends in several environmental toxins, including glyphosate, correlated with increasing diagnoses of autism in US children. Epidemiology has linked autism and developmental disorders to cholinesterase-inhibiting organophosphate insecticides. However, the use of these insecticides declined about 30% between 1995 and 2005 and does not correlate with autism trends. At the same time, the use of glyphosate has steadily increased. Its mechanism of toxicity involves the shikimate pathway, used by human gut bacteria, which play an important role in the immune system and are often compromised in autistic children.

In addition, the metabolism of glyphosate depends on glutathione, which is significantly depleted in autistic individuals. Based on the consideration that the temporal trend of glyphosate use tracks the ongoing increase in autism, the author concludes that glyphosate could be interacting with other toxins to drive up the prevalence of US autism.

Immune function

Several studies indicate that glyphosate formulations may interfere with the immune system resulting in adverse respiratory effects including asthma, rheumatoid arthritis, and autoimmune skin and mucous membrane effects.

Laboratory studies

A study to explore the ability of glyphosate to cause airway asthma-like pathology and occupational lung disease, found exposure to glyphosate to result in airway barrier damage and to modulate the immune system in mice. Glyphosate-rich air samples from farms, and pure glyphosate, induced substantial type 2 airway inflammation in mice, over both short and longer time periods. They increased eosinophil and neutrophil counts, mast cell degranulation, and production of asthma-related cytokines (IL-5, IL-10, IL-13, IL-33, TSLP). The rates used were 100 ng and 1 μg glyphosate per air sample; increasing the dose of glyphosate 100-fold up to 100 μg did not substantially change the degree or character of inflammation, however, a longer exposure to glyphosate did significantly worsen histological pathology. Additionally, co-exposure with an allergen led to a profound inflammatory and antigen-specific innate and adaptive immune response (Kumar et al 2014).

Human evidence

Exposure to glyphosate was associated with both atopy (immune responses to allergens) and with atopic asthma (asthma resulting from exposure to allergens) in women, in the US's Agricultural Health Study involving 25,814 female (Hoppin et al 2008) and 19,704 male farmers (Hoppin et al 2009). The same study also found that rhinitis ('runny nose') is associated with use of both glyphosate and 2,4-D (Slager et al 2009). A more recent prospective study of male farmers in North Carolina and Iowa, USA, found an association between glyphosate exposure and both allergic and non-allergic wheeze (Hoppin et al 2016).

In a case-control study on the systemic autoimmune disease rheumatoid arthritis (RA) amongst female partners of pesticide applicators in USA, (275 cases, 24,081 non-cases), women with RA were more likely to have used glyphosate (OR = 1.5) (Parks et al 2016).

One man developed severe autoimmune blistering of skin and mucous membranes (pemphigus vulgaris) after exposure to the fumes of burning glyphosate (Fisher et al 2008).

Toxic Interactions

Synergistic effects between glyphosate and chlorpyrifos were found on enzyme levels in mosquitofish (Rendón-von Osten et al 2005).

A synergistic increase in acute toxicity was reported for 2 glyphosate formulations with cypermethrin formulations. The magnitude of synergism was approximately 2-fold for all concentrations of Glifoglex® and 4-9 times for Glifosato Atanor® (Broduer et al 2014).

Glyphosate and metsulfuron-methyl were synergistic in their phytotoxic effects on plants; the effect was more pronounced with Roundup (Kudsk & Mathiassen 2004).

Pre-exposure to diazinon increased the inhibitive effects of glyphosate and Roundup on the growth of 'neurite-like structures' (axons or dendrites (Axelrad 2003).

Simultaneous exposure of rats to glyphosate, dimethoate and zineb at subclinical doses (10 mg/kg bw) increased the effects of glyphosate on testicular tissues, in which it caused oxidative stress and decreased antioxidant defence systems, resulting in decreases in testosterone, follicle stimulating hormone and luteinizing hormone levels in plasma (Astiz et al 2009b).

Glyphosate and Roundup significantly increased the uptake of mercury by the freshwater flea *Ceriodaphnia dubia* (Tsui et al 2005).

Human Exposure

Exposure guidelines

The US EPA (2006) established an 'incidental oral exposure' and a chronic Reference Dose (RfD) of 1.75 mg/kg/day based on the NOAEL of 175 mg/kg/day in the rabbit developmental study.

Reference doses:

- US Chronic RfD = 1.75 mg/kg bw/day (US EPA 2006)
- EU ARfD = 0.5 mg/kg bw/day (EFSA 2015a)

Acceptable daily intake (ADI):

- FAO (2000) = 0.3 mg/kg bw
- EFSA (2015a) = 0.5 mg/kg bw/day

Acceptable Operator Exposure Level (AOEL):

- EFSA (20165a) = 0.1 mg/kg bw/day
- EU limit for drinking water = 0.1 mg/L (Buffin & Jewell 2001)
- WHO guideline for drinking water = none set

Occupational exposure

The US EPA (1993) recommended protective clothing (including protective eyewear) for mixer/loader/applicators. According to US EPA (2006), the "Roundup WeatherMax® label specifies that for application solutions of 30% or greater concentration, mixers, loaders, other handlers and applicators must wear personal protective equipment (PPE) consisting of a long-sleeved shirt, long pants, shoes with socks, and chemical-resistant gloves. If the application solution is 30% or less of the product, applicators must wear PPE consisting of a long-sleeved shirt, long pants, and shoes with socks." Roundup Weathermax contains 48.8% glyphosate as the potassium salt.

According to a UK exposure model, hand-held spraying of glyphosate without wearing a long-sleeved shirt and long trousers will result in operators being exposed at levels more than 5 and a half times higher than the AOEL (568%), although the German exposure model estimates it to be 115% of the AOEL (EFSA 2015a).

A farm family exposure study (Acquavella et al 2004) carried out in Minnesota and South Carolina, USA, found that 60% of the farmers who had applied and/or mixed glyphosate-based herbicides had detectable levels of glyphosate in their urine on the day of application; the mean level was 3 μg/L and the maximum 233 μg/L, the highest every measured in a person (Niemann et al 2015). Higher levels were associated with not wearing rubber gloves. Twelve percent of the children had detectable glyphosate (max 29 μg/L) and all but 1 of these had been present/involved during mixing, loading and/or spraying. As well, 4% of spouses, who had no involvement in the mixing or application, also had detectable levels in their urine (max 3 μg/L). The highest estimated systemic dose (i.e. the dose that would have been absorbed into the body) was 0.004 mg/kg (Acquavella et al 2004). Although this is considerably below the US EPA's reference dose of 2 mg/kg/day, one study reported earlier showed that glyphosate and Roundup can have damaging effects at much lower exposure levels: Benachour & Séralini (2009) demonstrated that glyphosate, Roundup, POEA, and AMPA all cause cell death in human umbilical, embryonic and placental cells, at 105 dilutions.

Another study, comparing urinary levels in mothers, fathers and children in Iowa, USA, found a high incidence in all groups but especially children, with the highest levels of residues (18 μg/L) occurring in children (Curwin et al 2007):

	% detection	max conc. (μg/L)
father		
• non-farm	66	5.4
• farm	75	18
mother		
• non-farm	65	5.0
• farm	67	11
child		
• non-farm	88	9.4
• farm	81	18

More recently, glyphosate levels were tested in the urine of a farmer in France who sprayed glyphosate, and his family, including 3 children. Glyphosate reached a peak of 9.5 µg/L 3 in the farmer 3 hours after spraying, and 2 µg/L were found in him and in one of his children living 1.5 km from the field, 2 days later. The farmer sprayed by tractor 55 L of a glyphosate herbicide at various concentrations on 3 fields. In addition, he sprayed 0.75 L with a hand sprayer.

During the dilution of the formulation, he wore a mask and gloves whilst mixing but not while spraying from a tractor, which had an open window. When hand-spraying, he wore gloves but neither a mask nor a protective suit (Mesnage et al 2012).

Non-occupational exposure

Non-occupational exposure to glyphosate is very common, resulting primarily from ingestion of residues in food, but also through contact and inhalation exposure from its widespread use in home gardens, and for the spraying of roadsides, parks and other public places.

Exposure via spray drift is likely to be significant, given the extent of drift reported below and the array of health effects reported in Ecuador up to 10 km away from the aerial application of glyphosate (see section on 'Poisonings').

Spray Drift

Off-target drift of glyphosate can be a considerable problem. Studies report that 10-37% of glyphosate applied to the foliage of weeds drifts to non-target plants (Cakmak et al 2009).

Cox (1998) reported a number of studies showing:

- seedling mortality occurred at 20 m, and sensitive species mortality at 40 m, downwind from glyphosate application with a tractor-mounted sprayer;
- residues have been measured 400 m downwind from ground applications; and 800 m from helicopter applications;
- plant injury has occurred 100 m downwind from fixed wing aerial application;
- one study calculated that buffer zones of 75-1,200 m would be required to protect non-target vegetation;
- Monsanto itself has reported a number of drift incidences after aerial application causing damage to 1,000 trees, 250 acres of corn, and in a third incident 155 acres of tomatoes.

Residues in food and drink

According to the US EPA (2006), uptake of glyphosate and AMPA by plants from the soil is limited, so residues in plants result largely from direct application.

In terms of animal products, low level residues are most likely to occur in kidneys and liver, with lower levels also being transferred to egg yolks, according to studies on laying hens reported by EFSA (2015a).

Residue analysis for glyphosate and its metabolite AMPA is difficult and expensive, and is not routinely included in residue monitoring. As both are translocated throughout plant tissue, residues are unlikely to be completely removed from produce by washing, peeling or removing the outer leaves. Minimal breakdown of glyphosate occurs in plant tissue and pre-harvest use can result in significant levels of residues; in grains, they are not destroyed by milling and much of it remains in the bran; nor are they lost during baking. Residues in malting barley are transferred to beer. Use of glyphosate on forage and animal feed can result in residues in the kidneys of animals, also residues in meat, milk and eggs. Residues are stable for up to one year in plant material and in water, and 2 years in animal products, in storage. In the wild, residues of glyphosate can persist for a long time (45 mg/kg found in lichens 270 days after application). Sampling of wild berries after forest spraying operations showed that residues remained above 0.1 mg/kg for the 61 days during which samples were taken (Roy et al 1989; Agriculture Canada 1991; IPCS 1994; US EPA 1993; Buffin & Jewell 2001). EFSA (2015a) reports that residues of glyphosate, AMPA, N-acetyl-glyphosate and Nacetyl-AMPA in crops are stable under processing and storage conditions. Studies show that glyphosate remains stable in potato tubers for 8 months (Hutchinson et al 2014).

EFSA (2015) reports that glyphosate-tolerant crops “may contain high amounts of AMPA or N-acetyl-AMPA”, whereas glyphosate is the major residues in non-tolerant crops.

Glyphosate applied pre-harvest to wheat also causes the accumulation of shikimic acid in the wheat and subsequently elevated levels in bread (Bresnahan et al 2003).

A wide range of trials carried out under conditions of “Good Agricultural Practice” have resulted in residues in many different fruits, vegetables, grains and animal fodder crops (FAO & WHO 2005), so it can be assumed that residues in food are highly likely, and despite the failure to include glyphosate in routine residue monitoring, they have been widely found in foods. Many of the residues result from the practice of pre-harvest desiccation – spraying grains just before harvest to burn down the plants; and use on crops genetically engineered to tolerate glyphosate (Roundup Ready). Many of the crops treated in these ways become animal feeds and hence residues can be passed on to dairy, poultry and meat products; or they enter a wide variety of processed foods. When glyphosate-tolerant soy was permitted on the market in Australia and New Zealand, the Maximum Residue Level had to be increased 100-fold in order to accommodate the increased residues expected from direct application of glyphosate to the soybean crops (ANZFA 2000; FSANZ 2009). In Europe the MRL was increased 200 fold, from 0.1 mg/kg to 20 mg/kg (Dibb 2000).

In 1999, Monsanto declared that glyphosate residue levels of 5.6 mg/kg in glyphosate-tolerant soybean were considered to be extreme high values (Bøhn et al 2013). Yet levels much higher than this are now routinely found. An analysis of 31 batches of soybeans from Iowa, USA, found high levels of glyphosate and AMPA

in the Roundup Ready beans, but none in the conventional or organically grown beans. The mean levels were for Roundup 3.3 mg/kg and AMPA 5.7 mg/kg; and the highest levels were for Roundup 8.8.mg/kg and AMPA 10 mg/kg (Bøhn et al 2014). AMPA has been found at levels as high as 25 mg/kg in other studies (with glyphosate at 7 mg/kg) (Duke et al 2003). In 2003, glyphosate was found at levels up to 1.8 mg/kg in GM soybeans in Argentina (Arregui et al 2004). More recently, GM soybeans collected from fields in Argentina were found to contain up to 25.9 mg/kg glyphosate and 47 mg/kg AMPA (Testbiotech 2013).

Monitoring by the Alliance for Natural Health USA found glyphosate residues in bagels, bread, breakfast cereals, eggs, and coffee creamer including organic ones, with levels up to 1327.1 µg/kg for instant oatmeal strawberries and creamer. Organic free-range eggs contained 169 µg/kg, more than 3 times the allowable level (ANH 2016). And in 2013, glyphosate residues were found in the candies 'Froot Loops' at a level of 0.12 mg/kg (GMO Free USA undated). These findings are supported by a scientist with the US Food and Drug Administration, who acknowledged that he had found glyphosate residues in 10 out of 14 oat-based cereals (Chamkasem 2016; Gillam 2016).

Glyphosate residues were found in samples of honey and soy sauce purchased in Philadelphia, US. Of the 69 honey samples analysed, 41 samples (59%) had residues with a concentration range of 17-163 µg/kg and a mean of 64 µg/kg. Eleven of the tested honey samples were organic; 5 of these (45%) contained glyphosate, with a range of 26-93 µg/kg and a mean of 50 µg/kg. Glyphosate concentrations above the LOQ (75 µg/kg) were also found in 10 of 28 soy sauce samples (36%), with a concentration range between of 88-564 µg/kg and mean of 242 µg/kg. The countries of origin of the contaminated honey included Brazil, Germany, New Zealand and USA (Rubio et al 2014).

In the UK, glyphosate has been found in strawberries, lettuce, carrots, lentils, pulses, rice (Buffin & Jewell 2001; EFSA 2009; Waters 2013); and a number of cereal-based foods: bread, wheat flour, wheat, barley, bran, oats, breakfast cereals, cereal bars, and polenta (Harris & Gaston 2004). Between 2000 and 2013, 2,951 bread samples were tested by the UK's DEFRA Expert Committee on Pesticide Residues in Food; the most frequently found residue was glyphosate (PAN UK 2014).

In May 2016, Taiwan recalled Quaker oats products imported from the US after finding glyphosate residues in 10 out of 16 products (at 0.1 to 1.8 mg/L). Taiwan does not permit residue levels of glyphosate to exceed 1 ug/L (Chow 2016).

In Australia, glyphosate residues were found in 20 food samples, with 75% returning positive samples from a pool of samples taken from pregnant women's diets (McQueen et al 2012).

In the most recent results of food residue monitoring in the EU (2013), glyphosate was one of the most commonly detected residues, found in 44% of oat samples, wine grapes (7%), rye (5%), and apples (1.61%). Levels were highest in rye (2.06 mg/kg) and oats (1.5 mg/kg) (EFSA 2015a).

Glyphosate has also been detected in the organs and urine of cows, indicating that people eating dairy products or beef may be exposed to glyphosate via this route. Compositional changes detected in the cows with glyphosate residues may also affect the quality of food for humans. In a study of 30 dairy cows from 8 Danish farms, glyphosate was found in the urine of all, in the range of 10-103.3 ng/mL. Unexpectedly low levels of manganese and cobalt were observed in all cows (Krüger et al 2013b). A second study compared German and Danish cows, and those that had conventional feed and those kept in a GM-free area. German cows and those 'GM-free' had significantly lower levels of glyphosate in their urine than Danish cows and those on conventional feed. Additionally, glyphosate was found in cow intestine, liver, muscles, spleen, lung and kidney. Levels in urine were 0.46-164 µg/mL and in organs at levels of 4.7-108 µg /mL (Krüger et al 2014b).

Commercial broiler chicken fed the normal commercial feed were killed and tested for residues at 30 days of age. Residues of glyphosate were found in liver, spleen, lung, intestine, heart, muscles and kidney (Shehata et al 2014a).

Residues were found, in the US, in Pediasure Enteral Nutritional Drink, used to feed babies and children in critical care in hospitals. Thirty percent of samples had levels of glyphosate above the detection level of 75 µg/kg, with the highest level being 111 µg/kg. Residues are presumed to come with the GMO corn and soy in the feeding tube liquid, which is given to children who are unable to take food by mouth (Honeycutt 2015). This method of exposure delivers glyphosate

directly to the gastrointestinal track where it may affect the microbiome.

Glyphosate has been found in alcoholic beverages too. In Germany, the Munich Environmental Institute found glyphosate in all of the 14 brands of beer tested, at 0.46 to 29.74 µg/L (Guttenberger & Baer 2016).

It has also been found in 10 wines in California, USA, including organic wines, the levels ranging from 0.659 µg/kg in organic to 18.74 µg/kg in conventional wine (Honeycutt 2016a).

Glyphosate is moderately persistent in water and not removed by normal drinking water processing (Agriculture Canada 1991); and residues have been found in drinking water in the UK (Buffin & Jewell 2001).

In the USA, Honeycutt & Rowlands (2014) reported glyphosate above the LOQ of 0.05 µg/L in 13 out of 21 samples of household drinking water, at levels up to 0.151 µg/L.

Glyphosate was recently found in the raw drinking water supply on the island of Guernsey, UK. Residues were found in 19 out of 24 samples, with 11 of the streams tested above the UK compliance standard for treated water, but no glyphosate was detected after treatment (BBC 2015; Guernsey Press 2016). The maximum level found was 0.517 µg/L (Guernsey Water 2016).

Residues in non-food products

People may also be exposed to glyphosate through residues in other products they come in contact with. For example, glyphosate residues were found in 85% of cotton personal hygiene products tested in Argentina, including gauze, cotton balls, baby wipes, and female pads and tampons. AMPA residues were found in 62% (Pietrowski 2016).

In a preliminary study, Moms Across America submitted samples of 5 vaccines to Microbe Inotech Laboratories Inc. of St. Louis, Missouri, In March 2016, for testing for pesticide residues. Using an enzyme linked immunosorbent assay (ELISA), glyphosate residues were detected in all 5 vaccines submitted. The highest amount detected was 2.671 µg/L in MMR vaccine. How the glyphosate got into the vaccines is not yet clear but presumed to be through the use of GM-derived plant and/or animal material (or pre-harvest sprayed plant material directly or via products derived from animals fed such material) in the vaccine preparation (Honeycutt 2016b).

vaccine	dilution	µg/L
MMR	undiluted	2.671
Influenza	undiluted	0.331
Hepatitis B	undiluted	0.325
T Dap	undiluted	0.123
Pneumococcal	undiluted	0.107

Residues in humans

Breast milk

Glyphosate was found in breast milk, above the rather high LOQ of 75 µg/L, in 3 out of 10 women tested in USA, at rates up to 166 µg/L (Honeycutt & Rowlands 2014).

Blood

In a study on exposure to herbicides and bacterial toxins through genetically modified food, in Quebec, Canada, glyphosate was found in the serum of 5% of 39 nonpregant women, at a maximum of 93.6 ng/mL, but not found in 30 pregnant women (Aris & Leblanc 2011).

Urine

Thirty-five urine samples obtained from women, men and children (4 to 71 years of age) from 14 US States were tested for glyphosate. Even though the LOQ was high (at 7.5 µg/L) it was exceeded in 13 samples, with levels ranging from 8.1 µg/L in a 6-year old boy to 18.8 µg/L in a 26-year old woman (Honeycutt & Rowlands 2014).

A study commissioned by the German action group “Ackergifte Nein Danke” found glyphosate in the urine of 99.6% of 2,009 people monitored. The highest levels were in children aged 0-9 and adolescents aged 10-19, particularly in people raised on farms. Meat eaters had higher levels of glyphosate contamination than vegetarians or vegans (Krüger et al 2016a).

In April 2016, 48 Members of the European Union (MEPs) from 13 member states had urine tests for glyphosate. All were found to be contaminated. On average, the MEPs had 1.7 µg/L in their urine, with a peak of 3.57 µg/L and a minimum of 0.17 µg/L (Greens & EFA 2016; Krüger et al 2016b).

In 2013, 182 human urine samples from 18 European countries were analysed for glyphosate and AMPA residues. Glyphosate was found in 43.9% of samples, and AMPA in

35.7%. Mean level of glyphosate was 0.21 µg/L with a maximum of 1.56; and the mean level for AMPA was 0.18 µg/L with a maximum of 2.63. Glyphosate was found in the urine from all countries, with the highest frequency of detection being for Malta (90%) and the lowest Bulgaria and Macedonia (10%) (FOE 2013; MLB 2013).

In a study reported by Niemann et al (2015), but not publically available, frozen urine samples collected between 1996 and 2012 from 22 of 40 students from the northern Germany city of Greifswald contained glyphosate at a maximum level of 0.65 µg/L. 10 of the 40 also had AMPA, with a maximum value of 1.31 µg/L. There was an increase in the levels of glyphosate, but a decrease in AMPA, as time progressed.

In a study that drew attention to both the diet and health of the participants, the urine of 140 people in Europe was analysed for glyphosate residues; 99 of these ate a conventional diet and 41 an organic diet. A further 201 samples were collected, 102 from healthy people and 99 from chronically diseased people. Chronically ill people had significantly higher levels of glyphosate in their urine than healthy people (Krüger et al 2014b).

In a study of more than 300 people, mostly from Germany, glyphosate was significantly higher in urine of humans with conventional feeding than organic; and chronically ill people had significantly higher residues in their urine than healthy population (no figures provided). The maximum value was 5 µg/L for conventional diet (Krüger et al 2014b).

In 2016, testing was carried out on urine samples from 10 people living in each of 2 communities in the Yucatan Peninsular in Mexico (Ich Ek and Suc Tuc, in the Holpechen Municipality, State of Campeche). Glyphosate was found in 7 of the 20 smaples, at up to 0.5104 ug/L (Rendón von Osten et al 2016).

Poisonings

There have been many cases of intentional ingestion of glyphosate-containing products, which have led to comprehensive descriptions of acute symptoms. Involuntary occupational or bystander exposure has also resulted in a long list of both acute and chronic effects.

Acute effects observed in humans

Ingestion

Symptoms reported following ingestion of glyphosate formulations include:

- corrosive effects on the gastrointestinal system with sore throat, mouth ulcers, difficulty swallowing, abdominal pain, massive gastrointestinal fluid loss, gastrointestinal haemorrhage, gastric and duodenal ulcers, nausea, vomiting, diarrhoea;
- kidney and liver impairment, renal failure, with decreased urine and severe hypoxia (inadequate oxygen in body), pancreatitis;
- respiratory distress, nasal, bronchial and lung congestion, bronchial constriction, swelling of the lungs, pleuritic chest pain, pneumonia, lung dysfunction;
- metabolic acidosis, elevated potassium in the blood (hyperkalaemia);
- pulmonary oedema, arrhythmias, low blood pressure, slowed heart rate (bradycardia), red blood cell destruction, leucocytosis (raised white cell count), abnormal electrocardiograms, hypotensive shock, cardiogenic shock;
- impaired consciousness, and death (US EPA 1980; Sawada et al 1988; Talbot et al 1991; IPCS 1994; Cox 1995a; Chang et al 1999; Lin et al 1999; Bradberry et al 2004; Stella & Ryan 2004; Sampogna & Cunard 2007; Hsiao et al 2008).
- In 13 cases of acute intoxication with glyphosate (mostly suicidal), in France, the most common symptoms were oropharyngeal ulceration, nausea and vomiting. The main altered biological parameters were high lactate and acidosis. There was also respiratory distress, cardiac arrhythmia, hypercalcemia, impaired renal function, hepatic toxicity and altered consciousness. In fatalities, the common symptoms were cardiovascular shock, cardiorespiratory arrest, haemodynamic disturbance, intravascular disseminated coagulation and multiple organ failure (Zouaoui et al 2013).

A 56-year-old woman ingested about 500 ml of herbicide containing glyphosate isopropylamine salt. Symptoms included hypotension, coma, hyperkaliemia, and respiratory and renal failure. The patient survived the acute phase of poisoning, but she developed vigil coma (a state in which the patient appears awake with eyes

open but is in an unresponsive coma) (Potrebic et al 2009).

A 36-year-old male patient who attempted suicide by drinking approximately 300 ml of a glyphosate formulation presented as somnolent, normotensive, acidotic and hyperkalemic. He became hypotensive, and hypoxic with oliguric acute renal failure. After a single 27.5-hour dialysis treatment, clinical condition and renal function parameters indicated he did not require further dialysis, and there was complete recovery of renal function on day 5 (Knežević et al 2012).

A 62-year-old man who drank a bottle of Ortho TotalKill (41% glyphosate plus POEA) was found unresponsive. He may also have taken ethanol and an opioid. He became bradycardic and obtunded with respiratory depression. Haemodialysis 16 hours after the ingestion resulted in improvement in his clinical status and he was transferred from intensive care on day 3 (Garlich et al 2014).

Malhotra et al (2010) reported the case of a 71-year-old male who attempted suicide with a glyphosate formulation and developed a prolonged but reversible encephalopathy suggestive of acute central nervous system toxicity. He was in cardiogenic shock with severe metabolic acidosis. Neurologic investigations were performed to exclude structural pathology. CT brain scan was normal. An EEG reading on day 8 revealed generalised slow wave activity with triphasic sharp and slow wave complex consistent with an encephalopathy although non-convulsive seizures could not be excluded. Transferred from intensive care on day 10, he was discharged with full clinical recovery on day16.

In Japan, a 58-year-old woman who ingested approximately 150 ml of a formulation containing 41% glyphosate and 15% POEA was admitted to an emergency centre in a semicomatose state. Acute respiratory distress syndrome, circulatory collapse, acute renal failure, and disseminated intravascular coagulopathy were diagnosed. Meningitis was also suspected. All signs and symptoms suggesting meningitis resolved as the concentration of glyphosate in the cerebrospinal fluid decreased. She was discharged on day 39 of hospitalisation (Sato et al 2011).

A case study of an 89-year-old male who attempted suicide by ingesting a large amount of a glyphosate formulation reported acute pancreatitis as an effect not previously covered by other case reports (Hsiao et al 2008).

One woman in Taiwan developed painful swelling and rhabdomyolosys (breakdown of muscle tissue) in her arm after injecting herself with a glyphosate formulation in a suicide attempt (Weng et al 2008).

Adverse effects that have been attributed to the effect of surfactants include hypotension, tachycardia, renal failure, respiratory distress, metabolic acidosis, and electrolyte imbalances (Gil et al 2013).

Fatalities

A number of intentional ingestions of glyphosate formulations have been fatal: a 57-year-old woman in Taiwan suffered metabolic acidosis, respiratory failure, shock, and finally death, after drinking nian-nian-chun, a Chinese formulation containing 41% glyphosate and 15% POEA (Chang & Chang 2009). Severe poisoning following ingestion of lethal amounts involves respiratory and kidney failure, cardiac arrest, coma, seizures, and death (IPCS 1994; Cox 1995a).

A 29-year-old man who ingested approximately 300 ml of Roundup Ace developed severe and persistent lactic acidosis, hyperkalaemia, hypotension, torrential watery diarrhoea and abdominal distension in the first 24 hours. The clinical course was complicated by cardiac arrhythmia and an episode of cardiac arrest. On day 3 there was evidence of bone marrow failure with falling whole blood cell and platelet counts, and liver and respiratory failure, and he died that day (Beswick & Milo 2011).

A 37-year-old woman in Thailand died after ingesting approximately 500 ml of Roundup formulation. Toxic effects included erosion of mucous membranes and linings of the gastrointestinal and respiratory tracts. A mild degree of pulmonary congestion and oedema was observed in both lungs (Sribanditmongkol et al. (2012).

Of 80 cases of ingestion in China between 1980 and 1989, 7 died (Talbot et al 1991); of 56 cases reported in Japan between 1984 and 1986, 9 died (Sawada et al 1988). In the latter report, fatality occurred after ingestion of only 206 ml (about of a cup). Of 2,186 cases in Taiwan between 1986 and 2007, 146 people died including one from injection of the herbicide. The most common causes of morbidity and/or mortality in this Taiwanese study were shock and respiratory failure. The majority of the exposures were due to attempted suicide (1,631 of 2,031

cases). The authors reported that case fatality rates are relatively high among glyphosate formulation-poisoned subjects in many Asian countries, likely due to the high prevalence of intentional self-poisoning using these products (Chen et al 2009).

In a study conducted in Taiwan of 58 patients suffering from glyphosate/surfactant poisoning, of whom 50 had attempted suicide, 17 patients died from the intoxication. The authors concluded that poisoning by glyphosate/surfactant is multiorgan toxicity, with pulmonary and renal toxicity seeming to be responsible for mortality (Lee et al 2008).

Death occurs much more rapidly with ingestion of glyphosate-trimesium. One accidental ingestion, of a mouthful of glyphosate-trimesium resulted in the death of a 6-year-old boy within minutes. A 34-year-old woman who died after ingesting 150 ml of the trimesium suffered erosion of the gastrointestinal mucus membranes, pulmonary oedema, cerebral oedema, and dilated right atrium and ventricle of the heart (Sorensen & Gregersen 1999).

Occupational and bystander exposure

A wide-range of symptoms have also been observed following occupational and bystander exposure:

- irritation, swelling, tingling, itching or burning of the skin, photo-contact dermatitis, recurrent eczema, blisters, rashes;
- numb face, swelling of the eye and lid, face, and joints;
- conjunctivitis, painful eyes, corneal injury, burning eyes, blurred vision, weeping eyes;
- oral and nasal discomfort, unpleasant taste, tingling and irritation of throat, sore throat;
- difficulty breathing, cough, coughing of blood, inflammation of lungs;
- nausea, vomiting, headache, fever, diarrhoea, debilitation;
- rapid heartbeat, palpitations, raised blood pressure, dizziness, chest pains (IPCS 1994; Cox 1998; Gallardo 2001; Bradberry et al 2004).

Doctors in Argentina report acute effects from the aerial spraying of glyphosate to include vomiting, diarrhoea, respiratory problems and skin rashes (Robinson 2010).

A 59-year-old farmer who sprayed a glyphosate formulation without protective equipment over approximately 3 hours suffered from laboured breathing, cough and fever. A biopsy showed alveolitis and bronchiolitis (EFSA 2015).

A case of severe chemical burns leading to injury to nerves and muscles, resulting from accidental exposure to a glyphosate herbicide, was reported in Denmark. A 43-year-old male accidentally spilled the herbicide when diluting it. On the following day his left arm began to swell, and 2 days after exposure he was hospitalised with vesicles, bullae and exuding wounds on his left arm and hand, chest and leg; and periorbital oedema and redness on the left side of his head where he had touched it. The arm and fingers were swollen, affecting the range of motion, and especially impeding full extension of the fingers. He subsequently lost all sensation in his left hand. After 2 months, the swelling had decreased but the medial, ulnar and distal axons of the hand were still severely affected. After 4 months, ostoepenia and oedema were noted. After 9 months, sensation in the hand was near normal but there was still pronounced atrophy of the muscles, loss of strength and decreased range of motion with the hand (Mariager et al 2013).

One woman developed severe skin problems when her backpack sprayer leaked. She had sprayed Touchdown Premium (36% ammonium salt of glyphosate) diluted to 1.6%, 2 hrs/day for 3 consecutive days. She developed redness on her arms, which became eczematous on the second day. Five days later, reddish lesions appeared on her arms, as well as 'target-like' lesions with lymph-filled vesicles on her abdomen, armpits and groin (Heras-Mendaza et al 2008).

An agricultural worker was hospitalised with severe inflammation of the lungs, shortness of breath, irritative cough, dizziness, sore throat, and coughing of blood following exposure to Roundup: he had been cleaning and repairing tractor mounted spray equipment which contained residues of the herbicide (Pushnoy et al 1998).

One person developed acute dermatitis after the herbicide soaked through the seams of a shoe (Horiuchi et al 2008). Another person, a 78-year-old woman, received extensive chemical burns on her feet, legs and back from contaminated clothing. She had first knelt on ground that had recently been sprayed with a glyphosate

herbicide, and then put on clothing that had also lain for some time on the sprayed ground. After several hours she developed burning, blistering rashes, with extensive erosions and necrotic skin falling off in sheets. It took 4 weeks to recover (Amerio et al 2004).

An occupational exposure, in which the glyphosate was reputedly applied correctly, resulted in the applicator suffering severe dysphonia (loss of voice), with decreased vocal fold mobility suggesting damage to the laryngeal nerve (Ptok 2009).

Jamison et al (1986) reported increased respiratory problems in people handling flax that had been retted with glyphosate before harvest, as opposed to those handling flax that had not been so treated. They concluded that the acute bronchoconstrictor response to flax dust is increased by the glyphosate, causing increased shortness of breath, wheezing and coughing.

Ho & Ching (2003) reported "widespread disturbances of many body systems . . . after exposures at normal use levels. These include balance disorder, vertigo, reduced cognitive capacity, seizures, impaired vision, smell, hearing and taste, headaches, drops in blood pressure, body-wide twitches and tics, muscle paralysis, peripheral neuropathy, loss of gross and fine motor skills, excessive sweating and severe fatigue".

Dr Ricky Gorringe of New Zealand estimated, based on cases presenting to his clinic, that probably 1 in 20 New Zealanders were sensitive to Roundup. The most commonly occurring symptoms are unnatural fatigue, a band-like headache, a strange "spaced-out feeling with loss of confidence", a skin rash, and an otherwise unexplainable sudden increase in blood pressure (Watts 1994). The exposure route thought to give rise to these problems is largely that of micro-droplet inhalation.

A previously healthy 44-year-old woman presented with rigidity, slowness and resting tremor in all 4 limbs with no impairment of short-term memory, after sustaining long-term exposure to glyphosate for 3 years as a worker in a chemical factory exclusively in the glyphosate production division. Physical examination revealed a parkinsonian syndrome. There was no known family history of neurological or other relevant disorders (Wang et al 2011).

Other poisoning incidences

Latin America

Many of the observations of adverse effects from exposure to glyphosate have come from Latin America, where populations have been repeatedly exposed to the herbicide from aerial spraying campaigns to eradicate coca in Colombia and along its border with Ecuador since 1997 (Solomon et al 2009), or for weed control in GM soybean fields in Argentina.

Argentina

In Argentina numerous health effects have been linked to exposures to glyphosate resulting from the aerial spraying of GM soybean fields, over the last 5 years. These include cancers, birth defects, lupus, kidney disease, and respiratory and skin ailments (Valente 2009). Clinical studies have identified high rates of cancer, birth defects and neonatal mortality. In the small town of Ituzaingó in Cordoba, which borders soybean farms, there was reported to be 300 cases of cancer in a population of 5,000, by 2009. At 6% of the population, this rate is 41 times the national average of 0.145% (Trigona 2009).

Brazil

Data collected by up to 10 Brazilian poison centres between 2010 and 2012 identified more than 650 cases of poisoning or adverse effects ascribed to ingestion of or contact with glyphosate-based herbicides, according to an overview provided by the Brazilian National Health Surveillance Agency An even higher number of poison centres in Brazil did not provided data and, thus, this figure is likely an underestimate of the real incidence (EFSA 2015).

Colombia

Symptoms observed after direct exposures from aerial spraying included red eyes, dizziness, vomiting, diarrhoea, abdominal pain, gastrointestinal infections, itchy skin, skin rashes and infections (particularly prevalent in children), respiratory infections, headaches, and fever. One baby was observed to have blood in its urine and kidney problems 3 months after the spraying (Oldham & Massey 2002; pers com, Elsa Nivia, RAPAL, Colombia, November 2006).

In February 2001, the Health Department in Putumayo published a preliminary report on health effects in the municipalities of Orito, Valle del Guamuez, and San Miguel, which had

been sprayed between December 22, 2000 and February 2, 2001. Three local hospitals reported "increased visits due to skin problems such as dermatitis, impetigo, and abscesses, as well as abdominal pain, diarrhoea, gastrointestinal infections, acute respiratory infection, and conjunctivitis following spraying in the rural areas surrounding their respective municipalities" (Oldham & Massey 2002).

CBS News reported in 2002 that "a Colombian health department worker . . . Nancy Sanchez, also says illnesses like fever, diarrhoea and allergies were up 100% in the spraying areas and that 2,300 families have complained of sicknesses" (Kroft 2002).

In the town of Aponte, department of Nariño, a physician reported that "aerial spraying on indigenous people's lands had caused an epidemic of rash, fever, diarrhoea and eye infections" (Oldham & Massey 2002).

By July 2002, of the 800 complaints about the aerial spraying presented to the personero of La Hormiga, Putumayo, 73% included claims of impact on health, according to the Colombian Comptroller General's office (LAWG undated).

The human health problems have been accompanied by reports of large-scale destruction of legitimate food crops such as bananas, beans, and corn, as well as fish kills and sickness and death of livestock, contaminated water supplies, and severe environmental impacts in sensitive tropical ecosystems. A police investigation in the area of Valle del Guamuez (population 4,289), in the Province of Putumayo found that, of the 17,912 acres sprayed by February 21, 2001, < 12% was dedicated to coca cultivation. Crop and animal losses in the 59 settlements affected included:

- 2,263 acres of bananas, 1,030 acres of yucca, 1,032 acres of corn, 7,064 acres of pasture, 1,665 acres of other crops (coffee, peanuts, fruit trees, timber, and vegetables),
- 1,112 acres of forest,
- 38,357 domesticated chickens and ducks, 719 horses, 2,767 cattle, 6,635 guinea pigs, 128,980 fish (from aquaculture), and 919 other animals (pigs, cats, dogs).

A similar review for La Hormiga municipality, also in Putumayo, reported the destruction of 20,239 acres of food crops and adverse effects in 171,643 farm animals including livestock, poultry, and farmed fish (Oldham & Massey 2002).

Costa Rica

In 2011, 69 out of the 699 cases of acute occupational poisoning reported to Sivigila, Costa Rica's Public Health Surveillance System, involved glyphosate (CRC 2016).

Ecuador

A number of acute symptoms, as well as DNA damage, have been reported from people exposed to aerial spraying of Roundup-Ultra near the Ecuador-Colombia border area between December 2000 and March 2001. Shortly after the spraying began, 44 people from one community reported stomach and skin problems (Gallardo 2001). Symptoms included intestinal pain, vomiting, diarrhoea, fever, heart palpitations, headaches, dizziness, numbness, insomnia, depression, debilitation, burning eyes and skin, blurred vision, weeping eyes difficulty in breathing, dry cough, and skin rashes or blisters (Gallardo 2001; Paz-y-Miño et al, 2007). According to Paz-y-Miño et al (2007), the "Ecuadorian government data confirms the existence of health problems . . . in the spraying zone".

In October 2000, the health centre in Mataje, Esmeraldas, a community of 154, reported treating 44 residents and another 29 people from surrounding areas for skin and eye irritation, vomiting, and diarrhoea following the spraying (Oldham & Massey 2002).

In June 2001. the Ecuadorian press reported that the Marco Vinicio Iza hospital, in Sucumbios Province, which borders the Colombian province of Putumayo, was treating 10 to 15 patients a day for skin, respiratory, and other problems that local doctors attributed to the spraying (Oldham & Massey 2002).

Also in June 2001, the PAN Ecuador group, Acción Ecológica, undertook a clinical survey of 142 people from 6 communities within 10 km of the border zone. 100% of people within 5 km of the spraying were suffering acute poisoning symptoms, on average 6 symptoms. Those 8-10 km away were suffering an average of 4 symptoms. Schools in 2 farming cooperatives near the border had to close after 83 pupils became ill. Three months after the spraying, 33% of residents near the border, and 10% of those 5-6 km, were still suffering chronic poisoning symptoms, mainly dermatitis, fever, migraine and conjunctivitis (Gallardo 2001).

In addition to the human health impacts there have also been reports of deaths of domestic

animals and fish in hatcheries (Leahy 2007). The survey by Acción Ecológica found that 80% of poultry in the 0-2 km zone died, as did numerous cattle, pigs, horses, dogs and goats. Calves were aborted. Animal deaths occurred up to 10 km away. The entire coffee crop was lost, rice yields dropped by 90%, and production of coca, plantain, sugarcane, cassava and fruit was badly affected (Gallardo 2001). More than 6,500 complaints had been filed, by 2007, about damage to legal crops (Lubick 2007).

After aerial spraying between August 5 and 25, 2001, 242 families (<10% of the population) surveyed in the Cimitarra River Valley in Santander, had lost a total of 1,350 acres of food crops including corn, yucca, bananas, rice and yam. 600 acres of fruit trees and pasture suffered adverse effects. The deaths of a number of domestic animals, including cattle, mules, and chickens, were attributed to the loss of food and the contamination of water supplies, as secondary impacts of the spraying (Oldham & Massey 2002).

The government of Ecuador has asked Colombia to observe a “security strip” (no aerial spraying) of 10 km from their joint border (Sicard et al 2005).

Paraguay

In 2003, 11 year-old Silvino Talavera died after direct exposure to pesticides used on soybean fields. His mother and siblings were hospitalised for nearly 3 months. In all, 25 people suffered varying degrees of poisoning. In 2004, a court convicted two men of culpable homicide caused by the irresponsible and criminal use of agrichemicals sprayed on soybean, specifically glyphosate. Three family members had glyphosate residues in their bodies (Williamson 2004).

Europe

Germany

There have been 60 reports by physicians on cases of poisoning with glyphosate herbicides since 1990. Only slight health impairment was reported in 52 of the cases, but in 4 cases, health disturbances were considered “moderate”. One life-threatening case was the result of ingestion of 200 ml of a glyphosate formulation (EFSA 2015).

Italy

In 2005, glyphosate was the single largest cause of unintentional acute pesticide-related illness in Italy, responsible for 56 out of 625 cases (Settimi et al 2007).

UK

Glyphosate was one of the top 4 pesticides responsible for most severe cases of pesticide poisoning in the UK from 2002 to 2013 (the others were aluminium phosphide, paraquat and diquat). It was the top pesticide involved in adult unintentional poisonings (424 cases), 2nd for adult self-harm (72), 4th for adult unintentional chronic poisonings (11), and 5th for paediatric acute poisonings (151 cases) (Perry et al 2014).

Other countries

Sri Lanka

In a study of case fatalities from intentional ingestion of pesticides in 2 Sri Lankan clinics, glyphosate caused 21 deaths out of 887 ingestions, a fatality rate of 2.4% (Dawson et al 2010).

Carroll et al (2012) reported on hospital admissions for self-poisonings with glyphosate in Sri Lanka between 2002 and 2009. There were 1,499 admissions, resulting in 37 deaths, with the peak being 396 admissions and 10 deaths in 2007.

USA

The State of California registered 202 cases of glyphosate-related illness on their website, for the years 2000-2007. Of these, only 10 were from ingestion, the rest being unintentional occupational or bystander exposure; 94 were caused by non-agricultural uses (Cal EPA 2009).

Environmental Effects

Glyphosate is still regarded by its supporters as safe for the environment, including scientists such as Duke & Powles (2008) who described it as “very toxicologically and environmentally safe”, and as “environmentally benign” despite a wealth of information showing that it is far from benign, and is likely in fact to be causing significant environmental harm. The independent studies described here reveal the distance between regulatory statements of low toxicity and reality.

Indirect effects of wild plant elimination

Farmland biodiversity and ecosystem functions such as natural pest control, pollination services and functional soil structures are more and more jeopardised by today´s nearly complete elimination of weeds wild plants and their soil seedbanks in agriculture.

Glyphosate works against all plant species, even killing large trees. No other herbicide is so non-selective, and no other herbicide costs so little to buy. Hence, it is easy to destroy wild or semi-natural habitats, and easy to overdo weed control, as is increasingly happening (Oppermann 2015). This is not necessary: for example wheat and oilseed rape can tolerate many weeds per square meter without yield drag, and beet and potato can tolerate them between the rows. According to DEFRA (2001), Busche (2008), and Pallut & Jahn (2008), the tolerable number of weeds /m2 in wheat is between 20-30 in Northern Europe. Moreover, more sustainable weed control measures and expert knowledge on weed management that improves ecosystem functions are available (Storkey & Westbury 2007).

There is evidence of the effects of glyphosate on plant biodiversity from large-scale field tests and production sites. Herbicides reduce the density and diversity of the weed flora more than does mechanical weeding (Schütte 2003); and non-selective herbicides reduce it more than selective herbicides and herbicide mixtures used in conventional crops (with the exception of atrazine when compared to glufosinate) (Heard et al 2003 a,b). In glyphosate-resistant GM beet sprayed with glyphosate, the density, biomass and seed rain were between 1/3rd and 1/6th lower (relative to conventional herbicide mixtures). The seedbank abundance (for 19 out of 24 species) was overall 20% lower and the emergence of 8 species was lower (Heard et al 2003 a,b). Even wild plants at margins of fields sprayed with glyphosate were scorched, so that flowering and seeding was 34% and 39% less than with conventional herbicide regimes (Roy et al 2003).

The loss of one plant species is accompanied by on average 10-12 insect species, in Europe (Heydemann 1983). As the abundance of arthropods also changes in the same direction as the weed abundance (Hawes et al 2003), arthropods were also reduced when glyphosate was used: in glyphosate-treated herbicide resistant beet, numbers of within-field epigeal and aerial arthropods were smaller, due to forage reductions (Haughton et al 2003; Brooks et al 2003); and herbivores, pollinators, and beneficial natural enemies of pests were reduced (Hawes et al 2003) compared to other herbicide regimes. Fewer canopy arthropods and significantly fewer green lacewings have been observed in glyphosate-treated GM soybean than in soybean treated conventionally (Buckelew et al 2000).

In Canadian canola fields, wild bee abundance was highest in organic fields, followed by fields sprayed with conventional herbicides and lowest in crops where glyphosate was used (Morandin & Winston 2005), pollination decreased with decreased bee abundance.

The rapid adoption of herbicide-tolerant crops (most of which are glyphosate-tolerant) in the US has led to a drastic reduction of milkweed (*Asclepias syriaca*) populations, the main food plant of monarch larvae (Pleasants et al 2016). Milkweed plants in the Midwest, the main breeding ground of monarchs, may have declined by up to 60% and monarch propagation by about 80% (Pleasants & Oberhauser 2013). The monarch may also be the first sign that food webs in the US Midwest are being irrevocably disrupted as a side effect of widespread planting of herbicide-tolerant crops (most of which are glyphosate-tolerant. Monarchs are “the canary in the cornfield” (Wade 2014).

It may become more and more difficult to implement ecological farming and to secure pollination or control services in the long run because of the dimension and significance of indirect effects on beneficial species, for 2 reasons.

- Wild pollinators (which naturally depend on wild plants) are more important to agriculture than honey bees (Garibaldi et al 2011, 2013). In UK e.g. 2/3rd of pollination is done by wild pollinators (Breeze et al 2011).
- Most (e.g. 88% of 850 beneficial species identified in Switzerland) beneficial insect species that control pests (natural biocontrol agents) essentially depend on wild (weedy) plants either in winter as habitat or all year as sources of pollen, nectar and habitat (Keller & Häni 2000). Decreasing natural biocontrol could result in increased pesticide use as demonstrated by exclusion experiments (Edwards et al 1979, Thies et al 2011). The majority of (insect) pest species in agriculture on the other hand can survive without weeds.

The decline in farmland birds and other vertebrates and farmland biodiversity in general has partially been attributed to the use of

herbicides and broad spectrum insecticides and to their increased efficacy on their food sources (Chamberlain et al 2000; Schütte 2003).

Ecotoxicity – aquatic

Because glyphosate has high water solubility, and both it and its metabolite AMPA are increasingly found in the aquatic environment, effects on aquatic organisms are of growing concern (Contardo-Jara et al 2009).

Roundup and glyphosate are regarded as being of greater toxicity to fish than to mammals, yet they are widely applied to aquatic ecosystems for weed control. The US EPA (1993) concluded that "minimal risk is expected to aquatic organisms from technical glyphosate". However, exposure of aquatic organisms is rarely, if ever, to technical grade glyphosate; it is usually to formulated product and hence most of the independent toxicity testing has been carried out using Roundup formulations. Those that also include glyphosate generally show that Roundup is much more toxic than the active ingredient alone, probably because of its surfactant POEA. However, glyphosate has also been shown to be toxic in some tests.

Aquatic effects of pesticides are usually described in terms of acute toxicity to fish and invertebrates such as shrimps and the water flea Daphnia. But the healthy functioning of aquatic ecosystems depends on much more than these species. It depends on a wide variety of organisms including microorganisms, algae, and amphibia, effects on which are seldom if ever measured for registration purposes. The significant detrimental impact of glyphosate and its formulations on these less-regarded species is of far greater ecological importance than the singular measure of acute toxicity to fish.

Additionally, aquatic ecosystems are often exposed to pulses of glyphosate–based herbicides, and such regular exposures may have a cumulative effect that is only expressed after several generations (Mann & Bidwell 1999). Most tests miss this effect, but one reported here clearly shows 3rd generational effects on molluscs that were not apparent in the first 2 generations (Tate et al 1997).

Aquatic communities

The structure and composition of natural aquatic communities, the diversity of species and the balance and interactions between them are of profound importance for ecosystem functioning right through all the trophic levels (Pérez et al 2007); and glyphosate and formulations have been shown to have profound impacts on such communities. The effects on microorganisms, algae and amphibia vary considerably between species, raising concerns about how contamination of freshwater and marine environments with glyphosate can tip the ecological balance, possibly giving rise to harmful algal blooms (Pérez et al 2007) and reducing species richness (Relyea 2005a). Glyphosate can, on the one hand, act as a phosphate source stimulating growth of phytoplankton able to tolerate its herbicidal effects, and on the other have an inhibitory effect as a pesticide, (Qiu et al 2013), thus affecting aquatic community structure.

In an aquatic mesocosm study published in 2005, Rick Relyea of the University of Pittsburgh showed that Roundup reduced species richness by 22%. Two species of tadpoles were completely eliminated and a third one nearly so, whilst wood frog survival was only reduced by 2%, resulting in an overall 70% decline in species richness of tadpoles. Predator (insect and salamander) biomass was reduced, but algal biomass increased by 40%. The copepod (small crustacean) *Eurytemora affinis* was almost completely eliminated (Relyea 2005a).

In a second mesocosm study, Roundup affected the structure of phytoplankton and periphyton assemblages. Periphyton is the complex mixture of algae, cyanobacteria, microbes, and detritus attached to submerged surfaces in aquatic ecosystems. Total nano- and micro-plankton decreased, but picocyanobacteria increased by a factor of about 40. Diatoms were reduced. Amongst the periphyton there was an increased proportion of dead to living individuals, and increased cyanobacteria, leading the authors to warn that Roundup use may lead to algal (cyanobacteria) blooms, with consequent adverse effect on higher trophic levels (Pérez et al 2007).

A study carried out on a marine microbial community showed effects on microbial diversity and community composition after a 7-day exposure at levels of Roundup as low as 1 µg/L, concentrations described as "typical of those already observed in polluted coastal areas" (Stachowski-Haberkorn et al 2008).

Glyphosate affected the composition of the algal community in summer but not in spring, in a study on river microbial communities, reducing reproduction in the species *Asterionella, Cyclotella*, and *Oocystis.* This highlights the

importance of seasonally dependent ecosystem characteristics in determining effects (Pesce et al 2009).

In Lake Erie, Canada, glyphosate increased the abundance of the blue-green algae *Planktothrix* spp; some members of this species can cause harmful algal blooms. It also decreased the abundance of *Microcystis* spp., a cyanobacteria that can also cause algal blooms (Saxton et al 2012).

Glyphosate also modified the structure and function of experimental freshwater ecosystems (mesocosms) in Argentina, producing a long-term shift, and changing clear water to turbid water. It killed diatoms and favoured the growth of cyanobacteria, but the overall biomass production decreased. It also delayed the colonisation of the mesocosms by periphyton, one of the most significant microbial communities forming the base of food webs in shallow lakes, so the delay could have important consequences on the ecology of the whole freshwater system (Vera et al 2009).

In an experiment on amphipods (small crustaceans) in Brazil, all concentrations of Roundup (0.36 to 2.16 mg/L glyphosate) caused significant changes in all biochemical parameters, depressed reproduction and decreased survival rate, indicating a potential toxic effect at very low concentrations. Amphipods are an important link in the food chain and these changes can lead to significant changes in the structure of freshwater environments (Dutra et al 2011).

A single application of the formulated product Glifosato Atanor (mean concentration 3.45 mg/L) caused increased phosphate and chlorophyll-a concentrations, and a rapid increase in abundance of bacterioplankton, planktonic picocyanobacteria, and the rotifer *Lecane* spp., resulting in turbidity and rapid deterioration in water quality (eutrophication) (Vera et al 2012).

In a study on *Microcystis aeruginosa,* a freshwater cyanobacteria that can cause harmful algal blooms, Roundup had a dual effect: at low concentrations (< 1mg/L) it stimulated and at high levels (>1 mg/L) it inhibited cell density and chlorophyll-a content (hormesis). With glyphosate alone, both the cell numbers and chlorophyll-a content increased when the glyphosate concentration increased from 0.01 to 5 mg/L (Qiu et al 2013).

A 28-day experiment in New Zealand outdoor stream mesocosms demonstrated a synergistic adverse impact of Glyphosate 360 (containing POEA) and sediment load on algal populations (Magbanua et al 2013).

Microorganisms

Microorganisms underpin the entire aquatic ecosystem through nutrient cycling, decomposition and primary production (Annett et al 2014). As well as the studies described above, a number of other studies have demonstrated the effects of Roundup or glyphosate on microorganisms.

Glyphosate was described by the IPCS (1994) as being slightly toxic to aquatic microorganisms, but some formulations could be highly toxic. It can affect growth, greening processes, aromatic amino acid synthesis, and photosynthesis of blue-green algae (IPCS 1994).

More recent studies have shown that planktonic microorganisms are highly sensitive to glyphosate at environmentally relevant concentrations (Stachowski-Haberkorn et al 2008). Such concentrations also caused significant shifts in bacterial community composition in freshwater lake sediment in Sweden (Widenfalk et al 2008).

Glyphosate was highly toxic, at “expected environmental concentrations”, to freshwater diatoms (*Nitzschia* sp., *Cyclotella meneghiana*) and cyanobacteria (*Aphanizomenon flosaquae*), but not toxic to other cyanobacteria, algae or duckweed, in a Canadian study of aquatic organisms (Peterson et al 1994).

Glyphosate-based herbicides may result in an increase in growth rates of planktonic and biofilm phenotypes of the naturally occurring pathogenic (to humans) bacterium *Pseudomonas aeruginosa* in watercourses or reservoirs. At concentrations higher than 84.5 mg/L, glyphosate reduced the cell density of aerobic planktonic cultures, but favoured anaerobic growth. On the other hand, it slightly increased growth of biofilms in a concentration-dependent manner up to 84.5 mg/L. Above 169 mg/L biofilm growth was more pronounced. Glyphosate increased the growth of anaerobic biofilms regardless of concentration (Lima et al 2014).

Aquatic invertebrates

LC_{50} = the concentration of glyphosate in water that kills 50 percent of test animals.
TL_{50} = the median lethal time, or the average time interval during which 50% of a given population dies.

Glyphosate was originally described as being non-toxic to aquatic invertebrates based on a 48 hr LC_{50} for *Daphnia magna* of 780 mg/L in tests conducted for Monsanto in 1978. This interpretation of non-toxicity for *Daphnia* was then extrapolated through various regulatory processes and scientific reviews to become "glyphosate is non-toxic". It was the value used by US EPA, IPCS, the European Commission and other assessors. However, recent re-analysis of the original data, and similar reports at the time that supported it, found "unsuitable methodologies, evinced as flaws in experimental setup, misinterpretation of the data and miscalculation of endpoints", as well as "manipulation of conclusions" by US EPA staff (Cuhra 2015).

48 hr LC_{50} (mg/L) (IPCS 1994):

	glyphosate	formulations
Daphnia	780	5.3-930
Chironomus	55	44-5,600

The European Commission now gives the following LC_{50} values for Daphnia (EFSA 2015b):

- glyphosate acid = 40 mg/L
- AMPA = 690 mg/L

Cuhra (2015) reports the 48 hr LC_{50} of glyphosate and glyphosate herbicides for *Daphnia magna* to be in the range of 2-149 mg/L depending on herbicide type and test conditions, making it moderately toxic (US classification) and toxic (EU classification).

In chronic toxicity tests on *D. magnia*, spanning their whole life-cycle, Roundup was more toxic than glyphosate. Significant reduction of juvenile size occurred in the lowest test concentrations of 0.05 mg a.i./L, for both glyphosate and Roundup. At 0.45 mg a.i./L, growth, fecundity and abortion rate were affected in those exposed to Roundup. At 1.35 and 4.05 mg a.i./L of both glyphosate and Roundup, significant negative effects were seen on most tested parameters, including mortality. D. magna had a near 100% abortion rate of eggs and embryonic stages at 1.35 mg a.i./L of Roundup. The authors concluded that the toxicity of glyphosate and Roundup to aquatic invertebrates has been underestimated and that current European Commission and US EPA toxicity classification of these chemicals need to be revised (Cuhra et al 2013).

In a more recent study, Cuhra et al (2015) tested the effects of varying levels of glyphosate residues in GM soybean on *Daphnia*. The residues ranged from 1.1 mg/kg up to 15.1 mg/kg, well below current USA maximum residue limits of 40 mg/kg. The highest mortality was found with the highest level of residues. Impairment of animal growth, reproductive maturity and number of offspring also correlated with increasing residues.

Parasitic freshwater horsehair worms (*Chordodes nobili*) were adversely affected by Roundup "at glyphosate concentrations lower than those expected to be found in freshwater environments and those specified in the [Argentinean] legislation". It decreased the infective capacity of larvae exposed, or derived from eggs that had been exposed, to > 0.1 mg/L of glyphosate (Achiorno et al 2008).

The sediment-dwelling freshwater blackworm *Lumbriculus variegatus* was exposed for 4 days to both glyphosate and to Roundup Ultra. Both, but more so the formulation, caused oxidative stress. A low level of bioaccumulation was also found to occur, with a bioaccumulation factor for glyphosate varying between 1.4 and 5.9 for exposure to Roundup Ultra (higher than for exposure to glyphosate alone). The accumulated amount increased with increasing concentration in the surrounding medium (Contardo-Jara et al 2009).

Crustaceans

Roundup is toxic to freshwater shrimps *Caridina nilotica*. Mensah et al (2011) give the following values for lethality (96hr LC_{50} mg/L):

Neonates	Juveniles	Adults
2.5	7.0	25.3

The lethality for neonates is much lower than usual application rates (20-30 mg/L). Survivors, especially neonates, were erratic and slow in their movements (Mensah et al 2011).

Estuarine blue crabs in the US treated with levels of Roundup that caused 20% mortality, experienced significantly reduced time to metamorphosis and increased the frequency of juveniles dying within 6 hrs of moulting. Delays in moulting can reduce survival ability. The larvae were 50-fold more sensitive to Roundup than the juveniles (Osterberg et al 2012).

In a species of estuarine crab from Argentina, (*Neohelice granulate*), females carrying immature eggs were collected and exposed

to pure glyphosate at 2.5 and 5 mg/L, and a Roundup Ultramax treatment of 2.5 mg/L. These sublethal concentrations were chosen based on American Public Health Association guidelines and also observed in some runoff in Australia and the US. Higher levels of glyphosate are expected but not observed in the scant data available for Argentinean water and glyphosate contamination. Embryonic mortality was significantly higher in the Roundup Max treatment group. Morphological abnormalities such as hypopigmented eyes, hydropsy, or atrophied setae/spines (Roundup exposure group only) were increased in a significant proportion of larvae hatching from the females in the 2.5 mg/L glyphosate and the Roundup-exposed groups. Significant increases in the gonadosomatic index in both glyphosate exposure groups and hepatosomatic index in the lower dose of glyphosate were observed. (Avigliano et al 2014).

Developmental effects of Roundup were demonstrated in the studies on sea urchin embryos carried out by Marc et al (2005), in which they found impeded transcription of the hatching enzyme and a delayed hatching process, foreshadowing a potential adverse effect on the developmental process in humans.

Sublethal effects of glyphosate and POEA on freshwater crayfish *Cherax quadricarinatus* include oxidative stress, lower somatic growth and decreased muscle protein levels (Frontera et al 2011).

Molluscs

Developmental effects of Roundup formulations were documented in the Pacific oyster Crassostrea gigas by Mottier et al (2013), with far lower effective concentrations (e.g. eliciting an effect on 50% of the embryos) of exposure to Roundup (1.13 and 1.67 mg/L for Roundup formulations) as compared to exposure to glyphosate and its breakdown product AMPA (28.3 and 40.6 mg/L, respectively). The authors found that above the dose of 1.0 mg/L, a gradient of herbicide concentration exposure correlated to a gradient of severity of effect ranging from normal larvae to arrested development. They determined the following 48 hr EC_{50} values:

- glyphosate = 28.3 mg/L
- AMPA = 40.6 mg/L
- Roundup Express® = 1.13 mg/L
- Roundup Allées et Terrasses = 1.67 mg/L

Roundup, and especially POEA are toxic to the freshwater mussel *Lampsilis siliquoidea,* regarded as being representative of 300 species native to North America, one of the "most imperilled faunal groups in the world". The mussel is one of the most sensitive of aquatic species to glyphosate-based herbicides (Bringolf et al 2007). They give the following values for EC_{50} (mg/L):

	Glochidia 48hr	Juvenile (acute) 96 hr	Juvenile (chronic) 21 days
glyphosate	>200	>200	>200
isopropylamine	5.0	7.2	5.4
Roundup	2.9	5.9	6
POEA	0.6	3.8	1.7

Long-term exposure to sublethal concentrations of glyphosate affected reproduction and development of the freshwater snail *Pseudosuccinea columella* (Tate et al 1997). It had a delayed effect on growth and development, egg-laying capacity, and hatching – not in the first or second generations, but in the third generation. The number of egg masses increased but they contained a greater number of abnormal or deformed embryos.

Exposure of freshwater ram's horn snails, *Biomphalaria alexandrina*, for 6 weeks to Roundup at LC_{10} (concentration that kills 10% of snails with acute exposure) resulted in 85.6% abnormal egg masses and marked reduction in hatchability after 2 weeks (Barky et al 2012).

Omran & Salameh (2016) exposed an aquatic hermaphroditic species of snail to the formulation "Herfosate" (48% isopropylamine) at the LC_{10} and found significantly decreased whole-body testosterone and 17β estradiol, elevation of CYP450B1-like immunoreactivity in the exposed group and degenerative changes and lack of most stages of gametes found in the ovotestis. Degenerative changes included azoospermia and oocytes deformation. Treated snails maintained for 2 weeks in a recovery tank of water did not reverse the effects of treatment. The LC_{50} of the Herfosate was 41.6 mg/L.

In 2005, Marc et al showed that glyphosate inhibits RNA transcription in sea urchin embryos, at a concentration 25 times below the level that is recommended for commercial spray application. Transcription inhibition was dose-dependent and caused a 50% adverse effect at a concentration of 1 mM equivalent of glyphosate in a Roundup

formulation, resulting in delayed hatching of embryos.

Roundup caused DNA damage in sea urchin embryos (Bellé et al 2007).

Amphibians

Amphibians may be particularly susceptible to the effects of glyphosate herbicides because their preferred breeding sites are often shallow ephemeral pools that, by virtue of the small amount of water, can contain high concentrations of herbicides (Mann et al 2009). Studies show them to be particularly susceptible to formulations containing POEA. Sublethal effects include metabolic disturbance, oxidative stress, DNA damage, endocrine disruption, malformations, and behavioural changes that make them more vulnerable to predators.

The acute toxicity of Roundup and other formulations of glyphosate containing POEA to larval amphibia vary with species, pH, and developmental stage; the estimates for LC50 range from 0.4 to 11.6 mg a.e./L (Relyea & Jones 2009).

As reported earlier, Relyea (2005a), when examining the impact of pesticides on the biodiversity of aquatic communities containing algae and 25 species of animals, found that Roundup (at 3.8 mg a.e./L) completely eliminated 2 species of tadpoles (leopard frogs and gray tree frogs), and nearly exterminated a 3rd species (wood frogs), resulting in a 70% decline in the species richness of tadpoles.

Relyea (2005b) also exposed tadpoles in an outdoor mesocosm to Roundup at a concentration (3.7 mg a.e./L) representing those expected when aquatic habitats are sprayed for weeds. Within 3 weeks it had killed 98% of tadpoles. In laboratory studies on juvenile frogs (post metamorphosis), it killed 68-86% within one day.

Exposure to Roundup WeatherMax at 0.57 mg/kg glyphosate a.i. resulted in 80% mortality of western chorus frog tadpoles (Williams & Semlitsch 2010).

Bullfrog tadpoles (*Lithobates catesbeianus*) exposed to commercial formulations of glyphosate at environmentally relevant concentrations (36, 72, and 144 µg/L), experienced increased lipid peroxidation in all tissues; significant decrease in levels of glycogen and total lipids in gill, liver, and muscle; increased triglyceride levels in the gill and decreased in liver and muscle; and decreased cholesterol and total protein levels in liver and muscle (Dornelles & Oliveira 2014). Similar results were obtained with 18 *µ*g/L glyphosate using the same glyphosate-based formulation (Dornelles & Oliveira 2016). Likewise, 48 h exposure to Roundup Original at a glyphosate concentration of 410 *µ*g/L caused oxidative stress in another study with bullfrog tadpoles (*Rana catesbeiana*) (Costa et al 2008). Four commercial formulations of glyphosate caused oxidative stress in tadpoles of the Argentine toad, *Rhinella arenarum* in concentrations < 240 mg a.e./L (Lajmanovich et al 2011).

In the urnero frog (*Leptodactylus latinasus*), glyphosate promoted changes in hepatic tissue (increased vascularisation and vacuolisation of hepatocyte cytoplasm) at 10,000 µg/g; and erythrocyte nuclear abnormalities at 100 µg/g (Pérez-Iglesias et al 2016).

Roundup caused DNA damage in bullfrog tadpoles (*Rana catesbeiana*) at concentrations of 6.75 and 27 mg/L, but not at 1.69 mg/L (Clements et al 1997).

Glyphosate-based herbicides and glyphosate itself interfere with key molecular mechanisms, including endocrine mechanisms, which regulate early development in frogs, leading to congenital malformations. Incubating *Xenopus laevis* embryos with 1/5000 dilutions of Roundup (430 µM glyphosate) resulted in highly abnormal embryos, with marked alterations in cephalic and neural crest development and shortening of the anterior-posterior axis. Alterations in neural crest markers were later correlated with deformities in the cranial cartilages at tadpole stages. Embryos injected with glyphosate showed very similar effects; as did chicken embryos treated with Roundup (Paganelli et al 2010).

In a number of studies, exposure to sublethal concentrations of POEA or glyphosate/POEA formulations has caused delayed or accelerated development, interference with gill morphology accelerated development, reduced size at metamorphosis, developmental malformations of the tail, mouth, eye and head, and histological indications of intersex, avoidance behaviour, and symptoms of oxidative stress (Mann et al 2009).

Survival of tadpoles of the common hourglass tree frog (*Polypedates cruciger*), exposed to Roundup at concentrations of glyphosate found in wellwater in Sri Lanka (0.05, 0.10, 0.25 and 0.5 ug/L), was lower (75%). Survivors took

more time to metamorphose, were significantly smaller, and developed malformations at higher frequency. The LC_{50} was 14.99 ug/L. Of 4 pesticides tested (chlorpyrifos, dimethoate, propanil formulations), Roundup caused the greatest frequency of malformations (69%), including hunched back (kyphosis) and curvature of the spine (scoliosis). Oedema and skin ulcers were also observed (Jayawardena et al 2010).

Lajmanovich et al (2003) found that glyphosate formulations caused craniofacial and mouth deformities, eye abnormalities and bent curved tails in tadpoles of the species *Scinax nasicus.* Howe et al (2004) found that when tadpoles of a common North American amphibian, the Northern Leopard frog (*Rana pipiens*), are chronically exposed to environmentally relevant concentrations of POEA or glyphosate + POEA formulations, they developed decreased snout-vent length at metamorphosis and increased time to metamorphosis, tail damage, and gonadal abnormalities. The authors concluded that these effects may be caused by disruption of hormone signalling involving the thyroid hormone receptor.

One study has shown that gray treefrogs avoid laying their eggs in water containing residues of Roundup at concentrations "expected to be found in the field" (Takahashi 2007).

Exposure to Roundup also has implications for responses to stress: the addition of predator stress made Roundup twice as lethal, at least for one species, the wood frog (*Rana sylvatica*), something that is likely to occur frequently in the wild (Relyea 2005c). In an experiment on 3 frog varieties in North America in simulated outdoor habitats, 'environmentally relevant concentrations' of Roundup induced, in surviving tadpoles, morphological changes that appeared to mimic the adaptive changes induced by the presence of predators, such as relatively deeper tails. The tadpoles respond to Roundup as if it was a predator, triggering hormonal responses that resulted in tail growth (Relyea 2012). Wood frog (*Lithobates sylvatica*) tadpoles exposed to sublethal Roundup (0.5 mg a.e./L) showed reduced movement and impaired anti-predator response (Moore et al 2015).

Symptoms of acute toxicity of the formulation Glyphos and adjuvant Cosmo Flux, used in Colombia, include slow swimming and remaining on the bottom with no movement at lower concentrations; and uncontrolled rapid swimming and remaining in a vertical position at higher concentrations (Bernal et al 2009).

Reptiles

Roundup caused dose-dependent DNA damage and micronucleus induction in the erythrocytes of newborn broad-snouted caiman (*Caiman latirostris*) after exposure in ovo to concentrations of Roundup of 500 *µ*g/egg or higher (Poletta et al 2009). It also induced micronuclei in *C. latirostris* exposed at 20 days of age for 2 months, in concentrations that began at 11 mg/L decreasing to 2.5 mg/L, and at 21 mg/L decreasing to 5 mg/L. The authors of the study warn that "wild populations continuously exposed to low concentrations might be suffering adverse effects" (López González et al 2013).

When caiman eggs were exposed to glyphosate to simulate the likely effect on nests from neighbouring croplands, it caused genotoxic alterations, delayed growth, and enzymatic and metabolic disorders in caimans (Poletta et al 2011).

Fish

Adverse impacts on fish caused by glyphosate and more especially formulations that contain POEA include acute poisoning; structural effects on gills, kidney, liver and gut; oxidative stress; genotoxicity; metabolic, immune, endocrine, neurotoxic and reproductive effects; and behavioural outcomes that increase vulnerability to other environmental stressors including predators.

Fish breeding operations are reported to have been completely destroyed by aerial spraying of glyphosate formulations in Colombia (Sicard et al 2005).

Acute toxicity

A wide variety of acute toxicities have been reported for glyphosate and Roundup, as demonstrated by the LC/EC_{50}s given below. Toxicity varies with species and age of the fish, their nutritional status (more toxic to hungry fish), and the temperature, pH and hardness of water (toxicity increases with temperature and pH) (Cox 1995b). Young fish are particularly vulnerable when water temperatures increase in spring and summer (Folmar et al 1979).

FAO (2000) described glyphosate as moderately to slightly toxic, on the basis of the following values for 96 hr LC_{50}:

- bluegill sunfish 5.8-34 mg/L
- rainbow trout 8.2-26 mg/L

- channel catfish 39 mg/L
- coho salmon 22 mg/L
- chinook salmon 20 mg/L
- pink salmon 14-33 mg/L

However, EFSA (2015b) gives higher 96 hr EC_{50} values for glyphosate acid:

- rainbow trout 38 mg/L
- bluegill sunfish 47 mg/L
- zebrafish 123 mg/L
- common carp >100 mg/L

And for AMPA:

- rainbow trout 520 mg/L

The 96 hr LC_{50} values reported by IPCS (1994) show the significantly increased toxicity of some formulations, particularly Roundup:

	glyphosate	formulations
rainbow trout	10-197	3.2 >1,000 mg/L
bluegill sunfish	120-140	4.5 > 1,000 mg/L
coho salmon	27-174	13-33 mg/L

Servizi et al (1987) demonstrated that the acute toxicity of POEA to fish was more than 30 times that of glyphosate itself, and provided comparative toxicities for POEA (LC_{50}):

	Roundup	POEA
rainbow trout fry	25.5	3.2 mg/L
coho salmon fry	42.0	3.5 mg/L
sockeye fry	28.8	2.6 mg/L

Systemic effects

Roundup (at 3.5 and 4.0 mg glyph/L) caused moderate to severe irreversible damage to liver and reduced liver function in a neotropical fish species, *Piaractus mesopotamicus.* Effects included cytoplasmic vacuolisation, lipid accumulation, nuclear and cellular membrane alterations, and glycogen depletion, probably affecting their ability to detoxify and to repair tissues and contributing to fish death. It also caused hyperplasia and hypertrophy of lamellar epithelium in the gills (Shiogiri et al 2012).

Acute exposure of jundia fish, *Rhamdia quelen*, to sublethal concentrations of Roundup (1.2 mg/L) lowered their cortisol levels, and decreased their capacity to adequately cope with stress and to maintain homeostasis (Cericato et al 2008).

In carp, *Cyprinus carpio,* exposure to Roundup at concentrations 40- to 20-fold lower than those generally used (250-410 mg glyph/L) caused damage to liver cells (the appearance of myelin-like structures, swelling of mitochondria and disappearance of the internal membrane of mitochondria) (Szarek et al 2000). The authors concluded that Roundup is harmful to carp when used in generally applied concentrations. Higher, sublethal, doses of Roundup (5, 15 mg/L) modified liver enzymatic activity in Nile tilapia, *Oreochromis niloticus* (Jiraungkoorskul et al 2003).

Glyphosate, in sublethal concentrations (2, 5, 10 mg/L), increased the activity of alkaline phosphatase in liver and heart, glutamic-oxaloacetic transaminase in liver and kidneys, and glutamic-pyruvic transaminase in kidney in carp; and caused morphological changes in gill and liver tissue (Neskovic et al 1996).

Exposure to the formulation Excel Mera 21 (17.2 mg/L) of 2 Indian air-breathing teleosts, *Anabas testudineus* and *Heteropneustes fossilis* for 30 days under laboratory conditions caused serious alterations in the enzyme activities, resulting in severe deterioration of fish health. Lipid peroxidation was significantly increased in all tissues of both species. Catalase was enhanced in both the species, while GST activity in liver diminished substantially. Total protein decreased in all tissues (Samanta et al 2014).

Gholami-Seyedkolaei et al (2013) reported increases in liver enzymes AST, ALT, LDH, increase in the blood parameters mean cell hemoglobin and mean cell volume, and decreases in hemoglobin, hematocrit and red and white blood cell count, in *C. carpio* exposed to Roundup.

In the South American fish boga (*Leporinus obtusidens*), Roundup caused a significant increase in glycogen and glucose in the liver, but a significant reduction in muscle; and a decrease in all haematological parameters tested (Gluszcak et al 2006).

At 75% of its 96hr LC_{50}, Roundup caused alterations in gill structural and respiratory epithelium in juvenile tambaqui (*Colossoma macropomum*) (Braz-Mota et al 2015).

Acetylcholinesterase

Acetylcholinesterase was inhibited in the brain in C. carpio (Cattaneo et al 2011), the South American fish *Leporinus obtusidens* (Gluszcak

et al 2006), the Brazilian fish sábalo *Prochilodus lineatus* (Modesto & Martinez 2010a, 2010b), and juvenile tambaqui *Colossoma macropomum* exposed to Roundup (Braz-Mota et al 2015). Gholami-Seyedkolaei et al (2013) also report decreased AChE activity of muscle, brain and liver tissue in Roundup-exposed *C. carpio.* But Samanta et al (2014) report significantly increased AChE activity in all tissues of *A. testudineus* and *H. fossilis.*

Pure glyphosate can also inhibit AChE activity: exposure to environmentally relevant concentrations of glyphosate (1 mg/L) inhibited AChE activity in the anterior part of the fish *Cnestedoron decemmaculatus* (Menéndez-Helman et al 2012).

Genotoxicity

Roundup has caused DNA damage and/or micronucleus induction in a wide range of fish;

- Roundup caused DNA damage and micronucleus induction in the gill cells of the neotropical fish *Prochilodus lineatus*, and DNA damage but not micronucleus induction in its erythrocytes, at 10 mg/L concentration (Cavalcante et al 2008).
- Roundup caused micronucleus induction in the erythrocytes of the fish *Tilapia rendalli,* but not in mouse erythrocytes (Grisolia 2002).
- Roundup caused dose-dependent micronuclei induction, nuclear abnormalities, and DNA strand breaks in the erythrocytes of goldfish (*Carassius auratus*) at concentrations of 5, 10 and 15 mg/L (Cavaş & Könen 2007).
- Roundup caused DNA damage in blood, liver and gill tissue of the European eel (*Anguilla anguilla*) at 3.6 mg/L concentration (Guilherme et al 2009).
- Using the in vivo comet assay to blood cells of the European eel (*Anguilla anguilla*), genotoxic potential was confirmed for Roundup, as well as for glyphosate and POEA when tested separately (Guilherme et al 2012b).
- Roundup and glyphosate were tested in an *in vivo* comet assay at comparable concentrations of 0.48 and 2.4 mg/L glyphosate using the fish species *Prochilodus lineatus.* Genotoxic effects where observed in erythrocytes and gill cells at both concentrations after 24 hours of exposure, but in erythrocytes only after 96 hours 8 (Moreno et al 2014).
- Dose-dependent *in vivo* genotoxic effects were observed for Roundup at concentrations from 1.41 to 5.65 µl/L in guppy fish (*Poecilia reticulata*) using the comet and micronucleus assay (de Souza Filho et al 2013).
- Roundup caused DNA damage in sea urchin embryos (Bellé et al 2007).
- Roundup and Pondmaster "induced a very high frequency of lethals [sex-linked, recessive lethal mutations] in larval spermatocytes and in spermatogonia" of fruit flies (Kale et al 1995).
- Roundup, but not glyphosate, caused chromosomal damage in root-tips cells of onions (*Allium cepa*) (Rank et al 1993).

Oxidative stress

Roundup has caused oxidative stress in goldfish (Lushchak et al 2009), *C. punctatus* (Nwami et al 2013), *A. anguilla* (Guilherme et al 2010, 2012; Marques et al 2014), *C. macropomum* (Braz-Mota et al 2015), *C. carpio* (Cattaneo et al 2011), *R. quelen* (de Menezes et al 2011), *P. lineatus* (Langiano & Martinez 2008; Modesto & Martinez, 2010a, 2010b), and brown trout *Salmo trutta* (Uren Webster & Santos 2015). Glyphosate also caused oxidative stress in *S. trutta* (Uren Webster & Santos 2015).

Immune

Glyphosate adversely affected immune responses in *T. nilotica* (el-Gendy et al 1998) and *C. carpio*, in the later suppressing immunoglobulin M, complement C3, and lysozyme, at 52.08 mg/L (Ma et al 2015).

Kidney

Glyphosate at 104.15 mg/L damaged the kidney in *C. carpio*, causing vacuolisation of the renal paremchyma and intumescence of the renal tubule (Ma et al 2015).

Neurotoxicity

Exposure of zebrafish embryos, in the 5-24 hr post fertilisation time window which includes segmentation, somitogenesis and neurulation, to 50 µg/L glyphosate or Roundup formulation diluted to the same glyphosate concentration, are developmentally toxic to the forebrain and midbrain, but not the hindbrain. Concentrations between 50 and 75 µg/L resulted in developmental delays and general necrosis. Morphological changes in brain architecture included loss of

delineated brain ventricles and reductions in cephalic and eye regions; decreases in genes expressed in the eye, fore and midbrain regions of the brain; and a loss of retinoic acid expression in the retina. The authors noted that theirs is one of the first studies examining developmental neurotoxicity in zebrafish (Roy et al 2016).

Gut

C. punctatus were exposed at a dose of 4 mg/L – a dose "generally used by farmers to control weeds in water bodies" – for 45 days. In the gill, there was severe damage, shrinkage and degeneration of pentagonal cellular contour of stratified epithelial cells (SEC), the latter resulting in degeneration of microridges in the buccopharynx; in the oesophagus, slight necrosed and distorted SEC; in the stomach, severe mucus secretion and erosion on the apical surface of mucosal folds and columnar epithelial cells (CEC), and necrosis of CEC; and in the intestine, obliteration of CEC along the entire length from basement membrane. Protease activity was slightly reduced in stomach and intestine; and amylase activity reduced in oesophagus and intestine (Senapati et al 2009).

Reproduction

Ovaries of zebrafish (Danio rerio) were exposed for 15 days to glyphosate at 65 µg/L, the permissible concentration of glyphosate in Brazilian inland waters. No apparent changes were noted in general morphology. However, there were significant adverse ultrastructure effects on oocytes: a significant increase in diameter of oocytes; concentric membranes, appearing as myelin-like structures, associated with the external membranes of mitochondria and with yolk granules; and greater expression of steroidogenic factor-1, a major regulator of steroid hormone synthesis, in the oocytes. The authors expressed concern about the impact of these subtle adverse effects on fish reproduction (Armiliato et al 2014).

Sperm quality was assessed in zebrafish after 96 hours of exposure to glyphosate at concentrations of 5 and 10 mg/L, with reduction of sperm motility and period of motility observed at both concentrations (Lopes et al 2014). In a study of egg production, zebrafish were exposed for 21 days to 0.01, 0.5, and 10 mg/L Roundup and a treatment of 10 mg/L glyphosate, with some adverse effects on embryo survival and hatching observed at the highest doses of 10 mg/L. The gonadosomatic index indicating gonadal weight adjusted for body weight was significantly decreased and the number of eggs laid per female per day during the exposure period was significantly reduced with glyphosate treatment and a non-significant trend in reduction of eggs laid by females in all of the Roundup-treated females (Uren Webster et al 2014). These doses are however, relatively high except in the case of glyphosate being directly applied in control of algae.

When the New Zealand freshwater fish Galaxias anomalus was simultaneously exposed to glyphosate at "environmentally relevant concentrations" and a trematode parasite, Telogaster opisthorchis, juveniles developed spinal malformations not seen with either the parasite or the glyphosate alone (Kelly et al 2010).

Behaviour and survival

The presence of glyphosate-based herbicides in aquatic environments can alter physiological and behavioural endpoints critical to maintaining normal function, and cause adverse effects at the population level (Annett et al 2014).

Sublethal acute effects of Roundup include loss of mobility, complete loss of equilibrium, darkened pigmentation, rapid respiration (IPCS 1994), and erratic swimming (Cox 1998) – these effects can increase risk from predators, as well as affecting feeding, migration and reproduction (Cox 1998).

Glyphosate is neurotoxic to the olfactory system in fish (Tierney et al 2006) and exposure to Roundup alters behaviour, in particular provoking an avoidance response at > 10 mg/L, but eliminating preference behaviour at only 100 µg/L (Tierney et al 2007). Olfaction is critical for return migration, alarm response, feeding, and kin recognition, and impairment is an ecologically important effect (Tierney et al 2007).

Modesto & Martinez (2010a) postulate that, because Roundup at sublethal levels inhibits acetylcholinesterase in the muscle and brain of fish, the accumulation of acetylcholine due to reduction of enzyme activity "may affect the fleeing and reproductive behaviour of fish, interfering directly in the survival of the species".

Reduced survival was seen in *G. anomalus* simultaneously exposed to glyphosate at "environmentally relevant concentrations" and the parasite *T. opisthorchis.* Additionally, juveniles developed spinal malformations not seen with either the parasite or the glyphosate alone (Kelly et al 2010).

Plants

Sublethal glyphosate pollution of wetlands may result in decline of non-target species: at 30 mg/L glyphosate caused a 30% reduction in biomass of the wetland plant *Bolboschoenus maritimus*, through direct interaction with the roots resulting in photosynthetic stress and an alteration of essential nutrients (Mateos-Naranja & Perez-Martin 2013).

Glyphosate significantly decreased the wet and dry weights, the number and length of leaves, and chlorophyll content of *Ruppia maritima,* after 7 days of exposure in a laboratory study. *R. maritima* is a seagrass that grows in saline environments, forming 'meadows' which provide feeding and breeding sites for many species and perform nutrient cycling. The rapid growth and high biomass production of *Ruppia* seagrass meadows make it extremely valuable for sustaining wildlife in saline environments (Castro et al 2015).

The formulation Atanor caused oxidative stress in a tolerant strain of algae *Chlorella kessleri* (Romero et al 2011).

Ecotoxicity – terrestrial

In addition to the impacts that glyphosate has on bird, mammal and beneficial insect population losses through habitat and/or food supply destruction, as referred to earlier, it also has direct lethal and sublethal effects which can lead to individual deaths and changes in community and ecosystem dynamics.

Additionally, glyphosate and glyphosate-based herbicides can have very significant adverse effects on the agroecosystem, principally through effects on soil microorganisms and plant health.

Soil microorganisms

Numerous studies have demonstrated a wide range of effects of glyphosate on soil microorganisms—some positive, others adverse, depending on the species. Actual effects following glyphosate use depend on a number of factors including soil type, environmental conditions and interspecies interactions (Guiseppe et al 2006; Lupwayi et al 2009). Repeated applications appear to have a greater impact on soil microbes than single applications (Lancaster et al 2010). Monsanto acknowledges at least some of these in its application for a patent for glyphosate as an antimicrobial, mentioning in particular the *Bacillus* and *Pseudomonas* families, both important in soils (Williams 2010). Pseudomonas soil bacteria are phosphate mobilisers and suppressors of pathogenic *Fusarium* fungi. *Pseudomonas* and most other beneficial soil microbes also have an important function in making soil minerals available for plant use.

Carlisle & Trevors (1988) reported a study that showed 50 mg/kg glyphosate inhibited growth of 59% of randomly selected soil bacteria, fungi, actinomycetes and yeast; and, that of the 9 herbicides tested, glyphosate was the 2nd most toxic.

Results can be inconsistent—for example, a study of 2 Brazilian soils found that glyphosate increased the number of fungi and actinomycete bacteria, and decreased the number of other bacteria (Araújo et al 2003). However other studies have reported that actinomycetes are more sensitive to glyphosate than are other bacteria (Grossbard 1985). In fact the effect on soil bacteria varies between species—for example 5 *Pseudomonas* species were not inhibited by glyphosate, but a 6th species, *Pseduomonas fluorescens,* was (Kremer & Means 2009).

Increased microbial activity in soils has been reported as a result of exposure to glyphosate (e.g. Lupwayi et al 2009), but generally this appears to result from an increase in the number of microorganisms capable of metabolising glyphosate and using its phosphate ions as a nutrient (Lancaster et al 2009).

The concern therefore is not that glyphosate will diminish microbial activity, but that it will alter microbial community dynamics in ways that are harmful to plants and to ecological balance. In commenting on their study, which showed that pre-sowing application of a mixture of glyphosate and 2,4-D to a canola crop significantly decreased the diversity of species, Lupwayi et al (2009) concluded "such shifts in the structure of soil microbial communities can lead to successions that could have long-term effects on soil food webs and soil biological processes". As an illustration of this, glyphosate inhibited a number of saprophytic fungi that decompose dead plant material and hence are important for soil fertility (Grossbard 1985).

A review (Kremer & Means 2009) of published studies on the impact of glyphosate on soil microorganisms concluded that microbial groups and functions affected by glyphosate include "manganese transformation and plant availability; phytopathogen–antagonistic bacterial inter-

actions; and reduction in nodulation". The authors commented: "root-exuded glyphosate may serve as a nutrient source for fungi and stimulate propagule germination". These effects, together with the many studies showing that glyphosate increases the severity of soil borne fungal pathogens and the resulting disease in plants, are addressed in the following subsections on 'Plant diseases' and 'Other effects on plants'.

Food microorganisms

Glyphosate at concentrations lower than those used in agriculture inhibited the growth of three important food microorganisms: *Geotrichum candidum, Lactococcus lactis* subsp. *cremoris* and *Lactobacillus delbrueckii* subsp. *bulgaricus.* The first 2 are important in cheese processing, and *L. bulgaricus* is the starter bacteria commonly used in fermented food. This study, together with others showing effects on microorganisms in the soil and aquatic communities, has raised concern about the implications of widespread glyphosate use on microbial diversity and particularly those available for food culture (Clair et al 2012b).

Plant diseases

The authors of a review of glyphosate and plant diseases (Johal & Huber 2009) concluded that "extensive use of glyphosate in agriculture is a significant factor in the increased severity or 're-emergence' of diseases once considered efficiently managed".

There are a number of ways in which glyphosate increases disease severity in plants: by increasing populations of pathogens in the soil, by immobilising specific plant nutrients involved in disease resistance, by reducing vigour and growth of plants as a result of the accumulation of glyphosate in the plant, by altering physiological efficiency, and by modification of the soil microflora in ways that affect the availability of nutrients important to plants' disease resistance (Johal & Huber 2009).

<u>*Pathogens*</u>

Glyphosate can alter the balance between disease-causing and beneficial fungi in favour of the former: a number of studies have shown that it stimulates the growth of some pathogenic fungi such as *Aspergillus, Fusarium, Pythium, Phytophthora, Rhizoctonia*, and *Sclerotinia*, but inhibits beneficial fungi. For example:

- Glyphosate, at 1.5 to 5 mM, stimulated the growth of *Aspergillus flavus* and *A. parasiticus* strains, and in some strains there was a significant increase in production of a carcinogenic and mutagenic aflatoxin (Barberis et al 2013). However, the Roundup formulation R450 was toxic to *A. nidulans* at concentrations lower than recommended label rates (Nicolas et al 2016).
- Greenhouse studies on common waterhemp supported previous findings that glyphosate may predispose plants to soil-borne plant pathogens such as *Fusarium* (Rosenbaum et al 2014).
- At low concentrations glyphosate stimulated growth of *Fusarium solani* and *F. oxysporum* (Krzysko-Lupicka & Sudol 2008). Long-term exposure led to a fungal community dominated by *Fusarium* species.
- Glyphosate application to soil containing maize or peanut crop residues increased growth and sporulation of *Fusarium* and *Pythium*, in comparison with fallowed fields lacking crop residues, whilst the populations of the beneficial fungi *Trichoderma* and *Gliocladium* remained constant (Meriles et al 2006).
- Application of glyphosate in greenhouse studies resulted in increased disease severity caused by *Fusarium oxysporum* and *Rhizoctonia solani* in GM sugar beet (Larson et al 2006).
- Other studies have also shown increases in *Pythium* and *Phytopthora* (Kawate et al 1997; Descalzo et al 1998).
- Exposure of GM soybean to glyphosate increased the severity of Sclerotinia stem rot (*Sclerotinia sclerotiorum* (Lib.) de Bary) in some but not all cultivars (Nelson et al 2002).
- Sublethal doses of glyphosate inhibited resistance in beans to *Colletotrichum lindemuthianum*, in tomato to *Fusarium* spp, and in apple trees to root rot (Levesque & Rahe 1992).
- Exposure of GM soybean to glyphosate increased sudden death syndrome caused by *Fusarium solani* (Sanogo et al 2000).
- Pre-planting use of glyphosate reduced resistance of barley to *Rhizoctonia* root rot and lowered yields (Smiley et al 1992).
- Glyphosate injected into the sapwood around the root collar of lodgepole pine trees increased the growth and spread of the blue stain fungus *Ophiostoma clavigerum.* The fungus is symbiotic with the mountain pine beetle, *Dendroctonus ponderosae,* and the authors concluded that glyphosate

enhances brood development of the pest via enhancement of the fungus (Bergvinson & Borden 1992).

There has been a resurgence of *Fusarium* wilt in Roundup Ready cotton crops in Australia and "previous high levels of wilt resistance appear to be less effective under glyphosate management programs" (Johal & Huber 2009).

Fernandez et al (2009) have established an association between glyphosate use and cereal diseases, caused by *Fusarium avenaceum* and *F. graminearum,* in wheat and barley grown in the Canadian Prairies under minimum-till systems that are heavily dependent on glyphosate. *F. graminearum* produces a mycotoxin that can kill humans and animals.

In the US states of Washington, Idaho, and Oregon, production of peas, lentils and wheat in rotation has been in slow decline as nitrogen fixation (for the peas and lentils) has declined and *Fusarium* diseases increased, commensurate with the extensive use of glyphosate in no-till programmes (Johal & Huber 2009).

Summary of glyphosate predisposing crops to disease-causing pathogens (Johal & Huber 2009)

Plant	Disease	Pathogen
apple	canker	*Botryosphaeria*
banana	Panama disease	*Fusarium*
barley	root rot	*Magnaporthe*
bean	anthracnose damping off root rot hypocotyls rot	*Colletotrichum* *Pythium* *Fusarium* *Phytopthora*
canola	crown rot wilt	*Fusarium* *Fusarium*
citrus	chlorosis crown rot	*Xylella* *Phytopthora*
cotton	damping off bunchy top wilt	*Pythium* Mn deficiency *Fusarium*
grape	black goo	*Phaemoniella*
melon	root rot	*Monosporascus*
soybean	root rot target spot sudden death root rot cyst nematode white mould	*Corynespora* *Corynespora* *Fusarium* *Phytopthora* *Heterodera* *Sclerotinia*
sugar beet	yellows	*Fusarium*
	root rot	*Rhizoctonia*
sugarcane	decline	*Marasmius*
tomato	crown rot wilt	*Fusarium* *Fusarium*
various	canker	*Phytopthora*
weeds	(used as biocontrol)	*Myrothecium*
wheat	bare patch glume blotch root rot head scab take-all	*Rhizoctonia* *Septoria* *Fusarium* *Fusarium* *Gaeuman-nomyces*

Chelation, micronutrient deficiency and disease

Very low levels of glyphosate in the soil can greatly hinder the availability and uptake of the micronutrients manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn); and also impede their translocation within the plant. These nutrients are critical for plants' disease resistance mechanisms (Johal & Huber 2009). Bellaloui et al (2009) also found that glyphosate significantly reduces Fe content in soybeans.

Glyphosate binds Mn, so that Mn deficiency is now observed where once there were sufficient amounts of the micronutrient. Additionally, the glyphosate resistance gene in GM corn and soybean reduces the plant's uptake and use of Mn. There is now recognition of increased disease severity along with the Mn deficiency. *Corynespora* root rot, once considered minor, may now become economically damaging in Roundup Ready soybean because of the Mn effect, according to Johal & Huber (2009). Cereal take-all is another disease regarded as a Mn deficiency response to glyphosate, as are a wide range of diseases caused by the bacteria *Xylella fastidosa* (Pierce's disease of grapevine, plum scorch, almond scorch, citrus variegated chlorosis, coffee blight, citrus blight, alfalfa dwarf, pecan decline, etc). These diseases, which involve loss of vigour, slow decline, and reduced productivity, can be controlled by the elimination of glyphosate allowing the Mn and Zn micronutrient levels to be restored to sufficiency (Johal & Huber 2009). Glyphosate also forms complexes with Cu (Zhou et al 2013) and increases the adsorption of Cu in some soils, making it unavailable for plant uptake (Morillo et al 2002).

Nitrogen metabolism

Glyphosate modifies nitrogen metabolism resulting in changes, similar to high-temperature

changes, that lead to *Fusarium* head scab in cereals, so that the disease is now prevalent in cooler areas where it was rarely observed before extensive use of glyphosate (Johal & Huber 2009). Climate change is likely to further exacerbate this problem.

Other effects on plants

Phytotoxicity

Glyphosate can have inhibitory effects on desirable plants after it has been used on weeds. It can be also transferred from weeds to non-target plants through the roots: when weeds in orchards were sprayed, glyphosate was released from dying weeds and taken up by the neighbouring citrus trees (Yamada et al 2009). Glyphosate exudation by plant roots can also be considered as an environmental source of glyphosate exposure for non-target plants and should be included in risk assessment models (Gomes et al 2014). In a greenhouse study, glyphosate used to kill ryegrass strongly impaired the growth and micronutrient status of sunflowers planted, even 21 days after the ryegrass; it inhibited seedling growth, root development and manganese uptake (Tesfamraiam et al 2009).

In a study that simulated drift of glyphosate from ordinary applications onto potato crops, not only were the potato yields of that crop diminished, but subsequent yields from the tubers formed were also diminished. Additionally, the daughter tubers were damaged, with cracks and folds reducing their quality. The problem is worst if the drift occurs during the mid-bulking period of potato growth; the foliage can show no damage but the tubers will be most affected. The tuber is the main sink for glyphosate so it concentrates there, specifically in the eyes/buds affecting emergence and vigour next season (Hutchinson et al 2014). A media report from the UK states that there are a "handful" of legal cases each year resulting from the impact of glyphosate drift on the yields of daughter tubers (Pate 2014).

Roundup damaged the cytoskeleton of the microtubules of pollen from tobacco plants, potentially reducing pollen fertility (Ovidi et al 2001).

GM soybean was more susceptible to infection by soybean cyst nematode than the normal soybean after exposure to glyphosate (Giesler et al 2002).

Nutrient deficiency

There are a number of ways in which glyphosate can cause nutrient deficiencies in plants; for example it inhibits ectomycorrhizal fungi which help plants absorb nutrients and water (Estok et al 1989; Levesque & Rahe 1992; IPCS 1994).

As reported in the previous section, glyphosate can decrease uptake of micronutrients resulting in deficiency symptoms (Yamada et al 2009). In a greenhouse experiment that simulated glyphosate drift, Cakmak et al (2009) demonstrated that glyphosate significantly reduced the seed concentration of calcium (Ca), magnesium (Mg), Fe and Mn in soybean, most likely because glyphosate binds these minerals in poorly soluble complexes. In a similar experiment on sunflowers, Eker et al (2006) found that simulated drift caused substantial decreases in leaf concentration of Fe and Mn, and lesser decreases in Zn and Cu. Similarly glyphosate applications at 20% of normal application rates decreased Ca, Mg, Mn and Fe in turf grass shoots (Senem Su et al 2009). And in 1985 Duke et al reported that glyphosate reduced uptake and translocation of Ca and Mg, but not potassium (K), in soybean. These effects may have implications for the nutritional quality of soy and other food crops subjected to low levels of glyphosate through drift or soil residues.

As well as binding some minerals, glyphosate also interferes with the root enzymes involved in mineral uptake from the soil—for example the activity of ferric reductase which is involved in iron uptake is reduced by low levels of glyphosate, and Ozturk et al (2008) linked this with the iron deficiency that is "increasingly being observed in cropping systems with frequent glyphosate applications". The iron deficient soybeans also pose animal and human nutritional concerns, as human iron deficiency is widespread.

Greenhouse application of glyphosate to soybean seedlings reduced biomass production and photosynthesis, linked to lower chlorophyll content, probably because of reduced nutrients. Uptake and accumulation of Ca, Mg, N, sulphur (S), K, and phosphate (P) were all reduced (in that order of magnitude), as were micronutrients Fe, Mn, Cu, molybdenum (Mo), and boron (B), also in that order, with increasing exposure to glyphosate (Zobiole et al 2010b).

Nitrogen cycle

Glyphosate is toxic to the nitrogen-fixing bacteria in legume root nodules that make nitrogen available to legumes (Jaworski 1972; Zablotowicz & Reddy 2007). A number of studies have reported that the herbicide curtailed nodulation in soybean, inhibited growth of the bacteria, reduced nodule biomass and leghaemoglobin (an oxygen carrying protein), and reduced nitrogen fixation and/or assimilation in the plant (Grossbard 1985; King et al 2001; de Maria et al 2006; Zablotowicz & Reddy 2007; Bellaloui et al 2008). Soybeans are most sensitive to these effects during early stages of growth (Bellaloui et al 2006), and under conditions of water stress (King et al 2001; Zablotowicz & Reddy 2007).

In laboratory studies, glyphosate also inhibited *Rhizobium* root nodulation and nitrogen-fixation in sub-clover, the inhibition being higher in sandy soils (Eberbach & Douglas 1983, 1989; Martensson 1992). Up to 70% reduction in nitrogen-fixing nodules occurred in clover planted 120 days after the glyphosate application at a concentration corresponding to typical application rates (Eberbach & Douglas 1983).

Glyphosate treatment of soil collected from a tea plantation in India reduced the population of nitrogen-fixing bacteria (Bezbaruah et al 1995).

Glyphosate has other effects on the soil's nitrogen cycle: in a Canadian study, application of glyphosate to a grass field resulted in a 20-30% increase in denitrification up to 49 days after application, and hence contributed to nitrous oxide emissions (which contribute to ozone destruction) and nitrogen loss from the soil (Tenuta & Beauchamp 1995).

Metabolic and compositional changes

Exposure to glyphosate (and AMPA) can cause a number of metabolic and hence compositional changes in plants. It can cause oxidative stress and may interfere with cytochrome P450 activity. It may interfere with plant hormone metabolism and biosynthesis (Gomes et al 2014)

Thirty one batches of soybean from Iowa, USA were analysed for nutrient and elemental composition. The samples were grouped into 3 categories:

(i) glyphosate-tolerant soy (GM-soy)

(ii) unmodified soy cultivated using a conventional ''chemical'' cultivation regime

(iii) unmodified soy cultivated using an organic cultivation regime.

Organic soybeans showed the healthiest nutritional profile with more sugars (glucose, fructose, sucrose and maltose), significantly more total protein, Zn and less fibre than both conventional and GM-soy. Organic soybeans also contained less total saturated fat and total omega-6 fatty acids than both conventional and GM-soy. GM-soy contained high residues of glyphosate and AMPA (mean 3.3 and 5.7 mg/kg, respectively; max of both = 15mg/kg). There were no residues in the conventional and organic soybeans. Using 35 different nutritional and elemental variables to characterise each soy sample, the authors demonstrated ''substantial non-equivalence'' in compositional characteristics for 'ready-to-market' soybeans. Previous findings of reduced mineral content with glyphosate use were not supported in this study (Bøhn et al 2014).

In greenhouse experiments, glyphosate resulted in significant decreases in linoleic acid and linolenic acid, and significant increases in monounsaturated fatty acids in soy (Zobiole et al 2010a).

Bellaloui et al (2008) found that glyphosate application to GM soybean at a rate of 3.36 kg/ha caused the beans to have a 10% increase in protein content, an increase in oleic acid, and a decrease in linoleic acids, suggesting effects on carbon and nitrogen metabolism.

Glyphosate decreased chlorophyll content in conventional soybeans and corn (Kitchen et al 1981), GM soybean plants (Pline et al 1999), in sunflower leaves and shoot tips (Eker et al 2006), and in the freshwater green algae *Chlorella pyrenoidosa* it reduced chlorophyll and carotenoids increasingly with higher temperatures (Hernando et al 1989).

Glyphosate reduced the production of lignin in asparagus and flax; and phenolic compounds in beans, soybean, the roots of tomato seedlings and the bulbs of the sedge *Cyperus esculentus* (Levesque & Rahe 1992). Both of these effects may reduce disease resistance in the plant (Johal & Huber 2009).

In carrots, it decreased amino acid content, specifically serine, glycine, methionine, tyrosine, phenylalanine, and tryptophan (Nafziger et al 1984).

Pre-harvest treatment of wheat with glyphosate increases the level of shikimic acid in the grain 2-fold (Bresnahan et al 2003). It also increases the level in conventional corn and soybean that have been exposed to glyphosate by drift (Henry et al 2005).

Glyphosate reduced the RNA content of petunia flowers and changed their shape (Shimada & Kimura 2007), and it caused oxidative stress in rice leaves (Ahsan et al 2008).

Earthworms

Earthworms are considered to be sentinels of soil health and integrity (Verrell & Buskirk 2004), so any adverse effects on these organisms are of considerable concern.

According to the IPCS (1994), glyphosate is of low toxicity to earthworms, but exposure to higher concentrations can result in thin, slack and lethargic worms with a dark skin.

- NOEC *Eisenia fetida* = 131.9 mg/kg dry soil (EFSA 2015b)

However Springett & Gray (1992) found that Roundup applied to the soil in repeated doses had a substantial adverse effect on the growth rate of the earthworm *Aporrectodea caliginosa* at all rates of application. The rates used ranged from 0.7 to 2.8 g a.i./ha, substantially less than recommended agricultural rates. According to the authors, the highest rate used was only 20% of the normal applied, yet at this rate no earthworms matured. They concluded: "the reproductive capacity and the total population in the soil could be expected to fall following repeated low doses of biocides."

Exposure of the earthworm *Eisenia fetida* to the glyphosate formulation Glycel 41% SL, at commercial application rates, caused a 50% reduction in weight but no significant reduction in numbers (Yasmin & D'Souza 2007).

Eisenia fetida was also found to avoid soil contaminated with the glyphosate formulation Ortho Groundclear Total Vegetation Killer (5% glyphosate) at "nominal concentrations" of "low to negligible acute toxicity". They rapidly migrated to the surface of the soil, and the authors expressed concern such an effect may compromise survival (Verrell & Buskirk 2004).

A study to compare the effects of 2 different formulations of glyphosate on earthworms showed that Roundup FG (monoammonium salt, 72% a.e.) was 4.5 x more toxic to *Eisenia andrei* than Mon 8750 (monoammonium salt, 85.4% a.e.), indicating toxicity of other ingredients in the formulation. Adverse effects included DNA and lysosomal damage at "environmental concentrations" (14.4 μg a.e./cm) (Piola et al 2013).

Beneficial arthropods

Some studies have shown that glyphosate and/or Roundup can have adverse effects on a number of beneficial organisms that are important to a properly functioning agroecosystem, including a number of predatory insects and parasitoids.

The following effects were observed in one study (Schneider et al 2009) on glyphosate ingestion by the predator insect *Chrysoperla externa,* which is associated with soybean pests and has potential as a biological control:

- severely reduced fecundity and fertility;
- most eggs were abnormal, smaller, dehydrated and became black 2 days after being laid;
- shorter development from 3rd larval instar to pupae;
- longer adult pre-reproductive period;
- reduced adult longevity;
- adults developed tumours in the abdominal region at 20 days after emergence, more drastic in females than males.

Other reported effects of glyphosate:

- airborne components of Roundup sprayed at normal applications rates interfered with the ability of male wolf spiders, a common predator in US and other agroecosystems, to locate mates, either by affecting their ability to detect or react to female pheromones. This could affect their reproductive success and hence, also success at reducing pests (Griesinger et al 2011);
- the formulation Glifoglex had a range of negative effects on the orb web weaver spider, an abundant predator species in Argentina's transgenic soy crops, including reduced prey consumption, delayed and less complex web building, abnormal ovaries and egg sacs, reduced fecundity and fertility, and longer developmental time of progeny (Benamú et al 2010);
- high doses caused 100% mortality of the predatory mite *Amblyseius fallacis*, which predates the spider mites *Panonychus ulmi*

and *Tetranychus urticae* (Hislop & Prokopy 1981);

- decreased longevity in the detritus-eating springtail (*Onychiurus quadriocellatus*) and woodlouse (*Philoscia muscorum* and *Oniscus asellus*) (Eijsackers 1985);
- carabid beetles moved out of glyphosate treated areas in wheat field experiments and numbers did not return to normal until 29 days after application, consequently lowering potential rates of predation on lepidopteran pests (Brust 1990);
- the common green lacewing (*Chrysoperla carnea*) experienced 53% mortality in its larval stage when exposed to low levels of glyphosate (0.7 kg/ha); at 3.7 kg/ha there was a 20% effect on mortality (EC 2002);
- significantly reduced abundance of the small field spider *Lepthyphantes tenuis* when glyphosate was sprayed in controlled experiments (Haughton et al 2001);
- 50% mortality of the parasitic wasp *Trichogramma*, the predatory mite *Typhlodromus pyri,* the lacewing *Chrysoperla* and a ladybird, *Semiadalia;* and 80% mortality of a predatory beetle *Bembidion* after exposure to freshly dried Roundup (Hassan et al 1988);
- carabid beetles crawling over residues of Roundup Biaktiv moved at significantly lower speed (Michalková & Pekár 2009).

Conversely, with the foliar-feeding nematode *Nothanguinea phyllobia,* glyphosate was shown to prolong larval survival by 50%, thus potentially increasing incidence of this pest nematode (Robinson et al 1977).

Bees

Glyphosate was described by the IPCS (1994) as being slightly toxic to honeybees.

- Oral toxicity LD_{50} = >100 μg/bee
- Dermal toxicity LD_{50} = >100 μg/bee

(EFSA 2015b; FAO 2000)

However, sublethal exposure can have implications for honeybee survival. Exposure to levels "commonly found in agricultural settings" (10 mg/L) was found to impair honeybees' cognitive capacities needed to retrieve and integrate spatial information for a successful return to the hive. As a result, honeybee navigation was affected by ingesting traces amounts of glyphosate with potential long-term negative consequences for colony foraging success (Balbuena et al 2015).

Honeybees chronically exposed to glyphosate concentrations at low doses (2.5 mg/L) experienced reduced sensitivity to sucrose and reduced learning performance; additionally elemental learning and short-term memory retention decreased significantly. Non-elemental associative learning was impaired by an acute exposure to glyphosate traces. However, no effect on foraging-related behaviour was found (Herbert et al 2014).

Birds

Glyphosate is described by the IPCS as slightly toxic to birds, with an LD_{50} of >3851 mg/kg body weight (IPCS 1994).

Little information appears to be available about chronic effects on birds. However Roundup can cause endocrine-disrupting effects in animals just as in humans. Exposure to Roundup caused disruption of the male genital system in mallard ducks: it altered the structure of the testis and epididymis, serum levels of testosterone and oestradiol, and the expression of androgen receptors in the testis (Oliveira et al 2007). Glyphosate-based herbicides and glyphosate itself interfere with key molecular mechanisms, including endocrine mechanisms, which regulate early development in chickens leading to congenital malformations (Paganelli et al 2010).

Other animals

Glyphosate was found in the urine of all 30 dairy cows from each of 8 Danish dairy farms. The cows had increased levels of glutamate dehydrogenase, glutamate oxaloacetate transaminase and creatinine kinase in their blood serum, indicating a possible effect of glyphosate on liver and muscle cells. All animals also had very low levels of manganese and cobalt, which may have resulted from the strong mineral chelating effect of glyphosate. Cows from some farms had high serum urea, indicating possible nephrotoxicity (Krüger et al 2013b).

Accidental ingestion of glyphosate formulations by 25 dogs in France between 1991 and 1994 caused vomiting, hypersalivation, diarrhoea, and prostration, but no deaths (Burgat et al 1998).

"Nausea, vomiting, staggering and hindleg weakness have been seen in dogs and cats that were exposed to fresh chemical on treated foliage" (HSDB 2006).

In a small study in New Zealand, the common skink *Oligosoma polychroma* was sprayed once with glyphosate, glyphosate + POEA, or water. Their thermoregulatory behaviour, sprint speed, and weight were then monitored for 6 weeks. The skinks sprayed with glyphosate + POEA selected warmer microclimates and had slower sprint speeds than skinks that had been sprayed with glyphosate only or water. Sprint speed is an important predictor of lizard health and survival; and selecting hotter microclimates can lead to dehydration and greater predation rates, as skinks are more likely to be basking in exposed areas (Carpenter 2013).

Many animal deaths have been recorded after aerial spraying of glyphosate-based herbicide. As reported in the section on 'Human poisonings', aerial spraying of coco crops in Colombia has resulted in crop and animal losses in 59 settlements affected in Valle del Guamuez in the Province of Putumayo, including 38,357 domesticated chickens and ducks, 719 horses, 2,767 cattle, 6,635 guinea pigs, 128,980 fish (from aquaculture), and 919 other animals (pigs, cats, dogs). A similar review for La Hormiga municipality, also in Putumayo, reported adverse effects in 171,643 farm animals including livestock, poultry, and farmed fish (Oldham & Massey 2002).

In Ecuador, there have been reports of deaths of domestic animals and fish in hatcheries (Leahy 2007). A survey by Acción Ecológica found that 80% of poultry in the 0-2 km zone died, as did numerous cattle, pigs, horses, dogs and goats. Calves were aborted. Animal deaths occurred up to 10 km away (Gallardo 2001). Aerial spraying in the Cimitarra River Valley in Santander is reported to have resulted in the deaths of a number of domestic animals, including cattle, mules, and chickens, from loss of food and the contamination of water supplies, as secondary impacts of the spraying (Oldham & Massey 2002).

Animal deaths, including dogs, goats, and rabbits, following exposure to glyphosate drift have been reported in Germany (PAN Germany 2015).

Climate Change

Increased levels of atmospheric carbon dioxide increase the tolerance of 3 out of 4 mature invasive grass species to glyphosate, indicating that as climate change progresses, grasses may become less susceptible to the herbicide. The species were Chloris gayana, Eragostis curvula, Paspalum dilatatum, and Sporobolus indicus (Manea et al 2011).

Environmental fate

Soil persistence, residues and mobility

Glyphosate's persistence, degradation and leaching can be very different from soil to soil (Borggaard & Gimsing 2008). Generally residues remain in the top few centimetres of soil (e.g. Yang et al 2015; EFSA 2015a).

EFSA (2015b) describes glyphosate persistence in soil as being low to very high, and that of AMPA as being moderate to very high, with a half-life varying from less than a week to more than a year and a half, depending on the extent of soil binding and microbial breakdown. Glyphosate is broken down by microbial degradation, principally into AMPA, about 50% (EFSA 2015a).

Residues were detected in soil in Alberta, Canada, 10 months after spraying (Humphries et al 2005); and in Sweden up to 3 years after application (IPCS 1994). Glyphosate residues were found in soil where GM soy is grown in Argentina, at levels up to 4.45 mg/L (Peruzzo et al 2008).

AMPA accumulates in soil; modelling showed that after 20 years of application, levels of 4.9 kg/ha, or 8.5% of the total amount of glyphosate applied, were expected; and these results are consistent with various field studies (Mamy et al 2010).

Because degradation is largely microbial, glyphosate breaks down much more quickly under aerobic than anaerobic conditions:

- DT_{50} = 142.2 days (range 2.8 - 500) (EFSA 2015a, b)
- DT_{50} lab 20oc anaerobic 3-1699 days (EC 2002)

Half-life is not influenced by pH, according to EFSA (2015a).

AMPA has a median half-life of 240 days, ranging up to 958 days in some soils according to the US EPA (1993). EFSA (2015a) gives the laboratory half-life of AMPA as 38.98 - 301 days, and a field half-life of 283.6 to 633 days.

Glyphosate is adsorbed (bound) onto soil particles, making it biologically unavailable. Soils containing higher levels of clay minerals, iron, and aluminium increase adsorption of both glyphosate and AMPA (Piccolo et al 1994; Gerritse 1996). Higher levels of organic matter decrease adsorption (Gerritse 1996) and increases desorption (Aslam et al 2013). Cooler climates tend also to increase persistence (US EPA 1993), as does soil acidity (Albers et al 2009). However, in a study carried out in Hawaii in 1976 on behalf of Monsanto, on the loss of 14C from 14C-labelled glyphosate, the half-life of 14C (from degradation of glyphosate and AMPA) was calculated to be 22 years in clay soils derived from volcanic ash soils from Kukaiau, which had high levels of organic matter (9.71%) and low ph (5.5); but only 18 days in alluvial-derived clay soils from Honouliuli (organic matter 2.1% and ph 6.9) (Nomurra & Hilton 1977).

Glyphosate is frequently reported to be strongly adsorbed onto soil particles, and therefore to be biologically inactivated i.e. not available for microbial breakdown, and to have low potential to leach (IPCS 1994; EFSA 2015b); but this is not always the case. Agricultural soils to which phosphate fertilisers have been added can be high in unbound glyphosate because the soil sorption sites are occupied by the competing phosphate ions from the fertiliser. So unbound glyphosate remaining in the soil is available for root uptake, microbial metabolism, and leaching into groundwater (Kremer & Means 2009). The risk of leaching is greater in fertilised soils (Simonsen 2008). Conversely, studies have shown that the presence of glyphosate in some soils can reduce retention and availability of phosphate (Caceres-Jensen et al 2009), hence reducing fertility. EFSA (2015a) acknowledges that sorption is reduced in phosphate-rich soils, and this may lead to leaching in these soils.

Other reviews and studies have also concluded that glyphosate, and to a greater extent AMPA, leaches through soils (e.g. Landry et al 2005), especially after high rainfall (Vereecken 2005). The California Department of Pesticide Regulation (Fossen 2007) described glyphosate as a “potential leacher”. Studies in Sweden found that glyphosate applied to railway embankments did leach to the groundwater in some places (Torstensson et al 2005).

There is also evidence that glyphosate, once bound to soil particles, does not always remain bound, that adsorption is not permanent. Desorption can occur readily in some soils and the desorbed glyphosate becomes highly mobile in the environment. Piccolo et al (1994) and Piccolo & Celano (1994) showed:

- a high percentage of bound glyphosate can be returned to the soil solution: the least adsorbing soils desorbed up to 80% of the adsorbed herbicide, and the high adsorbing soils released 15-35%;
- desorption readily occurred in soil with a high clay mineral but low iron oxide content;
- desorbed glyphosate can leach to lower soil layers;
- glyphosate can adsorb onto water-soluble humic substances (soil components primarily responsible for the mobility of pesticides in soil) and be transported with humic substances to lower soil depths.

Albers et al (2009) found that glyphosate sorbed to humic substances is more easily desorbed than that sorbed to iron and aluminium oxides.

Simonsen et al (2008) demonstrated in laboratory studies that glyphosate that had aged in soils for 6.5 months, before seeds were sown, was subsequently taken up by plants.

The presence of Btk toxins increases the persistence of glyphosate in soils (Accinelli et al 2004), as does the addition of the herbicide diflufenican (Tejada 2009).

Residues of glyphosate and AMPA in soil are not uncommon, as an analysis of monitoring results in 2 US states show (Battaglin et al 2014):

	No. samples	% glyph detection	max (µg/L)	% AMPA detection	max (µg/L)
soil/ sediment	45	91.1	476	93.3	341
soil water	116	34.5	1	65.5	1.91

Glyphosate residues in soil have also been reported from Argentina, where concentrations in agricultural soils in the southeast of the Province of Buenos Aires, reached 1502 µg/kg and 2256 µg/kg for AMPA (Aparicio et al 2013).

Glyphosate residues in soil may affect subsequent crops. In a greenhouse study, sunflower seedling growth and biomass production were strongly inhibited by glyphosate application pre-sowing, even when sown 21 days after glyphosate application, with application to weeds having a greater impact than application directly on soil. Impairment was associated with increased levels of shikimic acid and decreased

manganese uptake (Tesfamariam et al 2009). A field trial in Finland found that glyphosate does not totally degrade during the growing season and that glyphosate applied in spring is still detected (about 10%) after winter and before the first spraying the following spring. Residues were detected down to 50 cm in the soil profile (below the plough layer). Results from the authors' previous pot experiments indicate that glyphosate is transported below the topsoil by translocation in weeds; and is further released into the soil when roots decompose (Laitinen et al 2006).

Glyphosate also contributes to wider pollution of the environment. The use of glyphosate as a post-emergent herbicide in French vineyards, was found to induce an increase in soil erosion and consequently to lead to a release of DDT residues, which had been previously stored in vineyard soil, back into the environment (Sabatier et al 2014). And in a study on hydrocarbon retention in pervious pavement systems involving geotextile, glyphosate disrupted the retention of hydrocarbons from mineral oil and much higher concentrations of lead and copper (>100%) were released into effluent (Mbanaso et al 2013).

Persistence in water

Glyphosate is soluble in water, resistant to hydrolysis (US EPA 1993) and, although it does break down by photolysis and microbial degradation, it can be persistent for some time in the aquatic environment, with a half-life up to nearly 5 months, and still be present in the sediment of a pond after 1 year.

- Solubility = 10.5 g/L at 200C (FAO 2000)

Photolytic degradation (EC 2002):

- DT_{50} = 33 days at pH 5 and 77 days at pH 9

Biological degradation (EC 2002):

- DT_{50} water = 1-4 days
- DT_{50} whole system = 27-146 days

Degradation of glyphosate in water/sediment (EFSA 2015b):

- DT_{50} whole system = 8.47-210.66 days
- DT_{50} water phase = 1-13.15 days
- DT_{50} sediment = 383.56

Degradation of AMPA in water/sediment (EFSA 2015b):

- DT_{50} whole system = 10.54-77.36 days
- DT_{50} water phase = 0.69 days
- DT_{50} sediment = no reliable information

The aquatic half-life of POEA has been estimated as 21-41 days (Mann et al 2009).

Half-life of glyphosate in seawater from the Great Barrier Reef area of Australia:

- 25°C, low light = 47 days
- 25°C, dark = 267 days
- 31°C, dark = 315 days

Glyphosate is highly persistent in seawater in the dark, and moderately persistent in low light (Mercurio et al 2014).

Glyphosate dissipates from water into sediment or suspended particles (IPCS 1994). It has been found to then dissipate from the sediment of a farm pond with a half-life of 120 days; and to be still present in pond sediment at 1 µg/kg one year later (US EPA 1993).

Distribution in water/sediment systems (EC 2002):

- after 1 day: 47-64% in water, 31-44% in sediment
- after 100 days: 3% in water, 29-44% in sediment

Residues in surface waters

Glyphosate and AMPA have been found in surface waters in many countries, including Argentina, Canada, China, France, Germany, Mexico, Netherlands, Norway, UK, and USA (Frank 1990; IPCS 1994; Buffin & Jewell 2001; Zhang et al 2002; Wan et al 2006; Kolpin et al 2006; Peruzzo et al 2008; Struger et al 2008; Botta et al 2009; Mamy et al 2010; Szekacs & Davas 2011; Battaglin et al 2014; Ronco et al 2016; Rendón von Osten et al 2016).

An analysis of US monitoring results in 38 states reveals widespread glyphosate pollution (Battaglin et al 2014):

	No. samples	% glyph detection	max µg/L	% AMPA detection	max µg/L
streams	1508	52.5	73	71.6	28
drains	374	70.9	427	80.7	397
rivers	318	53.1	3.08	89.3	4.43
lakes/ ponds/ wetlands	104	33.7	301	29.8	41
WWTP outfall	11	9.09	0.30	81.8	2.54

WWTP = waste water treatment plants

And residues of glyphosate continue to be found: Smalling et al (2015) reported a 64% detection frequency in water from 6 wetlands in Iowa, USA.

A 2012 report by Monsanto acknowledged that residues have been found in surface waters throughout Europe[6] at a detection rate of 33%, with 23% being above 0.1 μg/L, the EU drinking water limit. The highest level was found in Sweden (370 μg/L), Ireland (186 μg/L) and Belgium (139 μg/L). AMPA was found in 54% of samples with 46% over the 0.1 μg/L threshold, maximum concentration of 200 μg/L (Horth et al 2012).

Glyphosate residues were found in surface waters where GM soy is grown in Argentina, at levels up to 0.56 mg/L and in sediment at 1.85 mg/L (Peruzzo et al 2008).

It has been recorded in elevated levels in surface water, soil and sediment samples in Argentina as a result of GM soy cultivation in several monitoring studies (Szekacs & Davas 2011). In 2011-12, Ronco et al (2016) found glyphosate residues in 15% of samples from the Paraná River and its distal tributaries, but at 37% frequency in the tributaries of the middle stretch of the river, at up to 07 μg/L in water, 0.21 μg/L in suspended matter and 3,004 μg/kg/dw in sediment. AMPA was also found at up to 0.21 μg/L in suspended matter, and 5,374 μg/kg/dw in sediment. In 2013, Aparicio et al reported the following residues in the southeast of the Province of Buenos Aires:

Media	glyphos. % detection	Max value	AMPA % detection	Max value
surface water (μg/L)	15	7.6	12	2.3
suspended particulates μg/kg	67	298.4	20	118.7
sediment (μg/kg)	66	166.4	88.5	136.8

A study in southern Ontario, Canada found residues of glyphosate in a wide range of creeks, brooks, lakes, rivers, and drains, with the maximum detected level of 40.8 μg/L (Struger et al 2008). A previous study found it in "most of the wetlands and streams sampled" in Alberta (Humphries et al 2005).

A study of mid-western USA streams found glyphosate in 36% and AMPA in 69% of samples, with maximum levels of 8.7 μg/L glyphosate and 3.6 μg/L AMPA (Battaglin et al 2005).[7] Glyphosate has also been found in forest streams in Oregon and Washington, and in streams near Puget Sound (Cox 1998).

One study detected glyphosate in the runoff from no-till corn 4 months after application (Edwards et al 1980).

Glyphosate and AMPA were both found in vernal pools (pools that dry up in hot weather but reappear in wet weather, and are often critical for amphibians) in the US, with highest levels of glyphosate (328 μg/L) found in a national park (Battaglin et al 2009).

Urban

Runoff from hard surfaces in urban areas is increasingly being seen as a source of surface water pollution with glyphosate and AMPA, especially during storm events or after rainfall. In Switzerland, one study found that urban sources provided more than 60% of the loading in a small catchment with both urban and agricultural sources (Hanke et al 2010). Household use has also been found to contribute to surface water loading of glyphosate and AMPA in the UK (Ramwell et al 2014).

Analysis of water from urban streams in Washington (USA) over the period 1998-2003 found glyphosate in all samples (Frans 2004).

In another study of urban contribution to residues in US streams, AMPA was found in 67.5% and glyphosate in 17.5% of streams and with maximum concentrations of AMPA of 3.9 μg/L and glyphosate of 2.2 μg/L. There was a 2-fold increase in detections below urban wastewater treatment plants, indicating urban use is making significant contributions to stream contamination (Kolpin et al 2006).

A one-year monitoring project of 3 wastewater treatment plants and one composting unit near Versailles, France detected glyphosate and AMPA in all samples of sludge. The highest level of glyphosate was 2.9 mg/kg and of AMPA 33.3 mg/kg, although some of the latter may be attributable to household cleaning products containing aminophosphonates (Ghanem et al 2007a).

6 Austria, Belgium, Czech, Finland, France, Germany, Ireland, Italy, Norway, Slovakia, Spain, Sweden, Netherlands, UK

7 AMPA residues can also originate from the breakdown of detergents.

Glyphosate was found in 17.5% and AMPA in 67.5% of samples from 10 wastewater treatment plants in the US; and in Canada 21% of samples had glyphosate with concentrations up to 41 μg/L for glyphosate and 30 μg/L for AMPA (Szekacs & Davas 2011).

This is supported by a study in urban Paris which found levels in Ogre river water frequently exceeded the European standard for drinking water of 0.1 μg/L, with levels as high as 90 μg/L found after rainfall (60% of Paris drinking water comes from surface waters). The origin is believed to be use of glyphosate on road and rail sides (Botta et al 2009). Similarly, glyphosate was found in sewage sludge in the same area, at Ile-de-France, at levels of 0.1-3 mg/kg (Ghanem et al 2007b).

Landfill leachate

Glyphosate has been detected in landfill leachate in the UK (Slack et al 2005).

Groundwater

Glyphosate has been found in groundwater and wells in a number of countries, including Canada, European countries, Sri Lanka, and USA over a number of years, reported as long ago as 1992 (US EPA 1992) and still being reported today (van Steempvoort 2016).

In 2001/2002, 38 out of 193 private water plants supplying households in Denmark were found to contain glyphosate and AMPA. Of these 15 still had residues in 2005, with 10 exceeding the EU standard of 0.1 μg/L. The 15 plants extracted water from wells in shallow groundwater layers (Brusch 2006).

In 2002, the European Commission (EC 2002) warned that member states "must pay particular attention to the protection of the groundwater in vulnerable areas, in particular with respect to non-crop uses". Monsanto's report on glyphosate in water in Europe identified a 1% detection rate with 0.64% above the 0.1 μg/L drinking water threshold; The highest number of detections were in France (24 g/L) and Denmark (4.7 μg/L). AMPA had a 2.6% detection rate and 0.77% above the threshold. (Horth 2012).[8] Both Monsanto and EFSA (2015b) noted from this report that the high concentrations (39.3 μg/L) found in Wales warranted further investigation.

Since then glyphosate has been found in the groundwater in 11 different locations in Catalonia, Spain. It was detected in 41% of 140 samples, at concentrations as high as 2.5 μg/L, 25 times the legal limit for drinking water in the EU (Sanchis et al 2012). The authors of the study stated that few monitoring studies have looked for glyphosate in groundwater, or have found it, because it is difficult to analyse. However, their new more sensitive method, carried out in agricultural areas in peak application times, did find it extensively.

EFSA (2015b) referred to residues in Italy (in 2012) with concentrations >0.01 μg/L in 5 groundwater wells, and in France (2012) with 19 detections >0.01 μg/L.

In 2013, glyphosate and AMPA were found in shallow riparian groundwater at 4 out of 5 streams in urban catchments in Canada, with a detection rate of 13.2% and a maximum level of 43 ng/L for glyphosate and 11.7% detection with maximum level of 2870 mg/L AMPA (van Stempvoort et al 2014). Residues were also found in rural riparian (surface seeps) and upland groundwater in Ontario. Overall, glyphosate and AMPA were detected in 10.5% and 5.0%, respectively, of the groundwater samples. Highest concentrations were at upland sites (663 ng/L of glyphosate, 698 ng/L of AMPA) (van Stempvoort et al 2016).

The Battaglin et al (2014) analysis of groundwater monitoring results for the US revealed a 5.7% detection rate for glyphosate over 1,171 samples, with maximum 2.03 μg/kg (AMPA = 14.3% and max 4.88 μg/L).

Glyphosate was detected in of 94% of abandoned rural wells tested in Sri Lanka at a mean level of 3.5 μg/L (Jayasumana et al 2015a).

In 2016, glyphosate was found in all samples of water from wells in Mexico's Yucatan Peninsular (Ich Ek and Suc Tuc, in the Holpechen Municipality, State of Campeche), at levels up to 3.3637 μg/L (Rendón von Osten et al 2016).

Marine sediments and seawater

Detectable concentrations of glyphosate and AMPA were found in marine sediment at a number of sites in the Waitemata Harbour and Hauraki Gulf in New Zealand. The maximum glyphosate concentration detected was 1 mg/L,

8 Also in Austria, Belgium, Germany, Ireland, Sweden, Switzerland, Netherlands, UK (Wales)

while AMPA had a maximum level of 0.37 mg/L (Stewart et al 2008).

Glyphosate was detected in the waters of the Mareenes-Oleron Bay (France, Atlantic Coast), over an 11-day period in late spring 2004, at a peak concentration of 1.2 µg/L (Stachowski-Haberkorn et al 2008.

Bioconcentration/bioaccumulation

Bioconcentration is the accumulation of substances in an organism through uptake from water. The octanol-water partition coefficient (K_{ow}) is used to determine a chemical's ability to bioaccumulate.

Octanol/water partition coefficient (FAO 2000):

- log K_{ow} = < -3.2 at 25°C
- equivalent K_{ow} = < 6 × 10-4

The IPCS (2004) reported that bioconcentration factors for glyphosate were low in laboratory tests and there was no evidence of bioaccumulation, but residues of the metabolite had been found in carp 90 days after application.

EFSA (2015b) gives a bioconcentration factor (BCF) of 1.1 + 0.61.

However, bioaccumulation of glyphosate may be greater than predicted from the log KOW value alone (Annett et al 2014). The BCF for glyphosate is increased in the presence of POEA in the aquatic environment. The sediment-dwelling freshwater blackworm *Lumbriculus variegatus* was exposed for 4 days to both glyphosate and to the Roundup Ultra formulation. A low level of bioaccumulation was found to occur, with a BCF for glyphosate varying between 1.4 and 5.9. The accumulated amount increased with increasing concentration in the surrounding medium and was higher for Roundup Ultra formulation than for exposure to glyphosate alone (Contardo-Jara et al 2009). This may be because POEA, which is known to enhance glyphosate transport into plant cells, also facilitates increased permeability in animal cells (Hedberg & Wallin 2010).

Land snails (*Helix aspersa*) placed in a vineyard in France and subject to normal pesticide applications, were found to accumulate glyphosate and AMPA in their tissues 12 days after treatment. The concentration of AMPA was twice that of glyphosate showing that both metabolism and accumulation was occurring (Druart et al 2011).

A previous study had demonstrated a low level of accumulation of glyphosate in fish, carp (*Cyprinus carpio*) and tilapia (*Oreochromis mossambicus*) and the plant water hyacinth (*Eichhornia crassipes*) exposed at environmentally relevant concentrations (Wang et al 1994).

Together, "these results support the possibility of food chain contamination" (Annett et al 2014).

Findings by Professor Carrasco of Argentina indicate that glyphosate might be accumulating in amphibian cells (Valente 2009; Trigona 2009; Ho 2009).

Myers et al (2016) concluded that metabolism studies strongly point to bioaccumulation in the kidney and liver.

Richards et al (2005) proposed that the presence of adjuvants in Roundup may enhance glyphosate bioaccumulation in cells.

Benachour et al (2007) proposed that human embryonic and placental, and equine testis cells, exposed to non-toxic levels of glyphosate showed evidence of either bioaccumulation or time-delayed effect, suggesting a cumulative impact of very low doses of glyphosate approximating the Acceptable Daily Intake (0.3 mg/kg).

Atmospheric transport and deposition

EFSA (2015a) gives a photochemical oxidation degradation half-life in air as DT50 =1.6 hours and states no long-range transport is expected.

A Canadian study has identified glyphosate in particles in the air and has proposed that atmospheric transport of glyphosate is in association with particulate matter (dust) not vapour (Humphries et al 2005). Deposition is said to result largely from dust particles being washed out of the air. Because glyphosate is strongly adsorbed to the soil surface, it is prone to being transported with wind erosion of soils (Farenhorst et al 2015).

In a study on the drift of pesticides from areas of use into protected areas of the Maya Mountains in Belize, glyphosate was found in phytotelm (plant-held water) at all locations sampled, with mean concentrations at sites ranging from 0.22 to 1.7 µg/L (Kaiser 2011).

Volatility

EFSA (2015b) concluded there would be no significant volatilisation from plant surfaces or soil:

- Vapour pressure = 1.3 x 10-5 Pa at 250C
- Henry's law constant = 2.1 x 10-7 Pa/m3/mol (EC 2002)

Deposition

Interchange between air and water, which affects uptake into the atmosphere and redeposition in rain or snow, is described by Henry's Law constant (H)—the higher the value, the higher the deposition.

Henry's law constant for glyphosate (EC 2002):

- 2.1 x 10^{-7} Pa/m^3/mol

Glyphosate was one of the most frequently detected pesticides in rainwater in Belgium in 2001. It was found in 10% of rain samples at concentrations up to 6.2 µg/L, with AMPA found in 13% at a maximum concentration of 1.2 µg/L (Quaghebeur et al 2004). It was also measured in rain at Alberta, Canada, at all sites and throughout the "growing season", at a maximum concentration of 1.51 µg/m^2/day (Humphries et al 2005). In 2011, glyphosate was detected in roughly 10% of samples at levels up to 470 ng/L and AMPA up to 660 ng/L in rainfall in a small suburban catchment in Nantes, France. Levels were higher in summer. Roof runoff contained concentrations up to 980 ng/L of glyphosate and 120 ng/L of AMPA (Lamprea & Ruban 2011).

In the first ever report on ambient air monitoring for glyphosate in the US, the herbicide was found in 61 to 100% of samples of air and 63 to 92% of samples of rain in agricultural areas in 2 US states. The concentrations ranged up to 9.1 ng/m^3 in air and 2.5 µg/L in rain for glyphosate; and for AMPA 0.97 ng/m^3 in air and 2 µg/L in rain. Highest levels were recorded during weeks with rainfall following the application period. It was calculated that about 0.7% of the glyphosate applied in agricultural areas is removed from the air in rain (Chang et al 2011).

In an analysis of US monitoring results of 85 samples of precipitation taken in 3 states (Iowa, Indiana, Mississippi), glyphosate was found in 70.6%, at levels up to 2.5 µg/L, and AMPA in 71.8% with a maximum level of 0.04 µg/L (Battaglin et al 2014).

Not included in the above analysis were the results of monitoring in Mississippi by Majewski et al (2014) who reported that glyphosate and AMPA were detected in >75% of air and rain samples in the Mississippi Delta in 2007, in 86% of air and 77% of water samples.

Weekly bulk deposition monitoring from May to September in 2010 and 2011 in the Canadian city of Winnipeg identified glyphosate as the most common pesticide contaminant in rain. It was detected in 81% of samples, at a maximum weekly concentration of 16.9 µg/L, and it accounted for 65% of total pesticide deposition. AMPA was also detected at all sites but less frequently. Deposition was higher in dry years, indicating particle deposition was greater than that of residues in rain (Fahrenhorst et al 2015).

Deposition of glyphosate from the air was detected in wetlands in the Praire Pothole region of Canada every week over a 4-month period in 2008 at a weekly deposition rate up to 40 µg/m (Messing et al 2011). And wetland residues (max 21 ng/L) measured by van Steempvoort et al (2016) in the Nottawasaga River Watershed, Ontario, were believed to have resulted from atmospheric transport and deposition. Glyphosate was detected in 86.7% of precipitation samples (max = 135 ng/L) and AMPA in 26.7 % (max = 19 ng/L).

Herbicide resistance and weeds

Resistance to glyphosate has become a major problem in many countries. UK guidelines on minimising the risk of its development point to use on continuous monoculture annual crops, perennial crops, and amenity use as key risks; and recommend among other things to use nonchemical methods of weed management (Heap 2016).

Weed resistance to glyphosate was first recorded in 1996, in Australia, in the species *Lolium rigidum* (rigid ryegrass). Resistance is now recorded in 35 species of weeds and in 27 countries, most notably the USA (Heap 2016), up from 16 species in 14 countries in September 2009 (Heap 2009). There is now even a case of multiple resistance – to glyphosate, glufosinate, paraquat, and ACCase-inhibiting herbicides (e.g. fluazifop-p-butyl, haloxyfop, clethodim, sethoxydim), in *Eleusine indica* (India goosegrass) (Jalaludin et al 2015).

Resistance to glyphosate initially evolved very slowly: there were no reported cases until 20 years after the herbicide's introduction. However, the upsurge in repeated applications of glyphosate in no-till systems and GM crops greatly increased the risk of resistance developing (Dewar 2009).

Ten of the first 14 resistant species were recorded in USA, Argentina and Brazil, where glyphosate tolerant crops are widely grown, and are largely associated with Roundup Ready soybean, and to a lesser extent maize and cotton. The first glyphosate-resistant weed associated with a GM crop was horseweed, *Coryza canadensis,* which appeared in the US just 3 years after the introduction of Roundup Ready soybean and it rapidly became widespread across mid-west, southern and east coast USA (Dewar 2009).

In 2011, the following indicative figures were published (GM Freeze 2011):

- Palmer amaranth (*Amaranthus palmeri*) in maize, cotton and soy in the US since 2005; estimated at up to 1 million infested sites in North Carolina alone.
- Horseweed (*Conyza canadensis*) in cotton, soy and maize since 2000: up to 100,000 sites are infested in Delaware, USA, alone.
- Johnsongrass (*Sorghum halepense*) in soy in Argentina since 2005: up to 100,000 acres infested.

In 2012, the US EPA granted an emergency exemption for the use of the herbicide fluridone on cotton because of the extent of herbicide resistance (FR 2012). A survey of farmers in 13 cotton-producing US states in 2015 found that 69% of them had herbicide-resistant weeds on their farm (Zhou et al 2015).

Resistance to glyphosate continues to grow to such an extent that Monsanto has been forced to accompany its new GM technology with old herbicide packages to try to beat the weeds. For example, the Roundup Ready® Xtend Crop System consists of GM soybean seeds with tolerance to dicamba as well as Roundup (Monsanto 2012).

Resistant weeds in corn have spurred Dow to develop a GM corn resistant to both 2,4-D and glyphosate so that farmers can spray both herbicides on their crop (Schiffman 2012), and Monsanto to develop a soybean resistant to both dicamba and glyphosate (Monsanto 2016).

Country	Resistant weeds
Argentina	*Amaranthus hybridus (syn quitensis)* (smooth pigweed) *Amaranthus palmeri* (Palmer amaranth/pigweed) *Cynodon hirsutus* (couch grass) *Digitaria insularis* (sourgrass) *Echinochloa colona* (junglerice) *Eleusine indica* (goosegrass) *Lolium perenne* (perrenial ryegrass) *Lolium perenne ssp. multiflorum* (Italian ryegrass) *Sorghum halepense* (Johnsongrass)
Australia	*Brachiara eruciformis* (sweet summer grass) *Bromus diandrus* (ripgut broom) *Bromus rubens* (red broom) *Chloris truncata* (windmill grass) *Chloris virgata* (feather fingergrass) *Conyza bonariensis* (hairy fleabane) *Lactua serriola* (prickly lettuce) *Lolium rigidum* (rigid ryegrass) *Echinochloa colona* *Rhaphanus raphanistrum* (wild radish) *Sonchus olearaceus* (annual sowthistle) *Urochloa panicoides* (liverseed grass)
Bolivia	*Chloris elata* (tall windmill grass) *Eleusine indica*
Brazil	*Amaranthus palmeri* *Chloris elata* (tall windmill grass) *Conyza bonariensis* *Conyza canadensis* (horseweed) *Conyza sumatrensis* (Sumatran fleabane) *Digitaria insularis* *Eleusine indica* *Lolium perenne ssp. multiflorum*
Canada	*Ambrosia artemisifolia* (common ragweed) *Ambrosia trifida* (giant ragweed) *Amaranthus tuberculatus (A. rudis)* (common waterhemp) *Conyza canadensis*

	Kochia scoparia (kochia)
Chile	*Lolium perenne ssp. multiflorum*
China	*Conyza canadensis* *Eleusine indica*
Colombia	*Conyza bonariensis* *Eleusine indica* *Parthenium hysterophoris* (ragweed parthenium)
Costa Rica	*Eleusine indica*
Czech Repub.	*Conyza canadensis*
France	*Conyza sumatrensis* *Lolium rigidum*
Greece	*Conyza bonariensis* *Conyza canadensis* *Conyza sumatrensis*
Indonesia	*Eleusine indica*
Israel	*Conyza bonariensis* *Lolium rigidum*
Italy	*Conyza canadensis* *Lolium perenne ssp. multiflorum* *Lolium rigidum*
Japan	C*onyza canadensis* *Eleusine indica* *Lolium perenne ssp. multiflorum*
Malaysia	*Eleusine indica* *Hedyotis verticillata* (woody borreria)
Mexico	*Bidens pilosa* (hairy beggarticks) *Leptochloa virgata* (tropical sprangletop; Juddsgrass)
New Zealand	*Lolium perenne* *Lolium perenne ssp. multiflorum*
Paraguay	*Digitaria insularis*
Poland	*Conyza canadensis*
Portugal	*Conyza bonariensis* *Conyza canadensis* *Lolium perenne*
South Africa	*Lolium rigidum* *Conyza bonariensis* *Plantago lanceolata (buckthorn plantain)*
Spain	*Conyza bonariensis* *Conyza canadensis* *Conyza sumatrensis* *Lolium rigidum* *Lolium perenne ssp. multiflorum*
Switzer-land	*Lolium perenne ssp. multiflorum*
United States	*Amaranthus palmeri* *Amaranthus spinous* (spiny amaranth) *Amaranthus tuberculatus (A. rudis)* *Ambrosia artemisifolia* *Ambrosia trifida* *Conyza bonariensis* *Conyza canadensis* *Echinochloa colona* *Eleusine indica* *Kochia scoparia* *Lolium perenne ssp. multiflorum* *Poa annua* (annual ryegrass) *Lolium rigidum* *Parthenium hysterophorus* *Salsola traqus* (Russian thistle) *Sorghum halepense*
Venezuela	*Echinochloa colona*

Source: Heap 2016

The mechanisms involved in the development of this resistance are not well understood, but they appear to involve gradual changes within the exposed weed populations, in some cases involving herbicide sequestration by the weed in tissues, limited herbicide translocation within it, and slow metabolism (Johnson et al 2009).

However, there is another type of resistance—involving the spreading of the tolerance genes engineered into the Roundup Ready plants to wild relatives. This is known as gene flow, and it reduces the weed's susceptibility to glyphosate. Monitoring of GM sugar beet production in France lead to the discovery of resistant weeds that were descended from hybridisation of the GM sugar beet and weed beet, at up to 112 m distance from the nearest GM plant (Darmency et al 2007). Gene flow has also been found to occur from glyphosate-tolerant canola, corn, soybean, and creeping bentgrass. GE canola has been found growing as weeds along railways and roads in Canada. The glyphosate-tolerance genes in creeping bentgrass have been found in non-GM creeping bentgrass as far as 21 km distant from where the GM version was being grown under regulation (as it had not been commercially released) (Mallory-Smith & Zapiola 2008).

Constant use of glyphosate in cropping systems has affected the dynamics of weed populations, causing a species shift away from those that are

sensitive to glyphosate such as perennial grass and perennial broadleaf weeds, to weeds that are regarded as more difficult to control with herbicides, such as annual broadleaf weeds (Johnson et al 2009). It has also resulted in an increase in weed species richness in some crops, for example in a US corn-soybean rotation, in comparison with tillage and/or other herbicide regimes. Whilst these are not issues of resistance, they are important effects of glyphosate on weed dynamics, and they are reducing the utility of the Roundup Ready cropping systems (Johnson et al 2009).

There is also some evidence that glyphosate at normal rates of application can induce growth in weeds that have evolved resistance to the herbicide, a process known as hormesis, where a small dose (relatively) may enhance growth rather than kill the plant (Belz & Duke 2014).

Resistance to glyphosate of just 4 weeds – common ragweed, giant ragweed, common waterhemp and Palmer amaranth – is having a significant effect on corn and soybean production in the US corn belt (Johnson et al 2009). The same effects on cropping can be expected in other countries that have followed the US in adopting GM herbicide-tolerant crops.

Some scientists, and even Monsanto, recommend that growers use other herbicides as well as glyphosate to reduce the development of glyphosate resistance in weeds within glyphosate-tolerant crops. Herbicides recommended include alachlor, atrazine, 2,4-D, dicamba, diuron, flumioxazin, fomesafen, metolachlor, mesotrione, MSMA, and pendimethalin. In fact, one Monsanto suggestion for weed management in corn, cotton and soybean involve using glyphosate in only 2 out of 4 herbicidal treatments (Gustafson 2008). Fluometuron, prometryn, and trifluralin have also been suggested in Australia (Werth et al 2008). In Latin America, glyphosate is reported to be commonly mixed with other herbicides including atrazine, paraquat, and metsulfuron (Semino 2008).

Alternatives to Glyphosate

Attitudes to weeds have changed dramatically over the years, and are continuing to change. Once upon a time, many of the plants currently regarded as weeds were thought of as beneficial or at least not a problem. Ironically it was the emergence of chemical herbicides that created weeds out of some plants by altering attitudes towards them. For example, in his 1957 book *New Way to Kill Weeds*, US horticulturalist R. Milton Carleton stated that “lawn clover is now considered a weed”. Originally recognised for the ecological services clover provides (nitrogen fixation, bee food, etc), it then became vilified with the advent of a selected herbicide that would kill it without killing the grass. And now once again clover is widely understood to bestow great ecological benefits.

Therefore, the first step is to identify whether a non-crop plant is in fact a weed in a given situation. Generally there are 3 kinds of plants in a field: crop plants, non-crop plants and weeds that above a certain level reduce yields or cause other problems. Many of the plants that are currently called weeds are in fact better viewed as non-crop plants or even beneficial plants. Many provide valuable ecosystem services such as habitat for natural biological pest control agents.

‘Weeds’ can provide valuable ground cover, protecting the soil from sun and rain damage and erosion. Balanced weed populations can provide favourable microclimates that assist crop growth. Weed roots can help improve soil biological activity and structure. They can be useful green manures, and in compost. ‘Weeds’ can also produce chemicals that are beneficial to crop plants—for example, corncockle produces the chemical agrostemmin, which can increase the yield and gluten content of wheat (Lampkin 1990).

‘Weeds’ can attract insect pests away from crops and/or provide habitat for beneficial insects that control pest species, for example for ladybirds that control aphids (Lampkin 1990); or the use of the non-crop plant Napier grass in East African maize and sorghum systems: the grass produces an odour which attracts stem borer and a sticky substance which kills the larvae (Ho & Ching 2003).

Indian farmer Poorak Kheti, in Mohanpur, Uttah Pradesh uses the ‘weeds’ baru (*Sorghum halepense*), doob (*Cynodon dactylon*), tipatuiya and motha (*Cyperus rotundus*) to improve soil fertility and the yields of his sugar cane (Sciallaba & Hattam 2002).

‘Weeds’ can also be very useful as prized herbal remedies or valuable additions to the diet because of their nutritive quality. Plants that are called weeds by some are in fact highly valued plants for others – for example, the Napier grass

described above as a non-crop plant is also highly prized as a source of food and fodder by some African communities and can be a lifeline for them.

'Weeds' can be excellent indicators of problems with soil structure and fertility. 'Weed' species can be read to indicate problems with pH, poor drainage, compaction, low friability of soils and nutrient deficiency (Lampkin 1990). Spraying the weeds with glyphosate or any other herbicide will not fix the problem, but solving the soil health problem will control the weed as well as increase productivity and resistance to pests and diseases.

Weed problems in non-production areas are another issue. Glyphosate is used globally to control vegetation in public places such as parks and roadsides, schools, hospitals, utilities, and in natural forest and wild land regeneration. The starting point in identifying how to manage weeds in these situations is to first understand the benefits they may be bringing and to identify whether the negative effects of the weed in question may by outweighed by these benefits. Benefits can include erosion control, food for bees and birds, habitat for other beneficial or non-pest species, shade for the reestablishment of native species and for waterways, enhanced soil nutrients. Invasive species can help ecosystems, and people, adapt to climate change by maintaining ecosystem processes such as productivity, carbon storage, and nutrient cycling in a context of altered land cover (Davis et al 1999; Auckland Council 2013; Chandrasena 2014; Tassin & Kull 2015).

Alternative herbicides

There are many other synthetic chemical herbicides on the market, but these also have a range of adverse health and environmental effects, such as endocrine disruption, cancer, neurological damage, reproductive toxicity, groundwater contamination, persistence, etc. Hence their use is NOT recommended as replacements for glyphosate (PAN Germany and Agrarkoordination 2014).

There are some herbicides derived from natural plant extracts that can kill or suppress weeds, such as extracts from pine oil and coconut oil. But care must be taken to ensure that formulations do not include toxic surfactants, solvents or other inert or adjuvant ingredients. Some formulations are permitted in certain circumstances in organic growing systems.

Generally, however, even a natural herbicide should be regarded as the choice of last resort, with the primary focus being placed on alternative weed management practices that prevent the need for a spray.

Alternative weed management

Alternative weed management focuses on sustainable ecological solutions that minimise the incursion and build up of weeds. It takes a holistic approach to crop management that recognises weeds as an integral part of the whole agroecosystem, forming a complex with beneficial insects, weeds, and crops. The self-regulatory mechanisms of a highly biodiverse farming system help keep both weed and pest species in balance. Although weeds are generally regarded by the modern agricultural institution as reducing crop productivity and encouraging pests and diseases, there are many instances where the reverse is true. So-called weeds can play a vital role in suppressing pest and disease populations, improving soils and increasing yields.

Elements of alternative weed management in crops can include:

- designing a farm ecosystem that encourages biodiversity, providing habitats for beneficial insects, and utilising 'weeds' as an element of useful biodiversity whilst minimising the need for intervention to control them;
- understanding which weeds are a problem in the specific growing situation and then using physical, cultural and ecological methods to control the most harmful ones where needed and over time to alter the vegetation balance to favour more beneficial and neutral plants;
- in appropriate situations, e.g. coffee groves, planting good varieties at high density to provide a close canopy cover that shades out weeds;
- polycropping to reduce weed growth between rows; use of under-sown species; shade trees also help reduce weed growth in coffee groves;
- reading 'weeds' to identify soil problems and then making the necessary improvements to soil health;
- timely and appropriate cultivation prior to sowing the crop, to either bury weed seeds or encourage their germination before crop sowing, although mechanical disturbance of the soil should be minimised in order to protect the soil structure;

- crop rotations;
- selection of optimum planting dates with respect to crop choice, lunar cycles and weather patterns;
- increasing competitiveness of the crop through appropriate nutrient use and improving soil health;
- use of crop waste, grass cuttings and most herbaceous plant material as mulch to suppress weed seed germination and growth; such mulch is also moisture conserving and encourages beneficial ecosystem services from natural enemies of insect pests, including frogs, spiders, parasitic wasps and predatory insects, along with active microbes in the soil;
- sowing of green manures between crops helps prevent weed seed germination; then turning in the green manure and weeds before they seed provides added soil benefits;
- controlling the spread of weed seeds through good sanitation practices, such as cleaning machinery, cleaning seeds for saving, careful use of animal manures, good composting practices, and avoiding letting weeds go to seed;
- appropriate mechanical methods practices such as hand and mechanical weeders, smothering methods, thermal weed control such as hot water, steam, hot foam or flame weeders, solarisation;
- controlled grazing, e.g. introducing ducks into rice growing systems to eat weed seeds and seedlings; grazing sheep to control grassy weeds and herbs in coffee groves, vineyards and orchards;
- introduction of selected biological control organisms that target certain invasive, exotic weeds.

(Lampkin 1990; Ho 1999; BIO-GRO 2001; Watts & Williamson 2015; Williamson 2016)

Elements of alternative weed management in the built and natural environments can include:

- design of roads, paving, sports fields, parks and landscaping to reduce the potential for weed invasion and maximise ease of management;
- ongoing maintenance of infrastructure to prevent weed invasion;
- regular street-sweeping to prevent silt build-up in channelling inhibits weed growth;
- identify what level of weed and vegetation management is really necessary;
- selection of appropriate species in gardens and turf;
- increase mowing height on turf to reduce the potential for weed invasion;
- ensure appropriate fertility and drainage in turf to improve grass vigour;
- cultivation and re-sowing of sports fields to remove weeds and achieve a desired species mix in a grass sward;
- 'weed hygiene' to prevent spread of weeds by people or machinery
- use of thermal methods such as flame weeders, hot water or steam on hard edges of paths and roads and for turf replacement;
- use of mechanical methods such as mowers, slashers and strimmers;
- hand removal including grubbing, pulling, cutting, hoeing;
- plant-based products such as those based on pine, coconut or palm oils;
- mulching of gardens, ornamental trees, and in natural areas after weed removal;
- smothering, e.g. with weed mat, carpet or cardboard covered with mulch;
- introduction of selected biological control organisms that target certain invasive, exotic weeds;
- ensuring areas cleared are replanted with appropriate species to prevent reinvasion by weeds;
- use of stable and self-sustaining groundcovers, native grasses and shrubs that can reduce or eliminate management particularly on roadsides and right of ways;
- creation of native wildflower areas and corridors for wildlife, bees and other pollinating insects;
- use of browsing animals to control weeds in some situations.

(Davis et al 1999; Auckland Council 2013; Chandrasena 2014; Tassin & Kull 2014)

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Pesticide Action Network (PAN) is a global network of over 600 participating nongovernmental organizations, institutions and individuals in over 90 countries working to replace the use of hazardous pesticides with ecologically sound and socially just alternatives such as agroeoclogy. PAN was founded in 1982 and has five independent, collaborating Regional Centers that implement its projects and campaigns.

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