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Glyphosate: environmental contamination, toxicity and potential risks to human health via food contamination

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Abstract

Glyphosate has been the most widely used herbicide during the past three decades. The US Environmental Protection Agency (EPA) classifies glyphosate as ‘practically non-toxic and not an irritant’ under the acute toxicity classification system. This classification is based primarily on toxicity data and due to its unique mode of action via a biochemical pathway that only exists in a small number of organisms that utilise the shikimic acid pathway to produce amino acids, most of which are green plants. This classification is supported by the majority of scientific literature

on the toxic effects of glyphosate. However, in 2005, the Food and Agriculture Organisation (FAO) reported that glyphosate and its major metabolite, aminomethylphosphonic acid (AMPA), are of potential toxicological concern, mainly as a result of accumulation of residues in the food chain. The FAO further states that the dietary risk of glyphosate and AMPA is unlikely if the maximum daily intake of 1 mg kg^{-1} body weight (bw) is not exceeded. Research has now established that glyphosate can persist in the environment, and therefore, assessments of the health risks associated with glyphosate are more complicated than suggested by acute toxicity data that relate primarily to accidental high-rate exposure. We have used recent literature to assess the possible risks associated with the presence of glyphosate residues in food and the environment.

Keywords

NOAEL Withholding period Contamination AMPA Herbicide Toxicity

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Electronic supplementary material

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Introduction

Glyphosate [*N*-(phosphonomethyl) glycine] is the most widely used herbicide in the world (Duke and Powles 2008) with an estimated global demand of half a million tonnes per annum (Székács and Darvas 2012) and \$5.5 billion in sales in 2011 (Krebs 2011). Dr. Henri Martin synthesised glyphosate in 1950 (Parrot et al. 1995); however, it was not commercialised as a herbicide until 1974 (Duke and Powles 2008). The popularity of glyphosate revolves around its efficiency in killing weeds at low cost but is also due to its perceived low toxicity, rapid absorption by plants, and slow evolution of glyphosate resistance in weeds (Duke and Powles 2008).

Glyphosate is categorised as a non-selective, systemic, post-emergence herbicide (Duke and Powles 2008), which acts as an inhibitor of the enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), in the shikimate pathway (Duke and Powles 2008). The shikimate pathway produces aromatic amino acids used for synthesis of proteins and plays an important role in the production of secondary metabolites such as lignin (Haslam 2014). It is currently understood

that inhibition of EPSPS deregulates the shikimate pathway, resulting in uncontrolled carbon flow, mostly going into shikimate (Duke et al. 2003a). This depletes pools of compounds needed for carbon fixation, causing a general disruption of the organisms metabolism (Siehl 1997; Duke et al. 2003a; Duke and Powles 2008).

Glyphosate is categorised by the EPA as a 'least toxic' (category IV) substrate for animals (Williams et al. 2000). This is based primarily on toxicity data and also due to the unique mode of action of glyphosate being confined to a small range of organisms, primarily green plants. It is also due to the perception that glyphosate is rapidly mineralised from the environment (Mamy et al. 2005). In reality, the half-lives of glyphosate and its major metabolite, AMPA can be lengthy, ranging between 0.8–151 and 10–98 days, respectively (Table 1). Most likely, the relatively large range in persistence of glyphosate and AMPA resulted from varying soil properties and environmental conditions. For example, glyphosate and AMPA showed half-lives of up to 151 and 98 days, respectively, in one study based on clay soil in Sweden (Bergström et al. 2011) and 10 and 10 days, respectively, in a different study based on loamy soil in China (Zhang et al. 2015). Prolonged half-life and slow degradation may increase the risk of long-term environmental contamination (Al-Rajab and Schiavon 2010), particularly with frequent and repeated applications that are typical in agricultural settings. Therefore, this review summarises eco-toxicological effects of glyphosate on non-targeted species and possible human health risks posed by glyphosate contamination of food.

Table 1

Glyphosate and AMPA half-life ($T_{1/2}$) and glyphosate adsorption coefficients in different soil types

Site	Soil type	Soil depth (cm)	pH (H ₂ O)	Clay (%)	OC (%)	Glyphosate- $T_{1/2}$ (days)	AMPA- $T_{1/2}$ (days)	K_f	Referer
Agriculture	Clay loam	0–25	7.9	34.9	1.9	4	–	17	Al-Rajab Schiavor (2010)
Ozzano	Sandy loam	–	8.1	14	0.7	17.4	–	–	Accinelli al. (2004)
	Sandy loam	0–25	5.1	10.5	0.82	14.5	–	34	Al-Rajab Schiavor (2010)

	Silt clay loam	0–25	6.3	30.6	1.45	19	–	34	Al-Rajab Schiavor (2010)
Cardiano	Loam	–	7.9	24.5	0.92	12.3	–	–	Accinelli al. (2004)
Citrus	Loam	0–10	5.6	15.3	1.91	12.6	36.9	–	Zhang et (2015)
	Loam	0–10	7.3	38.2	2.65	11.7	–	–	Zhang et (2015)
	Loam	0–10	4.2	18.1	4.69	10	10	–	Zhang et (2015)
	Loam	0–10	6.3	29.5	3.72	7.5	–	–	Zhang et (2015)
	Loam	0–10	5.3	36.8	3.23	11.8	–	–	Zhang et (2015)
	Loam	0–10	5.5	6.57	0.81	14.2	–	–	Zhang et (2015)
	Loam	–	7.0	39.8	1.0	–	–	15.6	Shushko et al. (20
	Clay	–	7.0	54.5	1.6	–	–	18.7	Shushko et al. (20
	Clay	0–30	7.2	46.5	4.4	110	34.9	118	Bergströ al. (2011)
	Clay	30–60	7.4	56.1	–	151	97.6	165	Bergströ al. (2011)
	Sandy	0–30	7.4	7.7	2.0	16.2	60.4	40	Bergströ al. (2011)
	Sandy	30–60	6.4	–	1.0	36.5	93.1	28.7	Bergströ al. (2011)

China riparian zone	Sandy	–	7.9	8.3	0.65	14.1	–	–	Yang et al. (2013)
Sub-urban area		0–25	6.1 ^a	4.5	–	17	–	–	Grunewald et al. (2005)
		0–38	6.3 ^a	11.6	–	11	–	–	Grunewald et al. (2005)
Canola		0–15	5.2	25	3.97	8	–	63.66	Syan et al. (2014)
Agriculture –Chalons	Loam	0–10	8.2	9.3	2.0	1	25	34.8	Mamy et al. (2005)
Agriculture –Dijon	Loam	0–10	8.2	37.7	1.65	0.8	34	41.9	Mamy et al. (2005)
Agriculture –Toulouse	Loam	0–10	7.6	23.5	0.95	3.7	75	276	Mamy et al. (2005)
Rice		–	5.7	14.1	1.3	–	–	17	Cuervo a Fuentes (2014)
Forest		–	7.7	19.3	2.3	–	–	3.1	Cuervo a Fuentes (2014)
Grassland		–	6.3	30.5	4.3	–	–	1.8	Cuervo a Fuentes (2014)

OC organic carbon, K_f adsorption coefficients

^apH reported in salt rather than water

Fate pathways of glyphosate

Once applied, glyphosate may undergo mineralisation, immobilisation or leaching,

but it does not undergo volatilisation to a significant degree because it has an extremely low vapour pressure (Mamy et al. 2005; Al-Rajab and Schiavon 2010). Glyphosate mineralisation is considered the primary degradation mechanism, resulting in the production of AMPA, methylphosphonic acid, glycine and sarcosine (Fig. 1) (Mamy et al. 2005; Kwiatkowska et al. 2014). AMPA is the main metabolite of glyphosate and is further mineralised to methylamine and phosphate, with decomposition finally producing CO₂ and NH₃ (Borggaard and Gimsing 2008; Al-Rajab and Schiavon 2010). Bergström et al. (2011) propose that the degradation pathway to produce sarcosine might also be important.

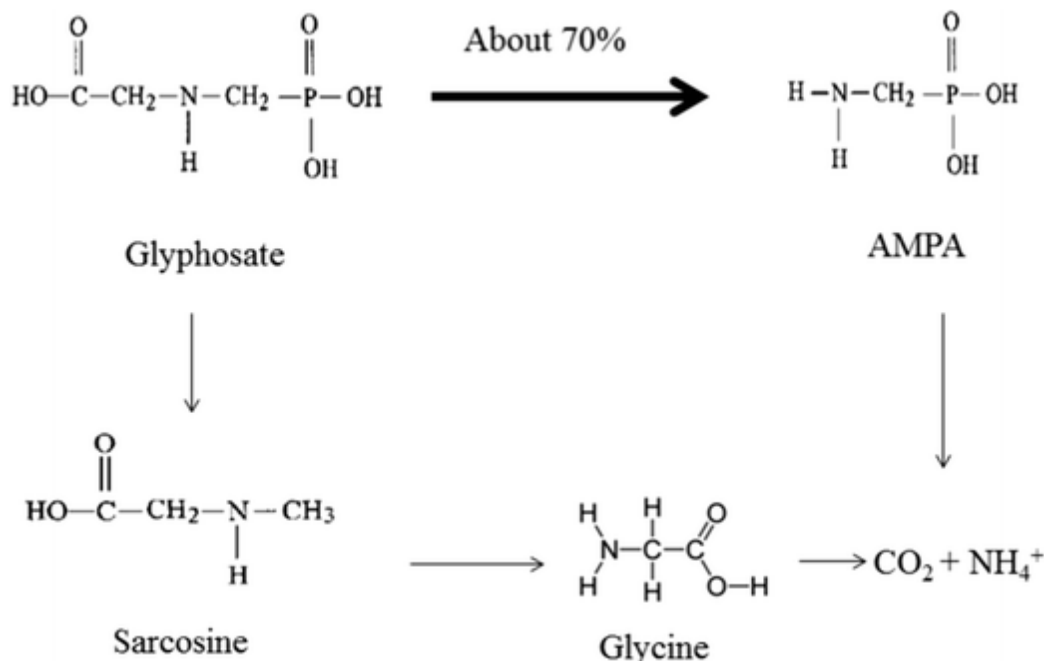


Fig. 1

The fate pathway of glyphosate (adapted from Borggaard and Gimsing (2008) and Mamy et al. (2005))

Mineralisation

Glyphosate and AMPA mineralisation are affected by soil biochemical properties and can occur within a very short period of time in certain situations (Mamy et al. 2005). Soil properties that accelerate glyphosate mineralisation include high soil pH and phosphate content or low soil Cu and Fe content, primarily driven by increased microbial mineralisation (Morillo et al. 2000; Mamy et al. 2005; Ghafoor et al. 2011; Zhang et al. 2015). Microbial activities are highly dependent on soil labile organic carbon (C) availability (Bai et al. 2012a; Bai et al. 2014). However, increased labile organic C may not always stimulate microbial activity and mineralisation because glyphosate sorption is often increased by organic matter in

soil (Mamy et al. 2005). Increased glyphosate adsorption to organic C is likely to be beneficial to the environment because it delays leaching and allows gradual release and degradation in soil. However, repeated glyphosate application may eventually lead to saturation of the organic C system. Ultimately, the combination of soil biochemical properties, microbial diversity and microbial activity drives glyphosate degradation through mineralisation.

Immobilisation and leaching

Glyphosate exhibits a high adsorption coefficient and is quickly immobilised following application in most natural situations (Bergström et al. 2011; Syan et al. 2014). Soil organic matter, clay and minerals are influential factors in glyphosate immobilisation. For example, adsorption to soil, of up to 20 % of the initial glyphosate quantity, can occur within 3 h of application, although no further immobilisation was observed in the following 3 days (Shushkova et al. 2009). High adsorption depends on low pH and phosphate concentration, high organic matter and clay content, and high Al and Fe concentrations in the soil (Gimsing et al. 2004; Laitinen et al. 2009; Shushkova et al. 2009; Syan et al. 2014). Conversely, soils with low organic matter, high phosphate, low Al and Fe and high pH are prone to glyphosate and AMPA losses, mainly due to decreased adsorption capacity and increased leaching (Laitinen et al. 2009; Shushkova et al. 2009).

Glyphosate leaching and resultant water contamination are a growing concern as both glyphosate and AMPA residues have been frequently reported in water sources (Fig. S1). For example, glyphosate and AMPA were detected in 40 and 55 %, respectively, of 3700 soil, water and sediment samples collected from 38 sites in the USA between 2001 and 2010 (Battaglin et al. 2014). All sample concentrations were under $700 \mu\text{g L}^{-1}$, which is below accepted maximum contamination levels (MCLs) (Battaglin et al. 2014) (Fig. S1). AMPA is also a degradation product of the artificial sweetener, acesulfame, and it is conceivable that this sweetener is a source of water AMPA. However, glyphosate and AMPA concentrations in water samples were strongly correlated, suggesting that AMPA was more likely to have originated from glyphosate rather than acesulfame (Van Stempvoort et al. 2014).

Ecological risks of glyphosate and AMPA residues in the environment

Both glyphosate and AMPA can be relatively persistent in the environment, which may result in a wide range of ecological risks. However, it is not easy to predict the significance and extent of those risks when there is a lack of toxicity, health and safety data on repeated and long-term exposures to glyphosate and AMPA. Thus

safety data on repeated and long-term exposures to glyphosate and AMPA. Thus, we have reviewed the potential presence of glyphosate and AMPA residues in soil, water and non-target crops, such as those that may be consumed by humans, and we discuss the potential risks for health in that context.

Glyphosate in soil

Despite glyphosate and AMPA being regarded as non-toxic to soil micro-organisms (Busse et al. [2001](#); Araújo et al. [2003](#)), studies that have investigated microbial composition and soil microbial diversity do not always support this perspective. For example, earthworms are a critical bioindicator of soil health, and earthworm biomass was reduced following glyphosate application to soils (Johnson-Maynard and Lugo-Perez [2006](#); García-Pérez et al. [2014](#)). In a coffee plantation subjected to repeated glyphosate application for 22 years, soil earthworm biomass was significantly lower than those plantations with no application in the past 7 years (García-Pérez et al. [2014](#)). However, other studies report no direct effects of glyphosate on earthworms (Pereira et al. [2009](#); Zhou et al. [2012](#); Fusilero et al. [2013](#)). For example, one study showed that earthworms may survive after glyphosate application but glyphosate may affect cocoon hatching leading to decreased earthworm populations in soil (Correia and Moreira [2010](#); Pelosi et al. [2014](#)). In another study, sub-lethal glyphosate application in a glasshouse did not affect the survival of earthworms but resulted in changed soil chemistry, which may have other implications for water quality and other soil dwellers (Santadino et al. [2014](#)). Interestingly, it has been reported that although AMPA may not increase earthworm mortality, juveniles produced in contaminated soil may have reduced body mass affecting their function in the system (Domínguez et al. [2016](#)).

The majority of studies that assess earthworm responses to glyphosate have been undertaken under laboratory conditions and responses may not necessarily be observed under field conditions. In general, the eco-toxicity of herbicides is difficult to assess under field conditions, as results will be confounded by numerous factors including levels of organic matter, nutrient application, soil type, soil cover and weather conditions (Yu and Zhou [2005](#); Fusilero et al. [2013](#)). Despite this, field studies must be undertaken such that the long-term effects of glyphosate on soil ecology can be thoroughly assessed. Furthermore, there is a need to create assessment criteria that allow laboratory data to be directly comparable to field data (Casabé et al. [2007](#); Pelosi et al. [2014](#)).

There are also conflicting reports on the impact of soil microbial diversity and biomass following glyphosate application (Kremer and Means [2009](#); Bai et al. [2012b](#); Zabaloy et al. [2012](#); Druille et al. [2013](#); Arango et al. [2014](#)). It seems clear that the effects of glyphosate on soil microbial biomass are dose-dependent, but recent evidence suggests that the effects may also be transitional (Nguyen et al. [2016](#)). However, it should be noted that microbial biomass and activity are not

indicative of microbial composition and it remains unclear as to the extent that soil microbial communities will respond to different management practises including repeated glyphosate applications (Nguyen et al. [2016](#)).

Some studies report that the observed shifts in soil community compositions due to glyphosate application altered soil nutrient availability and nutrient balance (Kremer and Means [2009](#)), which may influence plant performance (Wolmarans and Swart [2014](#)) and, ultimately, ecosystem productivity. However, many conflicting reports exist as to whether using glyphosate or glyphosate resistant species can result in nutrient imbalance (reviewed in Wolmarans and Swart [2014](#)). Glyphosate binds strongly to nutrients in the soil, which could significantly reduce nutrient availability in soil (Duke et al. [2012](#)). However, the concentrations of glyphosate added to soil even at the highest recommended doses are up to 500 times lower than concentrations of different soil nutrients. Therefore, even if all of the applied glyphosate is bound by the soil nutrients, the decrease in soil nutrient concentrations will be negligible and any impact on nutrient imbalance is unlikely to affect crop yield (Duke et al. [2012](#)).

Glyphosate in water

Detection of both glyphosate and AMPA residues in water sources is becoming frequent. Although runoff is one source of water contamination, some formulations are approved for the control of aquatic weeds (Annett et al. [2014](#)) and therefore, direct application can also result in contamination. In some cases, the reported concentrations are cause for concern. For example, glyphosate concentrations of over $400 \mu\text{g L}^{-1}$ are potentially toxic to some aquatic species including amphibians and fish (King and Wagner [2010](#); Annette et al. [2014](#); Braz-Mota et al. [2015](#)). The presence of glyphosate in marine ecosystems has also been reported and its persistence in seawater is now an area of active research (Mercurio et al. [2015](#)). However, several studies suggest that the levels of glyphosate residues in water are not capable of causing toxicity in aquatic species (Levine et al. [2015](#); Struger et al. [2008](#); Solomon and Thompson [2003](#)). Since the toxicity of glyphosate is both dose and species dependent (Annett et al. [2014](#)), there appears to be a need for additional research to assess the potential impacts of glyphosate in aquatic systems.

In terms of the risks posed to humans, the majority of the reported residue concentrations are below MCLs, and therefore, the acute toxicity risks posed to humans are minimal. The MCL for glyphosate before posing a risk to human health is considered to be $700 \mu\text{g L}^{-1}$ in the USA (EPA, [2015](#)) and $1000 \mu\text{g L}^{-1}$ in Australia (Australian Drinking Water Guidelines 6 [2011](#)). In Europe, the acceptable concentration in drinking water is less than $0.1 \mu\text{g L}^{-1}$ and the tolerable risk is reported to be $77 \mu\text{g L}^{-1}$ (Horth and Blackmore [2009](#)). Water treatment to reduce glyphosate concentrations is costly but, according to European guidelines, is

necessary to reduce the risk of glyphosate residues in human drinking water. These treatments do not impact on glyphosate levels in the source water and, therefore, the long-term effects of glyphosate on aquatic species remain a potential concern.

Glyphosate is not approved to be used in water to control weeds, and the majority of glyphosate exposures would be caused by runoff or accidental glyphosate spills. Hence, applying proper management practises may minimise eco-toxicity risks of glyphosate for aquatic species including reduced application frequencies and using vegetation buffers (Saunders and Pezeshki [2015](#)).

Glyphosate in non-target plant species

Both glyphosate and AMPA residues are found in non-target plant species (e.g. crops) following glyphosate application to weeds, even after the recommended withholding period in harvested crops (Table [S1](#)). Furthermore, both glyphosate and AMPA have also been detected in crop plants and native forest foliage following application of glyphosate to adjacent crops. The concentration of glyphosate and AMPA residues in different crop species and samples varies significantly (Table [2](#)). For example, glyphosate and AMPA residues were observed in 25 and 8.3 % of analysed cannabis samples, respectively (Lanaro et al. [2015](#)). Similarly, concentrations ranged from 1000 to 0.3 mg kg⁻¹ in tree foliage sampled within 3 days of glyphosate application (Table [2](#)). Such unusually high glyphosate residues (e.g. 1000 mg kg⁻¹) in tree foliage can be explained by direct absorption into tree leaves following drift contamination from aerial herbicide application (Newton et al. [1994](#)).

Table 2

Glyphosate and AMPA residues in different plant parts, food or feed

Matrix	Time and rate of application	Glyphosate	AMPA	Unit	Sampling time after application	References
Soybean						
Leaf and stem	1–1.7 kg ai/ha: annual application	2.1	0.6	mg kg ⁻¹	third year	Arregui et al. (2004)
Leaf: GR	6.7 kg/ha: greenhouse	37	1	µg g ⁻¹	22 days	Reddy et al. (2004)
Plant tissue GR	250 ^c g ai/ha	5826	119	ng g ⁻¹	7 days	Reddy et al. (2008)

Plant tissue non-GR	250 ^c g ai/ha	25,036	668	ng g ⁻¹	7 days	Reddy et al. (2008)
Seed	1–1.7 kg ai/ha: annual application	1.8	0.9	mg kg ⁻¹	second year	Arregui et al. (2004)
Seed	3 weeks after planting	0.18	0.6	μg g ⁻¹	After harvesting	Duke et al. (2003b)
Seed	8 weeks after planting	2.2	7.2	μg g ⁻¹	After harvesting	Duke et al. (2003b)
Whole soybeans: GR	Post plant application	3.3	5.7	mg kg ⁻¹		Bøhn et al. (2014)
Maize						
Shoot	0.8 kg ai/ha	15	ND	μg ai kg ⁻¹	56 days	Bernal et al. (2012)
Root	0.8 kg ai/ha	377	ND	μg ai kg ⁻¹	56 days	Bernal et al. (2012)
Shoot	1.6 kg ai/ha	22	ND	μg ai kg ⁻¹	56 days	Bernal et al. (2012)
Root	1.6 kg ai/ha	356	ND	μg ai kg ⁻¹	56 days	Bernal et al. (2012)
Corn						
Plant tissue GR	93 ^c g ai/ha	308	ND	ng g ⁻¹	7 days	Reddy et al. (2008)
Plant tissue non-GR	93 ^c g ai/ha	851	ND	ng g ⁻¹	7 days	Reddy et al. (2008)
Cannabis						
Leaf		0.15	ND	mg g ⁻¹	–	Lanaro et al.

						(2015)
Leaf		0.75	ND	mg g ⁻¹	–	Lanaro et al. (2015)
Leaf		0.55	0.36	mg g ⁻¹	–	Lanaro et al. (2015)
Cowpea	201 ^c g ai/ha	26,763	4765	ng g ⁻¹	–	Reddy et al. (2008)
Sickle pod	250 ^c g ai/ha	6414	1834	ng g ⁻¹	7 days	Reddy et al. (2008)
Coffee	75 ^c g ai/ha	5906	287	ng g ⁻¹	7 days	Reddy et al. (2008)
Honey ^a		17–163	–	ng g ⁻¹	–	Rubio et al. (2014)
Honey from NGM plants		26–41	–	ng g ⁻¹	–	Rubio et al. (2014)
Soy sauce ^b		88–580	–	ng mL ⁻¹	–	Rubio et al. (2014)
Cereal survey		11 % ADI				Harris and Gaston (2004)
Cattle						
Muscle	1.4/0.156; 4.0/0.48 and 12.8/1.4 mg eq/kg bw (glyphosate/AMPA)	<0.05	–	mg kg ⁻¹	–	EFSA (2015)
Milk	1.4/0.156; 4.0/0.48 and 12.8/1.4 mg eq/kg bw (glyphosate: AMPA)	<0.02	–	mg kg ⁻¹	–	EFSA (2015)

Milk	–	ND	–	–	Ehling and Reddy (2015)	
Milk	–	ND	–	–	Jensen et al. (2016)	
Kidney	100 mg/kg glyphosate and aminoglyphosate acid	1.4	–	mg kg ⁻¹	–	WHO (1994)
Kidney	1.4/0.156; 4.0/0.48 and 12.8/1.4 mg eq/kg bw (glyphosate/AMPA)	1.6	–	mg kg ⁻¹	–	EFSA (2015)
Liver	1.4/0.156; 4.0/0.48 and 12.8/1.4 mg eq/kg bw (glyphosate/AMPA)	0.07	–	mg kg ⁻¹	–	EFSA (2015)
Pig						
Muscle	1.08 mg/kg bw	<0.05	–	mg kg ⁻¹	–	EFSA (2015)
Liver and kidney	100 mg/kg glyphosate and aminoglyphosate acid	0.16 and 0.91	–	mg kg ⁻¹	–	WHO (1994)
Liver	1.08 mg/kg bw	<0.05	–	mg kg ⁻¹	–	EFSA (2015)
Kidney	1.08 mg/kg bw	0.12	–	mg kg ⁻¹	–	EFSA (2015)
Poultry muscle	0.24 and 2.2 mg/kg bw	<0.05	–	mg kg ⁻¹	–	EFSA (2015)
Eggs	0.24 and 2.2 mg/kg bw	<0.01	–	mg kg ⁻¹	–	EFSA (2015)

Meat, milk and egg	100 mg/kg glyphosate and aminoglyphosate acid	negligible	–	–	–	WHO (1994)
Composite food–maternal exposure	75 % of food detected residues	0.4 % ADI	–	–	–	McQueen et al. (2012)

GR glyphosate resistant, *ai* active ingredient, *NGM* not genetically modified, *ND* not detected, *ADI* acceptable daily intake

^{a,b}59 and 36 % of samples contained residues

^cThe concentration was based on I_{50} , the glyphosate rate required to cause a 50 % reduction in plant growth

In addition to the health risks potentially caused by food contamination, glyphosate contamination can have phytotoxic effects. Phytotoxicity can influence plant performance through reduced absorption of essential nutrients (Mateos-Naranjo and Perez-Martin [2013](#)), nutrient imbalance, yield reduction and compromised food quality (Bott et al. [2008](#); Zobiolo et al. [2010](#)). Plant biomass has been reduced up to 50 % in some non-target plant species following glyphosate contamination (Alister et al. [2005](#); Mateos-Naranjo and Perez-Martin [2013](#)). Therefore, the potential negative impacts of glyphosate contamination on non-target plant performance and productivity, especially reduced crop yield and quality, should not be dismissed (Alister et al. [2005](#); Reddy et al. [2008](#); Zobiolo et al. [2010](#)). However, other studies show no negative effects of glyphosate on plant yield (Bohm et al. [2014](#); Duke [2015](#)) and given the complicated influences of soil type, soil nutrient availability and environmental conditions, yield reduction and nutrient deficiency may not be directly caused by glyphosate (Duke et al. [2012](#)).

Toxicological effects of glyphosate and AMPA

Glyphosate and AMPA are considered low risk to mammals, primarily because of low skin and gastrointestinal absorption (Williams et al. [2000](#); Greim et al. [2015](#)).

Both glyphosate and AMPA are excreted in urine, with half-lives between 3 and 15 h without any changes in their structure (Anadon et al. [2009](#)). For these reasons, combined with a battery of acute toxicity data, glyphosate and AMPA are classified in the least toxic category (category IV; practically non-toxic and not an irritant) by the EPA (Williams et al. [2000](#)). However, given recent data regarding glyphosate contamination in the environment, acute toxicity may not be as important as chronic, sub-chronic and reproductive toxicity, which occur at lower concentrations. Drawing upon several case studies, it has been concluded that there is no robust evidence of cytotoxicity, genotoxicity, DNA damage, carcinogenicity or reproductive toxicity from glyphosate and AMPA (Williams et al. [2000](#); Greim et al. [2015](#); EFSA [2015](#)). However, much of the data referenced by those authors is relatively old and/or from unpublished data, and in this review, we summarise additional literature, which builds upon previous conclusions.

Acute poisoning

Worst case exposure causing acute poisoning in adult humans has been reported to be 125 and 5 $\mu\text{g kg}^{-1} \text{day}^{-1}$ for glyphosate and AMPA, respectively (Williams et al. [2000](#)). Fatalities caused by exposures of that order have occurred in 3.2 % of cases, with a median time to death of 20 h, mainly due to cardiorespiratory toxicity (Roberts et al. [2010](#)). Rat oral and dermal LD_{50} are reported to be much higher at $>5000 \text{ mg kg}^{-1} \text{bw}$, although there is also a lower LD_{50} value reported ($>2000 \text{ mg kg}^{-1} \text{bw}$) (Greim et al. [2015](#)). Importantly, the reported values may differ as a result of using different formulations of glyphosate. Most commercial formulations of glyphosate contain surfactants to facilitate penetration of the active ingredient and increase efficacy. As a result, recent research tends to focus on the toxicity of the formulation rather than the active ingredient (Currie et al. [2015](#)). For example, neat glyphosate had the least toxicity ($\sim 2 \text{ g L}^{-1}$) in vitro, whereas Roundup® 400 and 450 had the highest toxicity ($\sim 0.001 \text{ g L}^{-1}$) (Gasnier et al. [2009](#)). In another study, however, it was found that glyphosate itself, rather than the surfactants in the formulation, affected mechanisms of morphogenesis in vertebrate embryos (Paganelli et al. [2010](#)). It is therefore important that both commercial formulations and neat glyphosate are used to estimate acute toxicity parameters.

Chronic and sub-chronic toxicity

The no-observed-adverse-effect-level (NOAEL) for chronic toxicity is recommended to be $560 \text{ mg kg}^{-1} \text{bw day}^{-1}$ (Greim et al. [2015](#)) (Table 3). Williams et al. ([2000](#)) concluded that rodents can even tolerate a daily glyphosate uptake of $20,000 \text{ mg kg}^{-1} \text{bw day}^{-1}$. However, as summarised by Cox ([1995](#)), daily consumption of glyphosate between 60 and 2500 mg kg^{-1} for 90 days in rats and mice resulted in liver damage, increased bile acids, chronic kidney inflammation,

decreased body weight and increased potassium and phosphorous in the blood. In recent studies, there are reports of even lower rates causing irreversible damage to mammals (Table 4). For example, rats that were exposed to glyphosate at rates between 5 and 490 mg kg⁻¹ every 48 h for 75 days had irreversible damage to hepatocytes (Benedetti et al. 2004). In a separate study, mild liver damage was reported in rats following sub-chronic exposure of glyphosate (56 and 560 mg kg⁻¹) for between 35 and 90 days (Çağlar and Kolankaya 2008). Some studies show that even one exposure of glyphosate at very low concentrations is sufficient to change cell functions and cause cytotoxicity (Table 4). For example, sub-agricultural doses of both glyphosate and Roundup®400 caused disruption of the human endocrine system at 0.5 ppm, inhibition of transcriptional activities of oestrogen receptors at 2 ppm, and cytotoxicity at 10 ppm in vitro (Gasnier et al. 2009).

Table 3

No-observed-adverse-effect-level (NOAEL), adapted from Williams et al. (2000) and Greim et al. (2015)

Host	Duration of exposure	Active agent	NOAEL (mg/kg/day)	NOAEL (ppm)	References
Sub-chronic toxicity					
Mouse	90 days	Glyphosate	2310	10,000	Tierney (1979)
Mouse	90 days	Glyphosate	630	–	Chan and Mahler (1992)
Rat	90 days	Glyphosate	>1450	20,000	Stout and Johnson (1987)
Rat	90 days	Glyphosate	209	3125	Chan and Mahler (1992)
Rat	90 days	AMPA	400	–	Estes (1979)
Dog	90 days	AMPA	263	–	Tompkins (1991)

Chronic toxicity					
Dog	12 months	Glyphosate	≥500	–	Reyna and Ruecker (1985)
Mouse	24 months	Glyphosate	885	–	Knezevich (1983)
Rat	26 months	Glyphosate	≥33	–	Lankas (1981)
Rat	24 months	Glyphosate	409	–	Stout and Ruecker (1990)
Rat	12 months	Glyphosate	560	8000	Greim et al. (2015)
Dog	12 months	Glyphosate	500	–	Greim et al. (2015)
Developmental toxicity					
Rat	–	Glyphosate	1000	–	Tasker (1980a)
Rabbit	–	Glyphosate	175	–	Tasker (1980b)
Rat	–	AMPA	400	–	Holson (1991)
Rat	–	Glyphosate	300	–	Greim et al. (2015)
Rabbit	–	Glyphosate	50	–	Greim et al. (2015)
Reproductive toxicity					
Rat	–	Glyphosate	≥30	–	Schroeder (1981)

Rat	–	Glyphosate	694	–	Reyna (1990)
Rat	–	AMPA	>4.2	–	Reyna (1990)
Rat	–	Glyphosate	300	–	Greim et al. (2015)
Genotoxicity (results summarised from Greim et al. (2015))					
Rat	26 months	Glyphosate	31 ♂ / 34 ♀	≥300	Monsanto (1981)
Rat	24 months	Glyphosate	940 ♂ / 1183 ♀	8000	Monsanto (1990)
Rat	24 months	Glyphosate	595 ♂ / 886 ♀	10,000	Feinchemie Schwebda (1996)
Rat	24 months	Glyphosate	104 ♂ / 115 ♀	3000	Arysta Life Sciences (1997)
Rat	24 months	Glyphosate	3614 ♂ / 437 ♀	6000	Syngenta (2001)
Rat	24 months	Glyphosate	17 ♂ / 19 ♀	–	Chruscielska et al. (2000)
Mouse	24 months	Glyphosate	157 ♂ / 190 ♀	1000	Monsanto (1983)
Mouse	24 months	Glyphosate	≥1000	–	Cheminova (1993)
Mouse	18 months	Glyphosate	838 ♂ / 153 ♀	8000 ♂ / 1600 ♀	Arysta Life Sciences (1997)
Mouse	18 months	Glyphosate	150 ♂ / 151 ♀	1000	Feinchemie Schwebda

MOUSE					SCHEWENIA (2001)
18 months Mouse	Glyphosate	810 ♂ / 1081 ♀	≥5000		Nufarm (2009)

Table 4
Examples of disorders observed following glyphosate exposure

Exposed compounds	Duration of exposure	Concentrations	Observed disorders	Host	References
Damages caused by one exposure or short incubations					
Glyphosate	–	>0.25 mM	Negligible hemolysis and haemoglobin oxidation	In vivo human	Kwiatkowska et al. (2014)
Glyphosate	–	>0.5 ppm	Human cell endocrine disruption	In vivo human liver	Gasnier et al. (2009)
	–	>2 ppm	Transcriptional activities disruption on both oestrogen receptors, inhibition on HepG2	In vivo human liver	Gasnier et al. (2009)
	–	>10 ppm	Aromatase transcription and activity disruption	In vivo human liver	Gasnier et al. (2009)
	–	>10 ppm	Cytotoxicity	In vivo human liver	Gasnier et al. (2009)
Glyphosate	–	>10 ppm	Suppression	Kidney of	Ma et al. (2015)

Glyphosate	–	52.08 or 104.15 mg L ⁻¹	Suppressing the expressions of IgM, C3, and LYZ	Kidney of common carp (<i>Cyprinus carpio</i> L.)	Ma et al. (2015)
			Damaging the fish kidney		
Glyphosate	–	Minimal inhibition varied between 75 and 5000 ppm for different strains	Disruption of gut bacterial community	Gut bacterial strains	Shehata et al. (2013)
Chronic and sub-chronic toxicity					
Glyphosate- biocarb®	75 days with 48 h intervals	5–490 mg kg ⁻¹	Irreversible damages to hepatocytes	Rat	Benedetti et al. (2004)
Roundup®	35 and 90 days	56 and 560 mg kg ⁻¹ (p.o.)	Mild liver damage	Rat	Çağlar and Kolankaya (2008)
	30 or 90 days	3–20-fold lower doses of glyphosate than the accepted oral reference dose of 2 mg kg ⁻¹ day ⁻¹	Induce oxidative stress	Rat	Çağlar and Kolankaya (2008)
Genotoxicity					
Glyphosate and Roundup®	–	500 µg egg ⁻¹	DNA damage	<i>Caiman latirostris</i>	Poletta et al., 2009
Glyphosate	–	>5 ppm	DNA damage	In vivo human liver	Gasnier et al. (2009)
Glyphosate	–	25 and 50 mg kg	Increased	Swiss mice	Prasad et al.

		⁻¹ .b.wt.	chromosomal aberrations (CAs) and micronuclei (MN) in bone marrow cells		(2009)
Reproductive toxicity					
Glyphosate	Pregnant mother exposed for 21–23 days	50–450 mg kg ⁻¹	Male offspring: abnormal sperm, decreased serum testosterone, sperm degeneration	Rat	Dallegrave et al. (2007)
			One female offspring: delay of vaginal canal-opening		
Glyphosate	–	1/5000 dilutions of commercial rates	Impairing retinoic acid signalling	Vertebrates	Paganelli et al. (2010)
Glyphosate	–	50 mg kg ⁻¹ , NOAEL for reproductive toxicity	Male offspring: disruption of the masculinization process and promoted behavioural changes and histological and endocrine problems in productive parameters	Rat	
Carcinogenicity					

Glyphosate	–	10^{-12} to 10^{-6} M	Affecting on human hormone-dependent breast cancer, T47D cells, in oestrogen withdrawal condition	Human	Thongprakaisang et al. (2013)
Glyphosate +7, 12-dimethylbenz [a] anthracene (DMBA)		25 mg kg ⁻¹ b.wt thrice per week	Tumour promotion in DMBA-initiated mouse skin cells	Mouse	George et al. (2010)

Genotoxicity

Genotoxicity caused by glyphosate has regularly been questioned and often rejected (Williams et al. 2000; Greim et al. 2015). The main reason for this conclusion was that the majority of previous studies reporting DNA damage used unreasonably high doses of glyphosate. However, other studies that used sub-agricultural doses of both glyphosate and Roundup® have observed DNA damage to human cells (Gasnier et al. 2009; Prasad et al. 2009; Koller et al. 2012). DNA damage was reported when buccal epithelial cells were exposed to glyphosate and Roundup® at concentrations between 10 and 20 mg L⁻¹ or between 225- and 1350-fold lower than recommended agricultural rates (Koller et al. 2012) and, in a separate study, following application at 5 ppm to human liver HepG2 cells (Gasnier et al. 2009). Exposure of caiman embryos to Roundup® at different sub-lethal rates also resulted in DNA damage (Poletta et al. 2009). Even considering this data, Kier and Kirkland (2013) concluded that glyphosate was not genotoxic, suggesting that the observed DNA damage was due to cytotoxicity rather than genotoxicity. Irrespective of the cause being direct or indirect, recent evidence indicates that DNA damage may occur at relatively low concentrations of glyphosate.

Reproductive toxicity

The potential for glyphosate to cause reproductive toxicity has been reported to be 'very slim' (Williams et al. 2000), with a NOAEL between 300 mg kg⁻¹ bw day⁻¹ (Greim et al. 2015) and 50 mg kg⁻¹ bw day⁻¹ (Tu, 1995). Other studies suggest that

(Strom et al. 2012) and 50 mg kg⁻¹ per day (Lu 1995). Other studies suggest that glyphosate exposure even at NOAEL concentrations may cause adverse effects on the reproductive function of offspring (Dallegrave et al. 2007; Romano et al. 2012). Rats exposed to Roundup® at rates between 50 mg kg⁻¹ (recommended NOAEL by Lu (1995)) and 450 mg kg⁻¹ for 21 days during pregnancy showed no adverse effects but, interestingly, male offspring had damage to their reproductive organs (Dallegrave et al. 2007). Similarly, treatment of female Wistar rats (50 mg kg⁻¹) caused reproductive toxicity in male offspring (Romano et al. 2012 with changes observed to male offspring behaviour and reproductive parameters; the result of hyper-secretion of androgens and increased gonadal activity (Romano et al. 2012). Given these findings, it seems that additional research is needed to improve our understanding of the effects of glyphosate and Roundup® on mammalian reproduction (Dallegrave et al. 2007).

Carcinogenicity

Whether glyphosate is carcinogenic or not, it is heavily debated in the literature. Some authors argue that given glyphosate genotoxicity has been rejected, carcinogenicity caused by mutations is not possible (Williams et al. 2000; Kier and Kirkland 2013). Furthermore, there are studies that indicate glyphosate is not carcinogenic when exposure is within acceptable NOAEL (Table 3). For example, carcinogenicity was not observed when rats drank water containing glyphosate at rates within NOAEL for 2 years (Chruscielska et al. 2000). Although the reliability of this study has been questioned, in general, data rejecting glyphosate induced carcinogenicity has been evaluated as reliable and Greim et al. (2015) concluded that there was no statistically significant relationship between carcinogenicity and glyphosate exposure. However, those authors acknowledged that further research was needed before the carcinogenic potential of glyphosate can be completely excluded. In contrast, there is a body of research that reports potential carcinogenic cases in mouse skin, breast, kidney, intestine, liver and thyroid tissues (Cox 1995; George et al. 2010). Furthermore, the possibility of glyphosate causing tumour promotion in skin cells and proliferation in breast cells has been reported in vivo mouse and in vitro human models, respectively (George et al. 2010; Thongprakaisang et al. 2013). In one of these studies, hormone induced breast cancer was stimulated at glyphosate concentrations as low as 10⁻¹² M (Thongprakaisang et al. 2013), 600-fold lower than the acceptable European glyphosate residue concentration in drinking water (Fig. S1). More recently, the International Agency for Research on Cancer (IARC) classified glyphosate as 'probably carcinogenic to humans' (group 2A) (Guyton et al. 2015). To arrive at this conclusion, the IARC Working Group considered previous findings from the US EPA, recent published scientific literature and publically available government reports. In contrast, the European Food Safety Authority (EFSA) (2015) reported that glyphosate is unlikely to be carcinogenic. EFSA believes that IARC has not considered all of the relevant literature and is open to further clarify their

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assessment to address all concerns raised by other parties

(<http://www.efsa.europa.eu/en/press/news/160113>

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Possible risks of glyphosate to human health via food contamination

Glyphosate and AMPA residues in food consumed by humans are of potential toxicological concern if the residues are above acceptable daily intake levels (FAO 2005). Traces of glyphosate and AMPA have been observed in both plant and animal material suggesting that residues do exist in different food sources (Reddy et al. 2004; Druart et al. 2011; Bernal et al. 2012) (Table 2). Maximum residue limits (MRL) of glyphosate have been reviewed by the European Food Safety Authority in 2015 and generally range from 0.025 to 2 mg kg⁻¹ in different food sources (EFSA 2015). However, a MRL up to 30 mg kg⁻¹ was proposed for some cereals including rye, wheat, oat and barley (EFSA 2015). Surprisingly, no MRL has been established for fish tissue consumed by humans (McQueen et al. 2012), most likely due to the fact that glyphosate is not applied directly to water, it is not lipophilic (Glyphosate–Renewal Assessment Report 2013) and also there are no legal testing requirements for bioconcentration of glyphosate in fish. However, one study was reported for different aquatic species achieving a maximum bioconcentration factor (BCF) of 10 (Glyphosate–Renewal Assessment Report 2013), which is below the BCF trigger value of 1000 provided in Annex VI of the Stockholm Convention on Persistent Organic Pollutants (<http://chm.pops.int/Default.aspx?tabid=2806> (<http://chm.pops.int/Default.aspx?tabid=2806>)). Based on this data, bioaccumulation of glyphosate was assessed as unlikely (Glyphosate–Renewal Assessment Report 2013). Unfortunately, the details of this study were not provided, including the species studied and therefore, it is difficult to interpret the applicability of this assessment. Considering these MRLs, the theoretical maximum daily intake (TMDI) for glyphosate of 23.8 µg kg⁻¹ day⁻¹ for adults as suggested by Williams et al. (2000) and the glyphosate residues reported in various studies (Table 2), the likelihood of a human experiencing toxic side effects following long-term consumption of contaminated food ranges from possible to improbable. For example, food consumed by approximately 40 expecting mothers was assessed for glyphosate residues and despite the fact that glyphosate residues were detected in 75 % of the food items studied, the total concentration was less than 0.4 % of acceptable daily intake (McQueen et al. 2012). Williams et al. (2000) argued that, in reality, less than 50 % of harvested crop samples will contain high residue levels, and these levels would be further decreased by food processing. In support of this, food composite residue assessments have shown lower glyphosate residues than

Food composite residue assessments have shown lower glyphosate residues than expected accepted daily intakes (Gimou et al. [2008](#); McQueen et al. [2012](#)). Therefore, glyphosate residues in food would decrease below the predicted TMDI, thus posing no risk for humans (Williams et al. [2000](#)). However, more recent studies demonstrate that food or feed produced from genetically modified glyphosate resistant (GR) crops contain significantly higher residue concentrations compared with non-GR crops, likely because of different application practises between crops (Bøhn et al. [2014](#); Swanson et al. [2014](#)). These changes in application practises also increase the chance of drift and contamination of other crops and therefore, become a substantial source of concern as GR-crops become more widely used.

It is important to note that MRLs are typically determined based on the sensitivity of relevant analytical methods rather than on toxicology or eco-toxicity data. Glyphosate falls into this category with Limit of Quantitation (LoQ) values between 0.01 and 0.05 mg kg⁻¹ for validated analytical methods, which vary in different tissues (Glyphosate–Renewal Assessment Report [2013](#)). Thus, available MRLs may not necessarily suggest a safe level of a pesticide residue in or on food or feed.

Harvested crops and/or food composites may contain glyphosate and AMPA residues at levels that are unlikely to result in exposure to the currently accepted TMDI through direct consumption. However, glyphosate and AMPA residues are clearly present in food that can be consumed by humans or livestock (Table 2), and chronic exposure to glyphosate or AMPA through consumption of contaminated products may be a potential risk to human health. Recent research even suggests that there has been an increase in glyphosate in human urine samples (an indication of dietary exposure). This could be explained by the improved sensitivity of analytical techniques, but is also potentially a result of increased glyphosate usage (Niemann et al. [2015](#)). Those authors suggested that the reported concentrations are not sufficient to be of concern to human health (Niemann et al. [2015](#)). However, it is important to note that the literature on the risks of low concentration chronic exposure to glyphosate is minimal; Mesnage et al. ([2015](#)) suggest that low glyphosate concentrations may result in risks to human health and call for further studies to be undertaken before conclusions regarding the safety of glyphosate are made. It would therefore seem prudent to modify glyphosate application practises such that residues in the food are minimised, and as a matter of priority to undertake additional testing to better understand the risks.

Conclusion

Glyphosate is the most widely used herbicide in the world and its demand continues to grow. Although, the majority of glyphosate is mineralised following

application, the half-lives of glyphosate and its metabolites are long under certain conditions, and glyphosate and AMPA residues can persist in soil, water and plants in some circumstances. In fact, recent research suggests that contamination of soil, water and some food occurs at concentrations that may pose ecological risks.

However, the majority of literature concludes that the levels of contamination do not pose a risk to most organisms and are unlikely to cause an environmental risk, if recommended application rates are followed and repeat applications are avoided.

In 2015, the EFSA reported that glyphosate and its major metabolite, AMPA, may be present in food consumed by humans. Whilst it is unlikely that human exposure will reach TMDI levels through consumption of contaminated crops or other food, this review showed not only that it is possible, but also that chronic glyphosate exposure at low concentrations can potentially result in risks to human health.

However, this review also revealed a striking dearth of glyphosate and AMPA food residues analysis in the peer-reviewed literature, including a complete absence of data for any species of fish. More recently, and despite conflicting reports in the literature, the carcinogenic classification of glyphosate was changed to 'probably carcinogenic to humans' by the IARC, a classification based on limited evidence of carcinogenicity on human studies but sufficient evidence from animal research (Guyton et al. 2015). However, this classification was rejected by European Food Safety authority (EFSA 2015). Taken together, completion of additional studies that analyse glyphosate and AMPA residues in food and that explore the potential of chronic glyphosate toxicity seems prudent.

Glyphosate is a valuable and important weed management tool for agricultural professionals and hobby gardeners alike. However, in the light of recent research, there is a need to identify the most sensitive environmental and toxicological scenarios to inform future best practice management for glyphosate use such that it can remain effective, whilst ensuring minimal environmental contamination and no impact on human health.

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Supplementary material

[11356_2016_7425_MOESM1_ESM.docx \(227 kb\)](#)

Fig S1(DOCX 227 kb)

[11356_2016_7425_MOESM2_ESM.docx](#) (15 kb)

Table S1(DOCX 15 kb)

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