Indirect effects of glyphosate on plant, animal and human health through its effects on microbial communities

Supplement

van Bruggen, A.H.C.[1], Finckh, M.R.[2], He, M.[3], Ritsema, C.J.[4], Harkes, P.[4], Knuth, D.[4], and Geissen, V.[4]

[1] Emerging Pathogens Institute and Department of Plant Pathology, University of Florida, Gainesville, FL 32610, USA

[2] Faculty of Organic Agricultural Sciences, Ecological Plant Protection, University of Kassel, 37213 Witzenhausen, Germany

[3] Department of Life and Environment Science, Hangzhou Normal University, Zhejiang, 310036, China

[4] Department of Environmental Sciences, Soil Physics and Land Management Group, Wageningen University, 6708 PB Wageningen, Netherlands

Table S1. Glyphosate concentrations found in drinking water, plant and animal products and the corresponding maximum residue limits. Concentrations of degradation products (mainly AMPA) are included on a glyphosate equivalent basis for GM soybean, maize and rapeseed as well as eggs and meat products (EPA, 2020), whenever those data were available.

Drinking water, Plant or Animal Products	**Concentrations found (mg/kg)**	**References**	**Maximum Residue Limits, MRLs (mg/kg)[a]**	**References**
Drinking water	ND – 0.039[b]	(Mas *et al*., 2020; Minnesota Department of Health, 2017; Parvez *et al*., 2018; Rendón-von Osten and Dzul-Caamal. 2017; Sjerps *et al*., 2017)	0.0001 - 1.0	(Dolan *et al*., 2013; EPA, 2000; Health Canada, 2020; Marques *et al*., 2021; NHMRC, 2017)
Fruits	0.0013 - 0.66[c]	(EFSA, 2019; FAO, 2005; Kolakowski *et al*., 2020; Stephenson and Harris, 2016)	0.05 - 0.5	(Codex Alimentarius, 2013; EC, 2020; EFSA, 2015; EPA, 2020; MOHW, 2020)
Vegetables	0.0002 - 0.665[d]	(EFSA, 2019; John and Liu, 2018; Kolakowski *et al*., 2020)	0.05 - 5.0	(EFSA, 2015; EPA, 2020; MOHW, 2020; Zoller *et al*., 2018)
Legumes (including soybeans)	<0.001 – 100[e]	(Arregui *et al*., 2004; Bøhn *et al*., 2014; Çetin *et al*., 2017; Cuhra, 2015; Duke *et al*., 2003; EFSA, 2019; FAO, 2005; FDA, 2017; Kolakowski *et al*., 2020; Stephenson and Harris, 2016; Testbiotech, 2013; Zoller *et al*., 2018)	0.5 – 40[f]	(Bøhn and Millstone, 2019; Codex Alimentarius, 2013; EC, 2020; EPA, 2020; MOHW, 2020)
Cereal grains and rice (not including maize)	<0.001 – 21.4[g]	(EFSA, 2019; FAO, 2005; Çetin *et al*., 2017; Cruz and Murray, 2021; Kolakowski *et al*., 2020; Stephenson and Harris, 2016; Zoller *et al*., 2018)	0.1 – 30[g]	(Codex Alimentarius, 2013; EFSA, 2015; EPA, 2020; MOHW, 2020)
Oil seeds and nuts	<0.05 – 85[h]	(Cessna *et al*., 2000; EFSA, 2019; FAO, 2005);	1.0 – 40	(Codex Alimentarius, 2013; EPA, 2020; MOHW, 2020)
Herbs and spices	0.002 - 2.6	(FAO, 2005; Kolakowski *et al*., 2020)	0.1 – 200[i]	(EC, 2020; EPA, 2020)
Plants for drugs and beverages	0.05 – 0.58	(FAO, 2005; John and Liu, 2018)	0.05 – 1.0	(EFSA, 2019; EPA, 2020; MOHW, 2020)

Sugar crops	0.07 - 3.4	(FAO, 2005; FAO and WHO, 2016)	2.0 – 15[j]	(Codex Alimentarius, 2013; EFSA, 2019; EPA, 2020; MOHW, 2020)
Farm animal feed	0.2 – 20[k]	(Gerlach *et al*., 2014; Jarrell *et al*., 2020; Krüger *et al*., 2014b; Shehata *et al*., 2014; von Soosten *et al*., 2016)	5 – 400[l]	(Codex Alimentarius, 2013; EPA, 2020)
Fodder and forage	1.0 – 1099[m]	(EFSA, 2019; FAO. 2005)	50 – 530[n]	(Codex Alimentarius, 2013; EPA, 2020)
Pet food	0.008 – 2.14	(Zhao *et al*., 2018)		
Eggs	ND – 0.76[0]	(FAO, 2005; FAO and WHO, 2016; FDA, 2017; Ruuskanen *et al*., 2020b; Ruuskanen *et al*., 2020c; van Eenennaam and Young, 2017; Zoller *et al*., 2018;)	0.05	(Codex Alimentarius, 2013; EC, 2020; EFSA, 2019)
Cow milk	ND – 0.0005[p]	(FDA, 2017; John and Liu, 2018; Schnabel *et al*., 2017; van Eenennaam and Young, 2017; von Soosten *et al*., 2016)	0.05	(Codex Alimentarius, 2013; EC, 2020)
Human breast milk	ND – 0.166[q]	(Bus, 2015; Ehling and Reddy, 2015; Honeycutt and Rowlands, 2014; Steinborn *et al*., 2016);		
Meat	0.0005 –0.2	(EFSA, 2019; FAO, 2005; John and Liu, 2018; Stephenson and Harris, 2016; Zoller *et al*., 2018)	0.05 – 0.1	(Codex Alimentarius, 2013; EFSA, 2019)
Edible offal (meat by products)	0.001 – 16[r]	(EFSA, 2019; FAO, 2005; FAO and WHO, 2016; Fu *et al*., 2020; Stephenson and Harris, 2016; van Eenennaam and Young, 2017)	0.5 – 5.0	(Codex Alimentarius, 2013; EFSA, 2019; EPA, 2020)

[a] Depending on the product and country
[b] Highest reported concentration in drinking water in Minnesota (Minnesota Department of Health, 2017)
[c] Highest concentration of glyphosate (AMPA not measured) in olives (FAO, 2005)
[d] Highest observed residues in potatoes (EFSA, 2019).

[e] Highest observed concentrations found in GR soybeans in Argentina (Testbiotech, 2013)
[f] Highest MRLs for soybeans (20 mg/kg in the EU, 40 mg/kg in the USA) (Bøhn and Millstone, 2019; Fu *et al*., 2020)
[g] Highest observed concentrations and MRLs in barley and wheat (FAO, 2005; Kolakowski *et al*., 2020)
[h] Highest observed concentrations in canola, applied early before maturity (Cessna *et al*., 2000)
[i] Highest MRLs for peppermint and spearmint (EPA, 2020)
[j] Highest MRL for sugar beetroots (EFSA, 2019); MRL for sugarcane molasses = 30 mg/kg (EPA, 2020).
[k] Highest observed concentration on soy meal (Jarrell *et al*., 2020).
[l] Highest MRL for animal feed, non-grass (EPA, 2020)
[m] Highest observed concentration in grass pasture (FAO, 2005)
[n] Highest MRL for alfalfa grass and pea fodder (Codex Alimentarius, 2013)
[o] Highest observed concentration in experimental quail hens (Ruuskanen *et al*., 2020c)
[p] No glyphosate found in most publications on glyphosate residues in in milk (Schnabel *et al.* 2017)
[q] Glyphosate residues in breast milk were documented in a report of Moms Across America (Honeycutt and Rowlands, 2014) but disputed by Bus (2015) and not found by (Ehling and Reddy, 2015) or (Steinborn *et al*., 2016)
[r] Highest observed concentrations in pig livers (Fu *et al*., 2020). Edible offal consists mainly of organs such as kidneys, liver and intestines, often included in sausages.

Table S2. Minimal inhibitory concentrations (MICs) of glyphosate acid or glyphosate acid in glyphosate-based herbicide (Roundup, Glyfonova 450Plus, or Jablo™ Glyfosat) for bacteria *in vitro*. Glyphosate field application rate commonly is 4.5 or 5 mg/ml.

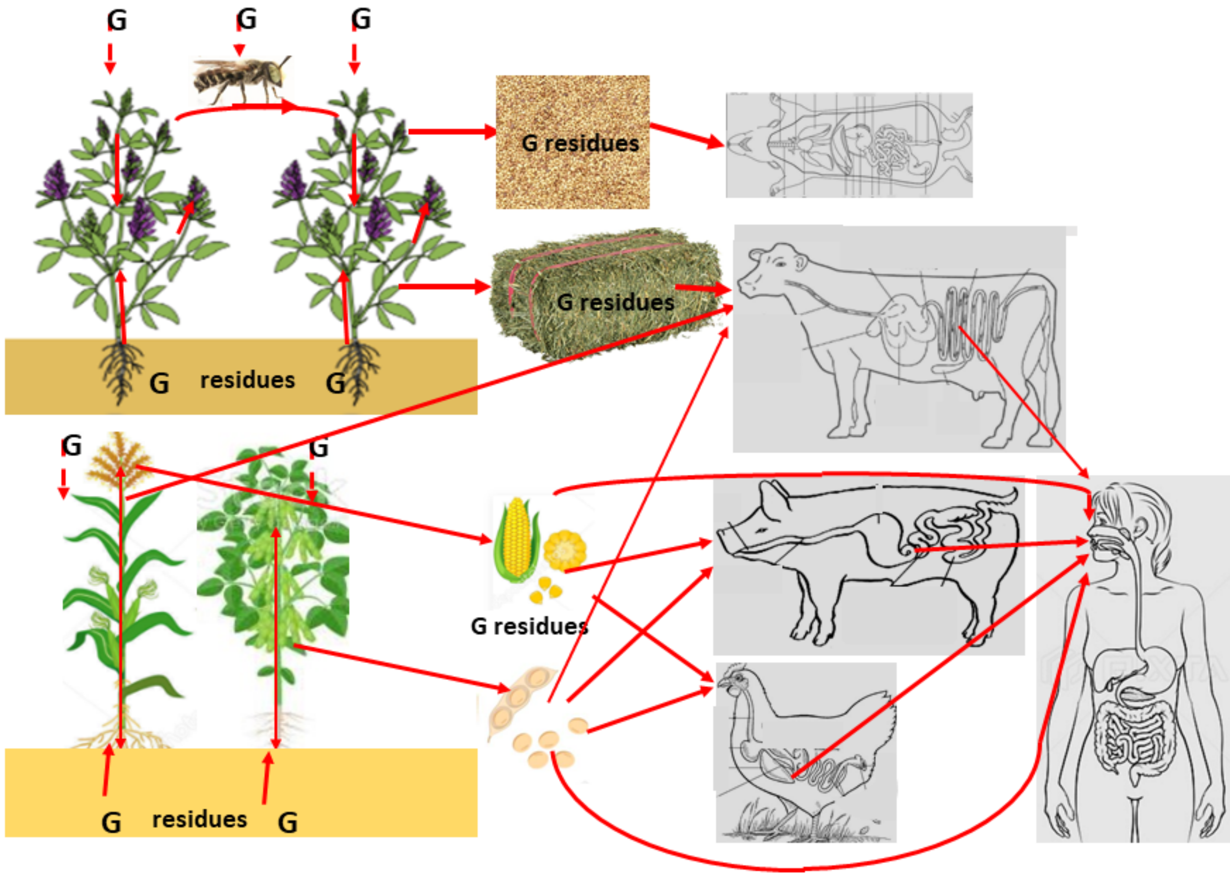
Glyphosate acid

Genus	**species**	**Niche**	**Pathogen**	**Incubation conditions**	**Turbido-meter**	**MIC (mg/ml)**	**Reference**
Escherichia	*coli* (90 strains)	human, pig, poultry or cattle	no	aerobic	no	5-20	Bote *et al*., 2019a
Escherichia	*coli* (83 strains)	pig, poultry or cattle	yes	aerobic	no	5-40	Bote *et al*., 2019a
Salmonella	*enterica* Typhimurium	pig	yes	aerobic	no	20-80	Pöppe *et al*., 2019
Salmonella	*enterica* Typhimurium	poultry	yes	aerobic	no	10-80	Pöppe *et al*., 2019
Salmonella	*enterica* Enteritidis/Infantis	pig	yes	aerobic	no	20-80	Pöppe *et al*., 2019
Salmonella	*enterica* Enteritidis/Infantis	poultry	yes	aerobic	no	10-80	Pöppe *et al*., 2019
Vibrio	*fischeri*	seawater	no	semi-aerobic	no	16-20	Tsui and Chu, 2003

Glyphosate-based herbicide

Genus	**species**	**Niche**	**Pathogen**	**Incubation conditions**		**MIC (mg/ml)**	**Reference**
Escherichia	*coli* (90 strains)	human, pig, poultry or cattle	no	aerobic	no	20-80	Bote *et al*., 2019a
Escherichia	*coli* (83 strains)	pig, poultry or cattle	yes	aerobic	no	20-80	Bote *et al*., 2019a
Escherichia	*coli*	cow	yes	anaerobic	no	40	Bote *et al*., 2019b
Salmonella	*enterica* Typhimurium	pig	yes	anaerobic	no	80	Bote *et al*., 2019b
Geotrichum	*candidum*	cheese	no	aerobic	yes	0.1-0.6	Clair *et al*., 2012b
Lactococcus	*lactis* subsp. *Cremoris*	cheese	no	aerobic	yes	0.3	Clair *et al*., 2012b
Lactobacillus	*delbrueckii* subsp. *bulgaricus*	yoghurt	no	aerobic	yes	1	Clair *et al*., 2012b
Escherichia	*coli*	human	no	aerobic	yes	7.4	Kurenbach *et al*., 2015
Salmonella	*enterica* Typhimurium	human	yes	aerobic	yes	6.2	Kurenbach *et al*., 2015
Bifidobacterium	6 species	human	no	anaerobic	no	10-20	Nielsen *et al*., 2018
Clostridium	3 species	human	yes	anaerobic	no	10-20	Nielsen *et al*., 2018
Enterococcus	*faecalis (2x)*	human	yes	anaerobic	no	40-80	Nielsen *et al*., 2018
Lactobacillus	*johnsonii*	human	no	anaerobic	no	20	Nielsen *et al*., 2018

Lactobacillus	3 species	human	no	anaerobic	no	40	Nielsen *et al*., 2018
Bacterioides	3 species	human	no	anaerobic	no	5-20	Nielsen *et al*., 2018
Bacterioides	*fragiles*	human	no	anaerobic	no	5-40	Nielsen *et al*., 2018
Escherichia	*coli (2x)*	human	no	anaerobic	no	20-80	Nielsen *et al*., 2018
Akkermansia	*muciniphila*	human	no	anaerobic	no	20	Nielsen *et al*., 2018
Salmonella	*enterica* Typhimurium	pig or chicken	yes	aerobic	no	20-80	Pöppe *et al*., 2019
Salmonella	*enterica* Enteritidis/Infantis	pig or chicken	yes	aerobic	no	20-80	Pöppe *et al*., 2019
Bacillus	*badius*	algae	no	aerobic	yes	0.3	Shehata *et al*., 2014
Bifidobacterium	*adolescentis*	chicken	no	aerobic	yes	0.15	Shehata *et al*., 2014
Enterococcus	*faecalis*	chicken	no	aerobic	yes	0.3	Shehata *et al*., 2014
Bacillus	*badius*	green algae	no	aerobic	yes	0.15	Shehata *et al*., 2013
Bacillus	*cereus*	green algae	no	aerobic	yes	0.3	Shehata *et al*., 2013
Bacteriodes	*vulgatus*	chicken	no	aerobic	yes	0.6	Shehata *et al*., 2013
Bifidobacterium	*adolescentis*	chicken	no	aerobic	yes	0.075	Shehata *et al*., 2013
Campylobacter	*coli*	chicken	yes	aerobic	yes	0.15	Shehata *et al*., 2013
Campylobacter	*jejuni*	chicken	yes	aerobic	yes	0.15	Shehata *et al*., 2013
Clostridium	*perfringens*	soil	yes	aerobic	yes	5	Shehata *et al*., 2013
Clostridium	*botulinum* A or B	canned food	yes	aerobic	yes	1.2	Shehata *et al*., 2013
Escherichia	*coli*	chicken	yes	aerobic	yes	1.2	Shehata *et al*., 2013
Escherichia	*coli* (1917 Nissle)	human	yes	aerobic	yes	1.2	Shehata *et al*., 2013
Enterococcus	*faecalis*	green algae	no	aerobic	yes	0.15	Shehata *et al*., 2013
Enterococcus	*faecium*	chicken	no	aerobic	yes	0.15	Shehata *et al*., 2013
Lactobacillus	*buchneri*	silage	no	aerobic	yes	0.6	Shehata *et al*., 2013
Lactobacillus	*casei*	human	no	aerobic	yes	0.6	Shehata *et al*., 2013
Lactobacillus	*harbinensis*	chicken	no	aerobic	yes	0.6	Shehata *et al*., 2013
Riemerella	*anatipestifer*	duck	yes	aerobic	yes	0.15	Shehata *et al*., 2013
Salmonella	*enterica* Enteritidis	chicken	yes	aerobic	yes	5	Shehata *et al*., 2013
Salmonella	*Gallinarum*	chicken	yes	aerobic	yes	5	Shehata *et al*., 2013
Salmonella	*enterica* Typhimurium	chicken	yes	aerobic	yes	5	Shehata *et al*., 2013
Staphylococcus	*aureus*	chicken	yes	aerobic	yes	0.3	Shehata *et al*., 2013
Staphylococcus	*haemolyticus*	chicken	yes	aerobic	yes	0.3	Shehata *et al*., 2013
Staphylococcus	*lentus*	chicken	yes	aerobic	yes	0.3	Shehata *et al*., 2013
Vibrio	*fischeri*	seawater	no	semi-aerobic	no	24-26	Tsui and Chu, 2003



Prevalent microbiota

	Normal	Glyphosate
Alfalfa	*Rhizobium*	Actinobacteria↓
	Pseudomonas	*Colletotrich.*↓
	Micromonospora	*Uromyces*↓
	(Actinobacteria)	
Maize	*Bacteroides*	*Fusarium*↑→
	Comamonas	*Aspergillus*↑
	Caulobacter	*Pseudomonas*→
Soybean	*Bradyrhizobium*	*Fusarium*↑→
	Rhizobium	*Pseudomonas*↓→
	Bacillus	*Rhizoctonia*↑
Bee	*Lactobacillus*	*Serratia*↑
	Snodgrassella	*Lactobacillus*↓
	Gilliamella	*Snodgrassella*↓
Cow	*Prevotella*	*Clostridium*↑↓
	Ruminococcus	*Lichtheimia*↑
	Clostridium	*Enterococcus*↓
Pig	*Lactobacillus*	*Clostridium*↑
	Prevotella	*Ruminococcus*→
	Escherichia	*Enterobacter*↓→
Chicken	*Bacteroides*	*Salmonella*↑
	Prevotella	*Clostridium*↑
	Stenotrophom.	*Lactobacillus*↓
Rats	*Bacterioides*	*Clostridium*↑
	Ruminococcus	*Ruminococcus*↑→
	Prevotella	*Eggerthella*↑
	Clostridium	*Lactobacillus*↓→
Human	*Prevotella*	*Clostridium*↑
	Bacterioides	*Helicobacter*↑
	Ruminococcus	*Prevotella*↓↑
	Bifidobacterium	*Ruminococcus*↑

Fig. S1. Relational diagram of the application of glyphosate on crops and soil and transfer of glyphosate residues in plant parts to various animal intestinal tracts. Humans receive glyphosate residues from animal offal products (like some sausages) as well as plant products. Three crop species are depicted as examples: insect-pollinated alfalfa, wind-pollinated maize and self-pollinated soybeans. Glyphosate resistant cultivars of these crops are widely used and sprayed with glyphosate. Products with glyphosate residues are traded globally. The animals selected for this diagram are: bees, rats, cows, pigs, chickens, and humans. For each of these plants and animals three or four typical rhizosphere or intestinal microorganisms are listed for conditions without and with glyphosate exposure. The microbial compositions are dependent on location (intestinal organs or plant parts) and time of sampling and nutritional conditions (not indicated here). Arrows up, down or horizontal indicate relative enhancement, decrease, or no effect of glyphosate. Effects of glyphosate on the human microbiome were derived from expected effects based on genomic analyses, not on direct measurements. References used for this diagram are listed on the next page.

Fig. S1 (continued). Microbiome information was obtained from the following references:

Alfalfa: (Martínez-Hidalgo *et al*., 2014; Nievas *et al*., 2021; Samac and Foster-Hartnett, 2012; Trujillo *et al*., 2015)
Maize: (Beirinckx *et al*., 2020; Benito *et al*., 2020; Carranza *et al*., 2019; Kepler *et al.,* 2020; Kremer and Means, 2009; Williams *et al*., 2015; Zhou *et al*., 2020; Zobiole *et al*., 2010)
Soybean: (Arango *et al*., 2014; Igiehon and Babalola, 2018; Johal and Huber, 2009; Kremer *et al*., 2005; Kuklinsky-Sobral *et al*., 2005; Liu *et al*., 2019; Lu *et al.,* 2018; Sanogo *et al.,* 2000)
Bees: (Baffoni *et al*., 2016; Graystock *et al*., 2017; Motta *et al*., 2018; Motta and Moran, 2020; Motta *et al*., 2020; Manirajan *et al*., 2018; Rayman and Moran, 2018)
Cows: (Gerlach *et al*., 2014; Krüger *et al.,* 2013; Mao *et al*., 2015; Schrödl *et al*., 2014; Tsiaoussis *et al*., 2019; Wallace *et al*., 2019)
Pigs: (Bergamaschi *et al*., 2020; Carman *et al*., 2013; Isaacson and Kim, 2012; Krause *et al*., 2020; Krüger *et al*., 2014b; Wang *et al*., 2018)
Chickens: (Krüger *et al*., 2013; Li *et al*., 2021; Ruuskanen *et al*., 2020a; Shehata *et al*., 2013; Shehata *et al*., 2014)
Rats: (Athari Nik Azm *et al*., 2018; Bilan *et al*., 2019; Čoklo *et al*., 2020; Hu *et al*., 2021; Mao *et al*., 2018; Mesnage *et al*., 2021; Nagpal *et al*., 2018; Nielsen *et al*., 2018; Tang *et al*., 2020; Tsiaoussis *et al*., 2019)
Humans: (Fan and Pedersen, 2020; King *et al*., 2019; Kurilshikov *et al*., 2021; Manor *et al*., 2020; Mesnage and Antoniou, 2020; Nagpal *et al*., 2018; Rinninella *et al*., 2019)

Images: Ekonomics (alfalfa), Dreamstime (soybean and maize), Alex Surcica, Pinterest (leafcutter bee), Josie McCartney (mouse), J.P. Rowan, Univ. of Florida (pig), Evolving Sciences (cow), Kathryn Born, Dummies (chicken), GeoImages (human).

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