Glyphosate and Gut Bacteria

A Historically-Overlooked Angle in Toxicology?

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Thesis Statement: The common herbicide glyphosate's ability to harm non-plant life has long been dismissed due to the absence of the chemical's target pathway in human and animal cellular physiology. However, mounting evidence suggests that the gut microbiome may be a mechanism through which the chemical creates health consequences in humans and animals.

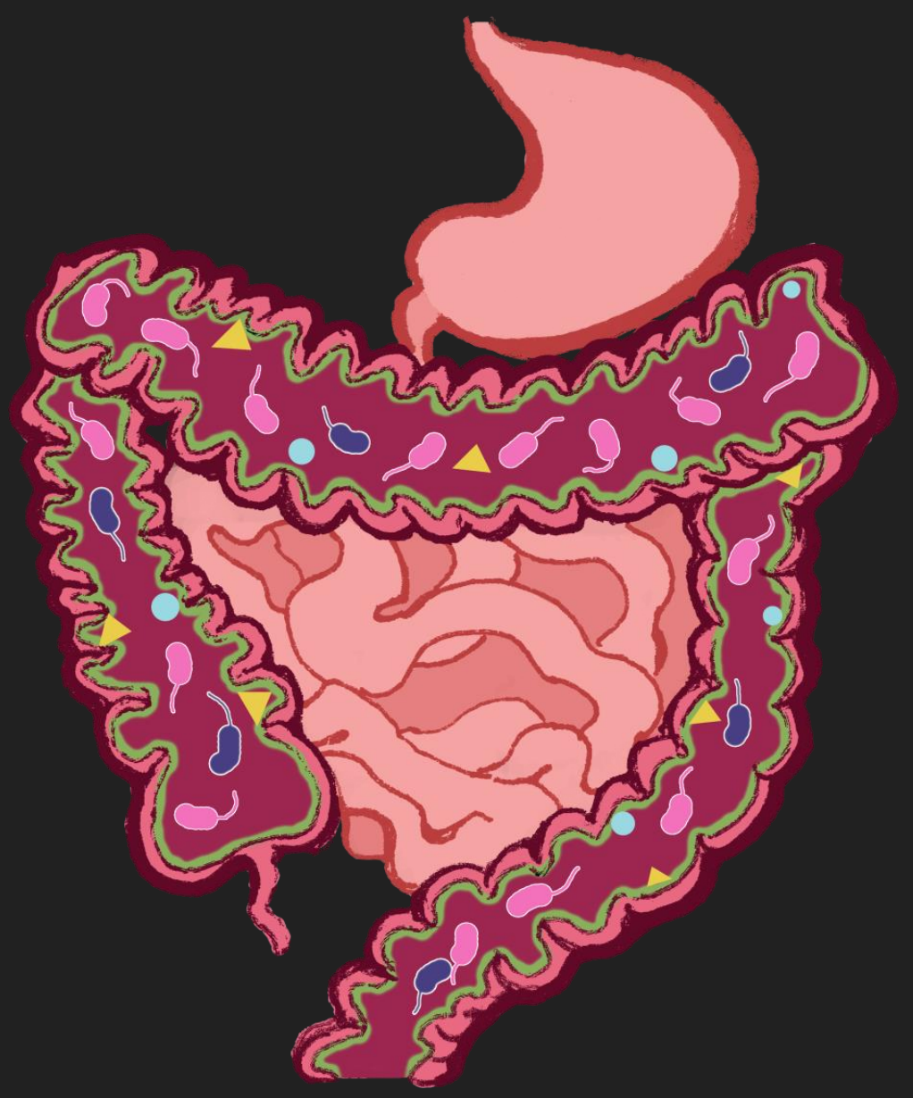
The Gut Microbiome Affects All Aspects of Human Health

"The Neglected Organ:"

- Human gut contains 10 bil.-100 tril. bacteria, >500 species, approx. 2kg (40% more than the liver)[1]
- >98% of microbial genes in the gut are bacterial; others are fungi, archaea, and viruses[1]

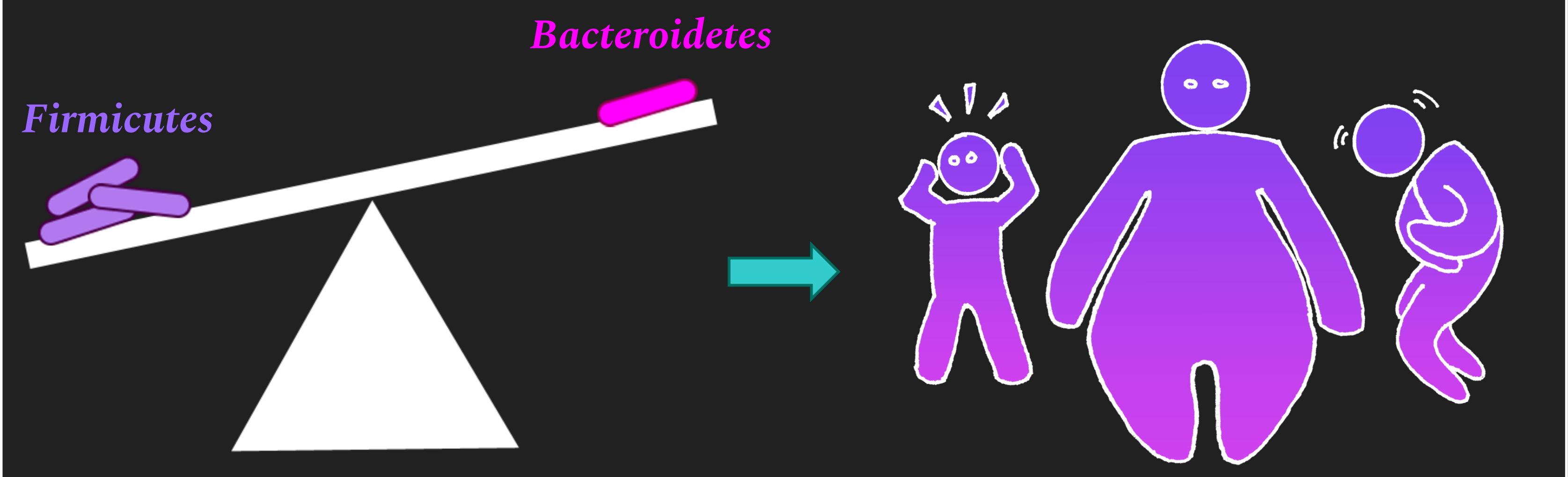
Population Balance:

- 90% of diversity in human gut is from phyla *Bacteroidetes* and *Firmicutes*[6]
- Absolute and relative population counts affect host health[2][5]
- Higher F:B ratio specifically linked to health conditions (see below)[3][6][7]



Roles in Health:

- Assists **digestion**[4]
- Assists **immune cells**[4]
- Synthesizes **vitamins** and **amino acids**[4]
- Aids in production of **neurotransmitters** (e.g., serotonin)[5]
- Maintains **gut mucus lining** → prevents leaky gut syndrome,[2][3] provides interface between microbes and host cells[4]

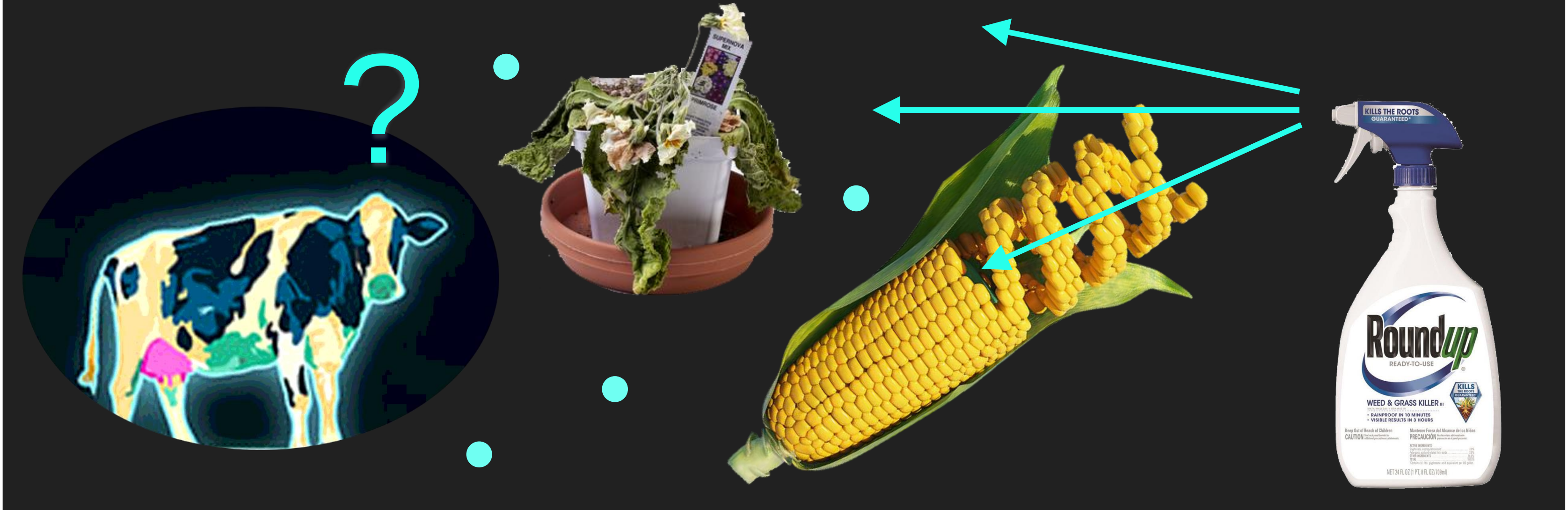


Dysbiosis is an imbalance of the gut microbiome, which is linked to chronic health conditions including obesity,[3][7] weakened immunities,[3][7] irritable bowel syndrome,[3][7][8] cancer,[7] diabetes,[3][7] heart disease,[3][8] depression,[5][8] autism,[2][7] and dementia.[7][8][9]

Glyphosate is the Most Common Weedkiller Worldwide

Usage and Distribution:

- Most popular herbicide since its introduction in 1974[10]
- Mixed with ***surfactants*** (synergistic chemicals) to make commercial weed killers, e.g., **Roundup**[11]
- Targets all plants, not just weeds [10]
- Applied liberally to **genetically engineered** (GE) "Roundup-Ready" crops since 1996[10]
- Residues found in food, water, dust, and animal tissues and products[10]
- Dosage and environmental residues increase as weeds gain resistance[14]

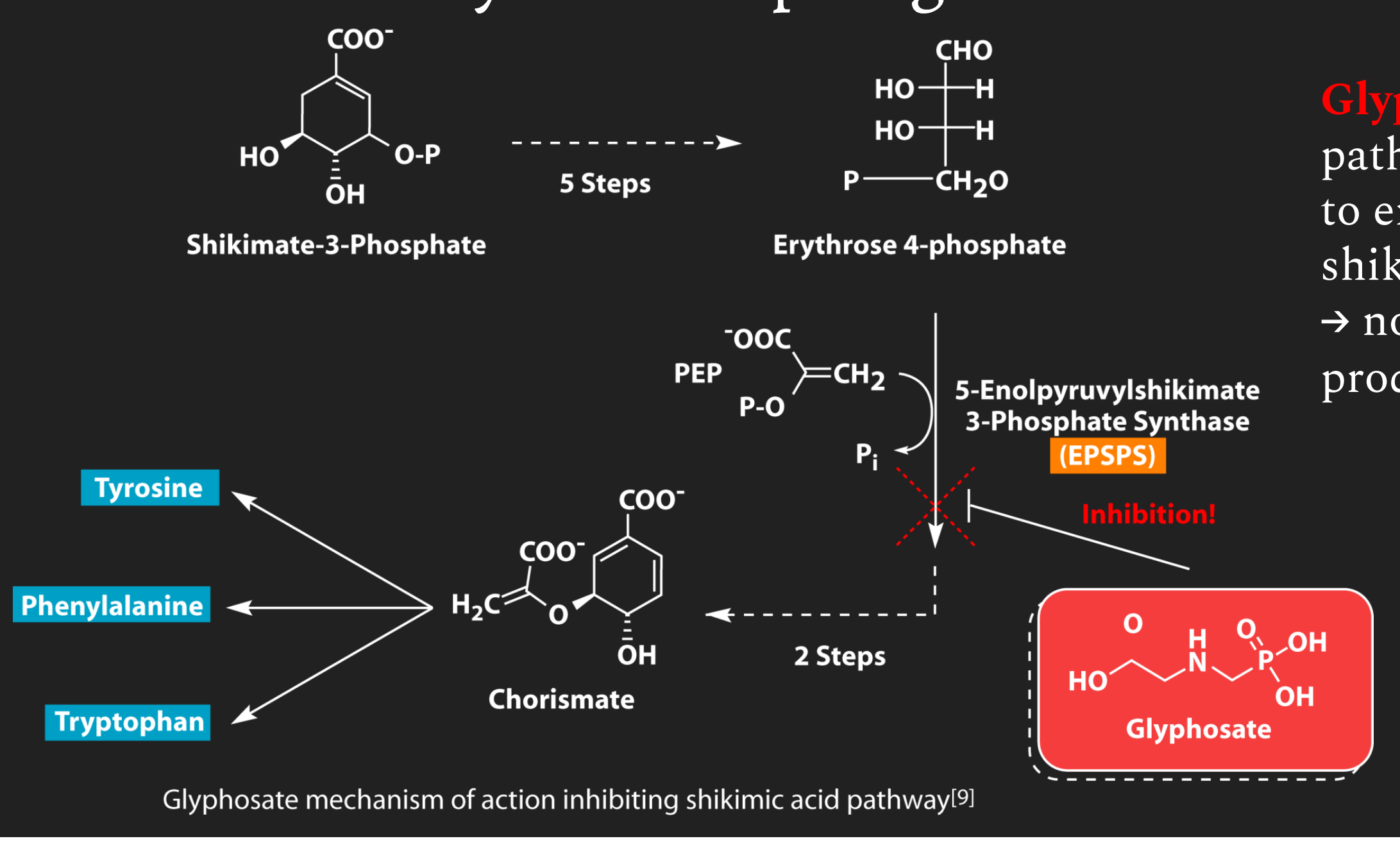


The Safety of Glyphosate Remains Controversial

- Glyphosate is asserted safe for humans based on differences between plant and animal physiology[2][8]
- Acceptable consumption levels vary by governmental agency[14][15][16]
- Status as carcinogen hotly contested[15][16]

Glyphosate Kills Plants by Interrupting the Shikimate Pathway

The **Shikimate pathway** synthesizes the **aromatic** (ring-shaped) **amino acids** *phenylalanine, tyrosine,* and *tryptophan*, which plants need to survive[2][11]



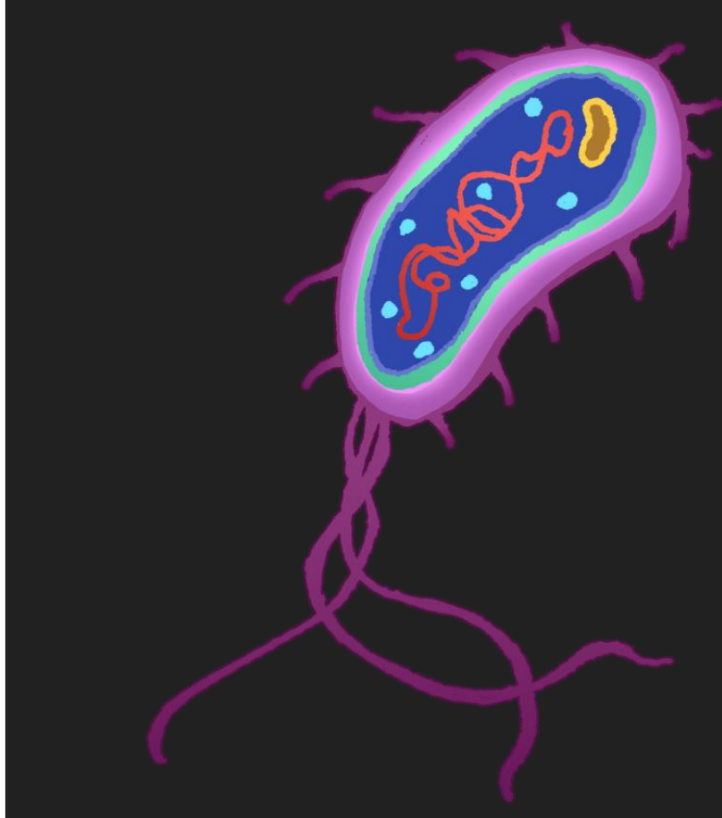
Glyphosate mechanism of action inhibiting shikimic acid pathway[9]

Glyphosate halts reaction pathway by binding to enzyme shikimate-3-phosphate → no **EPSPS enzyme** produced[12][13]

Glyphosate resistance results from mutant EPSPS genes which limit glyphosate's binding potential[13]

Could Glyphosate Affect Microorganisms?

- The shikimate pathway is present in bacteria, fungi, archaea, and other microorganisms[2][8]
- Glyphosate's potential to harm microorganisms already acknowledged: glyphosate patented as anti-microbial agent and anti-parasitic drug[4]
- Mechanisms for harming microbiota:
  - ○ Inhibiting **EPSPS** production (same as plants) → block essential amino acids[4]
  - ○ Compete for binding sites with enzyme MurA → limit production of ***peptidoglycan*** (bacterial cell wall)[4]
  - ○ Increase intestinal pH → deplete **manganese** → certain bacteria oxidized[5]

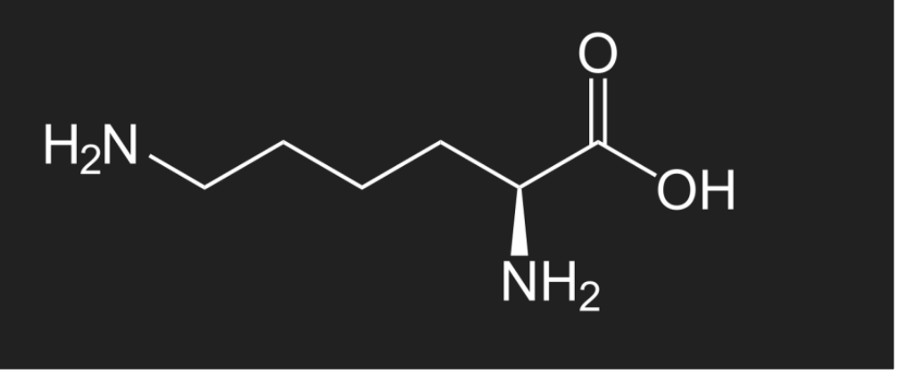


Some Bacteria More Sensitive than Others

Sensitive:
- ***Lactobacillus***: depends on manganese to prevent oxidation[5][8]
- ***S. alvi*** (main bacteria in honeybees): EPSPS enzymes especially prone to glyphosate binding[17]

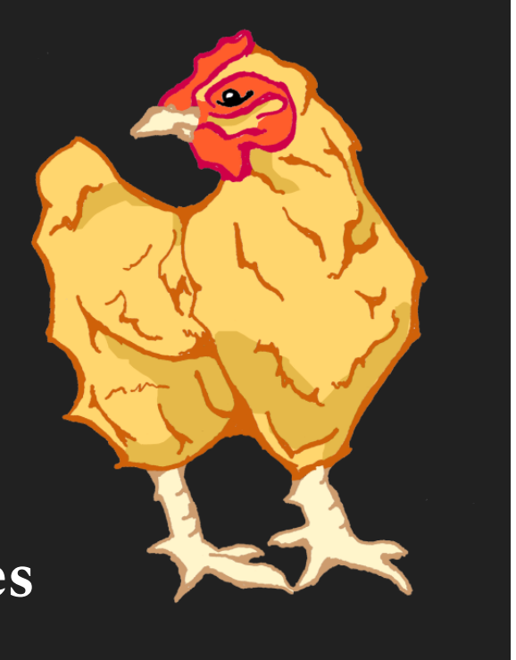
Resistant:
- ***Firmicutes***: EPSPS enzymes slightly resistant → less sensitive than *Bacteroidetes'*[17]
- ***Salmonella* and *E. coli*:** EPSPS is resistant,[8][18] or absent altogether[18]

Resistant EPSPS enzymes have lysine chain, which prevents glyphosate from binding[19]



Animal Studies Show Glyphosate—Gut Bacteria Interactions

Honeybees[17]**:**
- Main bacteria: *Lactobacillus*, *Proteobacteria*, and *Bifidobacteria*
- Glyphosate residues → ↓ *Proteobacteria* (esp. *Snodgrassella alvi*, which decreased by 5-13 times)
- Dysbiosis → vulnerability to parasites



Poultry[8]**:**
- Beneficial *Firmicutes* most strongly affected
- Pathogenic *E coli* and *Salmonella* show resistance

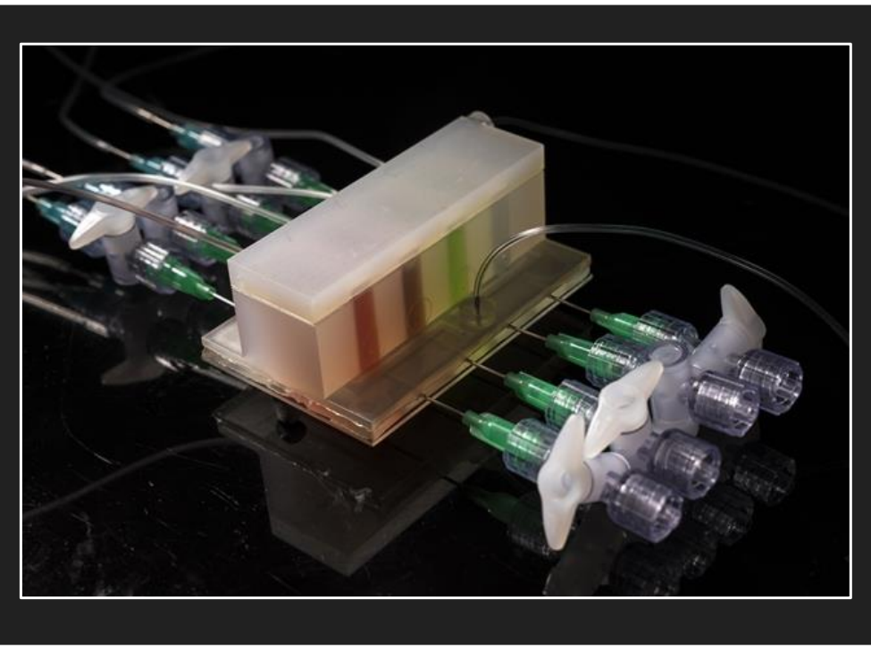
Cows (artificial rumens)[18]**:**
- Glyphosate inhibits growth of **ruminal bacteria**. increases success of **pathogenic species**

Mice:
- Gut bacteria 90% *Bacteroidetes* and *Firmicutes*—89% similarity to human gut microbiome[6]
- Chronic/sub-chronic glyphosate exposure → ↓*Firmicutes,* ↓*Lactobacillus,* ↓↓*Bacteroidetes*[5]

Rats:
- Glyphosate fed to nursing mother → infants suffer dysbiosis,[4] show distress signs[5]
- ↓*Lactobacillus*, ↨*Firmicutes*, ↑*Bacteroidetes* (opposite of mice studies)[4][18]; *E. coli* resistant[18]
- Dysbiosis mitigated by supplemental aromatic amino acids added to feed[20]

Shortage of Research

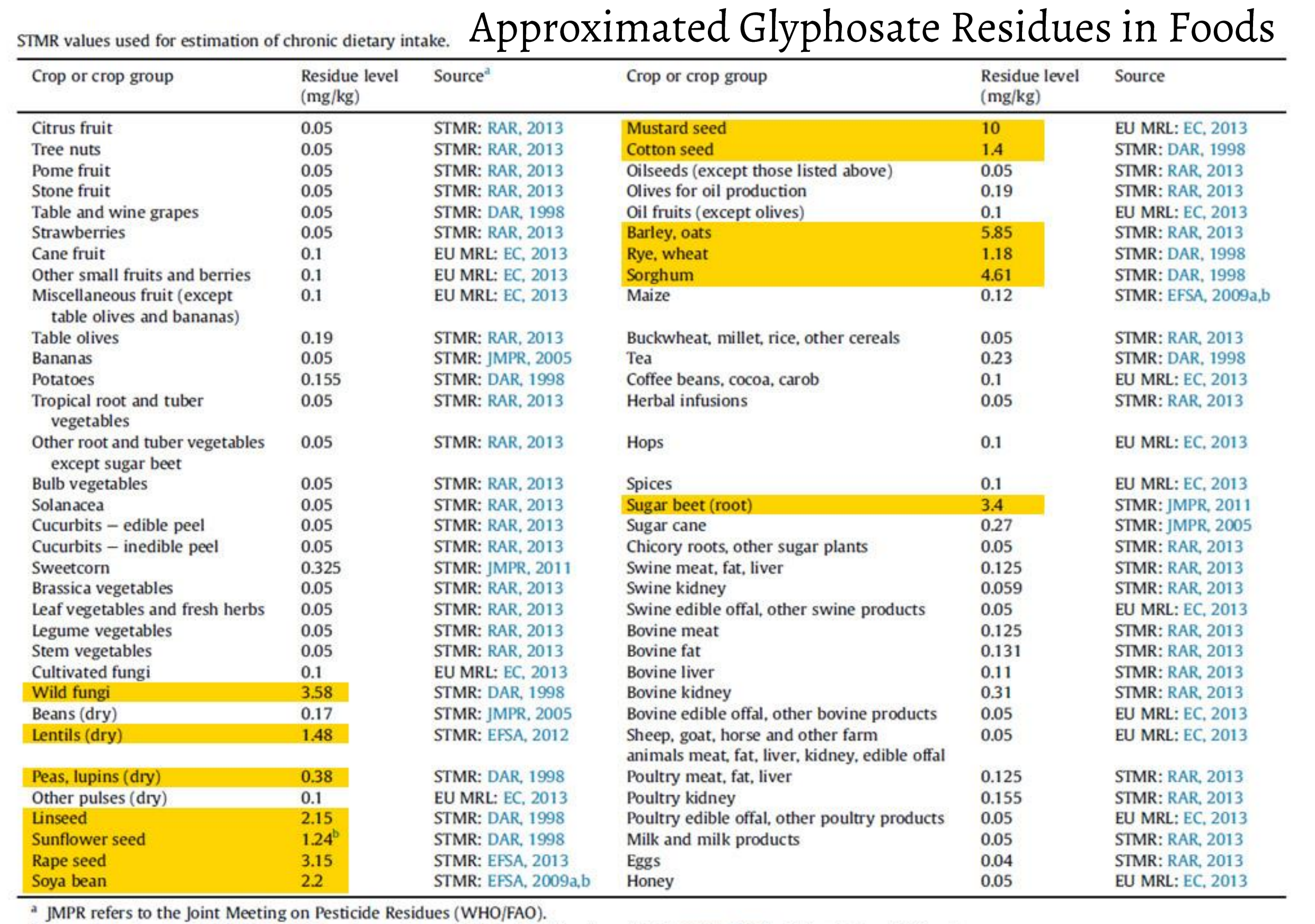
- Glyphosate's presumed safety → less incentive to document residues[2]
- Lack of studies comparing human exposure over time[10]
- Studies use conflicting methods, e.g., including or excluding surfactants[10]
- Unpredictability: animal microbiota varies by breed, supplier, housing[21]
- Solution: artificial gut, with mucus layer to simulate human GI tract[22]



An "artificial gut" unveiled by MIT in 2019, which could overcome the technological and ethical challenges of studying the human gut microbiome by allowing direct experimentation in a simulated environment.

Consumer Applications

- Support gut microbiome with probiotic and prebiotic foods[3]
- Mitigate effects of glyphosate by consuming proteins with aromatic amino acids[20]
- Consume adequate manganese to support *Lactobacilli*[5][8]
- Choose organic options for foods commonly sprayed with roundup[15]
- Avoid feeding glyphosate-heavy foods to children, as gut microbiome is most sensitive during early development[18]



Approximated Glyphosate Residues in Foods

STMR values used for estimation of chronic dietary intake.

Crop or crop group	Residue level (mg/kg)	Source[a]	Crop or crop group	Residue level (mg/kg)	Source
Citrus fruit	0.05	STMR: RAR, 2013	Mustard seed	10	EU MRL: EC, 2013
Tree nuts	0.05	STMR: RAR, 2013	Cotton seed	1.4	STMR: DAR, 1998
Pome fruit	0.05	STMR: RAR, 2013	Oilseeds (except those listed above)	0.05	STMR: RAR, 2013
Stone fruit	0.05	STMR: RAR, 2013	Olives for oil production	0.19	STMR: RAR, 2013
Table and wine grapes	0.05	STMR: DAR, 1998	Oil fruits (except olives)	0.1	EU MRL: EC, 2013
Strawberries	0.05	STMR: RAR, 2013	Barley, oats	5.85	STMR: RAR, 2013
Cane fruit	0.1	EU MRL: EC, 2013	Rye, wheat	1.18	STMR: DAR, 1998
Other small fruits and berries	0.1	EU MRL: EC, 2013	Sorghum	4.61	STMR: DAR, 1998
Miscellaneous fruit (except table olives and bananas)	0.1	EU MRL: EC, 2013	Maize	0.12	STMR: EFSA, 2009a,b
Table olives	0.19	STMR: RAR, 2013	Buckwheat, millet, rice, other cereals	0.05	STMR: RAR, 2013
Bananas	0.05	STMR: JMPR, 2005	Tea	0.23	STMR: DAR, 1998
Potatoes	0.155	STMR: DAR, 1998	Coffee beans, cocoa, carob	0.1	EU MRL: EC, 2013
Tropical root and tuber vegetables	0.05	STMR: RAR, 2013	Herbal infusions	0.05	STMR: RAR, 2013
Other root and tuber vegetables except sugar beet	0.05	STMR: RAR, 2013	Hops	0.1	EU MRL: EC, 2013
Bulb vegetables	0.05	STMR: RAR, 2013	Spices	0.1	EU MRL: EC, 2013
Solanacea	0.05	STMR: RAR, 2013	Sugar beet (root)	3.4	STMR: JMPR, 2011
Cucurbits – edible peel	0.05	STMR: RAR, 2013	Sugar cane	0.27	STMR: JMPR, 2005
Cucurbits – inedible peel	0.05	STMR: RAR, 2013	Chicory roots, other sugar plants	0.05	STMR: RAR, 2013
Sweetcorn	0.325	STMR: JMPR, 2011	Swine meat, fat, liver	0.125	STMR: RAR, 2013
Brassica vegetables	0.05	STMR: RAR, 2013	Swine kidney	0.059	STMR: RAR, 2013
Leaf vegetables and fresh herbs	0.05	STMR: RAR, 2013	Swine edible offal, other swine products	0.05	EU MRL: EC, 2013
Legume vegetables	0.05	STMR: RAR, 2013	Bovine meat	0.125	STMR: RAR, 2013
Stem vegetables	0.05	STMR: RAR, 2013	Bovine fat	0.131	STMR: RAR, 2013
Cultivated fungi	0.1	EU MRL: EC, 2013	Bovine liver	0.11	STMR: RAR, 2013
Wild fungi	3.58	STMR: DAR, 1998	Bovine kidney	0.31	STMR: RAR, 2013
Beans (dry)	0.17	STMR: JMPR, 2005	Bovine edible offal, other bovine products	0.05	EU MRL: EC, 2013
Lentils (dry)	1.48	STMR: EFSA, 2012	Sheep, goat, horse and other farm animals meat, fat, liver, kidney, edible offal	0.05	EU MRL: EC, 2013
Peas, lupins (dry)	0.38	STMR: DAR, 1998	Poultry meat, fat, liver	0.125	STMR: RAR, 2013
Other pulses (dry)	0.1	EU MRL: EC, 2013	Poultry kidney	0.155	STMR: RAR, 2013
Linseed	2.15	STMR: DAR, 1998	Poultry edible offal, other poultry products	0.05	EU MRL: EC, 2013
Sunflower seed	1.24[b]	STMR: DAR, 1998	Milk and milk products	0.05	STMR: RAR, 2013
Rape seed	3.15	STMR: EFSA, 2013	Eggs	0.04	STMR: RAR, 2013
Soya bean	2.2	STMR: EFSA, 2009a,b	Honey	0.05	EU MRL: EC, 2013

[a] JMPR refers to the Joint Meeting on Pesticide Residues (WHO/FAO).
[b] Sum of glyphosate STMR (DAR, 1998) and a maximum 10% contribution from AMPA (JMPR, 2005): 1.13 + 0.11 = 1.24 mg/kg.

(Stephenson & Harris, 2016)

Conclusions

- Presumed safety of glyphosate overlooks potential interactions with microbes
- Glyphosate disrupts gut bacteria balance in animals, even at concentrations accepted by government agencies
- Imbalanced gut microbiome linked to vast number of chronic illnesses
- Research is lacking because of glyphosate's asserted safety and the difficulty of studying the human gut microbiome
- Consumers can preemptively protect themselves by supporting a healthy gut microbiome and limiting consumption of glyphosate-heavy foods

Sources

1. Turner, P. V. (2018). The role of the gut microbiota on animal model reproducibility. *Animal Models and Experimental Medicine*(1).
2. Samsel, A., & Seneff, S. (2013). Glyphosate's Suppression [. . .] Biosynthesis by the Gut Microbiome: Pathways to Modern Diseases. *Entropy*(15).
3. Singh, R. K., et al. (2017). Influence of diet on the gut microbiome and implications for human health. *Journal of Translational Medicine*(15).
4. Mao, Q., et al. (2018). The Ramazzini Institute 13-week pilot study on glyphosate [. . .] rats: effects on the microbiome. *Environmental Health*(17).
5. Aitbali, Y., et al. (2018). Glyphosate [. . .] affects gut microbiota, anxiety and depression-like behaviors in mice. *Neurotoxicology and Teratology*(67), 44-49.
6. Turnbaugh, P. J., et al. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*(444), 1027-1031.
7. Rinninella, E., et al. (2019). What is the Healthy Gut Microbiota Composition? [. . .] Age, Environment, Diet, and Diseases. *Microorganisms*(7).
8. Rueda-Ruzafa, L., Cruz, F., Roman, P., & Cardona, D. (2019). Gut microbiota and neurological effects of glyphosate. *Neurotoxicology*(75), 1-8.
9. Saji, N., et al. (2019). Analysis of the relationship between the gut microbiome and dementia: [. . .] conducted in Japan. *Scientific Reports*(9)
10. Gillezeau, C, et al. (2019). The evidence of human exposure to glyphosate: a review. *Environmental Health*(18).
11. Van Bruggen, A.H.C., et al. (2018). Environmental and health effects of the herbicide glyphosate. *Science of the Total Environment*(616-617), 255-268.
12. Boocock, M.R., & Coggins, J.R. (1983). Kinetics of [. . .] synthase inhibition by glyphosate. *Federation of European Biochemical Societies*(154).
13. Funke, T., et al. (2006). Molecular basis for the herbicide resistance of Roundup Ready crops. *PNAS, 103*(35).
14. Benbrook, C.M. (2016). Trends in glyphosate herbicide use in the United States and globally. *Environmental Sciences Europe*(28).
15. Stephenson, C.L., & Harris, C.A. (2016). An assessment of dietary exposure to glyphosate [. . .]. *Food and Chemical Toxicology*(95), 28-41.
16. CEPA. (2017). *Proposed Amendment to: [. . .] Specific Regulatory Levels Posing No Significant Risk*. Office of Environmental Health Hazard Assessment.
17. Blot, N., Veillat, L., Rouze, R., & Delatte, H. (2019.) Glyphosate, but not its metabolite AMPA, alters the honeybee gut microbiota. *PLOS ONE*(4).
18. Tsiaoussis, J., et al. (2019). Effects of single and combined toxic exposures on the gut microbiome [. . .]. *Toxicology Letters*(312), 72-97.
19. Light, S.H., et al. (2016). An Unusual Cation-Binding Site and Distinct Domain–Domain Interactions Distinguish [. . .]. *Biochemistry*(55), 1239-1245.
20. Nielsen, L.N., et al. (2018). Glyphosate has limited [. . .] in the gut environment due to sufficient aromatic amino acid levels. *Environmental Pollution*(233).
21. Hugenholtz, F., & de Vos, W.M. (2018). Mouse models for human intestinal microbiota research [. . .]. *Cellular and Molecular Life Sciences*(75), 149-160.
22. McGovern, A. (2019). Artificial gut aims to expose the elusive microbiome. MIT News.