Title: Prenatal glyphosate exposure disrupts the gut-brain axis across several generations in mice.

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Abstract: Glyphosate, a widely used herbicide in North America, has become prevalent in the food supply due to preharvest applications, raising concerns about potential health impacts. This study investigated the effects of prenatal glyphosate exposure on the gut bacteriome, colitis, metabolic health, and behavior across generations in mice. In healthy mice, glyphosate decreased goblet cell numbers and mucin-2 expression in the colon and dysregulated cytokines in F1 and F2 progeny at levels below the EPA acceptable daily limit. Glyphosate also disrupted metabolism, including impaired glucose tolerance, increased insulin resistance and reduced serum GLP-1 levels. Moreover, prenatal glyphosate exposure induced behavioral deficits, including reduced locomotor activity and impaired working memory, which are associated with alterations in the gut microbiome composition and key gut-brain axis mediators. These data underscore the potential risks associated with glyphosate exposure, highlighting the need for further research and regulatory consideration.

Main Text: Glyphosate (tradename Roundup®) has emerged as a significant component of contemporary farming practices, yet its use has sparked debate regarding its impact on human health. North America is one of the largest consumers of glyphosate, with over 160 million kilograms applied annually.[1,2] The development of glyphosate-resistant crops and its use as a desiccant have led to increased glyphosate levels in foods consumed within North America including wheat, corn, soy, and oats.[3] In addition to being the largest user of glyphosate, North America also exhibits the highest prevalence of chronic inflammatory conditions, including inflammatory bowel disease (IBD), diabetes and cognitive disorders.[4,5] A growing body of evidence links the Western diet to rising disease rates in developed and newly industrialized countries.[6,7,8] However, the definition of this dietary pattern fails to recognize the significant contribution of environmental toxins, alone or in combination with other dietary factors, resulting from increased agrochemical usage.

Glyphosate targets the Shikimate pathway, a metabolic process restricted to plants, fungi, and bacteria. Historically, the absence of this pathway in mammals led to the assumption that glyphosate posed minimal risk to human health. However, recent advances in understanding the crucial role of the gut microbiome have prompted a reassessment of this notion. Previous studies examining glyphosate's impact on the gut microbiome used doses exceeding dietary exposure, raising questions about their applicability.[9,10] Additionally, studies have primarily examined healthy populations and have focused on direct exposure to glyphosate. This study sought to fill these research gaps by exploring the effects of prenatal glyphosate exposure on the gut bacteriome, colitis, metabolic health, and behavior across generations in healthy mice and mice genetically predisposed to developing colitis (*Muc2*$^{-/-}$ mice), using doses that mimic real-world exposure.

Determination of the Average American Diet (AAD) dose

To determine the amount of glyphosate the average North American is exposed to through diet, we calculated the Average American diet (AAD) dose based on a hypothetical menu for a 60kg female following the American food guide and reported values of glyphosate found within these foods (Table 1). The AAD dose was determined to be 0.01mg/kg/day, which is more than 100X lower than the acceptable daily intake (ADI) currently set by the Environmental Protection Agency (EPA) at 1.75 mg/kg/day. At the time of dose calculation, few studies existed detailing glyphosate levels in food. Therefore we relied on values collected from citizen science initiatives to calculate the AAD dose. To validate these reported levels of glyphosate in food, we developed a modified ultra-performance liquid chromatography method for quantification (figure S3). The values used to calculate the AAD dose were found to be similar to what has subsequently been reported in literature and our own measurements (Table 2).

Commercial rodent chow contains many items desiccated using glyphosate-based herbicides. Indeed, tandem MS analysis revealed that glyphosate was present in PicoLab® 5058 diet at a concentration of 0.381 parts per million (PPM) (Table 2). Considering all commercial rodent chows contain ingredients exposed to high levels of glyphosate, including wheat, soy, and corn, we assumed no commercially available food would be glyphosate-free. Therefore, we deemed this exposure negligible, given an estimated daily consumption of 4-5 grams of food per mouse, which would contribute approximately 0.001 mg of glyphosate per day to their diet.[17] While the control group was exposed to trace amounts of glyphosate, this exposure was well

below any current ADI. In addition to our calculated AAD dose (0.01mg/kg/day). In addition to the AAD dose, we also examined the ADI currently set by the EPA (1.75 mg/kg/day).

Prenatal glyphosate exposure induces colitis in healthy offspring and promotes intestinal inflammation.

To evaluate the impact of prenatal glyphosate exposure on intestinal pathology, we investigated commonly used markers of morbidity and inflammation. Healthy mice exposed to glyphosate through their parents or grandparents showed no overt signs of colitis. However, histopathological analysis revealed significant morphological changes (Fig. 1, A). Prenatal glyphosate exposure induced colitis in healthy mice, regardless of generation or treatment group, characterized by a near-complete loss of mucus in goblet cells and a reduction in mucin-2 gene expression within the colon (Fig. 1, B). F1 and F2 mice also displayed increased macrophage infiltration within the colonic submucosa (Fig. 1, C). While goblet cell depletion is not a standard diagnostic marker in humans, reductions in goblet cell abundance and *MUC2* gene expression are observed in IBD patients, particularly during the initial stages of disease.[18]

We then examined the cytokine environment within the colon. Healthy mice did not exhibit significant changes in individual cytokine gene expression, but cytokines function within complex networks, and their effects depend on the overall cytokine milieu. To analyze the cytokine profiles of each treatment group, we constructed a Principal Component Analysis (PCA) biplot, which revealed distinct cytokine network for each group, with the first and second principal components accounting for 77.7% of the variability (Fig. 1, D). The offspring of mice not exposed to glyphosate exhibit a cytokine profile which supports immunological tolerance, notably T regulatory cells, through the presence of TGF-β and Foxp3. Network analysis also revealed F1 offspring of mice exposed to glyphosate at the AAD level display a pro-inflammatory cytokines network characterized by TNF-α and IL-1β. Furthermore, F2 offspring of AAD-exposed mice showed an association with IFN-γ and IL-17, indicative of pathogenic TH17 cells. AAD F2 mice also showed associations with IDO1, which regulates inflammation through the kynurenine pathway and is upregulated by inflammatory cytokines such as IFN-γ. Both F1 and F2 offspring exposed to the EPA Upper Limit of glyphosate displayed associations with enzymes involved in reducing reactive oxygen species, including GPX2 and CAT3. These mice also exhibited connections with enzymes related to immune cell activity, including MCPT1 and MCPT2, markers of mucosal mast cell proteases, and iNOS, a marker of pro-inflammatory M1 macrophages. These findings suggest that prenatal glyphosate exposure drives a shift in healthy offspring towards pro-inflammatory TH1/TH17 pathways while simultaneously decreasing factors necessary for maintaining immunological tolerance within the gut.

Given that IBD has a strong genetic component we sought to explore if glyphosate exposure exacerbates colitis in a genetically susceptible host. To do this, we used *Muc2*$^{-/-}$ mice that are deficient in mucin 2, a protective mucin within the colon, resulting in the development of spontaneous colitis.[19] Changes in *MUC2* expression have been observed in human populations, making this a clinically relevant model.[20] Surprisingly, colitis-susceptible mice only exhibited a modest exacerbation in inflammation characterized by increased macrophage infiltration within the EPA Upper Limit F2 groups (Fig. 1, E) and an overall pro-inflammatory cytokine network within the colon (Fig. 1, F). Like their healthy littermates, colitis-susceptible mice within the AAD F2 and EPA Upper Limit F2 groups displayed increased expression of IL-17, and IFN-γ. Colitis-susceptible mice also exhibited increased expression of RELMβ, a driver of colitis in the *Muc2*$^{-/-}$ model (Fig. 1, G-J).[19]

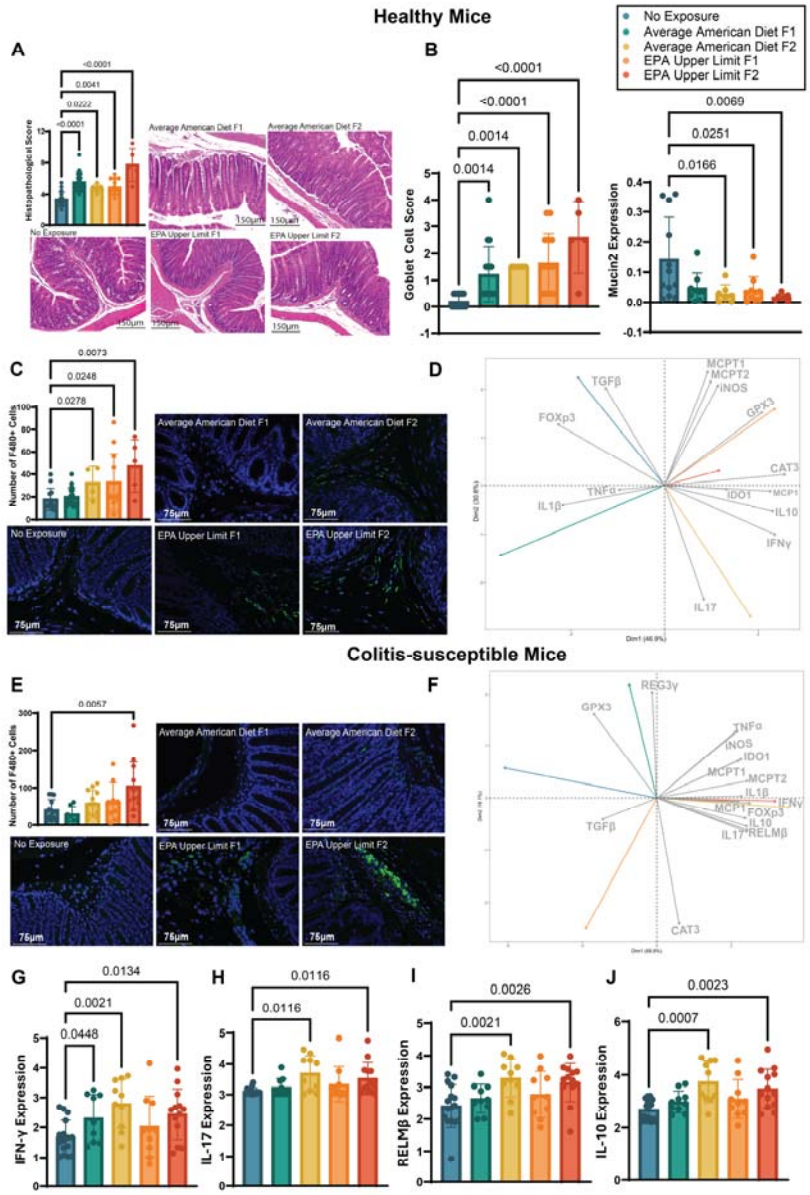

Fig. 1. Prenatal Glyphosate Exposure Induces Colitis and Exacerbates Colonic Inflammation in Mice. **[A]** Healthy offspring of glyphosate-exposed mice show increased histopathological damage in the colon compared to control (Kruskal-Wallis, $p<0.0001$, $\eta^2=0.493$, FDR-correction). Histological scoring criteria are found in tables S2 and S3. **[B]** Histopathological score is driven by a decreased abundance of mucus-producing goblet cells (Kruskal-Wallis, $p<0.0001$, $\eta^2=0.471$, FDR-correction), and reduced mucin-2 gene expression (Kruskal-Wallis, $p=0.025$, $\eta^2=0.178$, FDR-correction). **[C]** Healthy offspring exhibit an increased abundance of F480+ macrophages within the colonic submucosa (Kruskal-Wallis, $p=0.015$, $\eta^2=0.135$, FDR-correction). **[D]** Principal component analysis reveals that healthy offspring of glyphosate-exposed mice display a pro-inflammatory cytokine network in the colon, distinct from the No Exposure group. **[E]** Colitis-susceptible offspring in the EPA Upper Limit F2 group demonstrate an increased abundance of F480+ macrophages within the colonic submucosa (Kruskal-Wallis, $p=0.004$, $\eta^2=0.192$, FDR-correction). **[F]** Principal component analysis indicates that colitis-susceptible offspring of glyphosate-exposed mice display a pro-inflammatory cytokine network within the colon. **[G]-[J]** Colitis-susceptible offspring from both the AAD and EPA Upper Limit groups exhibit altered relative normalized gene expression in several cytokines including IFN-γ (ANOVA, $p=0.010$, $\eta^2p=0.214$, FDR-correction), RELMβ (ANOVA, $p=0.0009$, $\eta^2p=0.294$, FDR-correction), IL-17 (Kruskal-Wallis, $p=0.0036$, $\eta^2=0.195$), and IL-10 (Kruskal-Wallis, $p=0.0011$, $\eta^2=0.252$). Primer sequences used for cytokine analysis are found in table S4.

Prenatal glyphosate exposure impairs glucose metabolism, reduces insulin sensitivity, and alters metabolic hormone production in healthy mice.

Given the increased risk of metabolic syndrome in individuals with IBD, we examined whether glyphosate exposure influences metabolic health in offspring.[21] Healthy descendants of glyphosate-exposed mice exhibited significant metabolic disturbances. Second-generation mice exposed to EPA levels of glyphosate showed significantly decreased glucose clearance during an oral glucose tolerance test (OGTT) (Fig. 2, A), and F1 mice displayed significantly increased insulin resistance during an insulin-tolerance test (ITT) (Fig. 2, B). Additionally, both F1 and F2 offspring from AAD and EPA Upper Limit groups demonstrated a marked reduction in serum GLP-1, with a nonsignificant trend towards reduced GLP-1 within the AAD F2 group (Fig. 2, C). Additionally, mice within both the AAD and EPA Upper Limit F1 groups displayed reduced serum ghrelin (Fig. 2, D). Ghrelin is best known for its effects on promoting hunger. However, ghrelin has recently been implicated in numerous physiological processes including growth hormone release, gastric motility and metabolism. Ghrelin also plays a significant role in cognition by promoting neurogenesis and synaptic plasticity.[22] Mice in both the F1 and F2 AAD groups and EPA Upper Limit F1 group displayed significantly increased serum leptin (Fig. 2, E). At normal levels, leptin aids in regulating satiety. However, when leptin is present in excess, hyperleptinemia can form, resulting in impaired appetite regulation, metabolic dysfunction, including insulin resistance and impaired glucose metabolism, and increased systemic inflammation.[23]

Notably, colitis-susceptible offspring exhibited no metabolic deficits following prenatal glyphosate exposure. These findings suggest that, in addition to promoting damaging inflammation, prenatal glyphosate exposure results in metabolic disturbances in healthy offspring, including decreased glucose tolerance, insulin resistance, and alterations in metabolic hormones including GLP-1, ghrelin and leptin.

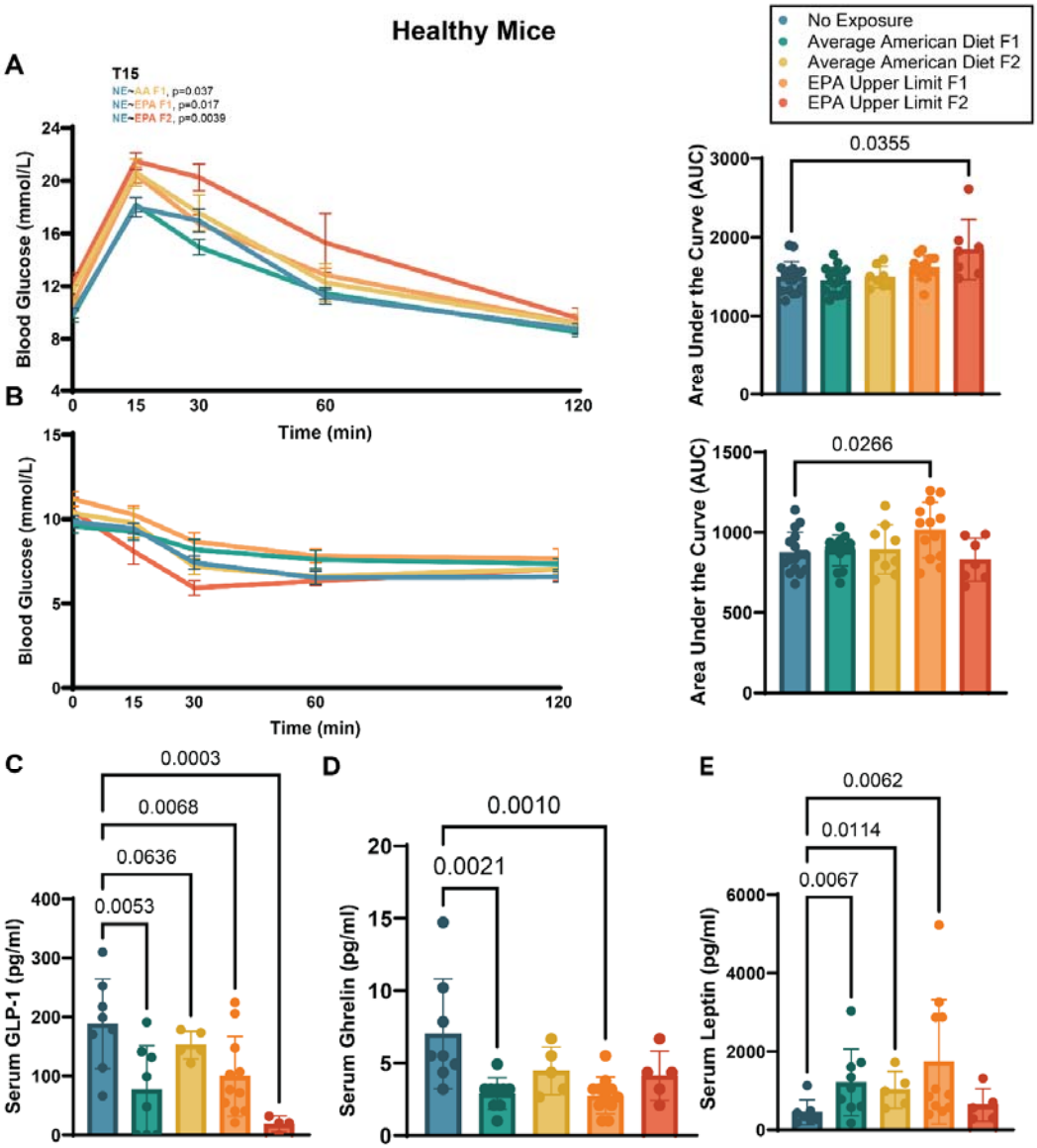

Fig. 2. Prenatal Glyphosate Exposure Impairs Glucose Metabolism, Increases Insulin Resistance and Alters Metabolic Hormones in Healthy Offspring. [A] Healthy mice whose ancestors were exposed to glyphosate display impaired glucose metabolism, characterized by increased blood glucose levels 15 min after glucose gavage (Two-Way ANOVA) and an increased area under the curve (AUC) in EPA Upper Limit F2 mice (Kruskal-Wallis, p=0.0106, η^2=0.129, FDR-correction). **[B]** Healthy mice within the EPA Upper Limit F1 group show increased insulin resistance, evidenced by an increased AUC (ANOVA, p=0.0326, η^2p=0.174). **[C]** Healthy mice within the AAD F1, EPA Upper Limit F1, and EPA Upper Limit F2 groups exhibit significant reductions in serum levels of GLP-1, while mice within the AAD F2 group show a non-significant trend towards reduction (Welch's ANOVA, p=<0.0001, ω^2=0.885, FDR-correction). **[D]** Healthy mice within the AAD and EPA Upper Limit F1 groups display reduced levels of ghrelin in their serum (Kruskal-Wallis, p=0.0040, η^2=0.0.345, FDR-correction). **[E]** Prenatal exposure to glyphosate induces leptin resistance in AAD group F1 and F2 offspring as well as EPA Upper Limit F1 offspring (Kruskal-Wallis, p=0.036, η^2=0.195, FDR-correction).

Prenatal glyphosate exposure reduces locomotor activity, impairs working memory and alters the gut-brain axis in offspring.

Individuals with IBD are at an elevated risk of developing psychological and cognitive impairments.[24] Additionally, experimental models of IBD, demonstrate decreased locomotor

activity.[25] Thus, we explored the transgenerational effects of glyphosate on behavior and cognition. Healthy F2 offspring within the EPA exposure group exhibited significantly decreased locomotor activity, characterized by reductions in overall distance travelled and average body velocity in the open field test (Fig. 3, A). Mice also showed changes in exploratory behaviors, with a significant reduction in unsupported rearing events (Fig. 3, B). This decrease in unsupported rearing could indicate anxiety or physical unsteadiness. However, anxiety-like behaviors were not significantly affected in the open field test or light-dark box, and no changes in serum corticosterone levels were detected (fig. S4).

Memory impairments are common in individuals with gastrointestinal disorders.[26] Healthy F1 offspring whose parents were exposed to glyphosate at levels found within the North American diet displayed impaired working memory during the radial arm maze test (Fig. 3, C). These behavioral changes were associated with decreased serum kynurenine levels in both AAD F1 and EPA Upper Limit F2 offspring (Fig. 3, D). While colitis-susceptible offspring did not show behavioral deficits, changes were observed in key components of the gut-brain axis. Mice within the AAD F2 and EPA Upper Limit F2 groups displayed increased alpha-synuclein gene expression in the colon (Fig. 3, E). Alpha-synuclein aggregates, often found in the digestive tracts of Parkinson's patients, are now believed to form years before clinical symptoms appear, facilitated by pathogenic bacteria that promote harmful aggregate formation.[27] Additionally, colitis-susceptible offspring in the EPA Upper Limit F2 group displayed decreased serum levels of serotonin (Fig. 3, F). Serotonin has been identified as a crucial gut-brain axis mediator, given its effects on mood, behavior, immune signaling, cytokine production, and gastrointestinal motility.

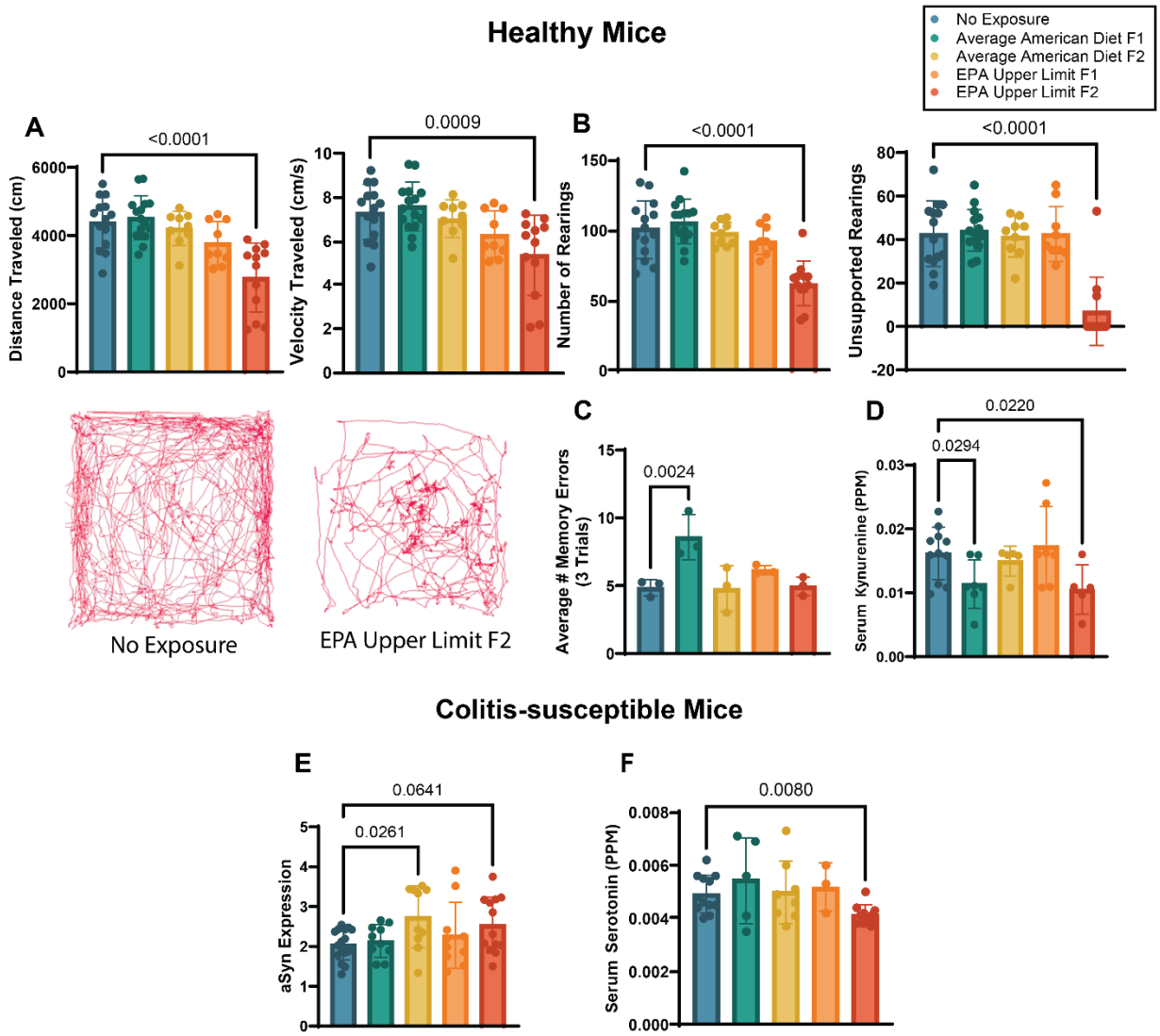

Fig. 3. Prenatal Glyphosate Exposure Alters Locomotor Activity, Working Memory, and the Gut-Brain Axis in Healthy and Colitis-Susceptible Mice. [A] Healthy offspring of glyphosate-exposed mice within the EPA Upper Limit F2 group exhibit reduced locomotor activity characterized by decreased distance traveled (ANOVA, p=<0.0001, η^2p=0.460) and slower body velocity (ANOVA, p=0.0004, η^2p=0.309) compared to mice within the no exposure group. **[B]** Mice within the EPA Upper Limit F2 group display decreased exploratory behaviors, including rearing (ANOVA, p=<0.0001, η^2p=0.533). When the total number of rearing events was broken down into supported and unsupported rearing, it could be seen that this was driven by reductions in the number of unsupported rearing events (ANOVA, p=<0.0001, η^2p=0.580, FDR-correction). **[C]** Healthy offspring within the AAD F1 group demonstrate impaired working memory during radial arm maze testing, with each point representing the average number of errors per trial over three trials conducted 24-hours apart (Two-Way ANOVA, Main Effect, n=6-20 mice/group). **[D]** Both AAD F1 and EPA Upper Limit F2 groups show decreased serum levels of kynurenine (ANOVA, p=0.0297, η^2p=0.293, FDR-correction). **[E]** Colitis-susceptible mice within the Average America Diet F2 and EPA Upper Limit F2 groups display increased expression of alpha-synuclein within the colon (ANOVA, p=0.053, η^2p=0.156, FDR-correction). **[F]** Colitis-susceptible mice display decreased levels of serotonin within serum of EPA Upper Limit exposed F2 offspring (Welch's ANOVA, p=0.0397, ω^2=0.193, FDR-correction).

Prenatal glyphosate exposure alters gut bacteriome composition, functional dynamics, and metabolite production in offspring.

Taxonomic differences were observed within the colon of healthy mice including an increased abundance of *Akkermansia muciniphila* within the no exposure (control) group. *Akkermansia* spp. have recently gained attention for their metabolic mediating effects through the secretion of a GLP-1-inducing protein, improving metabolic status in mice.[28] In addition to its effects on metabolism, GLP-1 has also been implicated in reducing colitis-associated inflammation through inhibition of the NF-κB signaling pathway and has been shown to improve learning and memory by promoting neurogenesis within the hippocampus.[29,30] Additionally, an increased abundance of *Parabacteroides distasonis* and *P. goldsteinii* was observed in the AAD F2 group. Like *Akkermansia* spp., *Parabacteroides* spp. are being explored for their potential probiotic effects; however, *P. distasonis* has been shown to induce depression-like symptoms within a murine model of colitis, highlighting how bacteria can elicit pleiotropic effects dependent on the environment.[31] Mice within the EPA Upper Limit F2 group displayed an increased abundance of Christensenellaceae (Fig. 4, A). Studies have identified increased Christensenellaceae with an elevated risk of developing Parkinson's disease, and members of the family Christensenellaceae are considered the most heritable taxon by forming intricate networks with other heritable taxa.[32,33]

Short-chain fatty acids (SCFA) are the most abundant microbial metabolite found within the gut. Gut-derived SCFA's can enter systemic circulation leading to a wide range of physiological effects on the host. Mice within the AAD F1 group displayed an increased abundance of the SCFA acetate within their cecum (Fig. 4, B). Generally, increases in SCFA are thought to be beneficial for host health. However, recent studies have linked excess acetate with the development of metabolic syndrome through increased activation of the parasympathetic nervous system.[34]

Colitis-susceptible offspring of glyphosate-exposed mice also showed taxonomic differences in the colon, including an increased abundance of non-photosynthetic cyanobacteria in the EPA Upper Limit F2 mice and increased *Bifidobacterium* spp. in EPA Upper Limit F1 mice (Fig. 4, C). While *Bifidobacterium* spp., are considered beneficial, biopsies collected from IBD patients during active disease often contain high levels of *Bifidobacterium* spp., again supporting the double-edged sword which comes with classifying microorganisms as "good" or "bad."[35] Additionally, colitis-susceptible F2 offspring within the EPA Upper Limit group exhibited an increased abundance of non-photosynthetic cyanobacteria, specifically Gastranaerophilales. In the environment, glyphosate promotes the growth of cyanobacteria as it provides a rich source of phosphorus needed for these organisms to thrive.[36] However, this study is the first to demonstrate that dietary glyphosate can promote the growth of cyanobacteria in the gut. Cyanobacteria within the environment can produce the non-coding amino acid β-Methylamino-L-alanine (BMAA). BMAA can be incorporated into proteins where it acts as a neurotoxin through the misfolding of proteins and has been associated with neurodegenerative diseases like Parkinson's. It has been suggested that glyphosate runoff may contribute to algae blooms, increasing BMAA levels and contributing to the recent increase in neurological diseases observed in coastal areas across North America.[37,38]

Predictive functional analysis of colon samples from colitis-susceptible mice indicated a shift towards a dysbiotic microbiome characterized by increased abundance of aerobic, pathogenic, and biofilm-forming bacteria within the colon of EPA Upper Limit exposed mice in both the F1 and F2 generations (Fig. 4, D). It has been suggested that dietary exposure to

glyphosate may promote pathobiont expansion within the colon as some bacteria have conferred resistance to glyphosate.[9] Glyphosate inhibits the enzyme 5-enol-pyruval-shikimate-3-phosphate synthase (EPSPS). All bacteria possess glyphosate-sensitive (EPSPS Class I) enzymes. However, some bacteria have developed glyphosate-resistant (EPSPS Class II) enzymes, and these resistant enzymes are more prevalent in pathogenic bacterial species.[9,10]

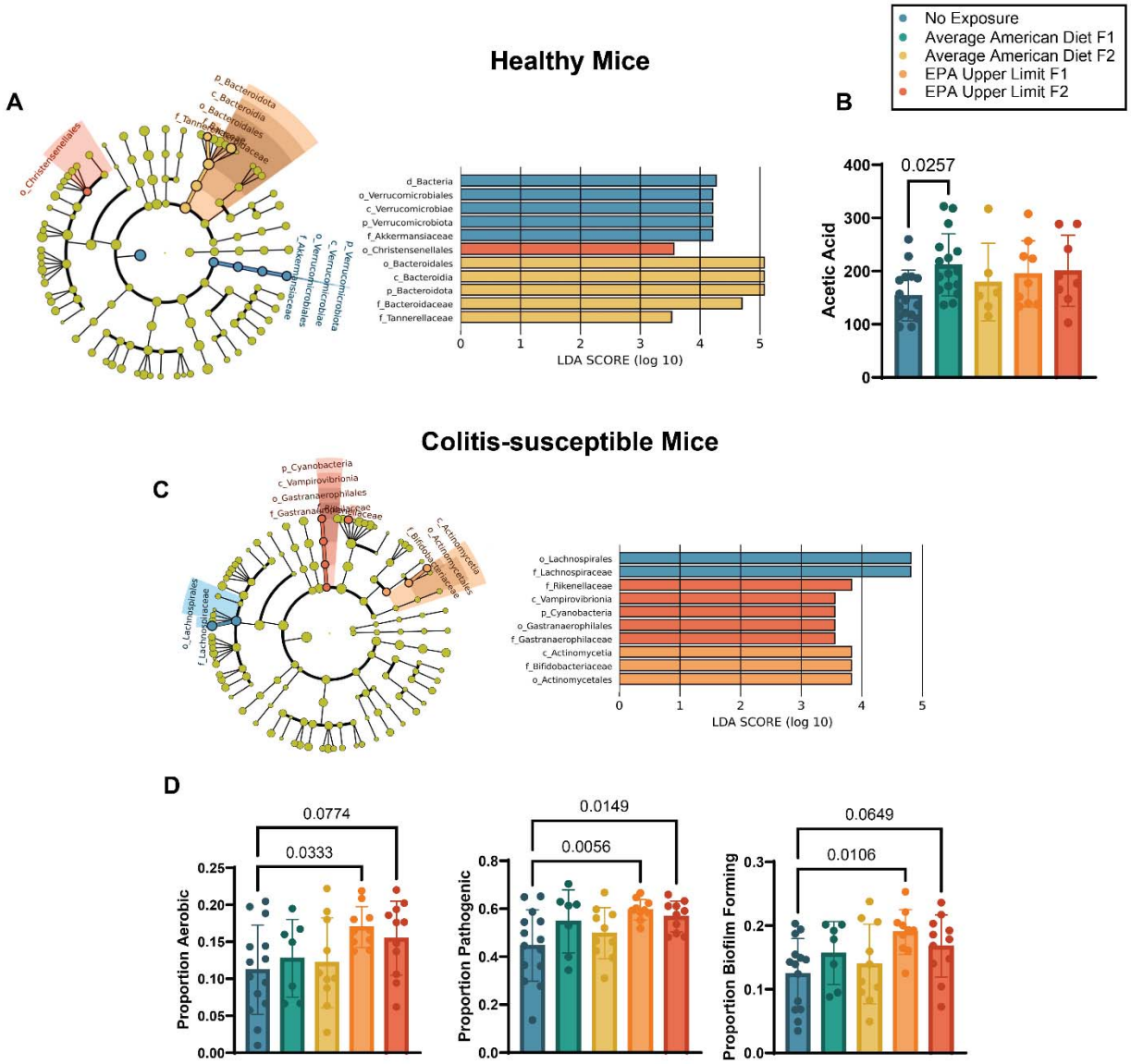

Fig. 4. Prenatal Glyphosate Exposure Induces Taxonomic Changes Within the Colon of Offspring. [A] Healthy mice display taxonomic changes, including an increased abundance of *Parabacteroides distasonis* and *P. goldsteinii* in the AAD F2 group, and increased Christensenellaceae in the EPA Upper Limit F2 group. **[B]** F2 mice within the AAD group display increased abundance of microbially derived acetic acid within the cecum (ANOVA, $p=0.064$, $\eta^2p=0.144$, FDR-correction). **[C]** Colitis-susceptible mice in the EPA Upper Limit F1 group show an increased abundance of *Bifidobacterium* spp., while those in the EPA Upper Limit F2 group show increased non-photosynthetic cyanobacteria, including Gastranaerophilales). **[D]** BugBase analysis predicts a shift in community function within colitis-susceptible mice, characterized by an increased proportion of aerobic bacteria (ANOVA, $p=0.0676$, $\eta^2p=0.167$, FDR-correction) and an increased abundance of biofilm-producing (ANOVA, $p=0.0365$, $\eta^2p=0.192$, FDR-correction) and pathogenic (Welch's ANOVA, $p=0.0137$, $\omega^2=0.370$, FDR-correction) bacteria in the EPA Upper Limit F1 group.

Discussion

Our findings reveal that prenatal glyphosate exposure, at levels previously deemed safe, induces significant health impacts in descendants. Both healthy and colitis-susceptible offspring

exhibited a shift towards a pro-inflammatory TH1/TH17 response resulting from prenatal glyphosate exposure. These findings contrast with other studies examining the generational effects of glyphosate within a murine asthma model, which showed reduced IL-17 and IFN-γ levels in the lungs of F1 generation female mice exposed to 0.5 mg/kg of glyphosate.[39] This discrepancy may be due to the pleiotropic nature of cytokines and their ability to elicit tissue-specific effects. Additionally, this divergence may be due to the non-monotonic dose response often seen with environmental toxins, including glyphosate, where effects appear at both low and high doses but may appear as a blended phenotype or disappear altogether at intermediate doses.[40]

Additionally, both F1 and F2 offspring from AAD and EPA Upper Limit groups demonstrated a marked reduction in serum GLP-1, as well as changes in serum leptin and ghrelin levels, and this was associated with altered glucose metabolism and increased insulin resistance. These findings align with a prospective cohort study using CHAMACOS data, which showed that early-life glyphosate exposure was associated with metabolic syndrome by age 18. Interestingly, urinary levels of glyphosate or AMPA at other time points, including 14 and 18 years of age, did not correlate with an increased incidence of metabolic disease, suggesting that a critical window of susceptibility may exist during fetal and early child development.[41]

Agricultural workers are at an increased risk of developing Parkinson's disease and individuals have developed Parkinsonian-like symptoms following acute high-dose glyphosate ingestion.[42,43] Currently, there are no animal experiments linking glyphosate with Parkinson's disease, though numerous animal studies have been conducted examining glyphosate toxicity. One plausible reason is the extent of damage necessary to elicit overt Parkinsonian-like symptoms. In the case of damage inflicted on the nigrostriatal system, parkinsonian signs arise after extensive damage. For example, animals appear healthy if 40% of cells have died within that region. It is not until 60-70% of cells have been lost in that region that animals begin displaying symptoms.[44] Thus, it is possible that prenatal glyphosate exposure induces sub-toxic changes within the brain, resulting in minor motor deficits.

Kynurenine is implicated in many processes, including behavior, memory, and inflammation. Insufficient levels of kynurenine may lead to reduced levels of kynurenic acid, an NMDA receptor antagonist with neuroprotective properties. Additionally, reductions in kynurenine can disrupt the balance of neuroactive metabolites, including quinolinic acid (neurotoxic) and kynurenic acid (neuroprotectant), leading to neuroinflammation and excitotoxicity, which are detrimental to cognitive functions, including memory.

Prenatal glyphosate exposure moderately exacerbated colitis in susceptible animals; however, unlike their healthy littermates, *Muc2*$^{-/-}$ mice did not exhibit any metabolic or behavioral changes. This may be attributed to the pre-existing abnormalities *Muc2*$^{-/-}$ mice display compared to healthy animals. Specifically, *Muc2*$^{-/-}$ mice demonstrate deficits in insulin signaling, leading to insulin hypersensitivity, which may mask the insulin resistance induced by glyphosate exposure.[45] Furthermore, *Muc2*$^{-/-}$ mice display increased exploratory behavior, including more frequent rearing and cover greater distances during the open field test compared to wild-type mice, again counteracting the phenotypes observed following glyphosate exposure.[46] Despite the absence of overt metabolic and behavioral changes, *Muc2*$^{-/-}$ mice were still able to transmit these phenotypic effects to their healthy offspring.

Considering our findings on prenatal glyphosate exposure, it is evident that current safety thresholds may not adequately protect against potential health risks. Our study highlights the urgent need for policy reassessment and stricter regulation to safeguard public health from the

cumulative effects of glyphosate exposure, particularly at levels considered safe under current guidelines. Addressing these concerns is crucial for ensuring comprehensive protection against the broad health impacts associated with agricultural chemical exposure.

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Table 1: Calculation of the AAD dose. A hypothetical menu for an active female > 20 years old (60kg) following the American food guide.

Meal	Amount	Glyphosate Detected (ppb)	Total (µg/serving)
Breakfast			
Cheerios	1 cup (27g)	577[11]	15.6
1% Milk	1 cup	-	-
Apple, whole	1 medium	-	-
Snack			
Ritz crackers	10 (32g)	569[11]	18.2
Fontaine Sante roasted garlic hummus	1/4 cup (60g)	760[11]	45.6
Water	No limit	-	-
Lunch			
PC Blue Menu Tortilla (100% whole grain)	2 (85g)	744[11]	63.2
Salsa	2 tbsp	-	-
Ground beef (grain fed and finished)	1/2 cup (75g)	5,000*[12]	375
Mozzarella cheese	2 ounces	-	-
Carrot/celery sticks	1 cup	-	-
Snack			
Tim Hortons glazed Timbit	6 (150g-25g each)	209[11]	31.4
Decaf coffee	1 cup	-	-
Honey	1 tbsp (21g)	64[13]	1.34
365 coffee cream	2 tbsp (30g)	104[14]	3.12
Dinner			
Grilled salmon	4 oz	-	-
Lentil salad	1/2 cup (80g)	284[15]	22.7
Green beans	1 cup	-	-
Teriyaki sauce (with soy)	2 tsp (8g)	242[16]	1.93
Apple crisp made with oatmeal	1/2 cup (50g)	135[11]	6.75
Water	No limit	-	-
Snack			
Ritz crackers	10 (32g)	569[11]	18.2
Cream cheese	2 tbsp	-	-
Apple, sliced	1/2 medium	-	-
Total (µg/day)			**603.02**
Total (mg/kg/day)			**0.01**

***Maximum Allowable Amount**

Table 2: Glyphosate levels in commercially available foods and laboratory rodent chow using our modified UPLC detection method.

Sample Matrix	Concentration of Glyphosate (µg/g)	Concentration Glyphosate (ppb)
Red Lentils	0.245	245
Picolab® 5053 Mouse Diet	0.381	381
Desiccated Wheat	2.445	2445
Nondesiccated Wheat	ND	ND

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