Autism and Glyphosate: Connecting the Dots.

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Outline

• Introduction
• Glyphosate as a Glycine Analogue
• Autism and the Gut
• Heparan Sulfate Deficiency
• Impaired Calcium Channels
• Other Issues
• Summary
Introduction

Roundup and GMO Crops

GMO Roundup-Ready corn, soy, canola, sugar beets, cotton, tobacco and alfalfa

What is glyphosate?
Glyphosate vs. Other Pesticides: Usage in the United States*


Glyphosate is Pervasive in Our Food Supply!
Main Toxic Effects of Glyphosate*

- Disrupts gut microbiome increasing pathogens
- Interferes with synthesis of aromatic amino acids and methionine by gut microbes
  - Leads to shortages in critical neurotransmitters (serotonin, dopamine, melatonin, epinephrine) and folate
- Interferes with function of cytochrome P450 (CYP) enzymes in the liver
- Chelates important minerals (iron, cobalt, manganese, etc.)
- Disrupts sulfate synthesis and sulfate transport

*Samsel and Seneff, Entropy 2013, 15, 1416-1463

Autism Prevalence: 6 year olds

glyphosate is total of year indicated + 3 previous years

$R = 0.9972$, $p <= 2.366e-07$

*Plot provided by Nancy Swanson, with permission

Data sources: autism: US Department of Education; Glyphosate: US Department of Agriculture
• Acetaminophen depletes sulfate and glutathione
• Oral antibiotics and glyphosate disrupt gut microbes
• Glyphosate inhibits aromatase that converts androgen to estrogen
• Placental/postnatal estrogens:
  – Dehydrate and mature brain myelin sheaths
  – Mature corpus callosum
  – Elevate brain serotonin and oxytocin
• Estrogen depletion leads to extreme male brain, low brain blood flow, hyperexcitability and social anxiety

Recapitulation

• Glyphosate is by far the most used herbicide on the planet
• Its exponential growth over the past two decades matches perfectly with the growth in autism in the US
  – Glyphosate is pervasive in the food supply
• Glyphosate disrupts mineral homeostasis, liver CYP enzymes and the gut microbiome
• Synergistic effects with antibiotics and Tylenol lead to estrogen insufficiency and impaired development of corpus callosum and myelin sheath in the brain
  – Hypermale brain, low serotonin, anxiety and excitability
Glyphosate as a Glycine Analogue

Glyphosate is a non-coding amino acid analogue of glycine

\[ \text{HO-CH₂-COOH} \]  

Glycine \textit{sate}
What If Glyphosate Could Insert Itself Into Protein Synthesis by mistake???

Any proteins with conserved glycine residues are likely to be affected in a major way.

Extra Piece Sticks Out at Active Site

Substrate no longer fits in active site.
Multiple species of bacteria and multiple species of weeds have developed resistance to glyphosate by swapping out a crucial glycine residue in the enzyme EPSP synthase in the shikimate pathway, replacing it with alanine.*

A bacterial gene coding for alanine instead of glycine is the basis of the GMO Roundup-Ready crops**


Inhibition of EPSPS by glyphosate:
Resistant E. coli mutant*

Wild type with glycine 96
Mutant with alanine substituted for glycine 96

*Figure 3, S Eschenburg et al. Planta 2002;216:129-135.
Quote from Monsanto Study (1989)*

- Study exposed bluegill sunfish to carbon-14 radiolabelled glyphosate
- Measured radiolabel in tissues greatly exceeded measured glyphosate levels
- Proteolysis recovered more glyphosate
  - 20% yield → 70% yield

"Proteinase K hydrolyses proteins to amino acids and small oligopeptides, suggesting that a significant portion of the 14C activity residing in the bluegill sunfish tissue was tightly associated with or incorporated into protein."


Autism and the Gut
Autism and the Gut*

“Prospective, controlled studies suggest that as many as 70% of autistic children exhibit chronic GI-related symptoms [1,5,6] including diarrhea, laxative-dependent constipation, abdominal distension, failure to thrive, weight loss, feeding problems, and abdominal pain related to extreme irritability, aggression, and self-injury.”

Glyphosate and the Gut: Pathogen Overgrowth*

- Glyphosate is an antimicrobial agent that preferentially kills beneficial microbes, allowing pathogens to flourish in the gut.
- Immune cells invade the gut and release inflammatory cytokines.
  - This causes increased risk to inflammatory bowel diseases such as Crohn’s and ulcerative colitis.


Pathogen Overgrowth in Poultry Microbes Exposed to Glyphosate*

* Plot provided by Dr. Martin Michener.
Glyphosate and the Gut: Digestive Enzymes

- Glyphosate has been found as a contaminant in digestive enzymes trypsin, pepsin and lipase*
- Trypsin impairment prevents proteins like gluten in wheat from being digested
- Undigested proteins induce release of zonulin which opens up gut barrier**
- Zonulin lingers because trypsin is defective


Trypsin, Pepsin and Lipase are all contaminated with glyphosate*

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Glyphosate (PPB)</th>
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<tr>
<td>Pepsin (ELISA)</td>
<td>&lt;40</td>
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<td>Pepsin (GC-MS)</td>
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<td>Trypsin (ELISA)</td>
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<tr>
<td>Lipase (ELISA)</td>
<td>24</td>
</tr>
</tbody>
</table>

* A Samsel and S Seneff. Journal of Biological Physics and Chemistry 2017;17: 8-32
Trypsin, Pepsin and Lipase are all contaminated with glyphosate*

Trypsin’s activation domain contains four crucial glycine rich subdomains:*  
- N-terminus to Gly 19  
- Gly 142 to Pro 152  
- Gly 184 to Gly 193  
- Gly 216 to Asn 223

*A Samsel and S Seneff. Journal of Biological Physics and Chemistry 2017;17: 8-32

A Scenario for Gluten Intolerance

Leaky brain barrier  
Autoimmune neurological disease  
Glycoside in wheat  
Zonulin released  
Leaky barrier  
Trypsin defective  
Gliadin  
Systemic immune response induces multiple complex symptoms
**Glyphosate and Celiac Disease**


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## Celiac Disease, Glyphosate and Non Hodgkin’s Lymphoma

- Glyphosate preferentially kills *Bifidobacteria* *
- Bifidobacteria are depleted in celiac disease **
- Celiac disease is associated with increased risk to non Hodgkin’s lymphoma ***
- Glyphosate itself is also linked directly to non Hodgkin’s lymphoma ****

**** M. Eriksson et al., Int J Cancer. 2008 Oct 1;123(7):1657-63.
Glyphosate Induces Antibiotic Resistance*

- Actinobacteria produce a free radical scavenger in response to glyphosate that provides resistance to a wide range of antibiotics, including penicillin
- E. coli exposed to glyphosate develop an "efflux pump" that increases resistance to the fluoroquinolone Ciprofloxacin and the aminoglycoside Kanamycin.
  – Same effect observed in Salmonella exposed to glyphosate

Glyphosate Usage and Papers on Antibiotic Resistance*

A BTBR Mouse Model of Autism*

These mice had all the mouse features of autism
They were fed “standard rodent chow” – glyphosate contaminated?

Some features in the gut:
• Reduced levels of bile acids (due to impaired CYP7A1 activity in the liver)
• Further reduced levels of secondary bile acids (impaired metabolism by gut microbes)
• Reduced levels of Lactobacillus and Bifidobacteria (microbes that metabolize bile acids)
  – These microbes are preferentially killed by glyphosate
• Serotonin deficiency (due in part to tryptophan conversion to kynurenine to fight infection)
  – Serotonin is derived from tryptophan, a product of the shikimate pathway which glyphosate disrupts

Glyphosate Disrupts Cytochrome P450 (CYP) Enzymes*

- Glyphosate has been shown to severely suppress CYP enzymes in rat liver
- CYP enzymes have a unique FXXGXRXCXG motif with two and sometimes three critical glycine residues**

**BTBR mice have low acetate, and glyphosate disrupts acetate synthesis in gut**

Children with autism had only 3.5 mg/ml acetate in stool samples compared to 5.1 in controls.

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Elevated pH linked to glyphosate exposure results in small intestinal bacterial overgrowth (SIBO)

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*LN Nielsen et al. Environmental Pollution 2018;233:364e376.*

*Adams et al. BMC Gastroenterology 2011; 11:22.*
Acetate synthesis by gut microbes depends on glycine and folate*

"The glycine is converted to serine by the addition of methylenetetrahydrofolate, and the resulting serine is converted to pyruvate, which is decarboxylated to form acetate."


Sulfur Reducing Bacterial Overgrowth with Diet High in Simple Sugars and Fat*

- Mouse study, two diets
  1. High fat, high simple sugars
  2. Low fat, high complex carbohydrates
- Mice fed diet (1) had overgrowth of Desulfovibrio due to extraction of sulfate from host mucins
  - Associated with low levels of short chain fatty acids, acetate and propionate
- High levels of H2S led to suppression of cytochrome c oxidase in the mitochondria of host colonic cells
  - Lower metabolic activity; Reduced uptake of nutrients.
- These results are consistent with observations of Desulfovibrio overgrowth, low acetate and propionate, and reduced nutrient uptake linked to autism

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Cheap vegetable-based fats (corn oil, soybean oil, cottonseed oil, canola oil) and simple sugars (cane sugar, beet sugar, high fructose corn syrup) can all be expected to be contaminated with glyphosate

Dissimilatory sulfate reduction induced by glyphosate

- **Multiple enzymes involved in **assimilatory** sulfate reduction in E coli are disrupted by glyphosate (PAPS reductase, APS kinase, sulfite reductase)**
  - Causes deficiency in sulfur-containing amino acids
  - Leads to increase in Desulfovibrio and Bilophila wadsworthia species
    - **Dissimilatory** sulfate reduction → excessive hydrogen sulfide gas → brain fog
  - Disrupted sulfur assimilation leads to impaired iron absorption**
    - Iron deficiency anemia is an epidemic worldwide

** BH Hudson et al. PNAS 2018 ePUB ahead of print.
Myosin in the Gut

• Myosin is a motor protein found in high levels in skeletal muscles
• Myosin is also essential for gut motility (peristalsis) and for the release of bile acids into the upper intestine
• Myosin contains a highly conserved glycine at position 699 *
  – If this is changed to alanine, the proteins’ contractile ability is reduced to less than 1%.
• Glyphosate has been shown in fish studies to suppress myosin expression**

**Ana Paula Rezende dos Santos et al., Chemosphere 2017;168:933e943.

Myosin in the Gut

• Myosin is a motor protein found in high levels in skeletal muscles
• SIBO (Small Intestinal Bacterial Overgrowth) is associated with impaired peristalsis*
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Evidence Linking Autism to Clostridia Overgrowth*

- 14 autistic children with gut disorder compared to 21 controls
- Significant increase in \textit{Clostridia} species in the gut in autistic children
- Associated with reduced tryptophan levels and increased expression of inflammatory markers
  - Tryptophan is a product of the shikimate pathway, which glyphosate blocks
  - Macrophages in inflamed tissue take up tryptophan, reducing bioavailability to the brain
- Proposed role for antibiotics
  - Glyphosate is a patented antimicrobial agent (2010)

*RA Luna et al., Cellular and Molecular Gastroenterology and Hepatology 2017;3(2): 218-230

CASE REPORT

Elevated Urinary Glyphosate and Clostridia Metabolites With Altered Dopamine Metabolism in Triplets With Autistic Spectrum Disorder or Suspected Seizure Disorder: A Case Study *

William Shaw, PhD

- Triplets: two boys, one girl. Both boys have autism and girl has seizure disorder
- Very high levels of glyphosate in urine in all three
- \textit{Clostridia} overgrowth due to glyphosate disruption of gut microbes
  - Clostridia produce toxins HPHPA and p-cresol, which block the conversion of dopamine to norepinephrine.
  - Damage to neurons in the brain through oxidative stress

*W. Shaw. Integrative Medicine 2017;16(1);50-57.
Recapitulation

- Autism is linked to multiple GI issues
- Glyphosate induces leaky gut and disrupts digestive enzymes, leading to autoimmune disease and gluten intolerance
- Glyphosate preferentially harms beneficial bacteria, particularly Bifidobacteria
- Glyphosate suppression of myosin and bile acids leads to impaired peristalsis and SIBO
- Glyphosate causes overgrowth of Clostridia and Desulfovibrio resulting in toxic metabolites
- Impaired supply of acetate, sulfate, sulfur-containing amino acids, folate and serotonin cause multiple symptoms
Heparan Sulfate Deficiency

Sulfate in Fetal Development*

- Fetus depends on mother for sulfate supply
- Sulfate is essential for transporting sterols (like estrogen and DHEA) and supplying extracellular matrix proteins everywhere with sufficient negative charge
- Sulfate detoxifies xenobiotics like acetaminophen (tylenol) and is essential for excreting toxins like aluminum and mercury
- Sulfate is severely deficient in autistic children (1/3 the normal level of free sulfate in blood stream)

“Heparan sulfate deficiency in autistic postmortem brain tissue from the subventricular zone of the lateral ventricles”*

“Aberrant extracellular matrix glycosaminoglycan function localized to the subventricular zone of the lateral ventricles may be a biomarker for autism, and potentially involved in the etiology of the disorder.”

*BL Pearson et al., Behav Brain Res. 2013;243:138-45

New neurons develop from stem cells in this zone through the action of “fractones” composed of heparan sulfate proteoglycans (HSPGs)**

**F. Mercier et al., Neuroscience Letters 506 (2012) 208–213
Impaired Dendrite Outgrowth in Autism could be Due to Glycine Substitution by glyphosate

- Major brain HSPG syndecan-2 mediates dendritic outgrowth in hippocampus*
- Heparan sulfate chains in syndecan 2 are attached to serine residues that have adjacent glycine residues**
  - Substitution of other amino acids for glycine here disrupts heparan sulfate binding
- Dendritic spines are defective in autism***

**L Zhang et al. JBC 1995; 270(45) Nov 10, 27127-27135.

The third ventricle is depleted in heparan sulfate in association with autism in both humans and mice*,**,***

*B.L. Pearson et al., Behav Brain Res. 2013 Apr 15;243:138-45.
Pineal gland normally supplies melatonin sulfate to the ventricles at night during sleep, but both melatonin and sulfate supplies are depleted by glyphosate

“Autism-like socio-communicative deficits and stereotypies in mice lacking heparan sulfate”*

- Experiment with “designer” mice: defect specifically led to impaired heparan sulfate synthesis in brain
- Mice exhibited all the classic features of autism – both cognitive and social

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- Experiment with "designer" mice: defect specifically led to impaired heparan sulfate synthesis in brain
- Mice exhibited all the classic features of autism—both cognitive and social

"Remarkably, these mutant mice recapitulate almost the full range of autistic symptoms, including impairments in social interaction, expression of stereotyped, repetitive behavior, and impairments in ultrasonic vocalization."


“Reduced sulfate plasma concentrations in the BTBR T+tf/J mouse model of autism”*

- BTBR mice were created through multiple generations of inbreeding among laboratory mice
  – Mice likely fed glyphosate-contaminated mouse feed
  – They exhibit autistic behaviors
  – They have multiple gut issues as previously discussed
- They also have low plasma sulfate and reduced heparan sulfate in the brain ventricles
- Their corpus callosum fails to develop

"Since previous work has suggested that blood sulfate concentration may determine the rate of sulfation [36], we hypothesize that BTBR deficiencies in plasma sulfate may disturb processes related to properly sulfated glycoaminoglycans and metabolism of neurotransmitters."*

**Vitamin D Prevents Sulfate Wasting***

- Activated vitamin D prevents sulfate wasting from the kidney in urine
- Mice engineered to have defective vitamin D receptors or with vitamin D deficiency had significantly reduced serum sulfate levels
- This was associated with sulfate depletion in the skeleton


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**Inflammation and Vitamin D: The Infection Connection***

- The problem appears to be a bottleneck in the production of 25(OH) vitamin D in the liver
  
  \[ \text{D3} \rightarrow \text{25(OH)D3} \rightarrow \text{1,25(OH)2D3} \]

  Liver \hspace{1cm} Kidney

- Both steps depend on CYP enzymes
- Liver CYPs are destroyed by glyphosate and aluminum??
  
  \[ \rightarrow \text{1,25(OH2)D3} >> \text{25(OH)D3} \]

* M. Mangin et al., Inflamm Res 2014; 63:803-819.
Taurine → Heparan sulfate

• Taurine is a non-coding amino acid and the only sulfonated amino acid
  – It is stored in large amounts in the heart and brain
• The heart releases taurine during a heart attack, and seizures induce taurine release by the brain
• Taurine is conjugated to bile acids in the liver and shipped to the gut
• Gut microbes (mainly Bifidobacteria) deconjugate taurine and metabolize it to sulfate
• Most melatonin and serotonin in the body is produced in the gut, and conjugated to sulfate before being shipped to the brain
  – Does this supply sulfate to the brain??


In a study on E coli exposed to glyphosate, the most highly suppressed enzyme observed was a taurine transporter*

Recapitulation

- Sulfate is essential in fetal development and to protect from toxic chemicals
- Heparan sulfate is deficient in the brain ventricles in both humans and mice with autism
- Sulfate supply to brain depends on serotonin sulfate and melatonin sulfate, which are disrupted by glyphosate
- Vitamin D protects from sulfate wasting
  - Activation by CYP enzymes in liver impaired by glyphosate
- Taurine buffers sulfate in the brain and heart
  - Gut microbes that are damaged by glyphosate (e.g., Bifidobacteria) metabolize it to sulfate

Impaired Calcium Channels
Timothy Syndrome* 

- A complex multisystem disorder
- A glycine mutation in the gene that encodes Ca(V)1.2 L-type calcium channel leads to loss of channel inactivation and intracellular calcium overload in various cell types.
- Multi-organ dysfunction:
  - Congenital heart disease, immune deficiency, irregular sleep patterns, hypoglycemia, cognitive abnormalities and autism


A Calcium Channel Glycine Mutation: Timothy Syndrome*

This single mutation of a glycine residue causes severe disability and early death

“CaV1.2 Calcium Channel Dysfunction Causes a Multisystem Disorder Including Arrhythmia and Autism”*

- Single mutation in calcium channel: G406R = Timothy Syndrome
- Long QT syndrome and life-threatening cardiac arrhythmias
  - *Disrupted timing of the cardiac action potentials*
- Intermittent hypoglycemia - life threatening
  - *Disrupted insulin signaling in the pancreas*
- Developmental issues: congenital heart diseases and syndactyly

- These children typically don't survive beyond early childhood
- *Very high rate of autism*


More Highly Conserved Glycines in Calcium Channel*

*Figure 1. J Teng et al. Biochimica et Biophysica Acta 2010;1798: 966-974.
If this glycine residue is replaced by a negatively charged amino acid (e.g., glyphosate), it causes a similar problem as Timothy Syndrome: excessive calcium entry.

Calcium mediates processes as diverse as synaptic transmission, muscle contraction, insulin secretion, fertilization, and gene expression.
"The pesticide increased intracellular Ca2+ concentration by opening *L-type voltage-dependent Ca2+ channels* ... leading to Ca2+ overload within the cells, which set off oxidative stress and necrotic cell death."

"In GBH [glyphosate-based herbicide]-poisoned persons, a high incidence of *QTc interval prolongation* and conduction blocks (from minimal to high grade) were reported along with arrhythmias, longer QTc and older age predicting mortality."

"Long-QT Syndrome!"
**Glyphosate, Calcium Channels and Glutamate***

- Acute exposure to the hippocampus in the brain activates NMDA receptors and *voltage-dependent calcium channels*
  - Oxidative stress and neural cell death
  - Increased glutamate release into the synaptic cleft → neurotoxicity

*Figure 9, D. Cattani et al. / Toxicology 320 (2014) 34–45*
Glyphosate and Glutamate

*Figure 9, D. Cattani et al. / Toxicology 320 (2014) 34–45

Glyphosate inhibits glutamine synthetase (GS) possibly through manganese chelation

Recapitulation

• Timothy syndrome is a rare disorder that can be caused by a glycine mutation in the L-type calcium channel
  – It is associated with congenital heart defects, immune deficiency, long QT syndrome and autism
• Three other highly conserved glycine residues in this calcium channel are also essential for its proper function.
  – Mutations lead to excessive calcium uptake
• Glyphosate has been shown to induce excessive calcium uptake in experiments on multiple cell types, Sertoli cells in the testes, cardiomyocytes, leading to long QT syndrome, and neurons, leading to glutamate toxicity
Other Issues

• Glyphosate chelates manganese
  – Autism is associated with low manganese in the hair, teeth and urine
  – Manganese is needed for glutamine synthesis, and glutamine is depleted in autism
  – Manganese deficiency also leads to impaired ammonia clearance by the liver, linked to autism
• Glyphosate’s antibacterial effects lead to yeast overgrowth, a common feature of autism
• Glyphosate interferes with folate synthesis and with synthesis of methionine by gut microbes, causing methylation deficiencies

Summary

• Glyphosate is pervasive in our environment and its exponential growth matches the alarming increase in autism in the US
• Glyphosate’s disruption of liver CYP enzymes impairs detoxification of other chemicals, disrupts bile acids, and prevents vitamin D activation
• Glyphosate may show an insidious cumulative toxic effect by erroneously substituting for glycine during protein synthesis
• Multiple mouse studies have demonstrated a link between gut dysbiosis and impaired neurodevelopment in autism
  – Severe deficiency in heparan sulfate in the ventricles and defective or nonexistent corpus callosum
• Overgrowth of gut pathogens leads to accumulation of toxic metabolites that disrupt neurotransmitter signaling and cause brain fog
• Glyphosate has been shown to overexcite calcium channels in studies on neurons, Sertoli cells and cardiomyocytes
  – Calcium channel glycine mutations are linked to autism