Glyphosate and Autism: An Indisputable Link and a Path Towards Healing

Stephanie Seneff
MIT CSAIL
AutismOne, May 2019

Outline

• Introduction
• Glyphosate as a Glycine Analogue
• Glyphosate and the Gut
• Glyphosate, Sulfate, Oxalate, Autism
• Glyphosate and Vaccines
• What You Can Do
Introduction

Autism Prevalence: 6 year olds*

glyphosate is total of year indicated + 3 previous years
\[ R = 0.9972, p \leq 2.366e-07 \]

\[
\begin{array}{c|c|c|c|c|c|c|c|c|c|c}
\hline
\text{Autism Age 6} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\text{Glyphosate (4 year total)} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\end{array}
\]

\[ R = 0.9972 \]

* Figure 15, Seneff et al., Agricultural Sciences, 2015, 6, 42-70
Some Foods Containing Glyphosate

Is Glyphosate Toxic?

• Monsanto has argued that glyphosate is harmless to humans because our cells don’t have the shikimate pathway, which it inhibits

• However, our gut bacteria DO have this pathway
  – We depend upon them to supply us with essential amino acids (among many other things)

• Other ingredients in Roundup greatly increase glyphosate’s toxic effects and are themselves toxic

• Insidious effects of glyphosate accumulate over time
  – Most studies are too short to detect damage
Main Toxic Effects of Glyphosate*

- Disrupts gut microbes leading to overgrowth of pathogens and inflammatory/leaky gut syndrome
- Interferes with function of cytochrome P450 (CYP) enzymes
- Chelates important minerals (iron, cobalt, manganese, etc.)
- Interferes with synthesis of aromatic amino acids and methionine
  - Leads to shortages in critical neurotransmitters and folate
- Disrupts sulfate synthesis and sulfate transport
  - Leads to disrupted bile flow and impaired fat digestion

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

Glyphosate vs. Other Pesticides:
Usage in the United States*

Sobering Statistics on Glyphosate Residues*

- Parts per *trillion* (*ppt*): increased proliferation of breast cancer cells in vitro
- 0.1 ppb:
  - Altered the gene function of over 4000 genes in the livers and kidneys of rats
  - Severe organ damage in rats
  - Permitted level for glyphosate and all other herbicides in EU tap water
- 10 ppb: demonstrated toxic effects on the livers of fish
- 700 ppb: Permitted level for glyphosate in U.S. tap water
- 11,900 ppb: found in Genetically Modified (GMO) soybeans

*http://detoxproject.org/glyphosate-in-numbers/

Some Biomarkers for Autism

- Disrupted gut bacteria; inflammatory bowel
- Low serum sulfate
- Methionine deficiency
- Serotonin and melatonin deficiency
- Defective aromatase
- Zinc and iron deficiency
- Urinary p-cresol
- Mitochondrial disorder
- Glutamate toxicity in the brain

These can all be explained as potential effects of glyphosate on biological systems
Dementia and Autism Have Much in Common

Deaths from Senile Dementia (ICD F01, F03 & 290) plotted against glyphosate applications on corn & soy (R = 0.9933, p <= 1.947e-09) sources: USDA NASS, CDC

Glyphosate as a Glycine Analogue
The Basics of Protein Synthesis

What if Glyphosate could Insert itself into Proteins during Synthesis???

-- Any proteins with conserved glycine residues are likely to be affected in a major way
This explains how glyphosate disrupts EPSPS in the shikimate pathway: Multiple bacteria have developed resistance by replacing active site glycine with alanine and this is the basis for GMO Roundup Ready crops.

*T Funke et al., Molecular basis for the herbicide resistance of Roundup Ready crops. PNAS 2006;103(35):13010-13015.
Inhibition of EPSPS by glyphosate: Resistant E coli mutant*

![Graph showing inhibition of EPSPS by glyphosate.](image)

Mutant with alanine substituted for glycine 96

Wild type with glycine 96

Activity (U/mg)

[Glyphosate] (μM)

0 0.1 1 10 100 1000 10000

*Figure 3, S Eschenburg et al. Planta 2002;216:129-135.

Only Glyphosate Works!*

“More than 1,000 analogs of glyphosate have been produced and tested for inhibition of EPSP synthase, but minor structural alterations typically resulted in dramatically reduced potency, and no compound superior to glyphosate was identified.”

Hypothesis:
These other molecules failed to work as an amino acid analogue of glycine, because they were not amino acids.

*T Funke et al. PNAS 2006; 103(35): 13010-13015.
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."


Some Predicted Consequences*

- Neural tube defects
- Autism
- Impaired collagen → osteoarthritis
- Steatohepatitis (fatty liver disease)
- Obesity and adrenal insufficiency
- Hypothyroidism
- Impaired iron homeostasis and kidney failure
- Insulin resistance and diabetes
- Cancer

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 release of bile acids into upper intestine
- Myosin contains a highly conserved glycine at position 699*
  - If this is changed to alanine, the protein’s contractile ability is reduced to less than 1%.
- Glyphosate has been shown to suppress myosin**

SIBO (Small Intestinal Bacterial Overgrowth) is associated with impaired peristalsis***

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

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**

CYP enzymes are needed to produce bile acids for digesting fats, to activate vitamin D and to detoxify many environmental toxicants

Protein Phosphatase I and Autism

- Glyphosate exposure to rat dams led to thyroid deficiency in pups*
  - Attributed to suppressed release of thyroid stimulating hormone (TSH) from the dam’s pituitary
- Thyroid deficiency in mom during pregnancy predicts significant increased risk to autism in child**
- Glyphosate link to impaired TSH due to disruption of protein phosphatase I***

*JS deSouza et al., Toxicology To Appear, 2017

Protein Phosphatase 1 Has Many Highly Conserved Glycines!*
Protein Phosphatase 1 Has Many Highly Conserved Glycines!*  

PP1 also depends on manganese as a catalyst, which glyphosate chelates, making it unavailable.

*Figure 1, Y. Shi, Cell 2009;139: 468-484.

Glyphosate 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>
</tr>
</thead>
<tbody>
<tr>
<td>Pepsin (ELISA)</td>
<td>&lt;40</td>
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<tr>
<td>Pepsin (GC-MS)</td>
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<tr>
<td>Pepsin (HPLC-MSMS)</td>
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<tr>
<td>Trypsin (ELISA)</td>
<td>62</td>
</tr>
<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
A Scenario for Gluten Intolerance

- Leaky barrier
- Zonulin
- Trypsin defective
- Zonulin released
- Gliadin
- Leaky brain barrier
- Autoimmune neurological disease
- Systemic immune response induces multiple complex symptoms

Glyphosate and Celiac Disease*

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.

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*Figure 20, NL Swanson et al. Journal of Organic Systems 9(2), 2014, p. 25.*
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
  – Serotonin is derived from tryptophan, a product of the shikimate pathway which glyphosate disrupts


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.**

*LN Nielsen et al. Environmental Pollution 2018;233:364e376.
**Evidence Linking Autism to Clostridia Overgrowth**

- 14 autistic children with gut disorder compared to 21 controls
- Significant increase in *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

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**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
- *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

• Glyphosate contamination in food proteins makes them hard to break down
  – This leads to autoimmune disease
• Digestive enzymes are contaminated with glyphosate
  – Undigested proteins induce Celiac disease and leaky gut
• Glyphosate is a key factor in the emergence of antibiotic resistant pathogens
• The BTBR mouse model of autism is consistent with glyphosate damage in the gut
• Glyphosate promotes Clostridia overgrowth
  – This induces inflammatory bowel disease, an epidemic today
  – Autism has been linked to Clostridia overgrowth
  – Clostridia release toxins that induce an inflammatory response

Glyphosate, Sulfate, Oxalate, Autism
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)


Thyroid and Sulfate

• Autism is associated with disrupted sulfate management → systemic sulfate deficiency*
• Glyphosate suppresses pituitary release of thyroid stimulating hormone (TSH) → hypothyroidism**
• Hypothyroidism in mom is linked to autism in child***
• Hypothyroidism causes sulfate loss in urine****

** JS de Souza et al. Toxicology. 2017 Feb 15;377:25-37.
Rosemary Waring on Autism (1990)*

“These results indicate that there may be a fault either in manufacture of sulphate or that sulphate is being used up dramatically on an unknown toxic substance these children may be producing.”


Rosemary Waring Found Extremely Abnormal Urinary Sulfur Products in Autism*

| TABLE 1. Excretion of urinary protein and anions in autism |
|-----------------|---------------|-----------------|---------------|
|                 | Autism (n = 232) | Controls (n = 68) |
| Age (years)     | 7.6 ± 2.4      | 8.5 ± 3.7       |
| Protein µg ml⁻¹ | 103.2 ± 89.9*  | 64.5 ± 27.5     |
| Sulphite        | 106.9 ± 162.9* | 2.1 ± 6.3       |
| Thiosulphate    | 130.8 ± 148.1* | 18.6 ± 25.0     |
| Thiocyanate     | 6.4 ± 16.9*    | 44.0 ± 101.0    |
| Sulphate        | 6819.0 ± 6712.3* | 3030.8 ± 1461.0 |

Anion excretion is given in nmol ml⁻¹, mean ± SD* p < 0.001 (Wilcoxon rank sum test).

Rosemary Waring Found Extremely Abnormal Urinary Sulfur Products in Autism*

50-fold increase in urinary sulfite suggests a deficiency in sulfite oxidase

<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>Protein µg ml⁻¹</th>
<th>Sulphite</th>
<th>Thiosulphate</th>
<th>Thiocyanate</th>
<th>Sulphate</th>
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Anion excretion is given in nmol ml⁻¹, mean ± SD* p < 0.001 (Wilcoxon rank sum test).


Glyphosate Plausibly Disrupts Sulfur Enzymes

Sulfite oxidase*
- Depends on molybdenum as catalyst (glyphosate chelation could make it unavailable)
- Changing glycine at residue 473 with aspartate destroys enzyme activity
  - Leads to severe impairment in ability to bind sulfite and 5-fold reduction in catalysis
  - Aspartate has similar properties as glyphosate, being bulky and negatively charged
- Defective SO leads to severe birth defects and neurological problems resulting in early death

The sulfotransferases**
- GxxGxxG motif required for binding PAPS

* H.L. Wilson et al., Biochemistry 2006, 45, 2149-2160 2149.
** K. Komatsu et al., Biochemi and Biophys Res Comm 1994;204(3): 1178-1185.
PCOS, Autism, PAPS Synthase

• PAPS synthase is essential for DHEA sulfate synthesis
• Defective PAPS synthase → polycystic ovary syndrome (PCOS) in women, high androgen*
  – Glycine 270 → aspartate mutation
• PCOS is a risk factor for autism in the woman and in her children**

*Cherskov et al. Translational Psychiatry 2018; 8:136.
**W Oostdijk et al. J Clin Endocrinol Metab. 2015;100(4):E672-80.

Autism Linked to Oxalate Crystals*

• Crystals of oxalate form kidney stones and cause great discomfort
• Study has shown at least 3-fold higher serum and urinary levels of oxalate in autistic kids**

*William Shaw, The Role of Oxalates in Autism and Chronic Disorders WAPF, March 26, 2010
Oxalate Causes Sulfate Flushing through Urine*

• Sulfate is essential for:
  – Synthesis of extracellular matrix glycoproteins
  – Synthesis of cerebroside sulfate, in myelin in nerve fibers
  – Detoxification of many environmental toxins

• Sulfate is flushed in the urine (lost) when kidney oxalate levels are high

• Oxalobacter microbes degrade oxalate but they are killed by antibiotics such as Cipro
  – Oxalate decarboxylase depends on manganese as catalyst**

**A Tanner et al. J Biol Chem. 2001;276(47):43627-34

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

• Experiment with “designer” mice: blocked heparan sulfate synthesis in brain ventricles
  – Mice exhibited all the classic features of autism – both cognitive and social

"Fractone-associated N-sulfated heparan sulfate shows reduced quantity in BTBR T+tf/J mice: a strong model of autism." **

**KZ Meyza et al., Behav Brain Res 2012;228:247–53.
“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.”

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

*BL Pearson et al., Behav Brain Res. 2013;243:138-45
**F. Mercier et al., Neuroscience Letters 506 (2012) 208–213

Is Encephalopathy a Mechanism to Renew Sulfate in Autism?*

Abstract: “This paper makes two claims:

(1) Autism can be characterized as a chronic low-grade encephalopathy, associated with excess exposure to nitric oxide, ammonia and glutamate in the central nervous system, which leads to hippocampal pathologies and resulting cognitive impairment, and

(2) Encephalitis is provoked by a systemic deficiency in sulfate, but associated seizures and fever support sulfate restoration. …”

Gut Microbes to the Rescue!

Glyphosate blocks taurine uptake into E coli microbes*

Safe Sulfate Transport: Carbon Rings

Glyphosate depletes serotonin and dopamine and disrupts enzymes involved with sterol sulfation: Imperiled sulfate transport
Recapitulation

- Sulfate plays many essential roles in the body
  - Sulfate deficiency is a core feature of autism
- Sulfate synthesis and transfer depend critically on both glycine residues and molybdenum
- PCOS due to glycine mutation is risk factor for autism
- Oxalate metabolism depends on microbial enzymes that are disrupted by glyphosate
  - High oxalate is linked to autism and causes sulfate flushing through urine
- Heparan sulfate deficiency in the brain is associated with autism in both humans and mouse models
- A low grade encephalopathy characterizes autism and may reflect the need to synthesize sulfate

Glyphosate and Vaccines
Patreon – Microbiome Vaccine Safety Project*

"Gut microbiota have a significant effect on host response to vaccination where a reduced or absent population of commensal flora coupled with an overgrowth of pathogenic strains may become a microbial predisposition to adverse vaccine reaction."

*thegutclub.org/patreon-microbiome-vaccine-safety-project/

Glyphosate in Vaccines?

- For MMR, flu vaccine, and rabies vaccine, live virus is grown on gelatin derived from ligaments of pigs
  - Pigs are fed GMO Roundup-Ready corn and soy feed
- The main component of gelatin is collagen
- By far the most common amino acid in collagen is glycine: glyphosate substitution is likely!
- There is also a significant amount of glutamate
  - Excite NMDA receptors in the brain
- Glyphosate’s known stimulation of NMDA receptors could cause neuronal burnout
### Glyphosate Contamination in Vaccines (Parts Per Billion)*

<table>
<thead>
<tr>
<th>Company</th>
<th>Vaccine</th>
<th>Glyphosate (ppb)</th>
<th>Disease(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merck</td>
<td>ZOSTAVAX</td>
<td>0.42</td>
<td>Shingles</td>
</tr>
<tr>
<td>Merck</td>
<td>MMR-II</td>
<td>2.90</td>
<td>Measles, Mumps and Rubella</td>
</tr>
<tr>
<td>Merck</td>
<td>VARIVAX</td>
<td>0.41</td>
<td>Varicella, Chicken Pox</td>
</tr>
<tr>
<td>MERCK</td>
<td>PNEUMOVAX</td>
<td>ND</td>
<td>Pneumococcal 18</td>
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<tr>
<td>MERCK</td>
<td>PROQUAD</td>
<td>0.43</td>
<td>Measles, Mumps, Rubella, Varicella</td>
</tr>
<tr>
<td>GSK</td>
<td>ENERGIX-B</td>
<td>0.33</td>
<td>Hepatitis B</td>
</tr>
</tbody>
</table>


### Measles Virus and Hemagglutinin*

- The measles virus synthesizes the protein hemagglutinin
  - Antibodies to hemagglutinin are essential following MMR vaccination to induce immunity
- Hemagglutinin bears a sequence resemblance to myelin basic protein (MBP) \( \rightarrow \) potential for autoimmune reaction
- MBP is essential for the formation of the myelin sheath surrounding nerve fibers
  - Children with a rare genetic defect involving deletion of MBP can suffer from microcephaly**
- Autoantibodies to MBP along with excessive levels of antibodies to measles hemagglutinin are linked to autism***

Autism and Measles Hemagglutinin*

• 125 autistic children and 92 control children
• 60% of the children with autism had high levels of antibodies to measles hemagglutinin specific to the MMR vaccine
  – 90% of these had autoantibodies to myelin basic protein (MBP)
• 0% of the control children had high antibody titers to either hemagglutinin or MBP
• There were no elevations in antibodies detected against any proteins in the mumps or rubella viruses


What you can do!
Go Organic!

Wholefoods:
Sign at Entrance

Foodland: organic shelf

Eat Natural Probiotic Foods

• Sauerkraut and apple cider vinegar contain acetobacter, one of the very few microbes that can metabolize glyphosate
• Kombucha and kimchi do too!
Eat Foods Containing Sulfur

Some Important Nutrients

- Curcumin
- Garlic
- Vitamin C
- Probiotics
- Methyl tetrahydrofolate
- Cobalamin
- Glutathione
- Taurine
- Epsom salt baths
Treating Glyphosate Poisoning in Animals (e.g., cows) *

Activated charcoal, bentonite clay, humic and fulvic acids, and sauerkraut juice have been shown to be effective in reducing urinary levels of glyphosate and improving animal health.

*H Gerlach et al., J Environ Anal Toxicol 2014, 5:2

Spend Time Outside in the Sunlight – Especially in the Ocean
Summary

• Glyphosate is pervasive in our food supply and in our environment
• I believe glyphosate is the most important factor in the autism epidemic
• Glyphosate may be substituting for glycine by mistake during protein synthesis, with enormous consequences
  – Insidious, cumulative toxicity
• Glyphosate disrupts the gut microbiome
• Glyphosate disrupts sulfur homeostasis
• Glyphosate causes hypothyroidism
• Glyphosate contamination in MMR may explain its observed link to autism
• Organic diet, fermented foods, sulfur-rich diet and sunlight exposure are beneficial for health