Glyphosate as a Glycine Analogue: Mechanisms of Toxicity
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Outline

• Brief Introduction
• Glyphosate as a Glycine Analogue
• Glyphosate, Sulfate, Oxalate, Thyroid, Autism
• Sulfate and Histamines
• Glyphosate and Vaccines
• Solutions
• Summary
Some Foods Containing Glyphosate

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

Extra Piece Sticks Out at Active Site

Substrate no longer fits in active site
Extra Piece Sticks Out at Active Site

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

Mutant with alanine substituted for glycine 96

*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 FXXGXRXXGXRXXG 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.


Glyphosate, Sulfate, Oxalate, Thyroid, 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.”


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Rosemary Waring Found Extremely Abnormal Urinary Sulfur Products in Autism*

**TABLE 1. Excretion of urinary protein and anions in autism**

<table>
<thead>
<tr>
<th></th>
<th>Autism (n = 232)</th>
<th>Controls (n = 68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>7.6 ± 2.4</td>
<td>8.5 ± 3.7</td>
</tr>
<tr>
<td>Protein µg ml⁻¹</td>
<td>103.2 ± 89.9*</td>
<td>64.5 ± 27.5</td>
</tr>
<tr>
<td>Sulphite</td>
<td>106.9 ± 162.9*</td>
<td>2.1 ± 6.3</td>
</tr>
<tr>
<td>Thiosulphate</td>
<td>130.8 ± 148.1*</td>
<td>18.6 ± 25.0</td>
</tr>
<tr>
<td>Thiocyanate</td>
<td>6.4 ± 16.9*</td>
<td>44.0 ± 101.0</td>
</tr>
<tr>
<td>Sulphate</td>
<td>6819.0 ± 6712.3*</td>
<td>3030.8 ± 1461.0</td>
</tr>
</tbody>
</table>

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

Glyphosate Disrupts Sulfur Enzymes

Sulfite oxidase*
• Depends on molybdenum as catalyst (glyphosate chelates it making 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.

Thyroid Deficiency and Glyphosate

• 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
• Glyphosate link to impaired TSH due to disruption of protein phosphatase 1 (PP1)**
  – PP1 is essential for TSH release

*JS deSouza et al., Toxicology To Appear, 2017.
Protein Phosphatase 1 Has Many Highly Conserved Glycines!*  

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

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. …”


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**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
- Maternal hypothyroidism is a risk factor for autism in the fetus
  - Glyphosate interferes with stimulation of thyroid gland
  - Glyphosate exposure to pregnant rats induced thyroid deficiency in pups
- 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
**Sulfate and Histamines**

**Heparan Sulfate Promotes Histamine Breakdown***

- Intestinal diamine oxidase (DAO) is synthesized in the mature enterocytes of the villus tip and is then transported to the binding sites on the intestinal vasculature.
- Heparin and heparan sulfate induce release of the bound DAO, so that it can break down histamine.
  - *Conclusion: deficiencies in heparan sulfate lead to build-up of histamines!*

Symptoms Caused by Histamines*

*Figure 1, L. Maintz and N. Novak, *Am J Clin Nutr* 2007;85:1185–96.

DAO Deficiency → Histamine Accumulation*

<table>
<thead>
<tr>
<th>System</th>
<th>Adverse Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central nervous system</td>
<td>Migraine, headache, hangover, sickness</td>
</tr>
<tr>
<td>Cardiovascular system</td>
<td>Hypotension, hypertension and arrhythmia</td>
</tr>
<tr>
<td>Epithelial system</td>
<td>Urticaria, atopic skin, psoriasis</td>
</tr>
<tr>
<td>Respiratory system</td>
<td>Nasal congestion, asthma</td>
</tr>
<tr>
<td>Digestive system</td>
<td>Irritable bowel syndrome, constipation, satiety, stomachache, vomits</td>
</tr>
<tr>
<td>Muscular system</td>
<td>Fibromyalgia, muscular pains</td>
</tr>
<tr>
<td>Skeletal system</td>
<td>Bone pains</td>
</tr>
</tbody>
</table>

*www.deficitdao.org/dao-deficiency/#.VRWYtWZGqDc
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. This may include reduced or absent protective microbiota such as Bifidobacteria, Lactobacillus and butyrate-producing Clostridia allowing immune dysregulating Bacteroides and Proteobacteria to be overgrown."

*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>Parts Per Billion</th>
<th>Description</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>
</tr>
<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>Hepatatis 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) → 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

Solutions

Go Organic!

Wholefoods: Sign at Entrance

Foodland: organic shelf
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.

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

Spend Time Outside in the Sunlight – Especially in the Ocean
Farming Solutions!*

Organic → agroecological → sustainable → regenerative agriculture

Vive la France!

4. A tax for those farmers using glyphosate amounting to 1 Euro per Kilo of glyphosate used. This is referred to as a “phytosanitary” tax on the use of a pollutant. It is anticipated that this tax will generate $50 million euros ($57 million dollars) annually to help farmers transition away from pesticide use.


Summary

• Glyphosate is pervasive in our environment and it is causing an epidemic in autism as well as many other debilitating diseases
• Hypothesis: Glyphosate has a unique mechanism of toxicity that involves substituting for glycine during protein synthesis
  – This leads to disruption of many biological pathways, most notably causing severe sulfate deficiency, systemically
  – This is a likely explanation for the strong correlations between glyphosate usage and autism rates
• Organic food, sunlight exposure, fermented foods and high sulfur diet can be effective to protect from or treat autism
• We need to go back to renewable organic agricultural methods to safeguard our families and the ecosystem