



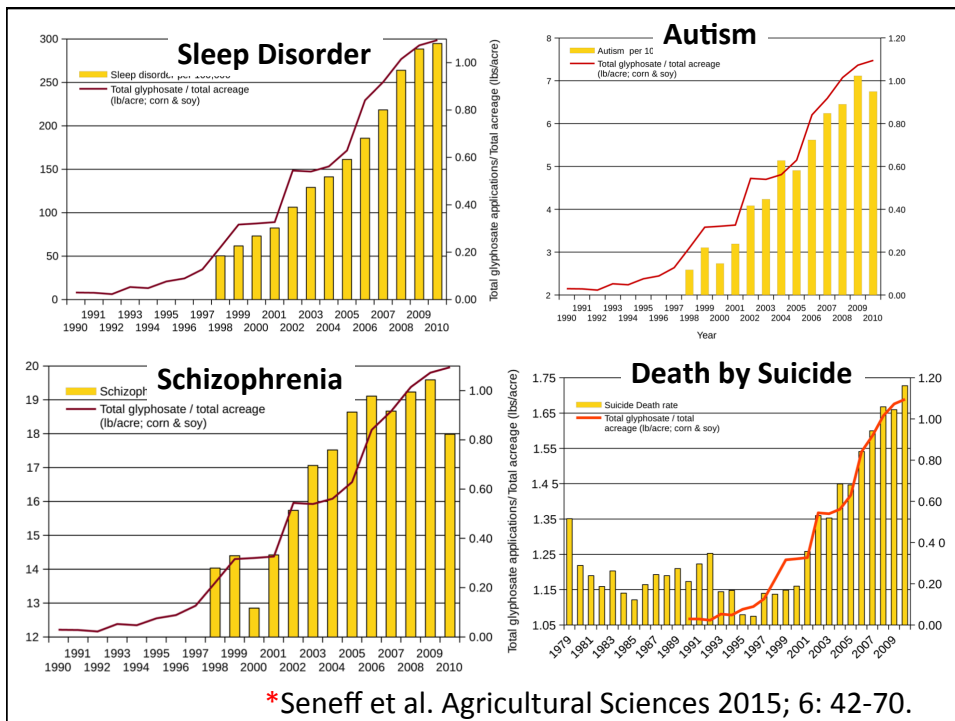
## Glyphosate as a Glycine Analogue: Mechanisms of Toxicity

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MIT CSAIL  
TACA Workshop  
March 22, 2019

### Outline

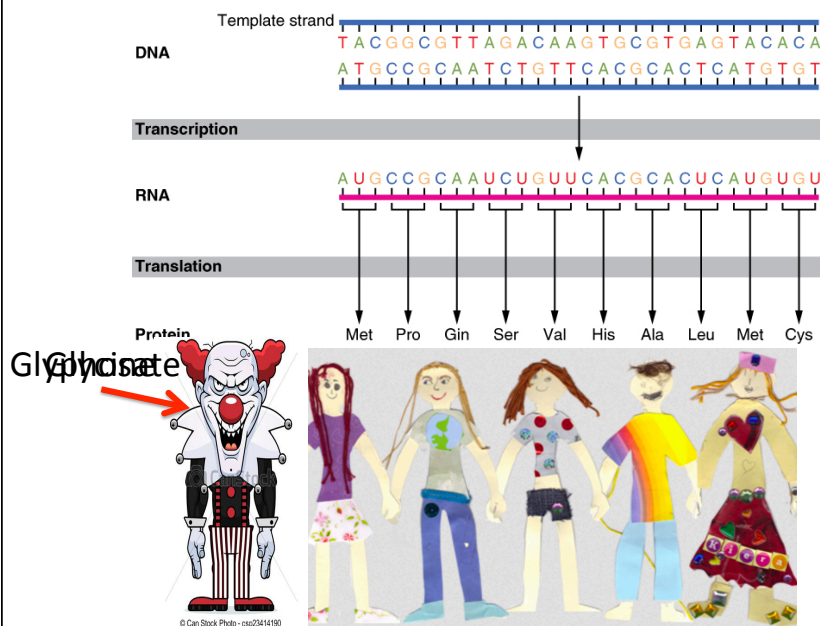
- Brief Introduction
- Glyphosate as a Glycine Analogue
- Glyphosate, Sulfate, Oxalate, Thyroid, Autism
- Sulfate and Histamines
- Glyphosate and Vaccines
- Solutions
- Summary

## Some Foods Containing Glyphosate

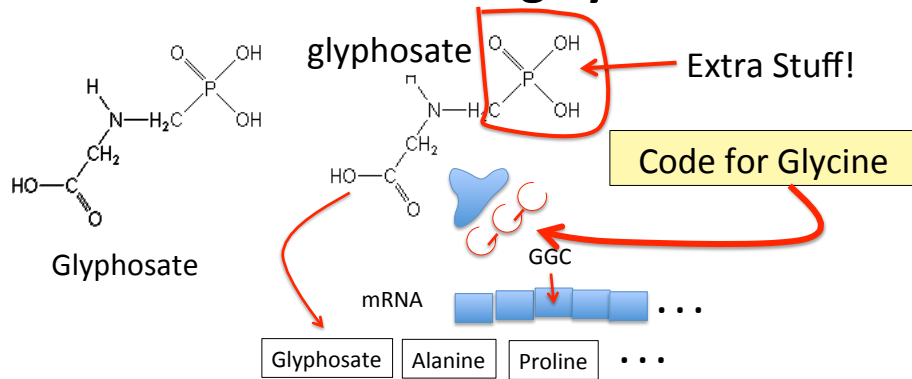


# Glyphosate as a Glycine Analogue

## The Basics of Protein Synthesis

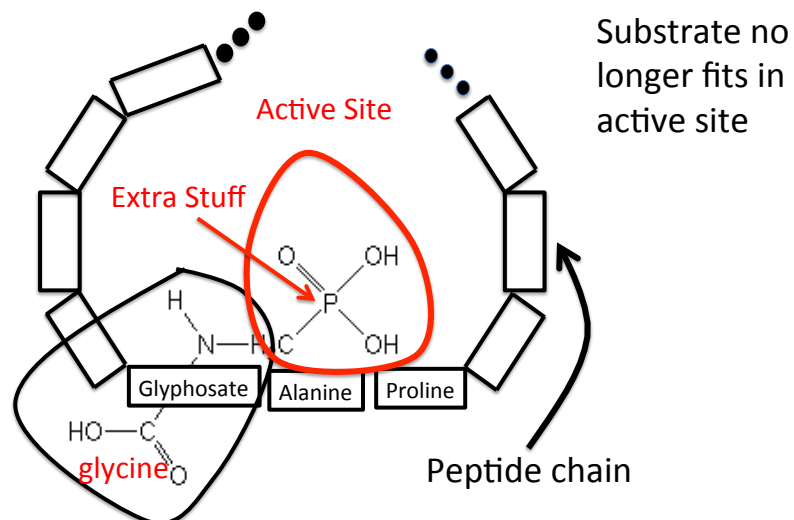


## What if Glyphosate could Insert itself into Proteins during Synthesis???



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

## Extra Piece Sticks Out at Active Site





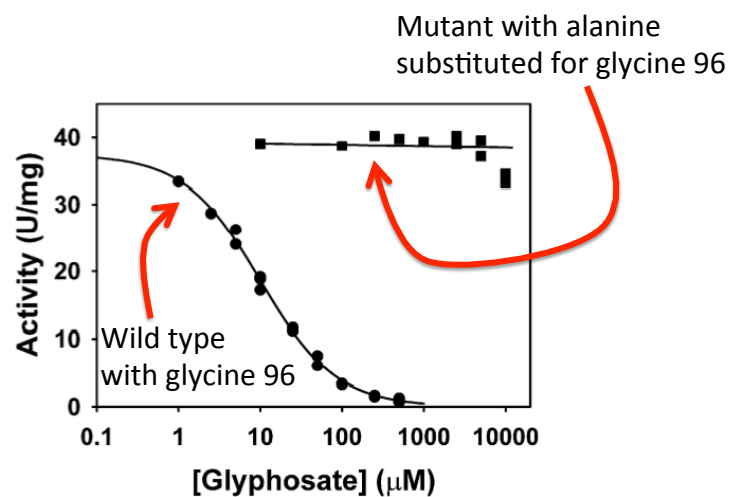
## Extra Piece Sticks Out at Active Site

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\*

Substrate no  
fits in  
site

\*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\*



\*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."

\*WP Ridley and KA Chott. Monsanto unpublished study. August, 1989.

## 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

\*A. Samsel and S. Seneff. Journal of Biological Physics and Chemistry 2016;16:9-46.

## 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\*\*\*

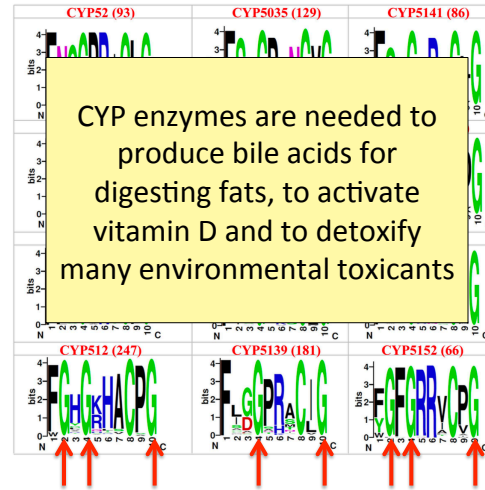
\*F Kinose et al. The Journal of Cell Biology 1996;134(4): 895-909.

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

\*\*\*AC Dukowicz et al. Gastroenterol Hepatol (N Y) 2007; 3(2): 112-122.

## Glyphosate Disrupts Cytochrome P450 (CYP) Enzymes\*

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



\*A Samsel and S Seneff. Entropy 2013; 15: 1416-1463.

\*\*K Syed and SS Mashele. PLOS ONE 2014; 9(4):| e95616.

**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)

\* PA Dawson, "Sulfate in Fetal Development,"  
Semin Cell Dev Biol 2011;22(6): 653-9.

## 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\*\*\*\*

\*RH Waring and LV Klovrza. J Nutr & Environ Med 2000; 10: 25-32.

\*\*JS de Souza et al. Toxicology. 2017 Feb 15;377:25-37.

\*\*\*GC Román, Ann Neurol 2013;74(5):733-42.

\*\*\*\*K Sagawa et al. Am J Physiol. 1999 Jan;276(1 Pt 2):F164-71.

## 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 .”

\*p. 198, O'Reilly, B.A.; Waring, R.H. Enzyme and sulphur oxidation deficiencies in autistic children with known food/chemical intolerances. *Xenobiotica*. 1990, 20, 117–122.

## 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 $\mu\text{g ml}^{-1}$	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  $\text{nmol ml}^{-1}$ , mean  $\pm$  SD\*  $p < 0.001$  (Wilcoxon rank sum test).

\* RH Waring and LV Klovrza. Journal of Nutritional & Environmental Medicine 2000; 10: 25-32.

## 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

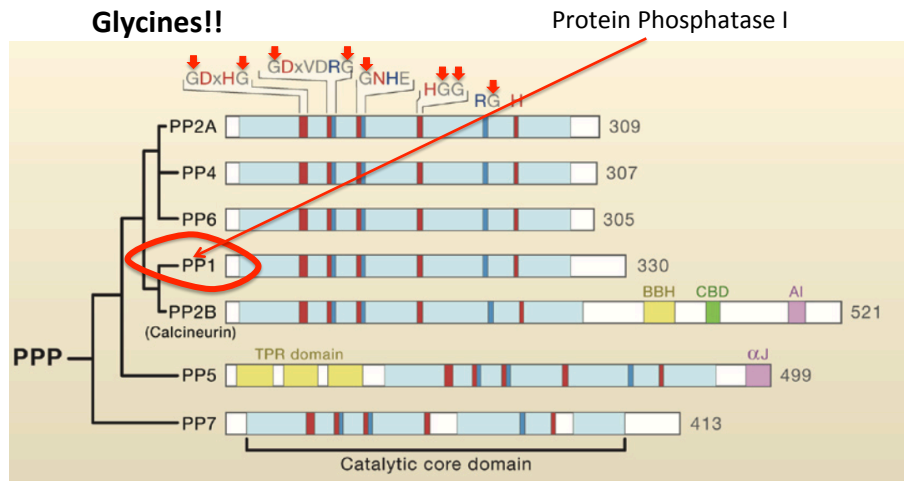
- Glyphosate exposure to rat dams led to thyroid deficiency in pups\*
  - Attributed to suppressed release of thyroid stimulating hormone (TSH) from the dam's pituitary
- Glyphosate link to impaired TSH due to disruption of protein phosphatase 1 (PP1)\*\*
  - PP1 is essential for TSH release

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

\*\*JE Beecham and S Seneff. *Journal of Autism* 2016;3:1.



## 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.

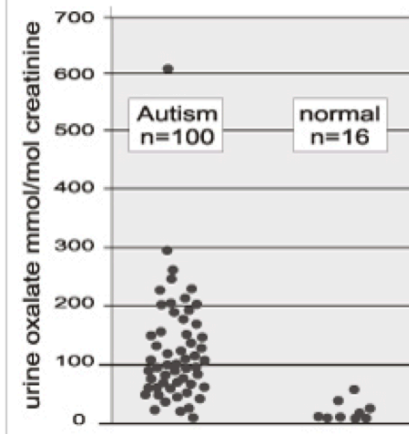
## Autism Linked to Oxalate Crystals\*

- 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

Figure 1. Comparison of urine oxalate values of children with autism and normal children.



\*\*J Konstantynowicz et al., European Journal of Paediatric Neurology 16(5), 2012, 485-491.

## 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\*\*

\*W Krick et al., Am J Physiol Renal Physiol 2009;297: F145-F154.

\*\*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." \*\*

\* F. Irie et al., PNAS Mar. 27, 2012, 109(13), 5052-5056.

\*\*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?\*

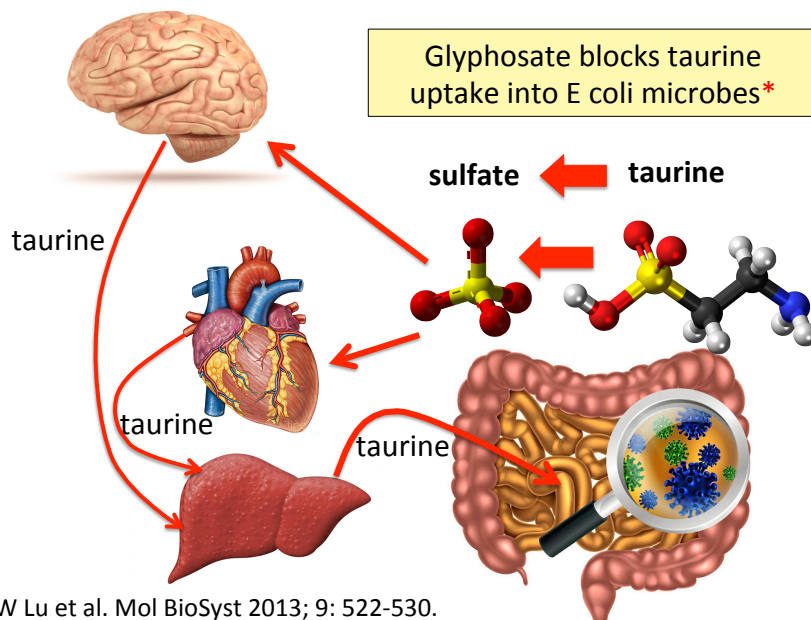
**Abstract:** “This paper makes two claims:

(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

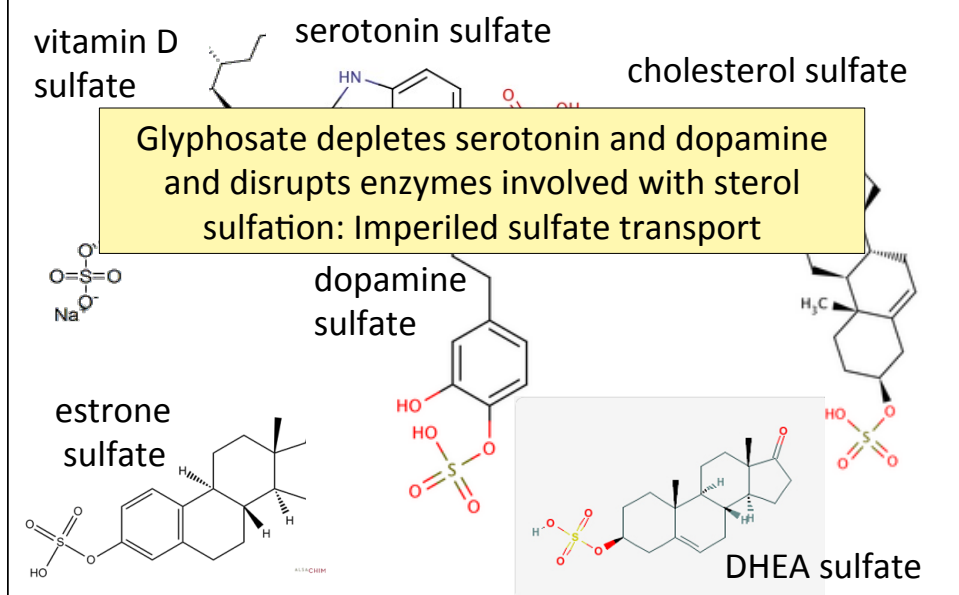
(2), encephalitis is provoked by a systemic deficiency in sulfate, but associated seizures and fever support sulfate restoration. ...”

\*S seneff et al., Entropy 2013; 15: 372-406.

## Gut Microbes to the Rescue!



## Safe Sulfate Transport: Carbon Rings



## Recapitulation

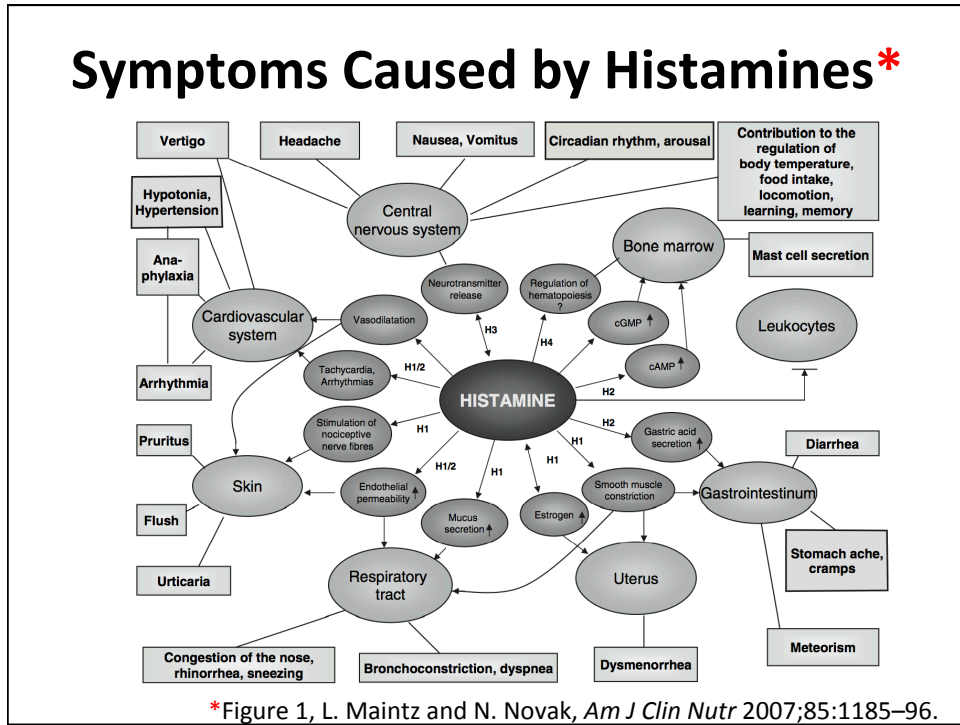
- Sulfate plays many essential roles in the body
  - Sulfate deficiency is a core feature of autism
- Maternal hypothyroidism is a risk factor for autism in the fetus
  - Glyphosate interferes with stimulation of thyroid gland
  - Glyphosate exposure to pregnant rats induced thyroid deficiency in pups
- 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!*

\* L. D'Agostino et al., *Biochim Biophys Acta* 1989;993(2-3):228-32.



## DAO Deficiency → Histamine Accumulation\*

Adverse effects derived from DAO Deficiency	
Central nervous system	Migraine, headache, hangover, sickness
Cardiovascular system	Hypotension, hypertension and arrhythmia
Epithelial system	Urticaria, atopic skin, psoriasis
Respiratory system	Nasal congestion, asthma
Digestive system	Irritable bowel syndrome, constipation, satiety, stomachache, vomits
Muscular system	Fibromyalgia, muscular pains
Skeletal system	Bone pains

\*[www.deficitdao.org/dao-deficiency/#.VRWYtWZGqDc](http://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/](https://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)\*

Merck	ZOSTAVAX	0.42	Shingles
<b>Merck</b>	<b>MMR-II</b>	<b>2.90</b>	<b>Measles, Mumps and Rubella</b>
Merck	VARIVAX	0.41	Varicella, Chicken Pox
MERCK	PNEUMOVAX	ND	Pneumococcal 18
MERCK	PROQUAD	0.43	Measles, Mumps, Rubella, Varicella
GSK	ENERGIX-B	0.33	Heptatitis B

\*A Samsel and S Seneff. Journal of Biological Physics and Chemistry 17 (2017) 8–32

## 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\*\*\*

\*Oldstone, MBA, Ed. Molecular mimicry: Infection inducing autoimmune disease. Springer Berlin Heidelberg; January 9, 2006.

\*\*AD Kline et al., Am J. Hum. Genet. 1993;52:895-906.

\*\*\*VK Singh et al., J Biomed Sci 2002;9(4):359-64.

## 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

\*VK Singh et al., J Biomed Sci 2002;9(4):359-64.

# 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



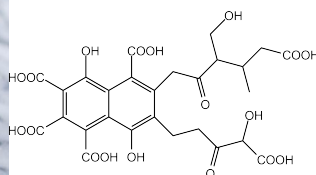
## Treating Glyphosate Poisoning in Animals (e.g., cows) \*



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



Bentonite Clay



Fulvic Acid



Activated Charcoal

\*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.

\*[www.independentsciencenews.org/health/how-france-and-germany-are-ousting-glyphosate-in-a-search-for-healthy-soils-and-pesticide-free-crops/](http://www.independentsciencenews.org/health/how-france-and-germany-are-ousting-glyphosate-in-a-search-for-healthy-soils-and-pesticide-free-crops/)

## 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