

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













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

#### 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









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# 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 Monsanto unpublished study.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.



#### 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.











#### 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."

\*SJ Walker et al. PLOS One March 2013; 8(3):e58058.



#### 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

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



#### 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

\*A Samsel and S Seneff. J Biol Phys Chem 2017;17:8-32 \*\* JJ Gildea et al. J Clin Nutr Diet. 2017, 3:1.

### Trypsin, Pepsin and Lipase are all contaminated with glyphosate\*

Enzyme	Glyphosate (PPB)	
Pepsin (ELISA)	<40	
Pepsin (GC-MS)	430	
Pepsin (HPLC-MSMS)	290	
Trypsin (ELISA)	62	
Lipase (ELISA)	24	



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





#### 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\*\*\*\*

\*A.A. Shehata et al., Curr Microbiol. 2013 Apr;66(4):350-8.

- \*\* M. Velasquez-Manoff, NY Times Sunday Review, Feb. 23, 2013.
- \*\*\* C. Catassi et all, JAMA. 2002 Mar 20;287(11):1413-9.
- \*\*\*\*M. Eriksson et al., Int J Cancer. 2008 Oct 1;123(7):1657-63.



#### 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

\*AV Glubeva et al. EBioMedicine. 2017 Oct;24:166-178.



#### 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

#### 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



- · 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.



#### 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\***

	Autism $(n = 232)$	Controls $(n = 68)$
Age (years)	$7.6 \pm 2.4$	$8.5 \pm 3.7$
Protein $\mu g$ ml <sup>-1</sup>	103.2 ± 89.9*	$64.5 \pm 27.5$
Sulphite	106.9 ± 162.9*	$2.1 \pm 6.3$
Thiosulphate	$130.8 \pm 148.1*$	$18.6 \pm 25.0$
Thiocyanate	6.4 ± 16.9*	$44.0 \pm 101.0$
Sulphate	6819.0 ± 6712.3*	$3030.8 \pm 1461.0$
Anion excretion i (Wilcoxon rank s	s given in nmol ml <sup><math>-1</math></sup> , um test).	mean $\pm$ SD* $p < 0.0$
* 01114	/aring and LV Klovrza	Iournal of Nutrition
* RH V		Journal of Natificion

#### **Rosemary Waring Found Extremely Abnormal Urinary Sulfur Products in Autism**\* 50-fold increase in urinary sulfite suggests a deficiency in sulfite oxidase $7.6 \pm 2.4$ $8.5 \pm 3.7$ Age (years) Protein $\mu g m l^{-1}$ $64.5 \pm 27.5$ 103.2 ± 89.9\* Sulphite $106.9 \pm 162.9^*$ $2.1 \pm 6.3$ Thiosulphate $130.8 \pm 148.1*$ $18.6 \pm 25.0$ Thiocyanate 6.4 ± 16.9\* $44.0 \pm 101.0$ 6819.0 ± 6712.3\* Sulphate $3030.8 \pm 1461.0$ Anion excretion is given in nmol ml<sup>-1</sup>, 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 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  $\rightarrow$  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.



#### 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 lowgrade 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.





#### 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



#### 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



ſ	Merck	ZOSTAVAX	0.42	Shingles	
ſ	Merck	MMR-II	2.90	Measles, Mumps and Rubella	
ſ	Merck	VARIVAX	0.41	Varicella, Chicken Pox	
ſ	MERCK	PNEUMOVAX	ND	Pneumococcal 18	
ſ	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					





- 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.











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





#### 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