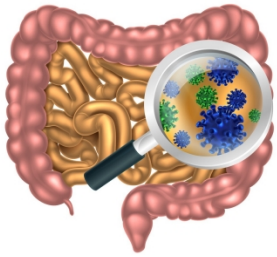
Autism and Glyphosate: Connecting the Dots.



Stephanie Seneff
MIT CSAIL
CHNC
May 5, 2018

Outline

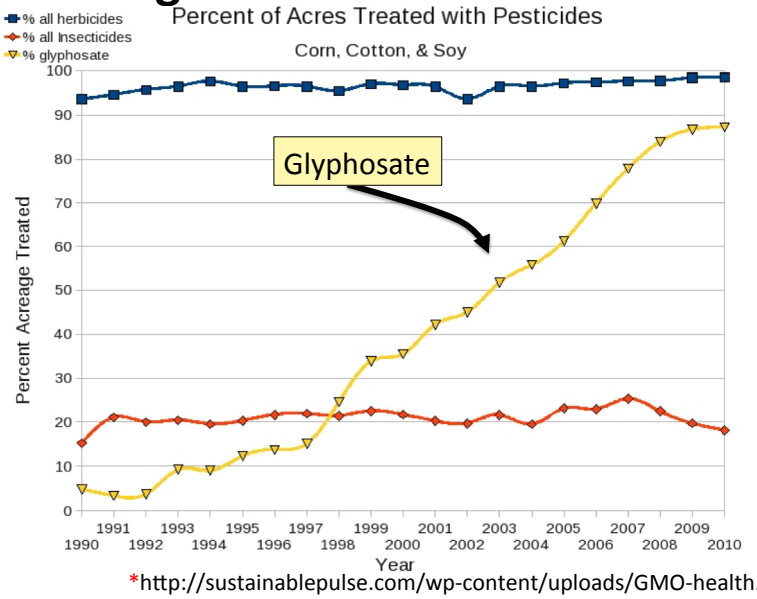
- Introduction
- Glyphosate as a Glycine Analogue
- Autism and the Gut
- Heparan Sulfate Deficiency
- Impaired Calcium Channels
- Other Issues
- Summary

Introduction

Roundup and GMO Crops

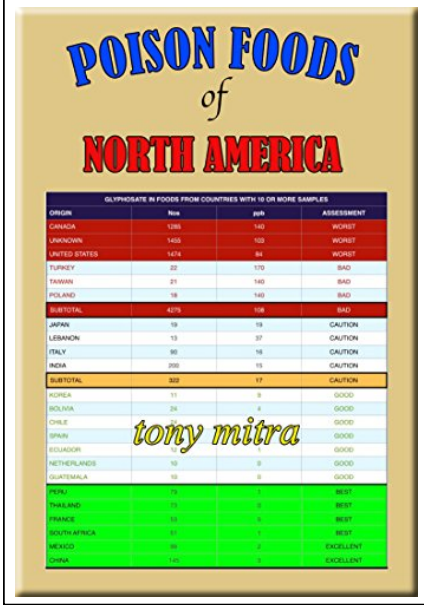
GMO Roundup-Ready corn, soy, canola, sugar beets cotton, tobacco and alfalfa

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



*http://sustainablepulse.com/wp-content/uploads/GMO-health.pdf

Glyphosate is Pervasive in Our Food Supply!

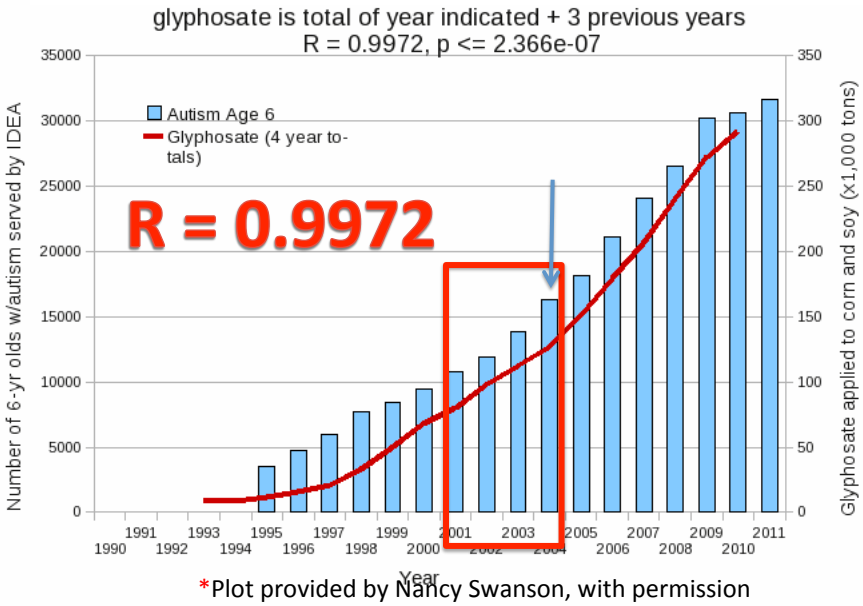


Main Toxic Effects of Glyphosate*

- 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



*Plot provided by Nancy Swanson, with permission

Data sources: autism: US Department of Education; Glyphosate: US Department of Agriculture

Contents lists available at ScienceDirect

Clinical Nutrition ESPEN

journal homepage: http://www.clinicalnutritionespen.com

Original article

Evidence the U.S. autism epidemic initiated by acetaminophen (*Tylenol*) is aggravated by oral antibiotic amoxicillin/clavulanate (*Augmentin*) and now exponentially by herbicide glyphosate (*Roundup*)

Peter Good [1]

- *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

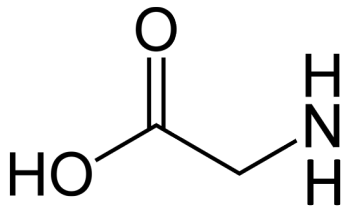
- Estrogen depletion leads to extreme male brain, low brain blood flow, hyperexcitability and social anxiety

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

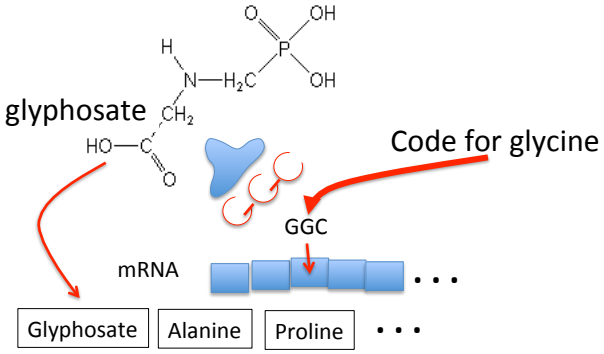
Glyphosate as a Glycine Analogue

Glyphosate is a non-coding amino acid analogue of glycine



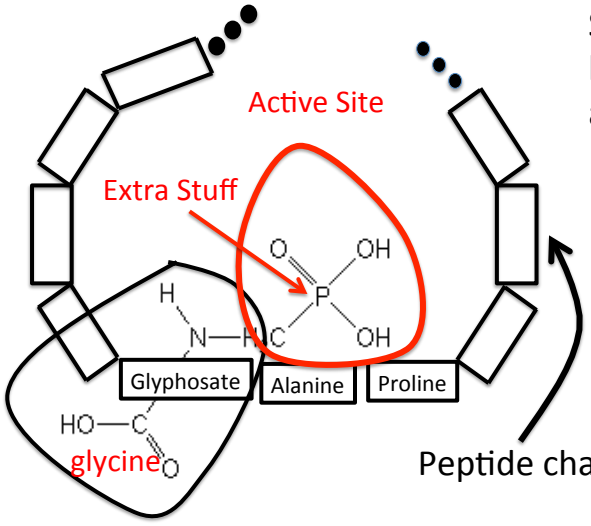
Glycine Glyphosate

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

Extra Piece Sticks Out at 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**

*S Seneff et al. J Bioinfo Proteomics Rev 2016; 2(3): 1-21.
**T Funke et al. Proc Natl Acad Sci U S A 2006; 103(35): 13010-13015.

Inhibition of EPSPS by glyphosate: Resistant E coli mutant*

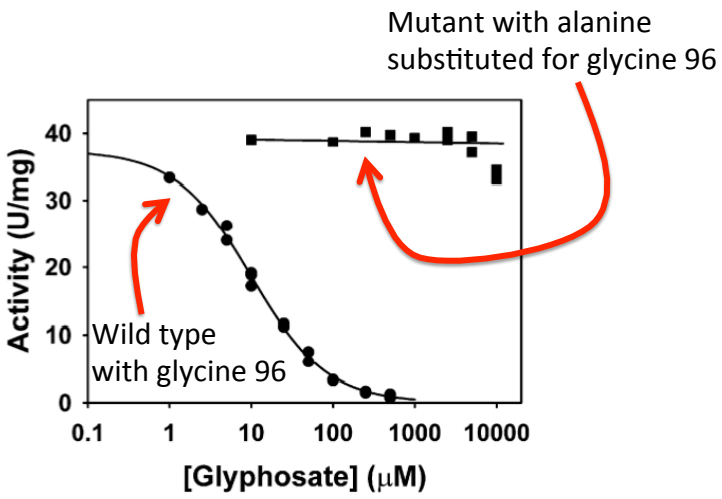
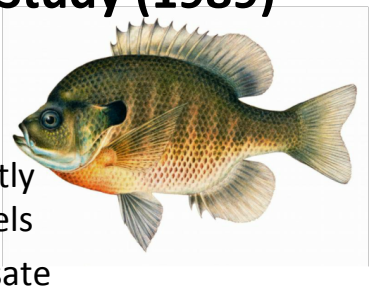


*Figure 3, S Eschenburg et al. Planta 2002;216:129-135.

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."

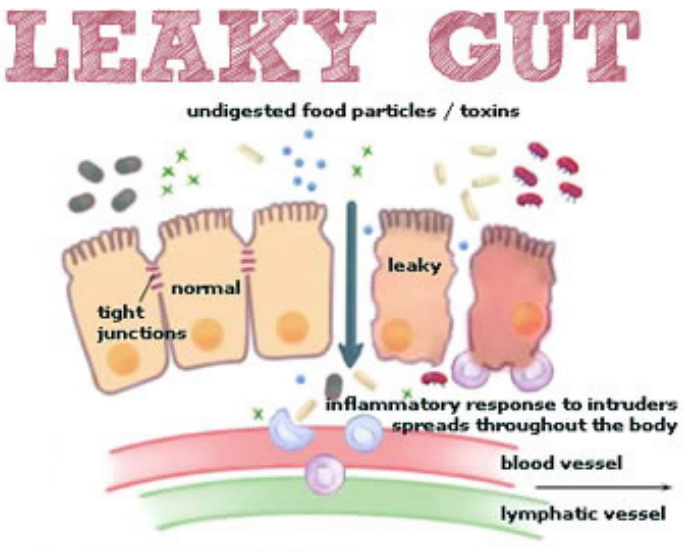
*WP Ridley and KA Chott. Monsanto unpublished study. August, 1989.

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."

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



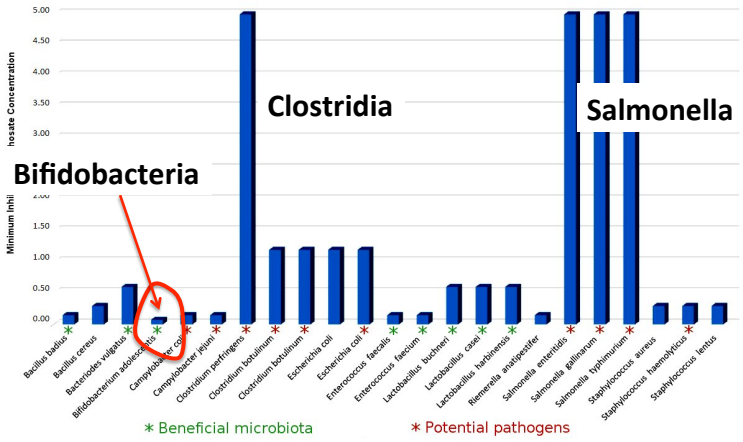
Glyphosate and the Gut: Pathogen Overgrowth*

- 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.

Pathogen Overgrowth in Poultry Microbes Exposed to Glyphosate*

Shehata AA, Schrödl W, Aldin AA, Hafez HM, Krüger M. The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro. Curr Microbiol. 2013 Apr;66(4):350-8.



*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

*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

Trypsin, Pepsin and Lipase are all contaminated with glyphosate*

Trypsin's activation domain contains four crucial glycine rich subdomains:*

N-terminus to Gly 19

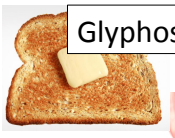
Gly 142 to Pro 152

Gly 184 to Gly 193

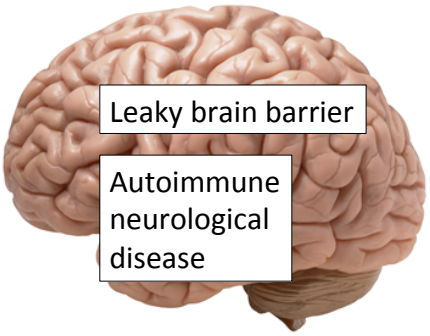
Gly 216 to Asn 223

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

A Scenario for Gluten Intolerance



Glyphosate in wheat

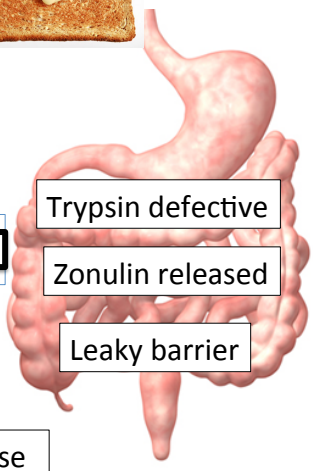


Leaky brain barrier

Autoimmune neurological disease

Zonulin

Gliadin



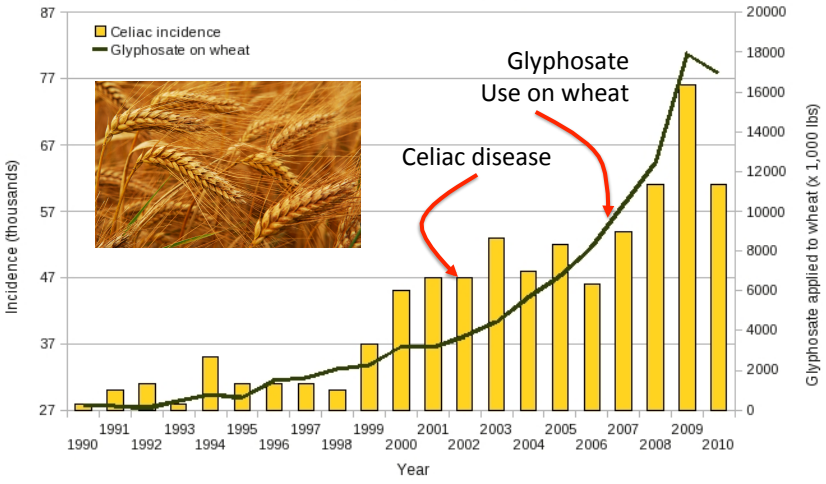
Trypsin defective

Zonulin released

Leaky barrier

Systemic immune response induces multiple complex symptoms

Glyphosate and Celiac Disease*



*Samsel and Seneff, Interdiscip Toxicol. 2013;**6**(4): 159–184.

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.

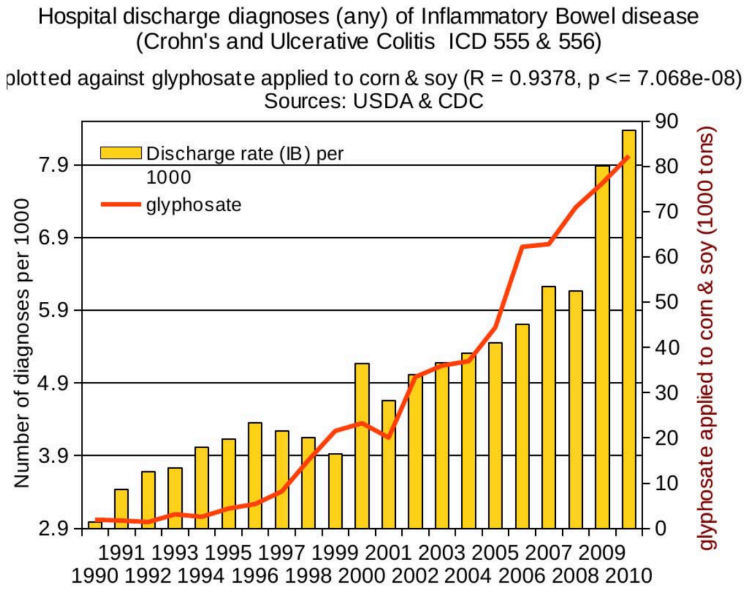


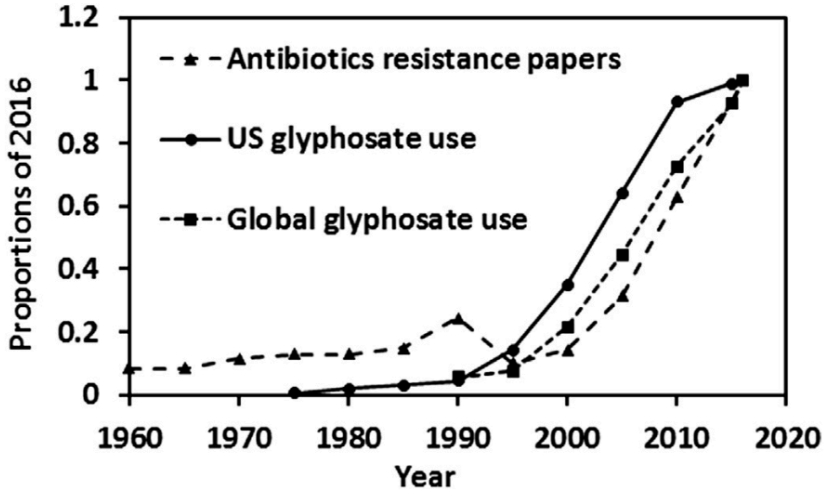
*Figure 20, NL Swanson et al. Journal of Organic Systems 9(2), 2014, p. 25.

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

*AHC Van Bruggen et al. Science of the Total Environment 2018;616-617: 255–268.

Glyphosate Usage and Papers on Antibiotic Resistance*



*AHC Van Bruggen et al. Science of the Total Environment 2018;616-617: 255–268.

A BTBR Mouse Model of Autism*

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

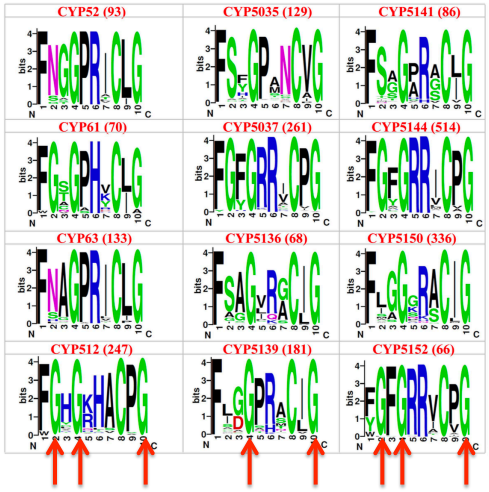
Some features in the gut:

- 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

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

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**

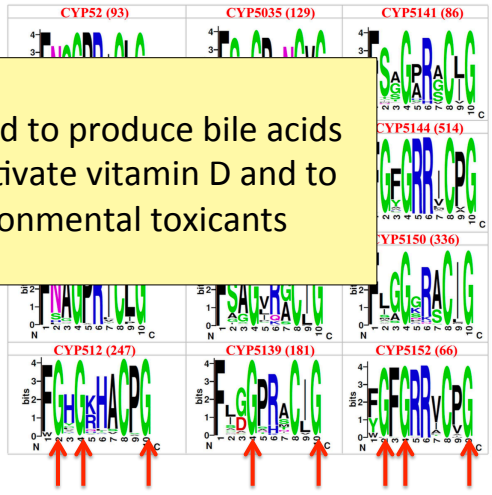


*A Samsel and S Seneff. Entropy 2013; 15: 1416-1463.
**K Syed and SS Mashele. PLOS ONE 2014; 9(4):| e95616.

Glyphosate Disrupts Cytochrome P450 (CYP) Enzymes*

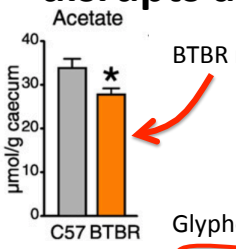
- Glyphosate has been
-
motif with two and sometimes three critical glycine residues**

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



*A Samsel and S Seneff. Entropy 2013; 15: 1416-1463.
**K Syed and SS Mashele. PLOS ONE 2014; 9(4):| e95616.

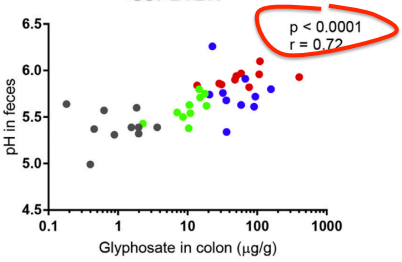
BTBR mice have low acetate, and glyphosate disrupts acetate synthesis in gut*



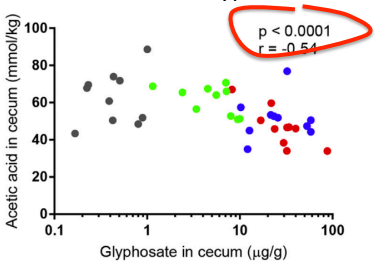
BTBR mice

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

Glyphosate and pH

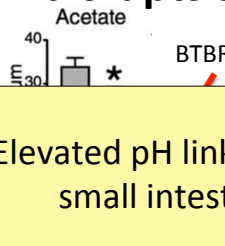


Glyphosate and Acetate



*LN Nielsen et al. Environmental Pollution 2018;233:364e376.
**Adams et al. BMC Gastroenterology 2011; 11:22.

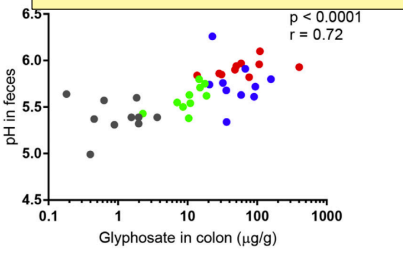
BTBR mice have low acetate, and glyphosate disrupts acetate synthesis in gut*

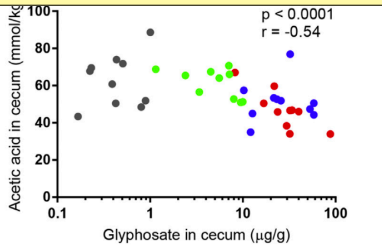


BTBR mice

Children with autism had

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





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*."

*LJ Waber and HG Wood. J Bacteriol. 1979 Nov; 140(2): 468–478.

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

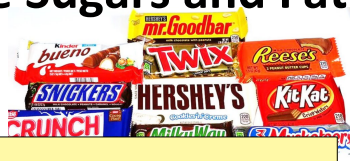
- Mouse study, two diets
  1. High fat, high simple sugars
  2. Low fat, high complex carbohydrates

- 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 H_2S 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

*FE Rey et al. Proc Natl Acad Sci U S A. 2013 Aug 13; 110(33): 13582-13587.

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



- M
  D
  –
- H
  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

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

*FE Rey et al. Proc Natl Acad Sci U S A. 2013 Aug 13; 110(33): 13582-13587.

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

*W Lu et al. Mol Biosyst. 2013 Mar;9(3):522-30.
**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**

*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.

Myosin in the Gut

- Myosin is a motor protein found in high levels in skeletal muscles
- [illegible] o
- [illegible]

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

  - 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**

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

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*

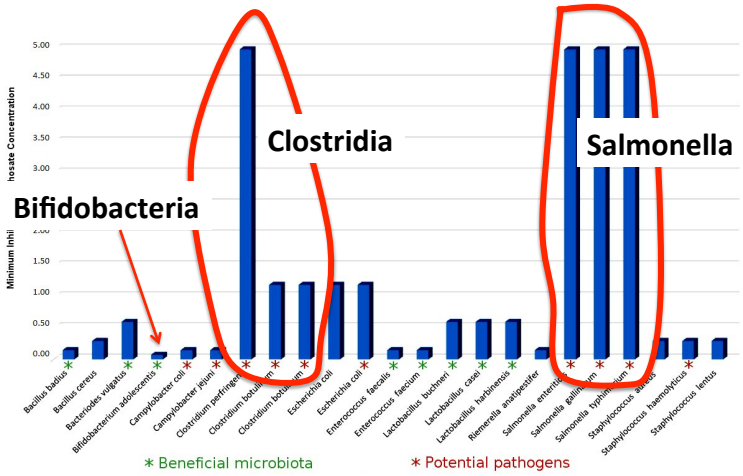
William Shaw, PhD

- 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.

Pathogen Overgrowth in Poultry Microbes Exposed to Glyphosate*

Shehata AA, Schrödl W, Aldin AA, Hafez HM, Krüger M. The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro. Curr Microbiol. 2013 Apr;66(4):350-8.



*Plot provided by Dr. Martin Michener

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)

* Dawson, "Sulfate in Fetal Development," Semin Cell Dev Biol 2011

"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

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

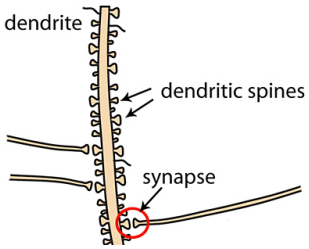
New neurons develop from stem cells in this zone through the action of "fractones" composed of heparan sulfate proteoglycans (HSPGs)**

the disorder."

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

**F. Mercier et al., Neuroscience Letters 506 (2012) 208–213

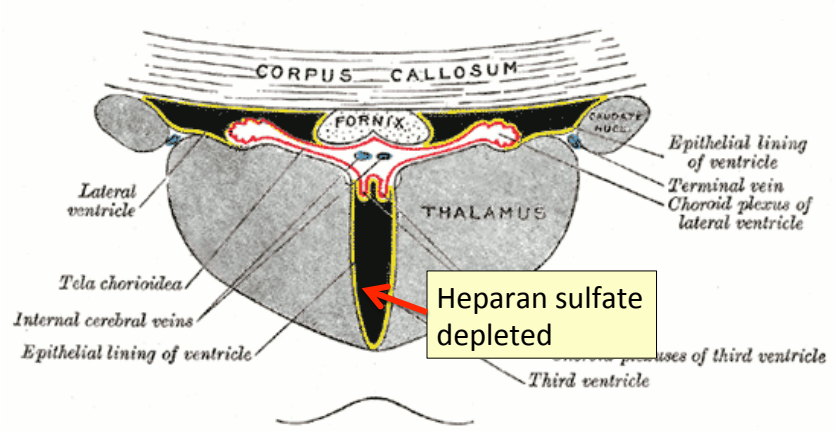
Impaired Dendrite Outgrowth in Autism could be Due to Glycine Substitution by glyphosate



- 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***

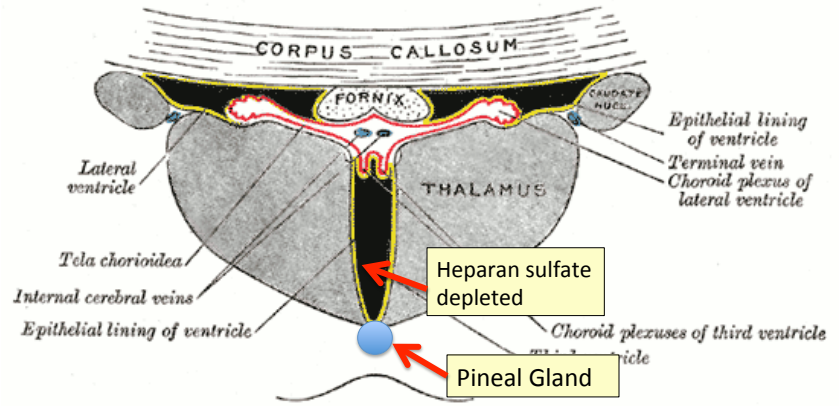
*IM Ethell and Y. Yamaguchi. J Cell Biol. 1999;144(3):575–86.
**L Zhang et al. JBC 1995; 270(45) Nov 10, 27127-27135.
***M Phillips and L Pozzo-Miller. Neurosci Lett. 2015; 601: 30-40

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.
**F Mercie et al., Neurosci Lett 506, 2012, 208-213.

Pineal gland normally supplies melatonin sulfate to the ventricles at night during sleep, but both melatonin and sulfate supplies are depleted by glyphosate



*B.L. Pearson et al., Behav Brain Res. 2013 Apr 15;243:138-45.
**F Mercie et al., Neurosci Lett 506, 2012, 208-213.

"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

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

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

- E
  "
  s
  i
  s
- M
  c
  a
  and social

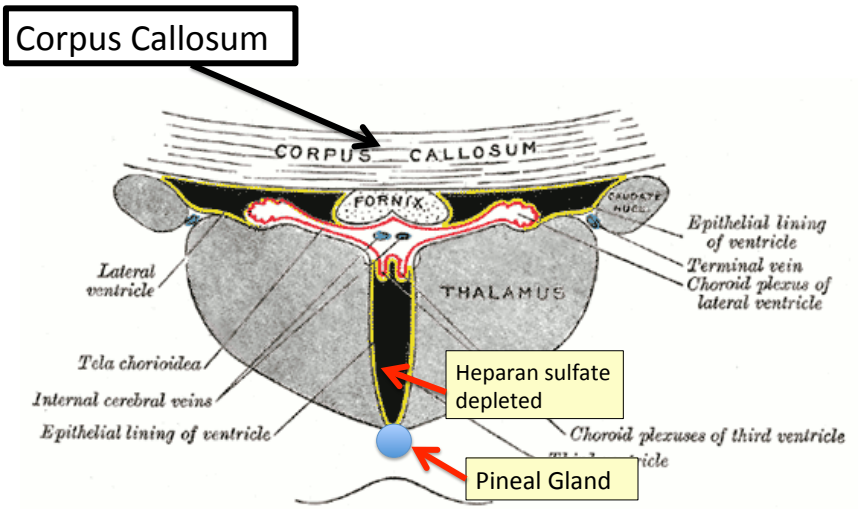
" Remarkably, these mutant mice recapitulate almost the full range of autistic symptoms, including impairments in social interaction, expression of stereotyped, repetitive behavior, and impairments in ultrasonic vocalization."

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

"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

*MJ Corley et al. Physiology & Behavior 107 (2012) 663–665



"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."*

*MJ Corley et al. Physiology & Behavior 107 (2012) 663–665.

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

*M.J.G. Bolt et al., Am J Physiol Endocrinol Metab 287: E744 –E749, 2004.

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

  D3 → 25(OH) D3 → 1,25(OH)2 D3

  Liver Kidney
- Both steps depend on CYP enzymes
- Liver CYPs are destroyed by glyphosate and aluminum??

  → 1,25(OH2) D3 >> 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??

*W Lu et al. Mol Biosyst. 2013 Mar;9(3):522-30.

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
- Taur
- Gut
- 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??

*W Lu et al. Mol Biosyst. 2013 Mar;9(3):522-30.

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

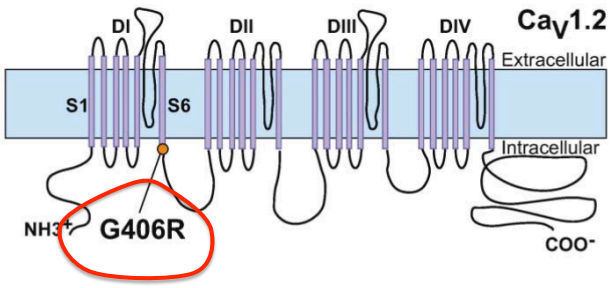
Impaired Calcium Channels

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*

*I Splawski et al.. Cell Vol 2004; 119: 19-31.

A Calcium Channel Glycine Mutation: Timothy Syndrome*



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

*I Splawski et al.. Cell Vol 2004; 119: 19-31.

"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*

*I Splawski et al.. Cell Vol 2004; 119: 19-31.

More Highly Conserved Glycines in Calcium Channel*

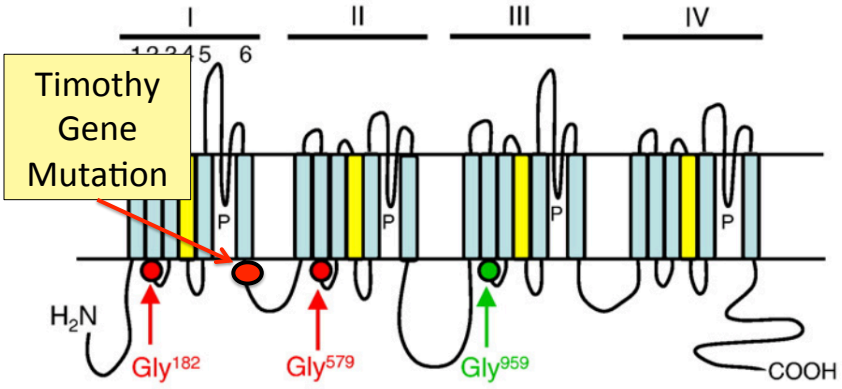


*Figure 1. J Teng et al. Biochimica et Biophysica Acta 2010;1798: 966-974.

More Highly Conserved Glycines in Calcium Channel*

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

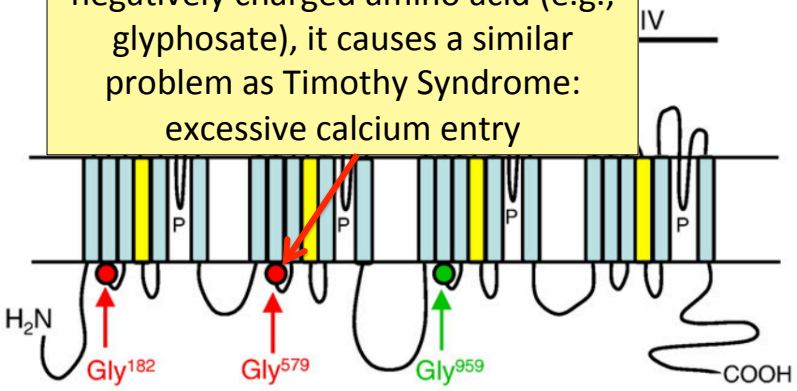


*Figure 1. J Teng et al. Biochimica et Biophysica Acta 2010;1798: 966-974.

More Highly Conserved Glycines in Calcium Channel*

Calcium mediates processes as diverse as synaptic transmission, muscle contraction, insulin secretion, fertilization, and gene expression

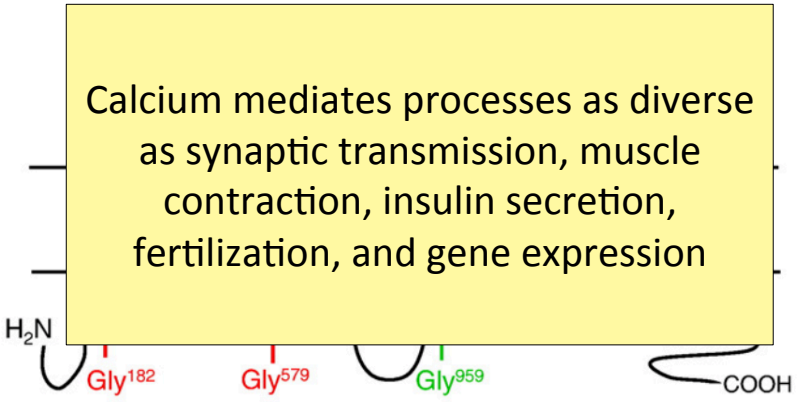


*Figure 1. J Teng et al. Biochimica et Biophysica Acta 2010;1798: 966-974.

Free Radical Biology and Medicine 65 (2013) 335–346

Contents lists available at ScienceDirect

Free Radical Biology and Me

journal homepage: www.elsevier.com/locate/fr

Original Contribution

Roundup disrupts male reproductive functions by triggering calcium-mediated cell death in rat testis and Sertoli cells

Vera Lúcia de Liz Oliveira Cavalli [a], Daiane Cattani [a], Carla Elise Heinz Rieg [a], Paula Pierozan [b], Leila Zanatta [a], Eduardo Benedetti Parisotto [c], Danilo Wilhelm Filho [c], Fátima Regina Mena Barreto Silva [a], Regina Pessoa-Pureur [b], Ariane Zamoner [a,*]

"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."

Cardiovasc Toxicol (2015) 15:324–335
DOI 10.1007/s12012-014-9299-2

Cardiotoxic Electrophysiological Effects of the Herbicide Roundup® in Rat and Rabbit Ventricular Myocardium In Vitro

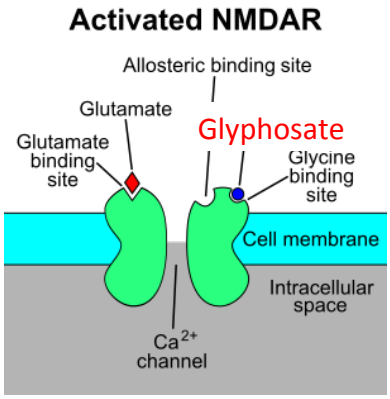
Steeve Gress · Sandrine Lemoine · Paolo-Emilio Puddu · Gilles-Eric Séralini · René Rouet

"In GBH [glyphosate-based herbicide]-poisoned persons, a high incidence of *QTc interval prolongat* 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*



Calcium uptake Through calcium channels

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

Glyphosate and Glutamate*

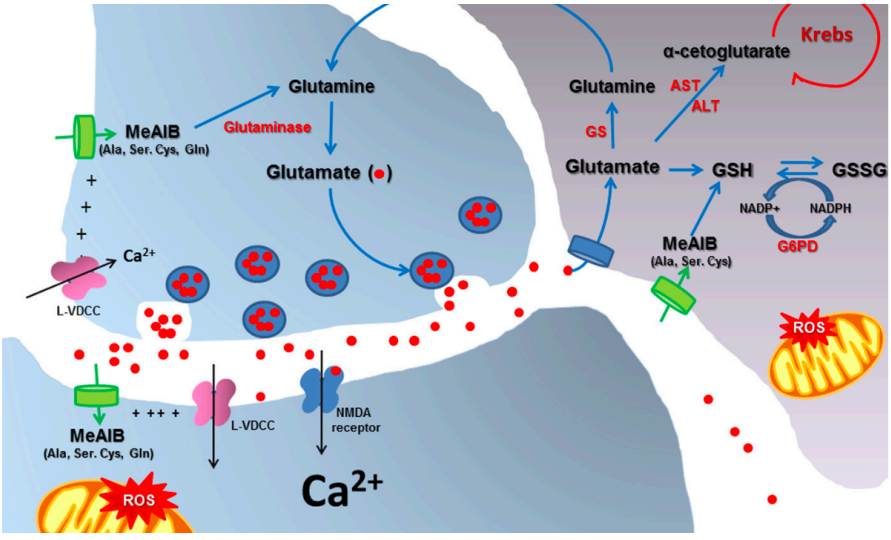


*Figure 9, D. Cattani et al. / Toxicology 320 (2014) 34–45

Glyphosate and Glutamate*

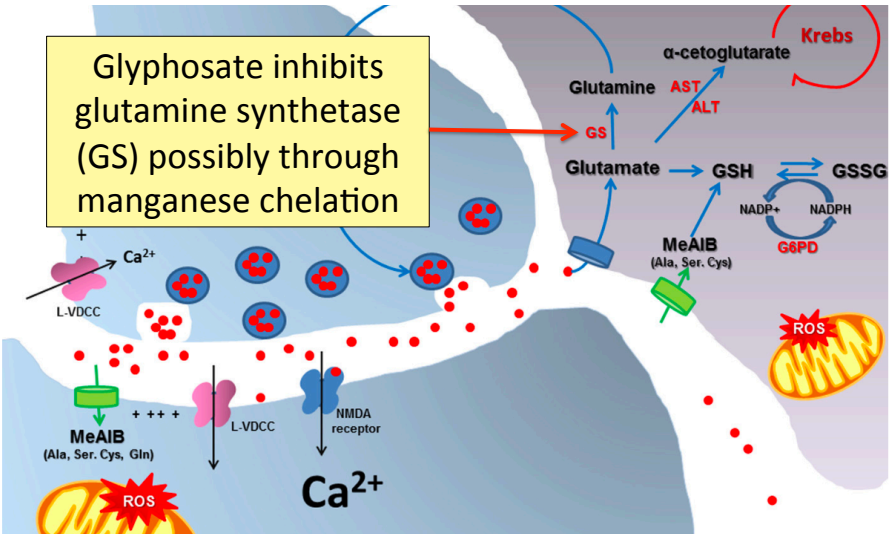


*Figure 9, D. Cattani et al. / Toxicology 320 (2014) 34–45

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