Zika, Glyphosate, Vaccines, Folic Acid, Neural Tube Defects and Autism

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AutismOne
Friday, May 27, 2016

The fatal tendency of mankind to leave off thinking about a thing when it is no longer doubtful, is the cause of half their errors.

— John Stuart Mill, 1859
British philosopher, political economist, and civil servant
Outline

• Introduction
• Glyphosate displacing glycine in protein synthesis
• Glyphosate and Zika
• Glyphosate, folic acid and pyriproxifen
• A role for vaccines
• A role for glufosinate (Liberty herbicide)
• Summary

Introduction
Neural tube defects occur in 1 in 1000 pregnancies and often lead to spontaneous or elected abortion.
Zika Virus and Microcephaly

• Mass hysteria has developed due to the fear that a new virus, Zika, spread by mosquitoes, is causing an epidemic in microcephaly in NE Brazil

• Could there be something more to the story?

Zika has been around since the mid 1900’s and it has never been known to cause microcephaly
**Some Statistics***

- 3,670 cases of potential microcephaly in Brazil investigated
  - 404 confirmed cases of microcephaly
  - 17 of these tested positive for Zika = 4.2%
  - 98% of the 404 cases were from the NE corner of Brazil (Pernambuco)

*What about the 96% who didn’t test positive for Zika??*

* http://noticias.uol.com.br/ultimas-noticias/agencia-estado/2016/02/02/pais-tem-404-casos-confirmados-de-microcefalia.htm

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**Both Macrocephaly and Microcephaly are linked to Autism***

- 126 autistic children studied
- 16.7% diagnosed with macrocephaly
- 15.1% diagnosed with microcephaly
- Both of these numbers are abnormally high, compared to the general population

These abnormal brain developmental features are milder forms of the more severe defects associated with neural tube disorders

Anecdotal Evidence

• Feb 8 PBS Evening News segment on Zika: interviewed young mother of a child with microcephaly
• The closing remark was that the mother would have to quit her job as an agricultural worker to tend to the child

“Why Brazil has a big appetite for risky pesticides”*

• “In the rural northeast, rampant use has led to sickness and violence”
• A series of irrigation canals in the arid NE sector have allowed farming to flourish
• Many of the region’s residents get their water from the same open-air canals that irrigate the farms
• In 2012, Brazil passed the United States as the largest buyer of pesticides

Some Other Potential Factors

- Pesticides: GMO Roundup-Ready crops plus a new GMO resistance gene to glufosinate
- Vaccines during pregnancy: Tdap, flu
  - Synergistic toxicity of glyphosate & (aluminum, mercury, glutamate)
- Measles outbreak in NE Brazil; aggressive MMR booster campaign?
- Insecticide to control the mosquitoes: Pyroproxifen
- GMO mosquito introduced into same area where microcephaly is highest
  - Supposed to reduce mosquito burden: fewer mosquitoes = more infection??

US CDC recommends flu vaccine for pregnant women (since 2004)

- GMO mosquito introduced into same area where microcephaly is highest
  - Supposed to reduce mosquito burden: fewer mosquitoes = more infection??
What If Glyphosate Could Insert Itself Into Protein Synthesis???

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

An Analogy: ALS in Guam

- An epidemic in ALS in Guam was traced to a natural toxin found in cycads
- BMAA is a non-coding amino acid that gets inserted by mistake in place of serine
- Defective versions of a glutamate transporter have been linked to ALS*
- The transporter has an essential serine-rich region in its sequence**

*Antioxidants & Redox Signaling 2009;11: 1587-1602.
Another Analogy: MS & Sugar Beets*

- Sugar beets contain an analogue of proline called Aze
- Remarkable correlation between MS frequency and proximity to sugar beet agriculture
- Myelin basic protein contains a concentration of proline residues that are absolutely essential for its proper function


Vulnerable Proteins: Resulting Pathologies

<table>
<thead>
<tr>
<th>Conserved Glycines</th>
<th>Disease Profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormone-sensitive Lipase</td>
<td>Obesity</td>
</tr>
<tr>
<td>Insulin Receptor</td>
<td>Diabetes</td>
</tr>
<tr>
<td>Amyloid Beta Plaque</td>
<td>Alzheimer’s Disease</td>
</tr>
<tr>
<td>OGG1</td>
<td>DNA Damage → Cancer</td>
</tr>
<tr>
<td>Lipocalin</td>
<td>Kidney Failure</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenal Insufficiency</td>
</tr>
<tr>
<td>Cytochrome C Oxidase</td>
<td>Mitochondrial Disease</td>
</tr>
<tr>
<td>Alpha Synuclein</td>
<td>Parkinson’s Disease</td>
</tr>
<tr>
<td>TDP-43</td>
<td>ALS</td>
</tr>
</tbody>
</table>
Glycine decarboxylase deficiency causes neural tube defects and features of non-ketotic hyperglycinemia in mice*

- Fascinating article about mice with a deficiency in a protein involved in generating methyl groups from glycine → methylfolate deficiency
- These mice have very high rate of neural tube defects
- Those that are viable have swollen ventricles (a feature of autism) and delayed development
- Methylation pathway impairment is a common feature of autism

*YJ Pai et al., Nature Communications 2015; 6:6388

Glycine decarboxylase deficiency causes neural tube defects and features of non-ketotic hyperglycinemia in mice*

- Fascinating article about mice with a deficiency in

Glycine decarboxylase has a glycine-rich region that maintains shape and flexibility of active site**

- Those that are viable have swollen ventricles (a feature of autism) and delayed development
- Methylation pathway impairment is a common feature of autism

**A Kume et al., JBC 1991; 266(5): 3323-3329
Sugar Cane and Roundup*  

• Roundup is used to ripen sugar cane in NE Brazil at harvest  
• The workers harvest by machete and chop off a small chunk of stalk to chew on as they harvest throughout the day  
• They suffer from end-stage kidney failure  
• Kidney failure is much lower in other regions where they have switched to a different ripening agent besides glyphosate  

• The workers also take stalk sections home for the family to eat  

* Prof. Don Huber, Personal Communication
Sugar Cane, Sugar Beets and Ethanol*

• Brazil is a leader in introducing flexible fuel vehicles (FFVs) into the market.
  – Designed to run on gasoline or gasoline-ethanol blends of up to 85% ethanol (E85)
• Brazil has a major industry converting sugar from sugar beets and sugar cane to ethanol
• Ethanol-based fuels produce toxic carcinogens: formaldehyde and acetaldehyde**
• There is likely glyphosate present in the fuel

*https://en.wikipedia.org/wiki/Pernambuco

Trucks in Brazil Lining Up Following Truckers’ Strike: February, 2015*

*kticradio.com/agricultural/deal-reached-in-brazil-truckers-strike-trucks-still-blocked/
GMO Mosquito*

- Grown in contained spaces in Cayman Islands
  - Fed sucrose as larvae followed by blood as adults
    (sucrose comes from sugar beets or sugar cane)
- Males engineered to become infertile without access to tetracycline
- They could be harboring more glyphosate than mosquitoes in the wild

*AF Harris et al., Nature Biotechnology 2011; 29:1034-1037.

Zika and Protein Mimicry

Studies from the Vaccine Induced Immunological Damage (VIID) Program

Zika E Protein: \(GWGNCGFLGKGSLV\)

IGG heavy chain: \(GYSSGCGYWQGTLV\)

- Lots of glycines! – These could get displaced by glyphosate during protein synthesis!!
- Likely to disrupt protein folding and cause resistance to breakdown
  \(\rightarrow\) Autoimmune reaction to similar protein (IGG heavy chain)
Zika and Protein Mimicry

Studies from the Vaccine Induced Immunological Damage (VIID) Program

A Zika virus growing in a GMO mosquito is likely to end up with glyphosate in its proteins

- Lots of glycines! – These could get displaced by glyphosate during protein synthesis!!
- Likely to disrupt protein folding and cause resistance to breakdown
  → Autoimmune reaction to similar protein (IGG heavy chain)

A Chromosomal Breakage Syndrome With Profound Immunodeficiency*

- Chromosomal Breakage Syndrome: case study
- Spontaneous chromosomal breaks, gaps and rearrangements in T-cells
- Failure to thrive, *microcephaly*, immune deficiency, neurological abnormalities
- Very low levels of immunoglobulins

A Chromosomal Breakage Syndrome With Profound Immunodeficiency*

- Chromosomal Breakage Syndrome: case study
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  - Failure to thrive, microcephaly, immune deficiency, neurological abnormalities
  - Very low levels of immunoglobulins

*Suggests a link between microcephaly and low immunoglobulins which can arise because of an autoimmune response to IGG heavy chain via Zika protein mimicry


PNKP & Microcephaly*

- The body relies on DNA repair mechanisms to repair chromosomal breaks and errors
- PNKP is intimately involved in DNA repair
  - PNKP is both a phosphatase and a kinase: it takes away and adds phosphate groups to DNA
- Mutations in PNKP have been shown to cause microcephaly, seizures and defective DNA repair
- Glyphosate substitution for conserved glycines in PNKP would disrupt both of its activities

Glyphosate, Folic Acid and Pyriproxifen

“Glyphosate-Based Herbicides Produce Teratogenic Effects on Vertebrates by Impairing Retinoic Acid Signaling”*

“Neuronal development and axon growth are altered by glyphosate through a WNT non-canonical signaling pathway”*

- Neurons grown in culture & exposed to glyphosate
- “They elicited shorter and unbranched axons and they also developed less complex dendritic arbors compared to controls”

*RP Coullery et al., NeuroToxicology 2016;52:150-161.

Glyphosate Could Cause Microcephaly through Impaired Methylation Pathway

- Disrupted folate one-carbon metabolism (methylation pathway)
  - Folate carries the methyl group that methylates DNA during development to regulate gene expression
  - Folate is produced for the host by gut microbes from the shikimate pathway
- Methyl group is provided by metabolism of glycine; a critical enzyme in this pathway depends on a glycine-rich region that glyphosate could disrupt
Folic Acid Fortification/Supplementation: A Bad Idea!

- U.S. started requiring flour to be fortified with folic acid in 1998
- Folic acid is a synthetic, unmethylated, oxidized form of folate
  - This stresses the liver, causing gluthathione loss and loss of methyl groups
  - Excess folic acid during pregnancy ends up in the bloodstream and binds to the fetal folate receptors in the brain, causing cerebral folate deficiency!

Glyphosate and Anencephaly*

- Yakima, Benton and Franklin counties in Washington State have an unusually high number of pregnancies affected by anencephaly
- 75 pesticides were analyzed in studying contamination due to surrounding agriculture
  - 47 (63%) of these were detected
  - Glyphosate was applied in large amounts, but was not studied
- 5% solution of glyphosate was also used heavily around irrigation ditches to control weeds
  - Main herbicide recommended due to its “low toxicity”

“Glyphosate, Brain Damaged Babies, and Yakima Valley - A River Runs Through It”*

Noxious aquatic weed control program with Glyphosate ‘Rodeo’


“Glyphosate, Three Rivers, and Anencephaly”*

Yakima Harold Republic
Slide thanks to Prof. Don Huber, with permission

*Farm Wars 3/6/14

Pyriproxifen: Insecticide*

• Pyriproxifen is a commonly used insecticide to control mosquitoes, fleas on pets and the whitefly in agriculture
• Brazil is the only country that puts pyriproxifen in the drinking water to control mosquitoes (since 2014)
• Pyriproxifen is a powerful juvenile hormone analog
• Juvenile hormone controls metamorphosis and reproduction in insects

*D Evans et al., A Possible Link Between Pyriproxyfen and Microcephaly.
http://necsi.edu/research/social/pandemics/pyriproxyfen
Pyriproxifen: Insecticide, Cont’d*

- Juvenile hormone and retinoic acid (vitamin A) are both lipid-soluble terpenoids
- Isotretinoin (a vitamin A analogue) causes microcephaly in human babies via maternal exposure
- Juvenile hormone and its analogs bind to the vertebrate retinoic acid receptor
  - This could prevent the normal retinoic acid from binding to the receptor and cause developmental disorders in mammals
- Liver CYP enzymes needed to detox pyriproxifen are suppressed by glyphosate

*D Evans et al., A Possible Link Between Pyriproxyfen and Microcephaly.
http://necsi.edu/research/social/pandemics/pyriproxyfen

A Role for Vaccines
A Role for Vaccines: Tdap

- In the spring of 2015, public health officials in Brazil mandated that pregnant women be vaccinated with the adult form of DTaP, called Tdap.
- Tdap viruses are grown on a protein source derived from bovine casein (protein in milk)
- Casein has significant amounts of glycine which could be displaced by glyphosate
- Tdap also contains aluminum, which is a neurotoxin
- Aluminum and glyphosate are synergistically toxic

Glyphosate and Aluminum: Partners in Crime*

- Glyphosate induces pathogens like C. difficile in gut, leading to *leaky gut syndrome*
  - C. diff produces *p-cresol* which promotes aluminum uptake by cells
  - p-Cresol is a known biomarker for autism
  - p-Cresol is an important factor in *kidney failure* which leads to aluminum retention in tissues → dementia
- Glyphosate *cages* aluminum to promote entry
- Glyphosate promotes *calcium uptake* by voltage-activated channels
  - Aluminum gains entry as calcium mimetic

*S Seneff et al., Agricultural Sciences 2015;6:42-70
"Measles Reemergence in Ceará, Northeast Brazil, 15 Years after Elimination”*

- CDC post: 21(9), September 2015
- Measles “eliminated” in Brazil in 2000 w/ massive vaccination campaign
- From December 2, 2013, through December 31, 2014, in the state of Ceará, NE Brazil, 681 measles cases were reported
  - 19% were patients aged 20 to 29
- Either measles infection or MMR vaccine could be a factor

*http://wwwnc.cdc.gov/eid/article/21/9/15-0391_article

**Autism, Glyphosate, Vaccine Reactions**

*Collaboration with Dr. Nancy Swanson*
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
• Gelatin (derived from collagen) contains significant amounts of both glycine and glutamate
  – These two neurotransmitters excite the NMDA receptors in the brain
  – Glyphosate substitution by mistake for glycine is a possibility!
• Glyphosate stimulation of NMDA receptors could cause neuronal burnout

Glyphosate and Glutamate*,**, *(http://www.greenmedinfo.com/blog/roundup-weedkiller-brain-damaging-neurotoxin
**D Cattani et al., Toxicology 2014; 320: 34-45.)
Symptoms of Adverse Reactions to MMR before and after 2002*

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<th>p-value</th>
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*Data analyzed from the VAERS database

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These are all characteristic symptoms of allergies to MSG

*Data analyzed from the VAERS database
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 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 antibodies to either hemagglutinin or MBP
- There were no antibodies detected against any proteins in the mumps or rubella viruses

Precedent: Glyphosate in Drugs?*

- Trasylol (aprotinin) is a protein derived from bovine lung used to reduce bleeding during open heart surgery
- Rare acute reaction to Trasylol involves precipitous drop in blood pressure, acute kidney failure, and sudden death
- Piglets injected with glyphosate salts had similar acute reaction with high mortality rate
- Cows fed GMO Roundup-Ready feed had highest residue levels of glyphosate in lungs

*S Seneff et al., Agricultural Sciences 2015; 6:1472-1501.

A Role for Glufosinate (Liberty Herbicide)
**Glyphosate Resistant Weeds**

- Widespread appearance of glyphosate-resistant weeds has necessitated ever-increasing applications of glyphosate on Roundup-Ready crops
- Liberty is an alternative herbicide to kill these resistant weeds – *glufosinate*!
- LibertyLink is a GMO modification to support glufosinate resistance

**Increased Use of Glufosinate after 2010**

- **LibertyLink** is a Bayer-owned brand of genes for use in agriculture providing tolerance to **Liberty** herbicide and glufosinate
- LibertyLink is the only GMO herbicide tolerant gene other than Roundup-Ready on the market.
- LibertyLink versions of cotton, corn and soybeans are approved in Brazil
  – LibertyLink soybeans approved in 2010
Glufosinate is a Glutamate Analogue!* 

- Its toxicity to plants is based on its disruption of glutamine synthetase by pretending to be glutamate  
  - This causes a build-up of excess ammonia and glutamate and a depletion of glutamine  
- As an analogue of glutamate, it could substitute for glutamate during protein synthesis – and this could lead to microcephaly!


### Protein Disruption Through Coding Errors

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Imposter</th>
<th>Protein</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>serine</td>
<td>BMAA</td>
<td>Glutamate transporter</td>
<td>ALS (Lou Gehrig’s)</td>
</tr>
<tr>
<td>proline</td>
<td>Aze</td>
<td>Myelin basic protein</td>
<td>Multiple Sclerosis</td>
</tr>
<tr>
<td>glutamate</td>
<td>glufosinate</td>
<td>Asparagine synthetase</td>
<td>Microcephaly</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Immune deficiency</td>
</tr>
<tr>
<td>glycine</td>
<td>glyphosate</td>
<td>Many proteins</td>
<td>Autism, microcephaly, etc.</td>
</tr>
</tbody>
</table>
Microcephaly and Asparagine Synthetase*

- Loss-of-function mutation in asparagine synthetase causes a severe condition
  - Congenital microcephaly, intellectual disability, progressive cerebral atrophy, and intractable seizures
- Mutant mice have enlarged ventricles, reduced thickness of cortex, and learning disabilities


Microcephaly and Asparagine Synthetase*

Asparagine synthetase has a conserved glutamate that is essential for its function**

- Loss-of-function mutation in asparagine synthetase causes a severe condition
- Mutant mice have enlarged ventricles, reduced thickness of cortex, and learning disabilities


** ME Meyer et al., Biochemistry 2010;49(43):9391-401.
A Theory Emerges: Glyphosate/Glufosinate Synergy

- Glyphosate disrupts glutamine synthesis through chelation of manganese
  - Glutamine is precursor to asparagine
- Glufosinate disrupts enzyme that synthesizes asparagine
- Both shortages of glutamine and impaired enzyme activity result in severe deficiency in asparagine → microcephaly

A Bit of Chemistry

Glutamate → Glutamine → Aspartate

Glutamine synthetase

Asparagine synthetase

Asparagine deficiency leads to microcephaly and impaired immunity
Glufosinate Induces Autistic Symptoms in Mice*

• 1 mg/kg administered three times a week to dam during gestation and lactation
• Offspring demonstrated autistic behaviors – socialization impairment, repetitive activities
• Dose was about 5 times lower than the EPA approved dose

* A. Laugeray et al., Frontiers in Behavioral Neuroscience 2014;8:390.

We can expect a dramatic rise in the use of glufosinate on core crops as more and more weeds become resistant to Roundup

• Dose was about 5 times lower than the EPA approved dose

* A. Laugeray et al., Frontiers in Behavioral Neuroscience 2014;8:390.
Summary

• Brazil’s microcephaly epidemic should be a lesson to us all!
  – The Zika virus likely plays only a minor role
• Exposure to toxic chemicals in agriculture is an unacceptable risk to our children and therefore to our future
• Multiple vaccines with dangerous toxicants now administered to pregnant women in Brazil
  – Some vaccines may also be contaminated with glyphosate
• I believe synergy between glyphosate and glufosinate is the key factor in the epidemic
  – These chemicals are both amino acid analogues and this is a very dangerous class of molecules
• We need to go back to sustainable organic agricultural methods to protect our children from disability