Fetal Exposure to Glyphosate

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The Investigators of this study have no financial relationships to disclose or Conflicts of Interest (COIs) to resolve.
• 300 million pounds of glyphosate (GLY) (Roundup®) are applied each year in the US.

• Though glyphosate has been in use since 1974 no previous measures of GLY exposure in US pregnancies have been published.

• Rodent models of pesticide exposure in pregnancy correlate with adult and transgenerational disease through epigenetic mechanisms.

• We measured GLY in pregnant women to estimate fetal exposure and potential adverse effects on pregnancy outcomes.
Environmentally Induced Epigenetic Transgenerational Disease

- Chemicals (or stressors) in Environment
- Induce DNA methylation of Genome (in child, adolescent or fetus)
- Which alters adult disease risk (gene expression shifts)
- And is transmitted to future generations
Is Vinclozolin Safe?

- High doses don’t kill.
- High doses for 1 week don’t cause fetal loss or fetal anomalies, or low birth weight or preterm birth.
- Endocrine disruption does occur but male rats aren’t deformed at birth.
- 90% of V-exposed male rats had adult diseases: (immune, kidney, prostate, cancer, cholesterol, low sperm counts, accelerated aging, sexually undesirable)
- Diseases were heritable! DNA methylation patterns changed gene expression across generations!

Michael Skinner
U.Washington, Pullman
Pregnant Rat

Vinclozolin Exposure (PC days 8-14)

Offspring

Vinclozolin
Methoxychlor

F0
F1
F2
F2
F3
Failed Experiment!
The Baby Rats Were Normal

Adult Rats had Diseases
Fetal Exposure: Adult disease

- Low sperm count
- Infertility
- Cancer
- Kidney
- Prostate
- Pregnancy abnormalities
- Immune dysfunction
- High cholesterol
- Accelerated aging
- Non “Sexy” Scent
- Anxiety Prone
Transgenerational Effects of Fetal Pesticide Exposure
The chromosome location and physical mapping of each candidate (numbers) are indicated for each chromosome (Chr), with those unknown (UN) sequences not mapped indicated.
Vinclozolin ; Heritable Anxiety

F3 generation males had a decrease in anxiety-like behavior, 92 genes in the hippocampus and 276 genes in the amygdala.

Females had an increase in anxiety-like behavior, 1,301 genes in the hippocampus and 172 genes in the amygdala.

Brain transcriptome microarray analysis from F3 generation control (con) and vinclozolin (vin) animals.

Younger Age at Menarche

![Bar Chart: F3 Female Puberty]

- Control
- Pesticide
- Plastics
- LD Plastics
- Dioxin
- Jet Fuel

* indicates significant difference from Control group.
Declining Age of Menarche (Japan)

**Bar Chart 1: Age of Menarche**
- **Y-axis:** Mean age of Menarche (11-14 years)
- **X-axis:** Decade (1930s-1980s)

**Bar Chart 2: % Girls Menarche <11 Years**
- **Y-axis:** Percent <11 yrs (0-2.5%)
- **X-axis:** Decade (1930s-1980s)

BMC Women’s Health 2012 Jul 16;12(1):19
Hosokawa, Imazeki, Mizunuma, Kubota, Hayashi
Males Developing 2 years Earlier Too!

Age to 3ml Testicular Volume
(US white male) Pediatrics 2012
Polycystic Ovarian Disease

Premature Ovarian Failure (POF)
DDT; Transgenerational Obesity

DDT induced transgenerational obesity and associated disease through the female (egg) germline. Thus, female germline can also transmit transgenerational disease.

DNA Methylation

Cytosine \rightarrow 5\text{-Methylcytosine}

SAM \rightarrow \text{Dnmt}
DNA Methylation at CpG Site

TTTCGATTACGA
Contaminants Produced New DNA Methylation Sites
Transgenerational (F3) Sperm Epigenome Alterations

(Ancestral Exposure Specific Epimutation Biomarkers)

Dioxin

Plastics

Vinclozolin

Hydrocarbons (JP8)

A (198)
B (50)
C (33)
D (367)
E (45)

28
80
24
228

0
0
1
1
0
2
1
0
1

0
0
0
0
1
0
1
0
1
1

0
1
1
1

20
Prenatal Atrazine Causes Transgenerational Disease
Objectives

This is a prospective cross-sectional birth cohort study with the following aims:

• How many Midwest pregnant women are exposed to glyphosate (GLY) ?
• Is drinking water an important exposure source?
• What risk factors and adverse pregnancy outcomes correlate with exposure?
Design/Methods

- Pregnant women between ages 18-40 years and their newborn infants resulting from this pregnancy were enrolled prospectively at a private obstetrical practice.
- Same day urine and household water samples were collected during a subsequent clinical visit.
- Specific gravity in urine samples was measured by certified hospital laboratory technicians.
- Urine and water samples were frozen and stored at -80°C.
- Urine and water samples were measured for GLY in ng/mL with liquid chromatography-tandem mass spectrometry (LLOQ 0.5 ng/mL).*
Design/Methods

• Electronic medical records were reviewed and clinical and pregnancy outcome data were collected.
• Questionnaires relating to food and water consumption during pregnancy were administered electronically or paper form.
• Subjects received a $20 gift card compensation for their time and effort.
• Statistical linear models were used to assess relationships between GLY level and clinical outcomes of gestation age and adjusted birth weight as well as pregnancy related risk factors.
Study Population

- A total of 69 pregnant women with live-born infants were studied.
- 69 drinking water samples were tested.
- Mean maternal age was 29 years (range 18-39 years)
- Maternal race was 94.2% Caucasian, 7.8% Asian
- Maternal education, Household Income, Mean Pregnancy Length,
- Mean Birth Weight %ile, % NICU admissions are shown in Table 1.
# Demographic Information of Cases

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>N (%)</th>
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<tbody>
<tr>
<td>Residential Area</td>
<td>Rural 14 (20)</td>
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</tr>
<tr>
<td></td>
<td>Suburban 49 (71)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urban 6 (09)</td>
<td></td>
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<tr>
<td>Maternal Race</td>
<td>White 65 (94)</td>
<td></td>
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<tr>
<td></td>
<td>Asian 4 (06)</td>
<td></td>
</tr>
<tr>
<td>Maternal Age</td>
<td>Less than 20 years 2 (03)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20-35 years 63 (91)</td>
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</tr>
<tr>
<td></td>
<td>Greater than 35 years 4 (06)</td>
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<tr>
<td>Marital Status</td>
<td>Single 19 (28)</td>
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<tr>
<td></td>
<td>Married 50 (72)</td>
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<tr>
<td>Primip</td>
<td>No 49 (71)</td>
<td></td>
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<tr>
<td></td>
<td>Yes 20 (29)</td>
<td></td>
</tr>
<tr>
<td>Hypertension (CHTN &amp; PIH)</td>
<td>No 51 (74)</td>
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<tr>
<td></td>
<td>Yes 18 (26)</td>
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<tr>
<td>Diabetes (DM &amp; GDM)</td>
<td>No 64 (93)</td>
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<tr>
<td></td>
<td>Yes 5 (07)</td>
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<td>Maternal Drug Use</td>
<td>No 68 (99)</td>
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<td>Maternal Tobacco Use</td>
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<td>Yes 12 (17)</td>
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<td>Maternal Alcohol Use</td>
<td>No 66 (96)</td>
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<td></td>
<td>Yes 3 (04)</td>
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<td>Route of Delivery</td>
<td>C-section 20 (29)</td>
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<td>Vaginal 49 (71)</td>
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<td>Infant Sex</td>
<td>Male 36 (52)</td>
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<td></td>
<td>Female 33 (48)</td>
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<td>NICU care</td>
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<td>Yes 3 (04)</td>
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<td>Household Income</td>
<td>Less than $25,000 6 (09)</td>
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<td>$25,000 to $34,999 10 (14)</td>
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<td>$35,000 to $49,999 8 (12)</td>
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<td>$50,000 to $74,999 11 (16)</td>
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<td>$75,000 to $99,999 14 (20)</td>
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<td></td>
<td>$100,000 to $200,000 14 (20)</td>
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<td>$200,000 or more 1 (01)</td>
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<tr>
<td></td>
<td>I don’t know 5 (07)</td>
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<tr>
<td>County of Residence</td>
<td>Hamilton 1 (01)</td>
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<tr>
<td></td>
<td>Hendricks 1 (01)</td>
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<tr>
<td></td>
<td>Johnson 23 (33)</td>
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</tr>
<tr>
<td></td>
<td>Marion 42 (61)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Morgan 2 (03)</td>
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<tr>
<td>Maternal Education</td>
<td>≤ High School Diploma or GED 14 (20)</td>
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<tr>
<td></td>
<td>Some college or Associate Degree 23 (33)</td>
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<tr>
<td></td>
<td>Bachelors degree 32 (46)</td>
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</tr>
</tbody>
</table>
Results

• 65 of 69 pregnant women (91%) tested + for GLY (>LLOD)
• Mean GLY concentration was $3.6 \pm 0.12$ ng/mL
• When GLY was compared with risk factors, it was found negatively correlated to women’s BMI both @ beginning and end of pregnancy ($r$’s= -0.31, $p$’s=0.01)
• GLY levels were found higher in women who consumed more than 24 ounces of caffeine per day (means of GLY 4.61 vs. 3.33, $p=0.001$)
• GLY levels were found higher in women who lived in rural areas (means of GLY 4.21 vs. 3.30, $p=0.004$)
• None of the drinking water samples had detectable GLY.
Maternal Weight vs. Glyphosate Levels

GLYPHOSATE VS. MATERNAL BMI (PREPREGNANCY)

- Healthy weight
- Overweight
- Obese

GLYPHOSATE (ng/mL)

MATERNAL BMI

$p = .069$

$R = .286$

$p = .017$
Pre-pregnancy Obesity (≥30BMI) vs. GLY

Obese mothers had lower Glyphosate levels.
Do you currently consume beverages containing caffeine (coffee, caffeinated soda, tea, energy drinks)?
Results

• 91% prenatal urines tested positive for glyphosate (LOD>0.1ng/mL)
• GLY levels were negatively correlated to gestation age ($r= -0.25$, $p=0.0352$)
• GLY levels were negatively correlated to gestation adjusted birth weight ($r= -0.24$, $p=0.045$)
Pregnancy Length vs. Glyphosate

* Only includes singleton ≥37 week gestation infants.
Gestation-Corrected Birth Weight (Bwt %Ile) vs. Glyphosate

*B Only includes singleton ≥37 weeks gestation infants
Summary

• The most heavily used pesticide in the US is found at quantifiable levels in over 90% of pregnancies in the Midwest.

• Higher GLY levels were associated with shorter gestations and with lower gestation-adjusted birth weights.

• Since water samples were largely negative the source of exposure is probably food.

• Maternal pre-pregnancy weight and Caffeine intake were associated with higher GLY but Organic food intake was NS.
Conclusion

• The majority of fetuses must be assumed to have exposure to glyphosate during critical periods of fetal development.

• It is incumbent upon policy makers to ensure that such a large scale fetal exposure does not result in altered DNA methylation and potential multigenerational disease.
Limitations

• Funding prohibited examination of GLY variability by trimester and comparisons between blood, urine, concentrations. Small sample size and regional and demographic differences are also not addressed. Fetal epigenetic changes related to maternal levels likewise were not addressed. Food GLY residues and other exposure sources were not addressed.

• Specimen processing, transport etc. are also likely to affect measured levels. Laboratory validation of assays was rigorously completed prior to this study.(RG)
Acknowledgements

Franciscan St. Francis Health
- Laboratory Technicians
- OB/GYN Physicians
- Labor & Delivery Nurses
- Post Partum Nurses
- Neonatal Intensive Care Nurses
  Medicine

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Office Manager
- Medical Assistants
- Physicians
- Study Population

Fairbanks Public Health
- Grant Support
- Study Design Collaboration
- Co-PI

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- Medicine

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Neonatology Department
- Clinical Research Coordinator
OB GYN of Indiana, Collaborators

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Tara Debikey, MD

Stephanie R. Young, MSN AGPCNP-C

Thomas Wisler Jr., MD

Vicky Sherman, MD
*Glyphosate Assay*

- Glyphosate (N-(phosphomethyl)glycine) is directly analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Water and urine samples are prepared for analysis by solid phase extraction using an ion exchange column. Extracted samples are injected to the LC-MS/MS and the analyte is separated using an Obelisc N column (SIELC Technologies, Prospect Heights, IL) through isocratic elution. Ionization of glyphosate is achieved using an electrospray ionization source operated in negative polarity. The analyte is detected by multiple reaction monitoring using a 13C-labelled glyphosate as internal standard. Quantification of the analyte is done by isotope dilution method using an eight-point calibration curve.

- The assay has a limit of quantification of 0.1 ng/mL. The intra- and inter-day precision observed are 6-15% in concentrations that range 0.1-80 ng/mL. Recoveries for glyphosate range 70-80% at concentrations within the assay’s linear dynamic range. (RG, UCSF)
Drinking Water Sources

All water samples < LOD

Sources of Drinking Water
(N=69)
Organic Food Consumption

- 56% Never
- 28% Rarely
- 11% Frequently
- 5% Always

GLYPHOSATE VS. ORGANIC FOOD

Not significant
Maternal Education

- ≤ High School Diploma: 20%
- ≤ College Degree: 71%
- Masters, PhD, Professional: 9%

 Glyphosate vs. Maternal Education

- ≤ High School Diploma, GED, or equivalent
- Some College or Associate Degree
- Bachelors or Advanced Degree

Mean Glyphosate (ng/mL)
A critical review of glyphosate findings in human urine samples and comparison with the exposure of operators and consumers


# Previous Studies of Urinary Glyphosate

## Table 1 Glyphosate concentrations in human urine samples (mean and maximum values) and resulting estimates of previous exposure, compared to ADI or AOEL

<table>
<thead>
<tr>
<th>References</th>
<th>Analytical method, LOD/LOQ</th>
<th>Participants</th>
<th>Urine concentrations [µg/l]</th>
<th>Estimated exposure or systemic dose [µg/kg bw]</th>
<th>Percentage of ADI or AOEL [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acquavella et al.</td>
<td>HPLC following ion exchange LOD 1 µg/L</td>
<td>48 male farmers from Minnesota and South Carolina (USA), their spouses and 79 children</td>
<td>3.2</td>
<td>233 (farmer) 29 (child)*</td>
<td>8.3 (systemic dose) 8.3 % of AOEL (maximum value), ca. 0.1 % of AOEL (mean value)</td>
</tr>
<tr>
<td>Curwin et al.</td>
<td>Immunoassay (fluorescent microbeads) LOD 0.9 µg/L</td>
<td>48 women, 47 men, 117 children from &quot;farm&quot; and &quot;non-farm&quot; households in Iowa</td>
<td>1.1–2.7 (in different groups)</td>
<td>18 (&quot;farm child&quot;)*</td>
<td>0.5 (dietary exposure highest mean) 0.1 (systemic dose highest mean) 0.1 % of ADI</td>
</tr>
<tr>
<td>Mesnage et al.</td>
<td>HPLC–MS LOD 1 µg/L LOQ 2 µg/L</td>
<td>1 farmer, his wife and 3 children, presumably Europe</td>
<td>n.a. (only single values available)</td>
<td>9.5 (farmer) 2 (child)*</td>
<td>0.33 (systemic dose) &lt;0.4 % of AOEL</td>
</tr>
<tr>
<td>Hoppe (2013)</td>
<td>GC–MS/MS following derivatisation LOD 0.15 µg/L</td>
<td>182 citizens from 18 European countries</td>
<td>0.21</td>
<td>1.82</td>
<td>0.3–0.4 (dietary exposure) &lt;0.1 % of ADI</td>
</tr>
<tr>
<td>Markard (2014)</td>
<td>GC–MS/MS (presumably) LOD 0.15 µg/L</td>
<td>40 male and female German students</td>
<td>n.a. (22 samples above LOQ)</td>
<td>0.65</td>
<td>0.13 (dietary exposure) &lt;0.1 % of ADI</td>
</tr>
<tr>
<td>Krüger et al.</td>
<td>ELISA partly validated against GC–MS LOD/LOQ not given</td>
<td>&gt;300 (mostly from Germany)</td>
<td>≤2</td>
<td>5</td>
<td>0.83 (dietary exposure) &lt;0.2 % of ADI</td>
</tr>
<tr>
<td>Honeycutt and Rowlands (2014)</td>
<td>ELISA LOQ 7.5 µg/L</td>
<td>35 women, men and children from USA</td>
<td>n.a. (13 samples above LOQ)</td>
<td>18.8</td>
<td>3.3 (dietary exposure) 0.66 (systemic dose) &lt;0.7 % of ADI, &lt;0.7 % of AOEL</td>
</tr>
</tbody>
</table>

n.a. not applicable

* For children, comparisons to reference values were not performed since age, body weight and urine volume were not known.
Round Up ….. Is UP

Roundup Use up 11500% Increase in 17 years.
Glyphosate Use 1992-2014

20 X Increase in GLY Use

February 2016 FDA started testing for GLY
The tests conducted by Anresco were done on 29 foods commonly found on grocery store shelves. Glyphosate residues were found in General Mills’ Cheerios at 1,125.3 parts per billion (ppb), in Kashi soft-baked oatmeal dark chocolate cookies at 275.57 ppb, and in Ritz Crackers at 270.24 ppb, according to the report. Different levels were found in Kellogg’s Special K cereal, Triscuit Crackers and several other products. The report noted that for some of the findings, the amounts were “rough estimates at best and may not represent an accurate representation of the sample.” The food companies did not respond to a request for comment.

1,125.3 ppb
Organic Food Glyphosate

• 45% of Organic Honey samples tested + for GLY

• Abraxis and Boston U, 11 were organic and five of those tested above 15 ppb, results ranging from 26 to 93 ppb, with a mean of 50 ppb. (Sustainable Pulse, Henry Rowlands)

41 ppb
GLY
Ten out of 36 (28%) Oatmeal products + glyphosate

812.53 ppb GLY

Quaker Oats, owned by PepsiCo, has been sued over its "all natural" oats containing high levels of GLY.
<table>
<thead>
<tr>
<th>Nabisco (continued)</th>
<th>Glyphosate - 140.90* ppb</th>
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</thead>
<tbody>
<tr>
<td>Oreo Double Stuf Chocolate Sandwich Cookies</td>
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<tr>
<td>Oreo Double Stuf Golden Sandwich Cookies</td>
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<table>
<thead>
<tr>
<th>PepsiCo</th>
<th>Glyphosate - 812.53 ppb</th>
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<tbody>
<tr>
<td>Stacy's Simply Naked Pita Chips (Frito-Lay)</td>
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<tr>
<td>Lay's: Kettle Cooked Original</td>
<td></td>
</tr>
<tr>
<td>Doritos: Cool Ranch</td>
<td></td>
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<tr>
<td>Fritos (Original) (100% Whole Grain)</td>
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</table>

<table>
<thead>
<tr>
<th>Campbell Soup Company</th>
<th>Glyphosate - 18.40 ppb</th>
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<tbody>
<tr>
<td>Goldfish crackers original (Pepperidge Farm)</td>
<td></td>
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<tr>
<td>Goldfish crackers colors</td>
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<tr>
<td>Goldfish crackers Whole Grain</td>
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<thead>
<tr>
<th>Little Debbie</th>
<th>Glyphosate - 264.28* ppb</th>
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<tr>
<td>Oatmeal Creme Pies</td>
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<table>
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<th>Lucy's</th>
<th>Glyphosate - 452.44* ppb</th>
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<td>Oatmeal Cookies Gluten Free</td>
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<th>Glyphosate - 119.12* ppb</th>
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<td>365 Organic Golden Round Crackers**</td>
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<th>Back to Nature</th>
<th>Glyphosate - 327.22* ppb</th>
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<td>Crispy Cheddar Crackers</td>
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3/25/2017
<table>
<thead>
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<th>Brand</th>
<th>Product</th>
<th>Glyphosate</th>
<th>AMPA</th>
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<tr>
<td>Honey Nut Cheerios</td>
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<td>Glyphosate - 670.2 ppb</td>
<td>AMPA - 14.5</td>
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<td>Wheaties</td>
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<td>Glyphosate - 31.2 ppb</td>
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<td>Trix</td>
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<td>Glyphosate - 9.9 ppb</td>
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<td>Annie's</td>
<td>Gluten Free Bunny Cookies Cocoa &amp; Vanilla</td>
<td>Glyphosate - 55.13* ppb</td>
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<td>Kellogg's</td>
<td>Corn Flakes</td>
<td>Glyphosate - 78.9 ppb</td>
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<td>Raisin Bran</td>
<td>Glyphosate - 82.9 ppb</td>
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<td>Organic Promise**</td>
<td>Glyphosate - 24.9 ppb</td>
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<td>Special K</td>
<td>Glyphosate - 74.6 ppb</td>
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<td>Frosted Flakes</td>
<td>Glyphosate - 72.8 ppb</td>
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<td>Cheez-It (Original)</td>
<td>Glyphosate - 24.6 ppb</td>
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<td>Cheez-It (Whole Grain)</td>
<td>Glyphosate - 36.25* ppb</td>
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<td>Soft-Baked Cookies, Oatmeal Dark Chocolate</td>
<td>Glyphosate - 275.58* ppb</td>
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<td>Nabisco</td>
<td>Ritz Crackers</td>
<td>Glyphosate - 270.24 ppb</td>
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<td>Triscuit</td>
<td>Glyphosate - 89.68 ppb</td>
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<td>Oreo Original</td>
<td>Glyphosate - 289.47* ppb</td>
<td></td>
</tr>
</tbody>
</table>
Coffee Creamer, Eggs, Bagels, Bread

- Instant Oatmeal Strawberries & Cream: 1,327.1
- Whole Wheat Bagels: 491.9
- Organic Multigrain bagels: 151.5
- Whole Grain Bread: whole wheat: 403.0
- Organic Killer Whole Wheat Bread: 136.4
- Organic Cage-Free Antibiotic-Free Large Eggs: 169.0
- Organic Coffee Creamer: 104.0
Round up in PET FOOD! MEOW!

Research links 0.1 ppb glyphosate to Non-alcoholic Fatty Liver Disease

Pet food has tested at 300 ppb
Pet Food levels of Round UP

Cat Food
- Purina Cat Chow Complete Dry – 102 ppb glyphosate.
- Friskies Indoor Delights Cat Food Dry – 79 ppb glyphosate.
- 9 Lives Indoor Complete Cat Food Dry – 140 ppb glyphosate.
- Rachael Ray Zero Grain Whitefish and Potato Recipe Cat Food Dry – 22 ppb glyphosate.

Dog Food
- Kibble’s ‘n Bits Chefs Choice American Grill Dog Food Dry – 300 ppb glyphosate.
- Iams Proactive Health Toy and Small Breed Dog Food Dry – 65 ppb glyphosate.
- Rachael Ray Nutrish Real Beef and Rice Recipe Dog Food Dry – 140 ppb glyphosate.
- Purina Beyond Simply 9 White Meat Chicken and Whole Barley Recipe Dog Food Dry – 47 ppb glyphosate.
The New Mother is a Walking Chemistry Set
FDA and EPA No Longer Tracking Glyphosate.

- **ENVIRONMENTAL PROTECTION AGENCY** “WE ARE GOING TO GET RID OF IT IN ALMOST EVERY FORM.” TRUMP (NOV. 14, 2016)

- FDA SUSPENDS TESTING FOODS FOR GLYPHOSATE RESIDUES (NOVEMBER 24, 2016)

- MYRON EBELL, WHO LED TRUMP’S EPA TRANSITION TEAM, HAS DESCRIBED THE ENVIRONMENTAL MOVEMENT AS ‘THE GREATEST THREAT TO FREEDOM IN THE MODERN WORLD’