Glyphosate Biomonitoring: Challenges and Opportunities

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Target Analyte

N-(Phosphomethyl)glycine (PMG, generic name; “glyphosate”)

- Broad spectrum herbicide
- Most widely used pesticide in the world; 113.4 million kg used in the US in 2014 (Benbrook, 2016)
- Most widely used domestic pesticide

Uses

- In conjunction with PMG-tolerant crops
- As desiccation agent for drying grains and field defoliation agent
- Spot application

Safety Status of PMG

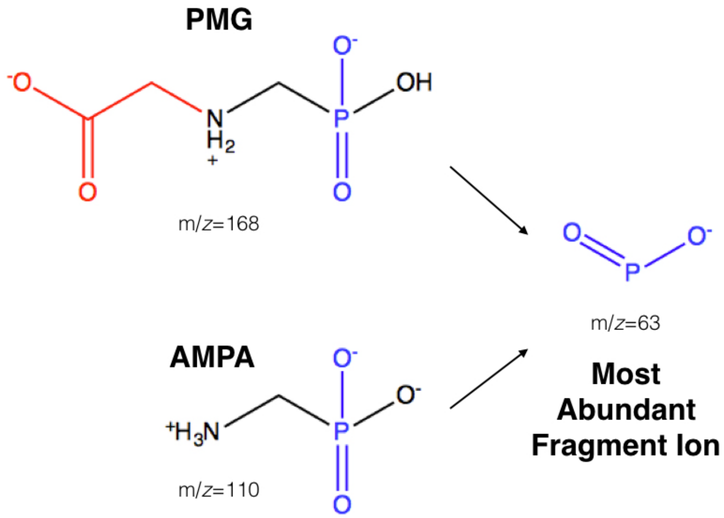
- Classified as “not likely to be carcinogenic to humans” by the U.S. EPA in 2015
- 2014: International Agency for Research on Cancer (IARC) meta-analysis finds an association with PMG exposure and Non-Hodgkin lymphoma (OR=1.5, 95% CI 1.1-2.0, I^2= 32.7%) (Schinasi et al. 2014)
- 2015: IARC classified PMG as a Group 2A “probable” carcinogen (Guyton et al. 2015)
- *In vitro* and *in vivo* evidence of endocrine disruption (Walsh 2000, Gasnier et al. 2009, Koller et al. 2012, Thongprakaisang et al. 2013)

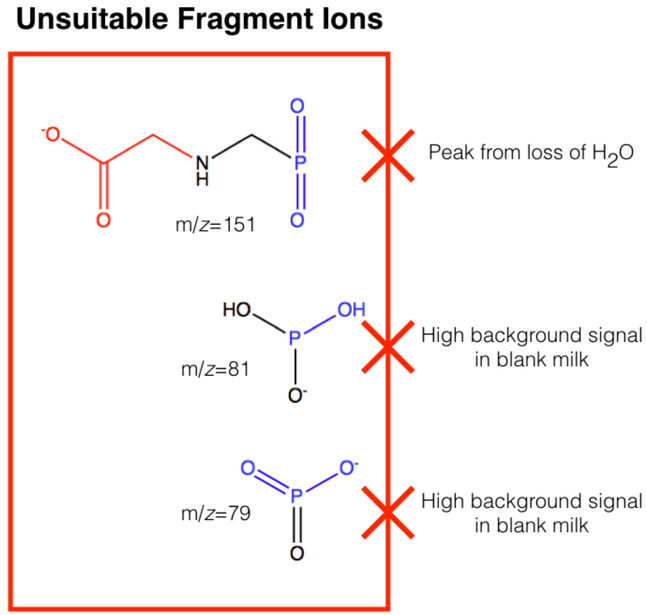
PMG Methods

- Most MS methods for measuring PMG are indirect
- Very few direct methods available-
  - Wang et al. 2008 (serum): LOQ of 5ng/mL with ion-pair chromatography
  - Yoshioka et al. 2011 (whole blood): LOD of 20ng/mL, LOQ of 90ng/mL
  - Jensen et al. 2016 (urine): LOD and LOQ reported per MRM, problematic method, not externally calibrated in blank urine

Chemical Features of PMG and AMPA

- PMG: secondary aminomethylphosphonic acid
  - Chemical features
    - Amphoteric
    - Small
    - Lacks analytically useful groups, e.g. fluoroph chromophore
    - pKa's 2.32, 5.86, 10.6
    - Chelates bivalent and trivalent cations
  - Stability
    - Water, DT50: 33 days at pH5, 69 days at pH7 77 days at pH9
    - Predominant degration in soil is cleavage of the C-N bond to produce AMPA





LC-MS/MS Overview

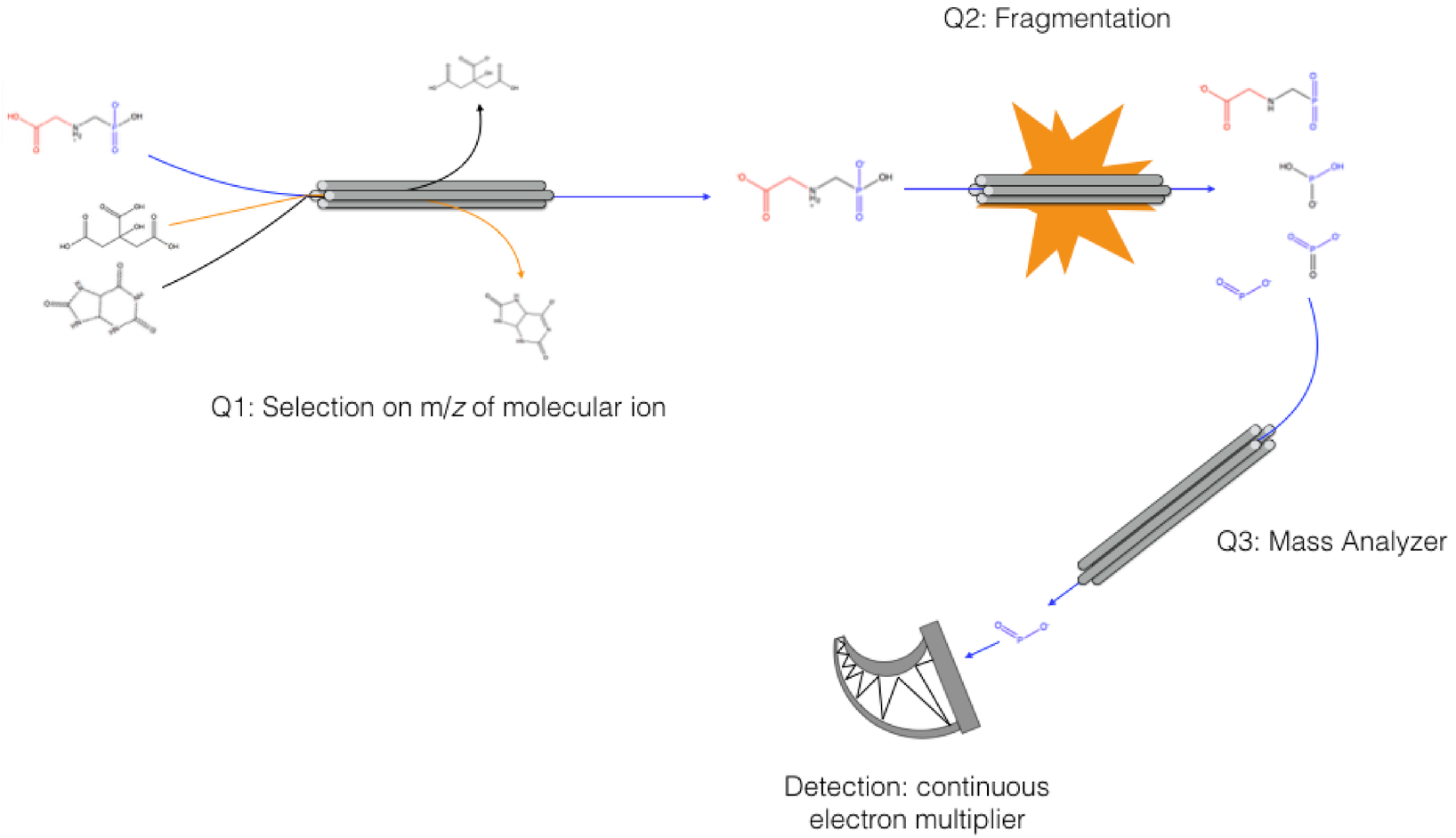
Liquid chromatography-tandem mass spectrometry; the gold standard of separation science for polar analytes and complex matrices

<u>Principles</u>

1. Separation of solution based upon polarity & stereochemistry/charge location (LC)

2. Separation of individual molecular ions (MS1)

3. Separation of individidual fragments (MS2)



Matrix: Tap Water

Matrix: Tap water from SF municipal water supply

1. Water acidified to pH 2.2
2. ^{13}C-PMG (internal std.) spiked at 5ng/mL
3. Externally calibrated - 10pt calibration curve ranging 10-0.02ngPMG-AMPA/mL

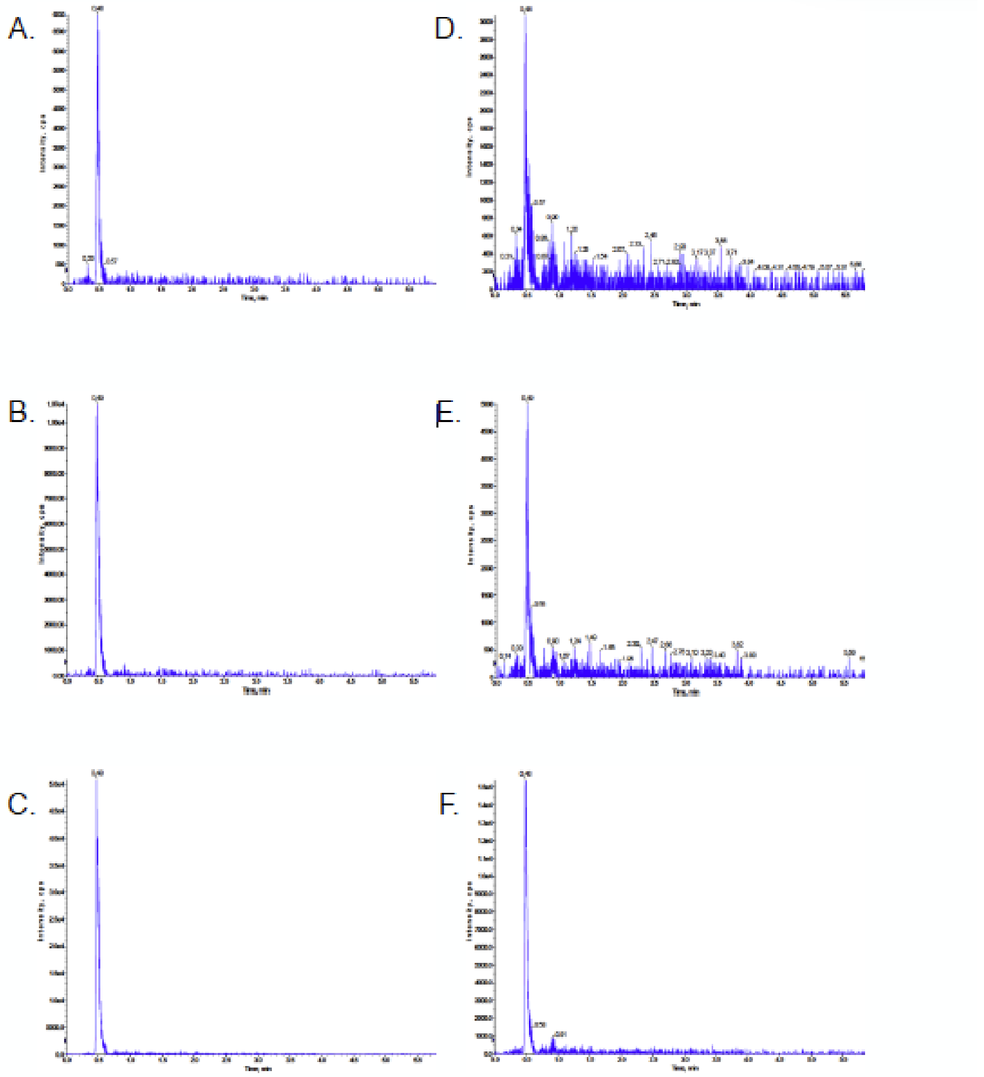


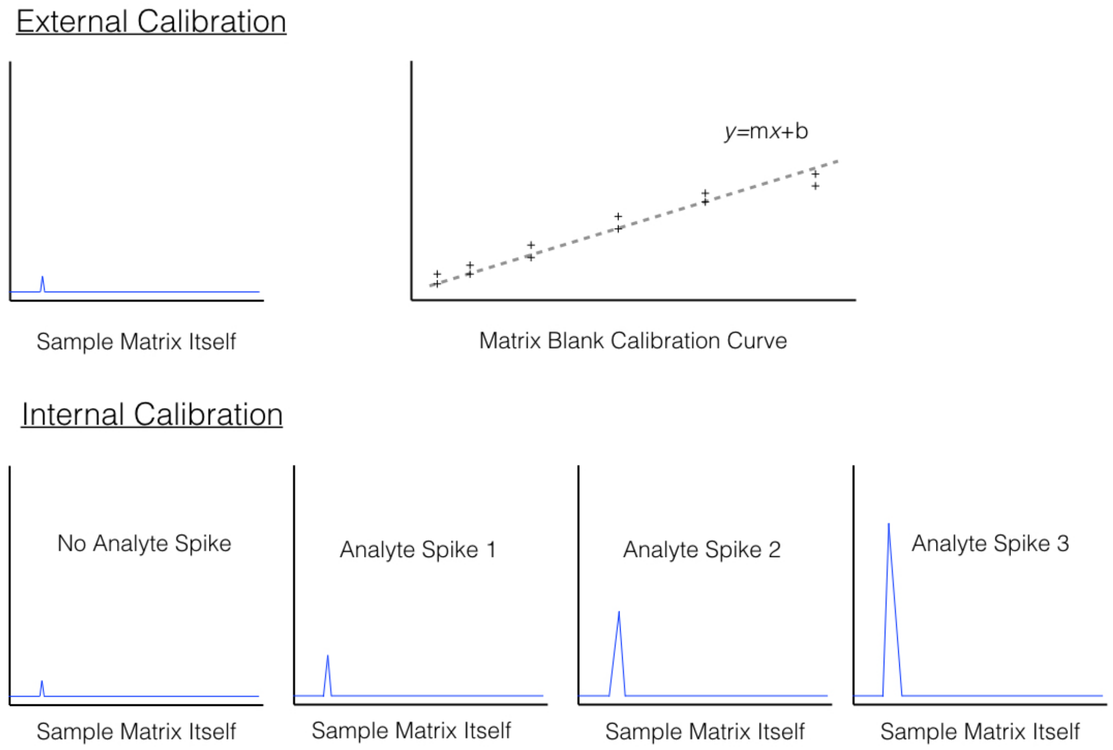
Figure 2. Extracted ion chromatograms for PMG in tap water. PMG (qt fragment 168.071/62.900) at 0.5, 1, and 5 ng/mL (A., B., C., respectively). PMG (ql fragment 168.071/81.000) at 0.5, 1, and 5 ng/mL (D., E., F., respectively).

Tap Water Validation

	Intraday Precision, %CV (n=6)	Intraday Accuracy (%) (n=6)	Interday Precision, %CV, (n=6)	Interday Accuracy, %CV, (N=6)	LOD (ng/mL)	LOQ (ng/mL)
PMG, Tap Water					0.02	0.08
0.5ng/mL	10.01	0.72	10.44	11.56		
1ng/mL	2.40	2.40	8.57	10.03		
5ng/mL	5.76	10.51	6.92	2.90		
AMPA, Tap Water					0.04	0.1
0.5ng/mL	3.08	6.47	9.50	2.71		
1ng/mL	4.41	14.37	15.34	7.53		
5ng/mL	4.61	4.153	16.18	7.40		

Method Transfer: Standard Addition

- Urine and breast milk are more complex than tap water
- Available matrix blanks not available or not suitable (e.g., background in drug-free human urine)
- Internal calibration is an alternate method for dealing with uncertainty in matrix effects
- PMG elutes at near the solvent front
- Can reduce rotational matrix effects but not translational matrix effects



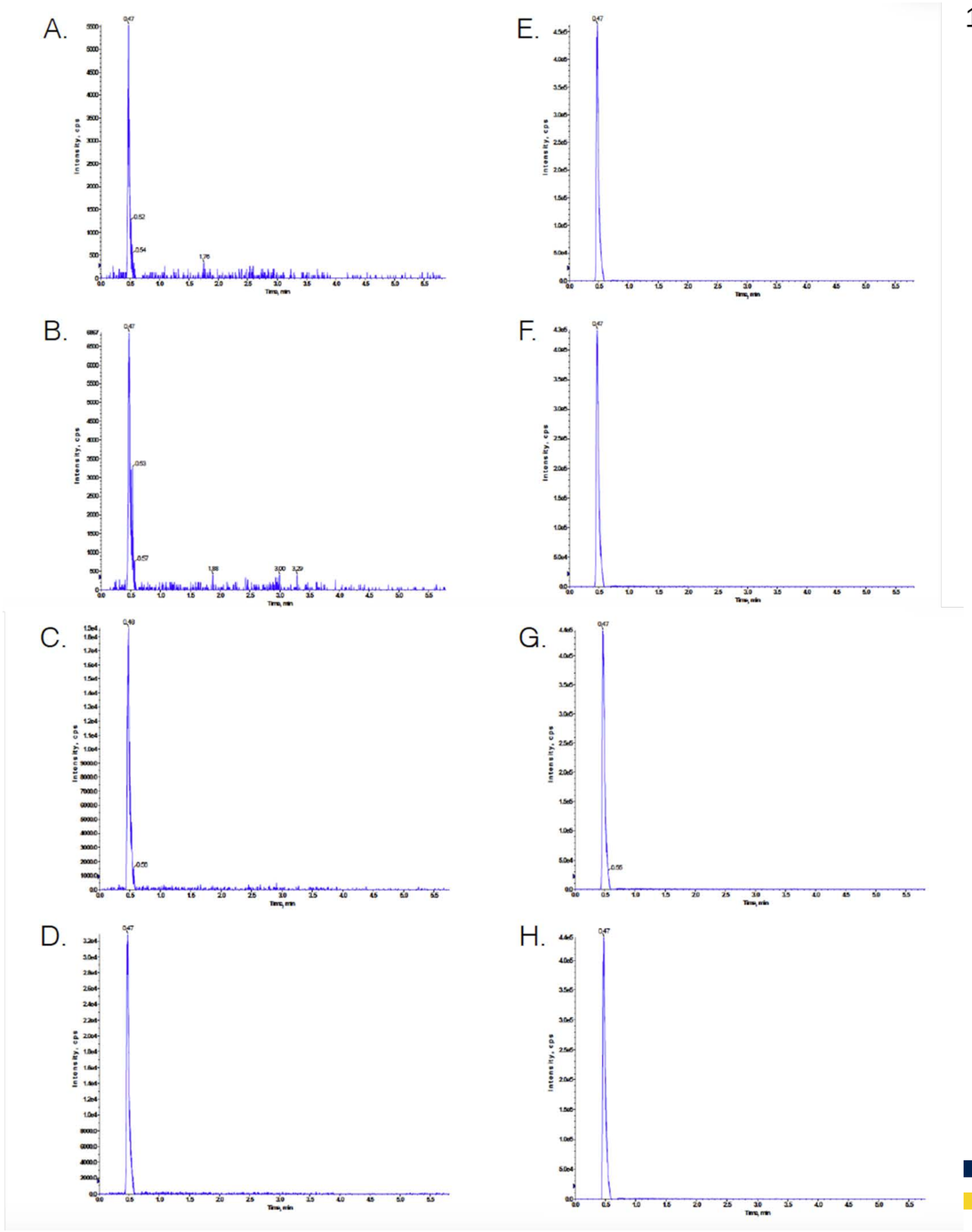
Matrix: Urine

Stock Matrix: Drug-free human urine & urine from three lab staff

1. Urine diluted 10x with HPLC-grade water and acidulated to 1%FA

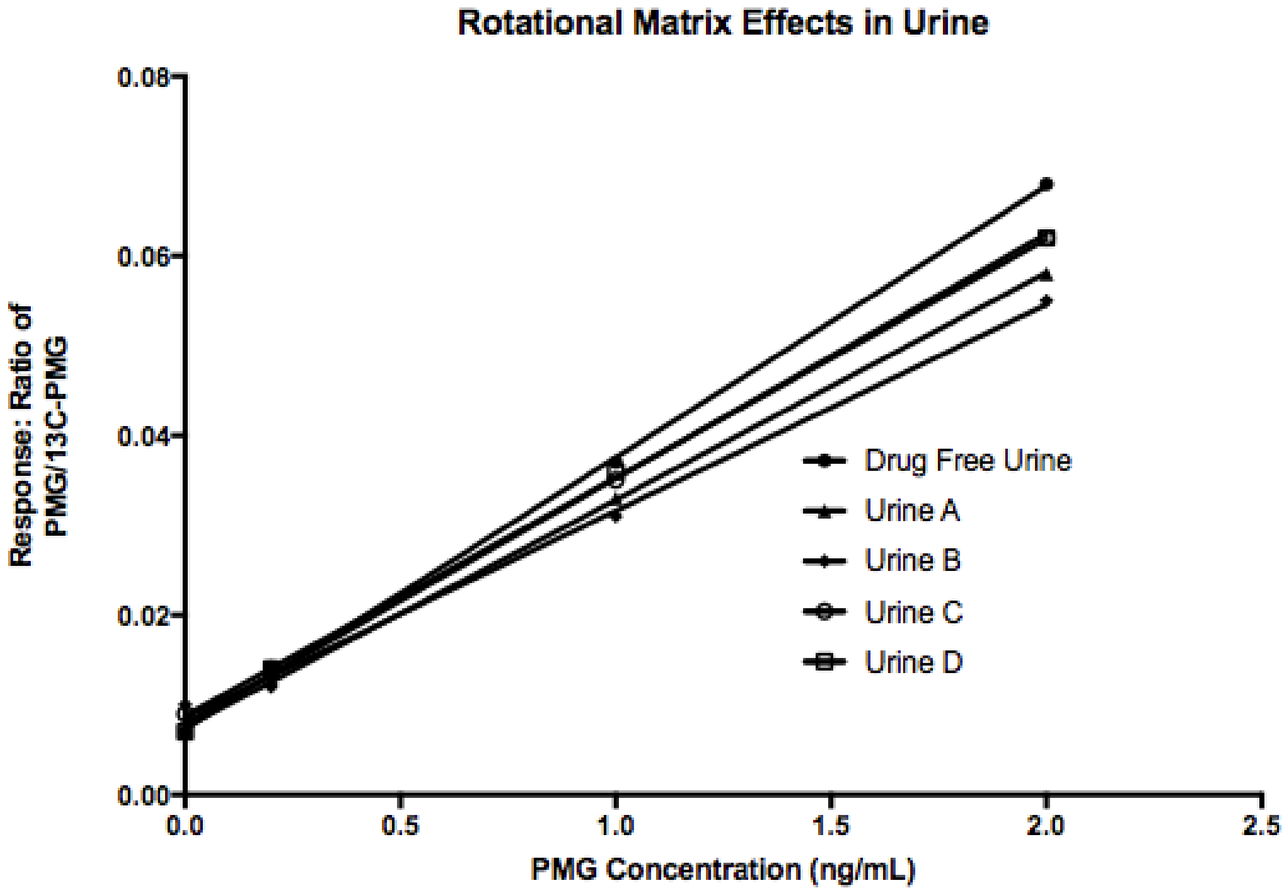
2. ^{13}C-PMG spiked at 50ng/mL of diluent

3. Standard addition curve prepared with fortifications at 0, 0.2, 1, and 2 ng/mL of diluent



Urine Validation

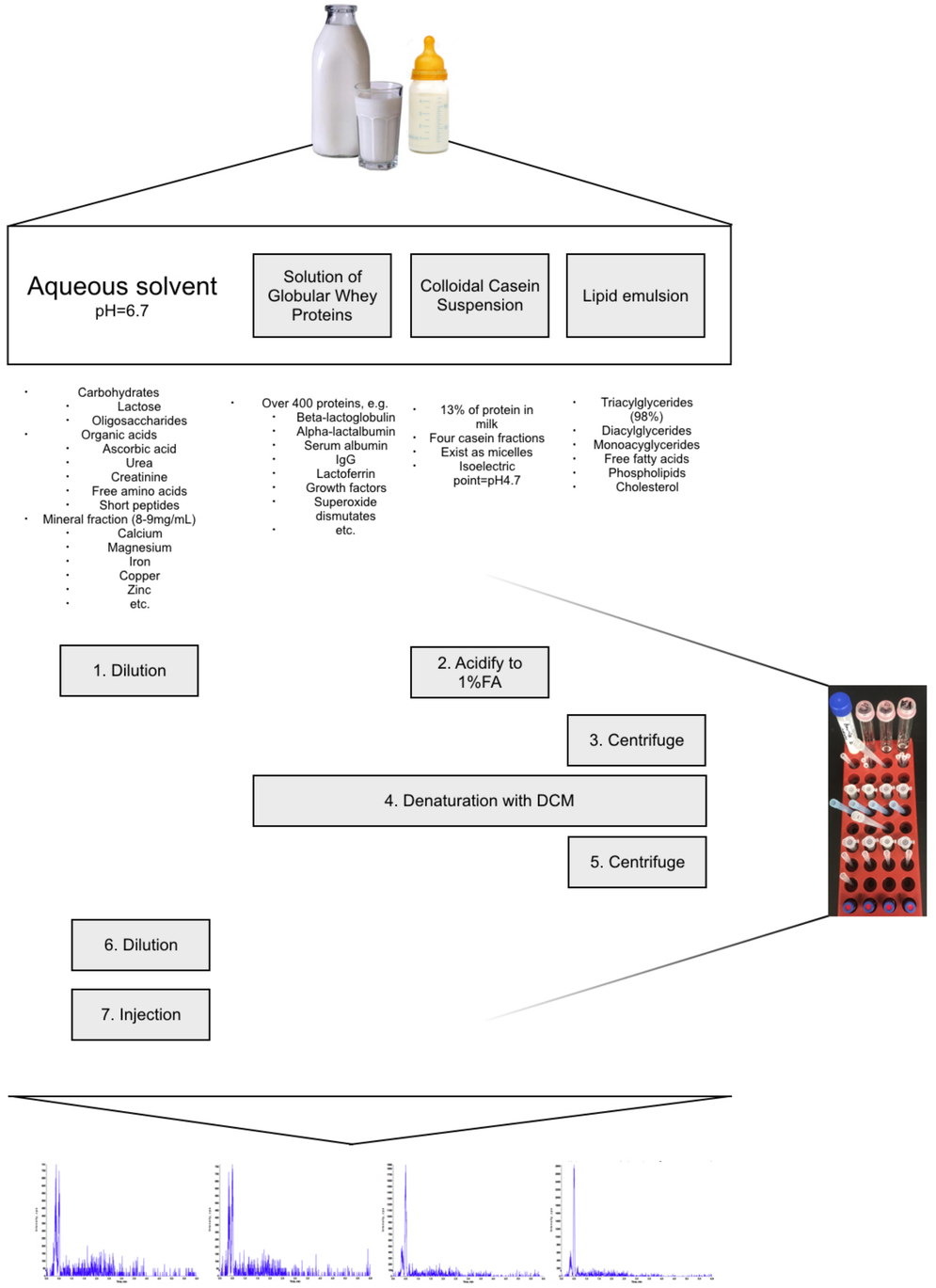
- Linear Range: 0.1-50ng PMG/ml urine (R^2: 0.9978-0.9997)
- Interday variation: 10.37% (n=4 individuals)
- Intraday variation: 8.51% (n=5 replicates)
- LOD: 0.1ng PMG/ml
- κ=31
- Q=6.45



Matrix: Milk

Matrix for method development: non-homogenized bovine milk (both pasteurized and unpasteurized) and human breast milk

1. Dilute 150µl of milk to 1 ml with 1% formic acid v/v in water
2. Spike C13-PMG to 50ng/mL diluent
3. Centrifuge to remove lipid and casein fraction
4. LLE with DCM 50:50; thoroughly mix
5. Centrifuge to remove globulin fraction and additional phospholipids
6. Dilute supernatant 2x with 1% formic acid in water



Milk Validation

- Linear Range: 1-40ng PMG/ml of milk (R^2: 0.9975-0.9991)
- Interday variation: 9.75%, (%CV), n=4 bovine milk samples
- Recovery:
  - 1ng/ml: 87.3%
  - 5ng/ml: 97.2%
  - 10ng/ml: 93.8%
- LOD: 0.167ng PMG/ml of milk (S:N>3)
- LOQ: 1ng PMG/ml of milk (S:N>10)
- K and Q are unknown

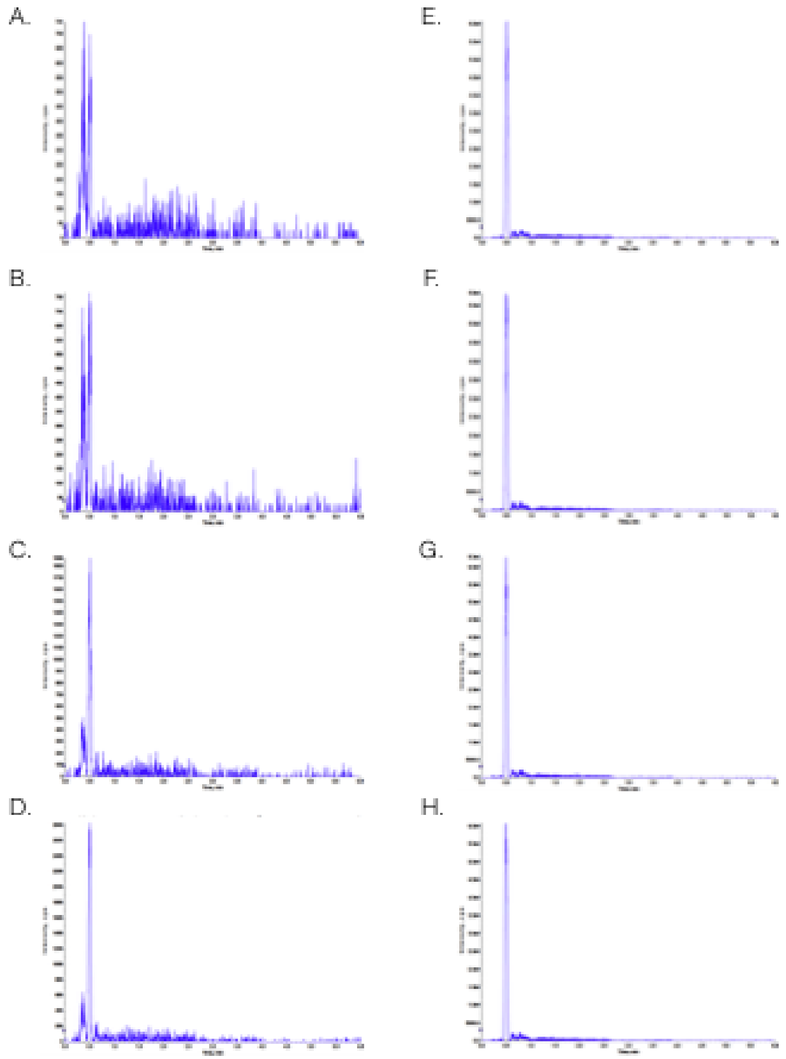


Figure 4. Extracted ion chromatogram for PMG and C13-PMG for one standard addition curve in raw, unpasteurized bovine milk. 0ng/mL, 0.2ng/mL, 1ng/mL, and 2ng/mL extract (A., B., C., and D., respectively), and C13-PMG at 25ng/mL extract in each sample aliquot corresponding to A., B., C., and D., (E., F., G., and H., respectively).

Methodological Limitations

1. Obelisc-N column subject to degradation (~1000 injections in milk extracts)

2. Standard addition - 4 aliquots/sample

   - No external calibration, however

3.With the current method, AMPA not detectable due to short RT

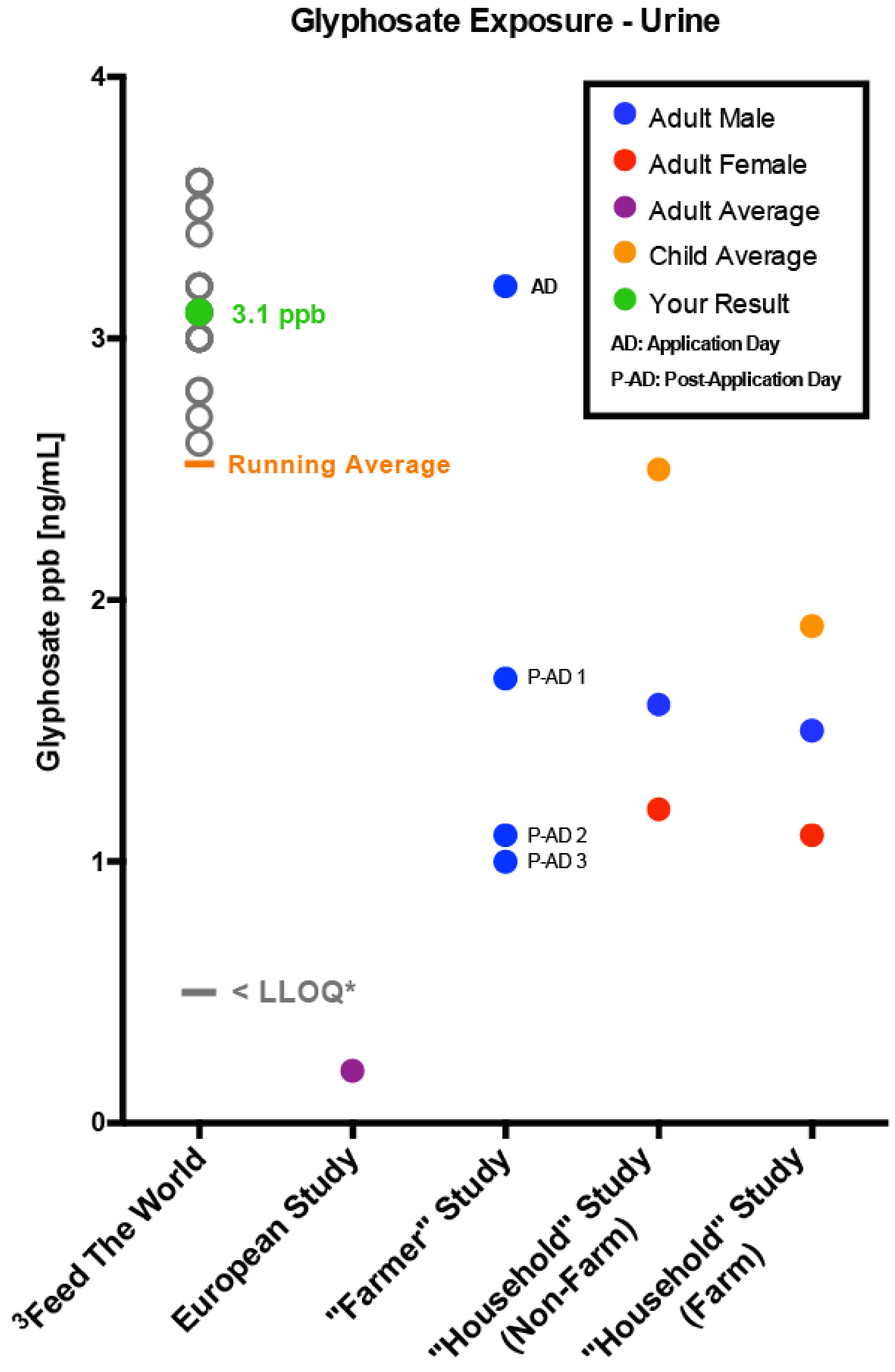
Application of Methods to Epidemiological Studies

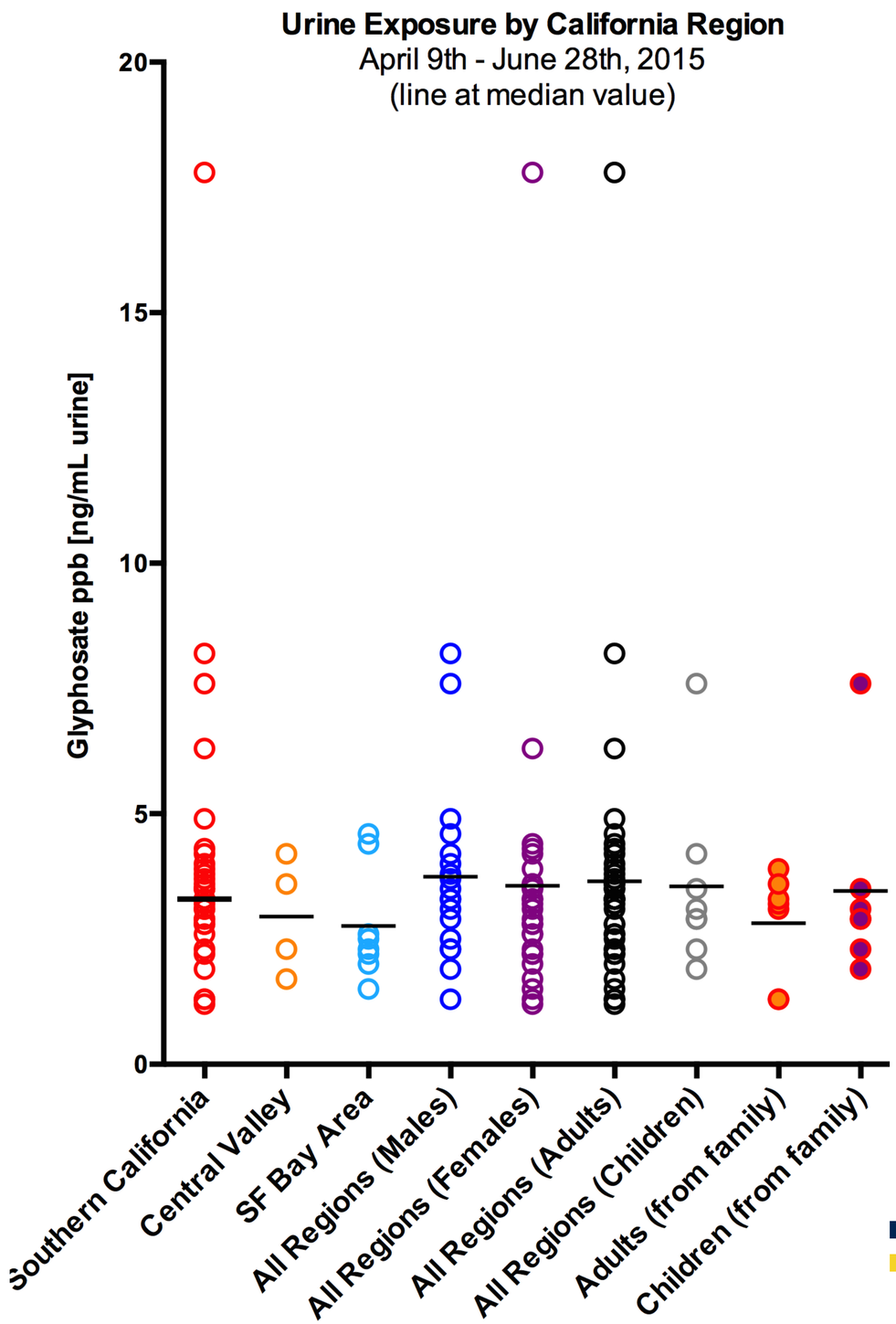
- Cohort 1: The Detox Project- Adult and Children (n=252): Completed
- Cohort 2: Indiana Pregnant Women (n=283): Sample Run Completed
- Cohort 3: Wisconsin Infants in First Year of Life (n=144): On-Going
- Cohort 4: Children with Autism (n=60, breast milk; n=180 urine): Samples for transport
- Cohort 5: Mother-Infant samples (n=200, matched serum, urine, breast milk and infant urine): Recruiting

Cohort 1: The Detox Project

252 Urine samples sent from across the United States

OVERALL STATISTICS						CANADA
STATISTICS	US	WEST	MIDWEST	SOUTH	EAST	
Number of Subjects	252	124	55	24	47	9
Detection Frequency	86.1%	85.2%	90.9%	83.3%	87.2%	88.9%
Mean	3.1	3.0	3.3	3.0	3.4	3.1
Median	3.3	3.1	3.4	3.4	3.4	3.1
Geometric Mean	2.5	2.3	2.8	2.4	2.8	2.2

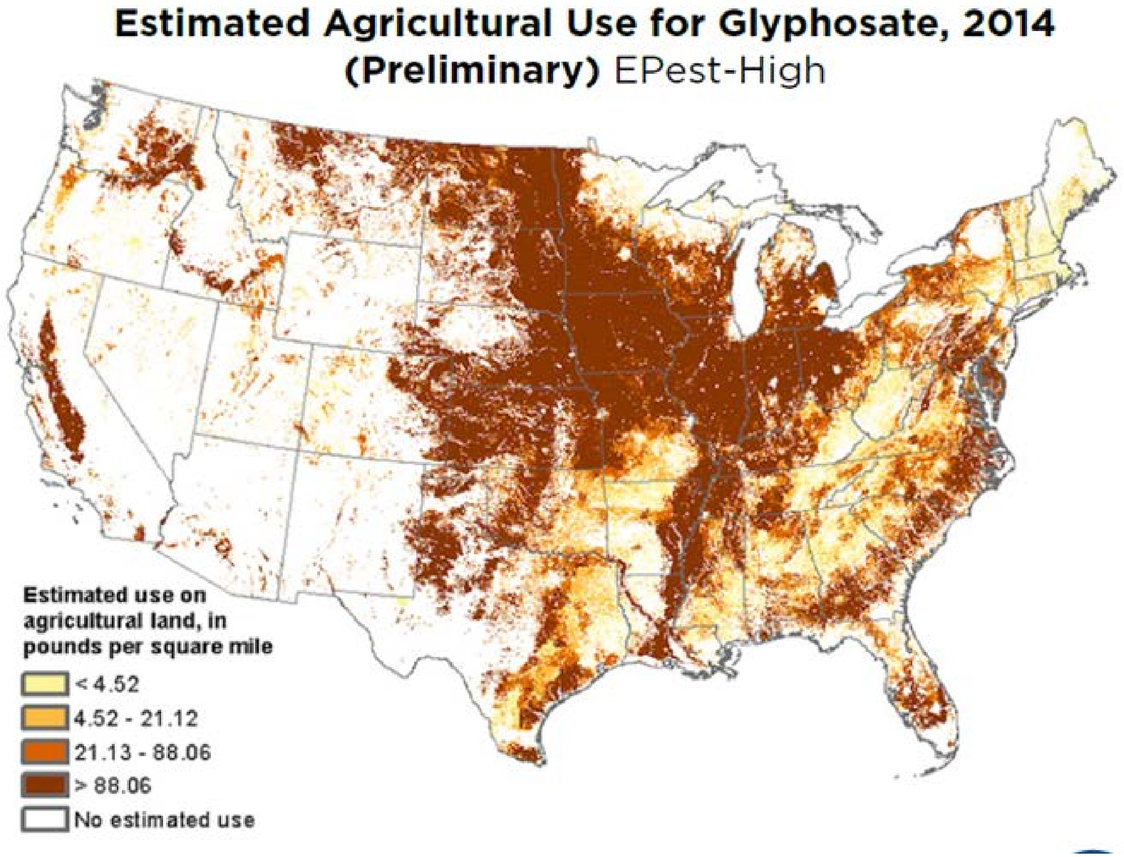

Glyphosate Exposure - Urine
Glyphosate ppb [ng/mL]
4
3
2
1
0
Adult Male
Adult Female
Adult Average
Child Average
Your Result
AD: Application Day
P-AD: Post-Application Day
3.1 ppb
Running Average
< LLOQ*
AD
P-AD 1
P-AD 2
P-AD 3
[3]Feed The World
European Study
"Farmer" Study
"Household" Study (Non-Farm)
"Household" Study (Farm)


Urine Exposure by California Region
April 9th - June 28th, 2015
(line at median value)
Glyphosate ppb [ng/mL urine]
20
15
10
5
0
Southern California
Central Valley
SF Bay Area
All Regions (Males)
All Regions (Females)
All Regions (Adults)
All Regions (Children)
Adults (from family)
Children (from family)

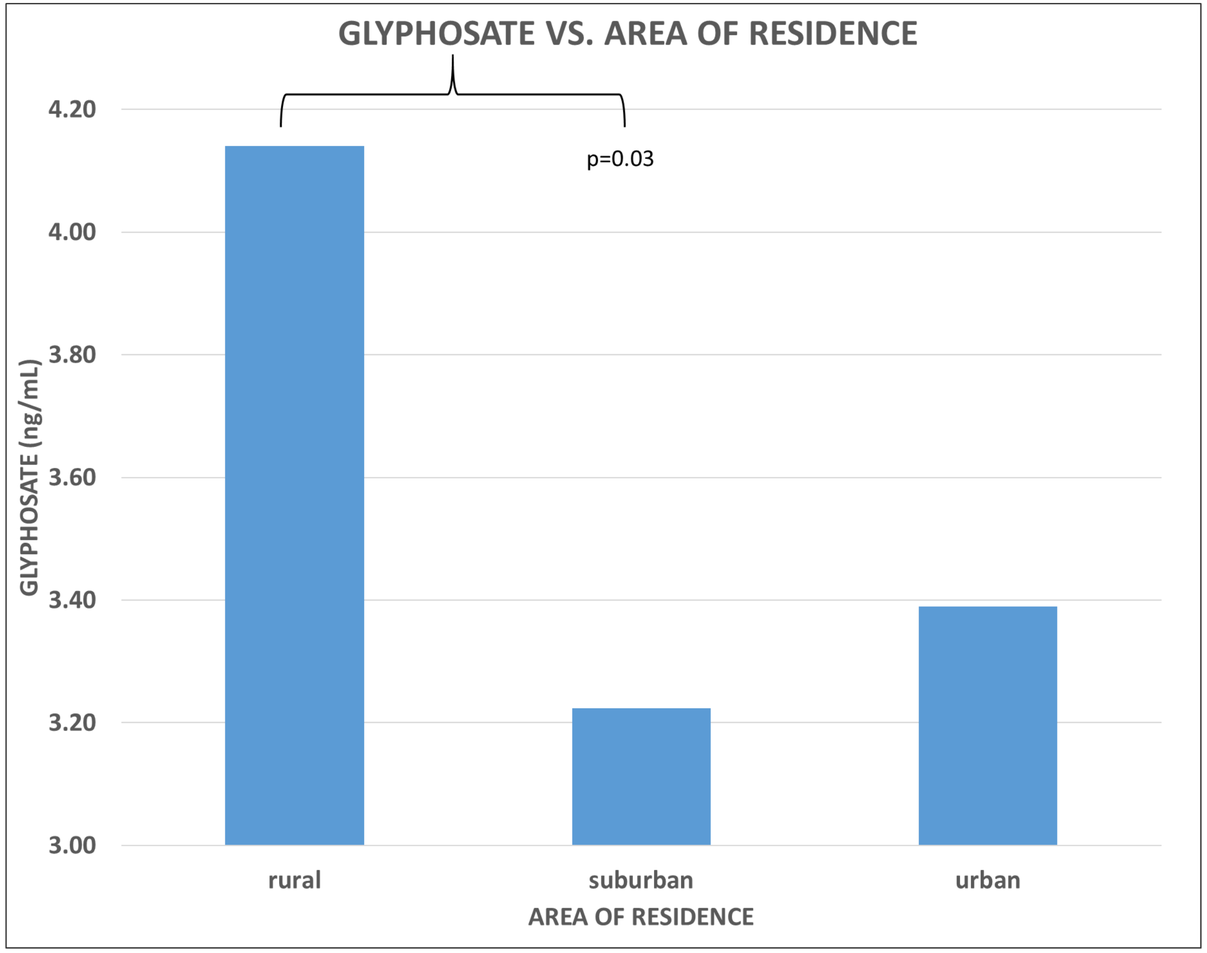
Cohort 2: Indiana Pregnant Women

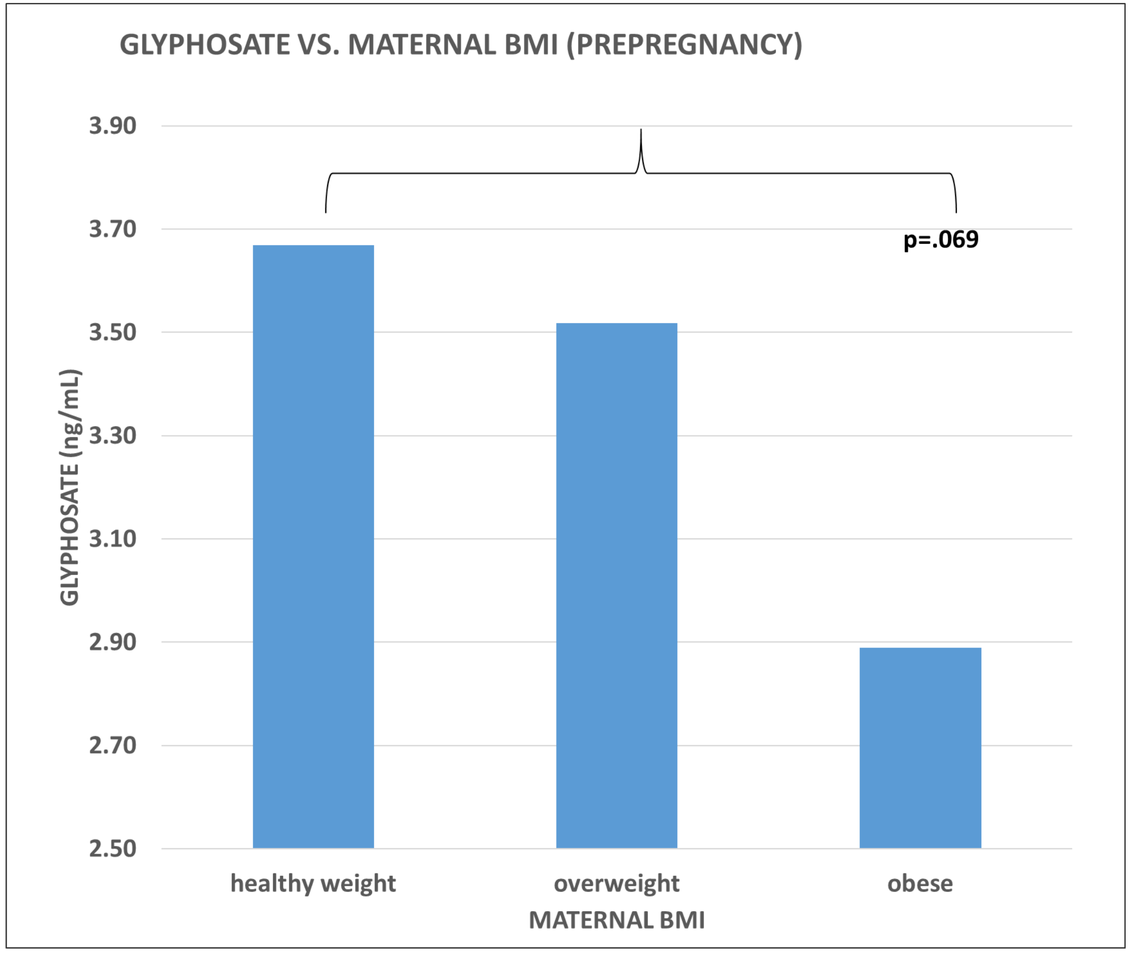
83 urine samples from Indiana pregnant women

OVERALL STATISTICS			
STATISTICS	US	MIDWEST	Indiana
Number of Subjects	252	55	83
Detection Frequency	86.1%	90.9%	91.5%
Mean	3.1	3.3	3.5
Median	3.3	3.4	3.3
Geometric Mean	2.5	2.8	3.0



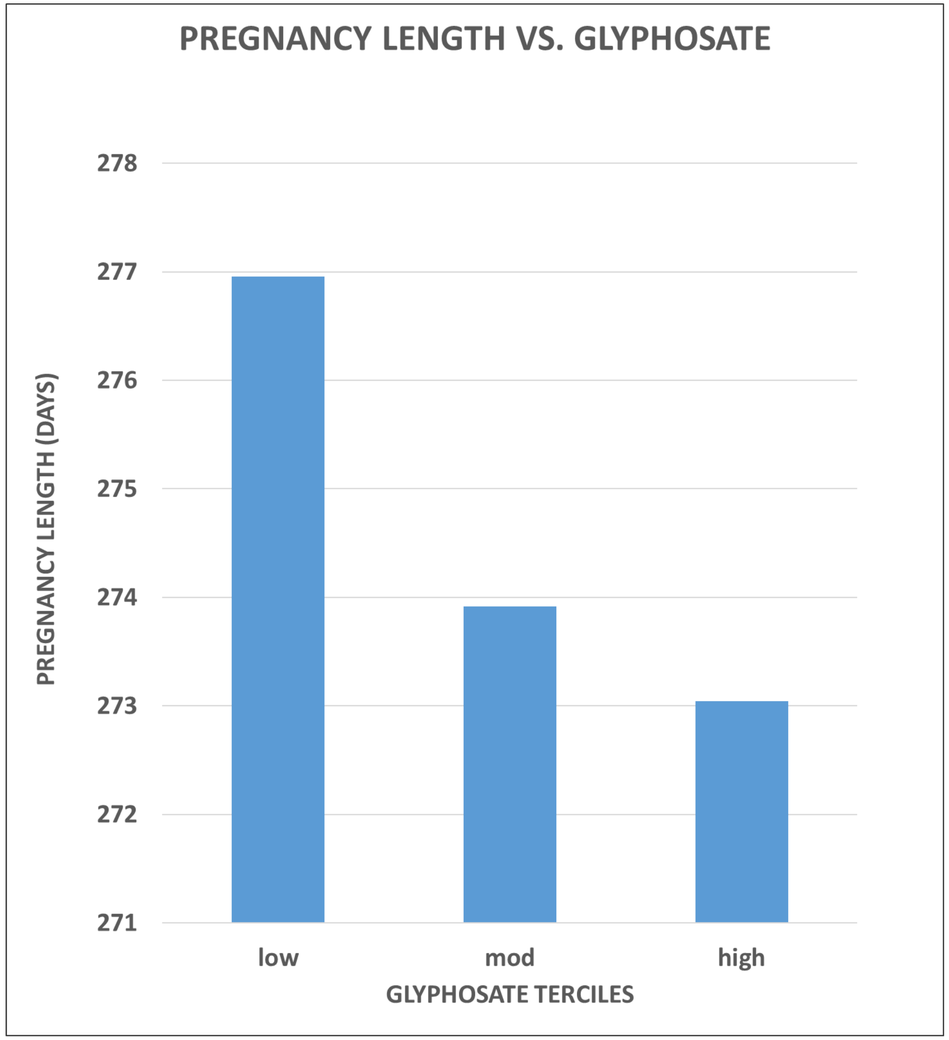
Cohort 2: Indiana Pregnant Women

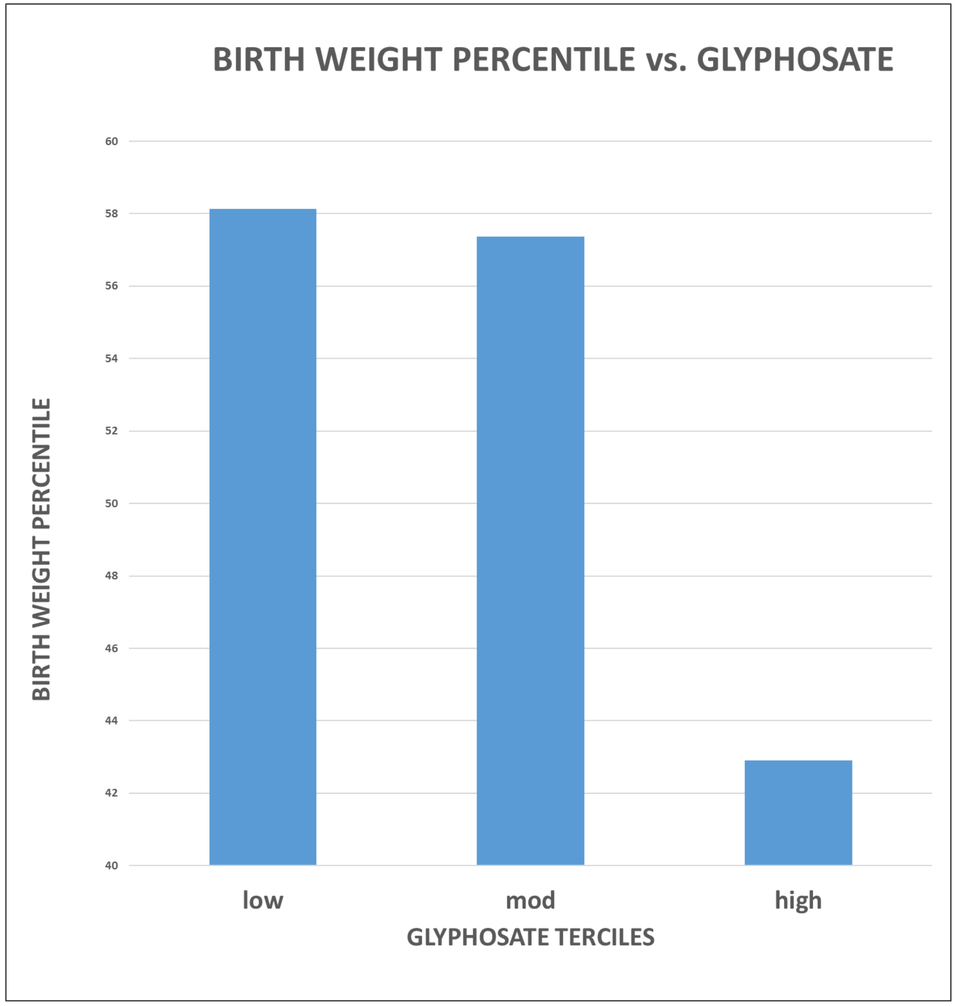




Slide courtesy of Paul Winchester (Indiana University)

Cohort 2: Indiana Pregnant Women





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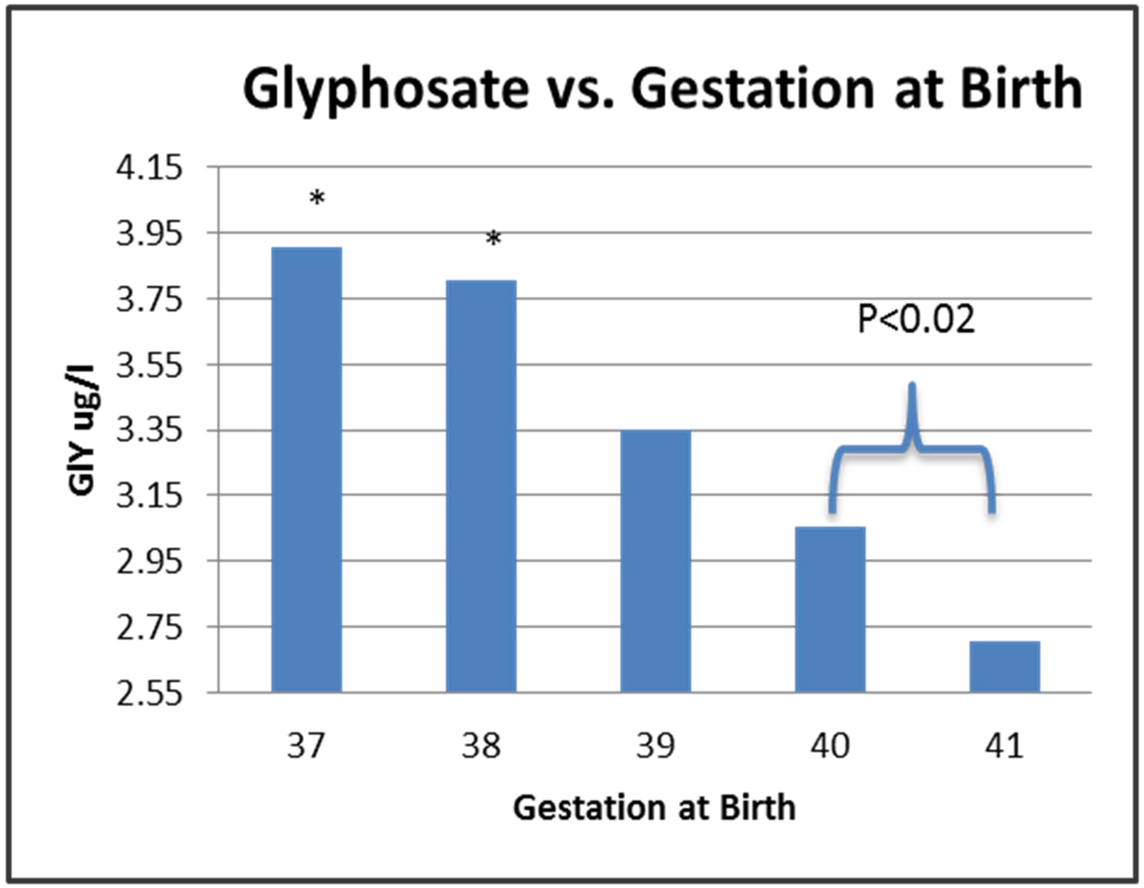
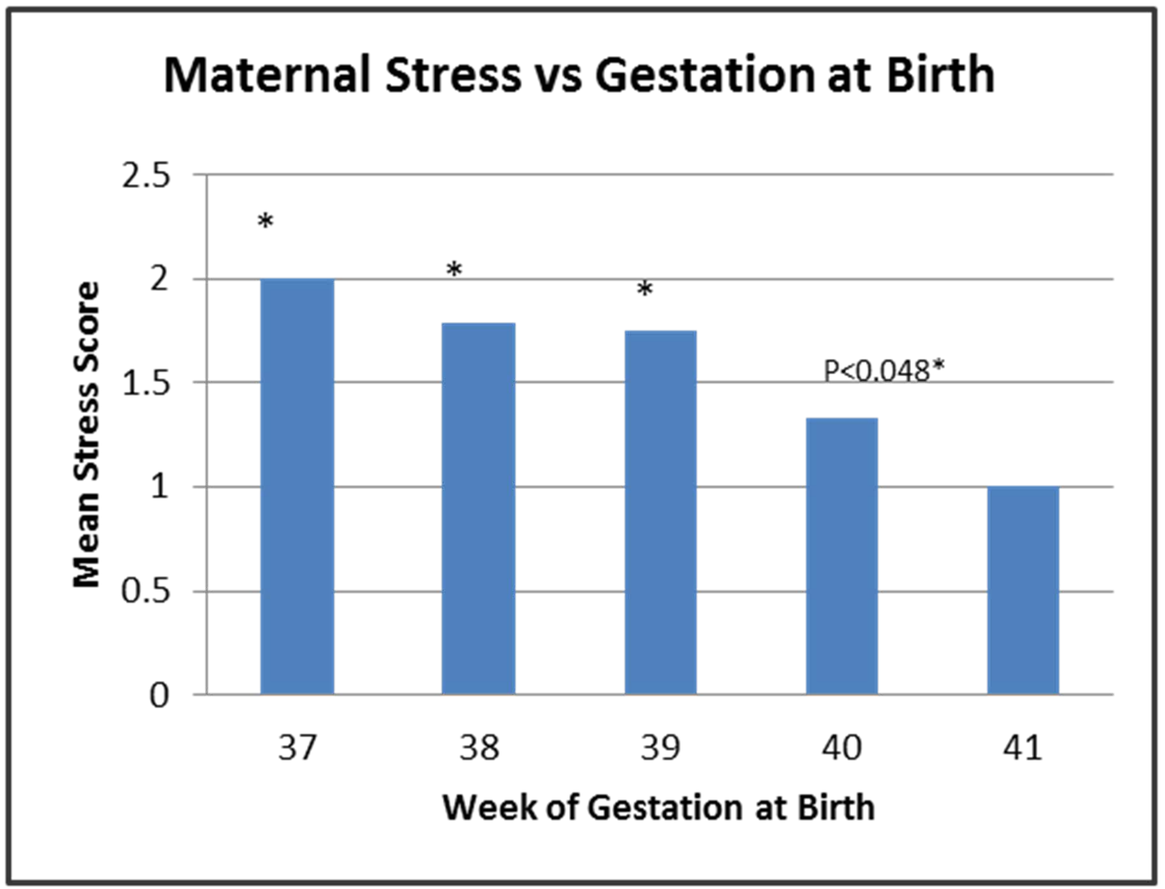
Cohort 2: Indiana Pregnant Women





Slide courtesy of Paul Winchester (Indiana University)

Future Directions

- Re-validate milk method in n=10 breast milk lot
- Finish current collaborative epidemiological studies
- Assist with method transfer to a collaborating laboratory
- Assist several groups with method application to epidemiological research pursuits
- Incorporate in a multi-analyte method for polar pesticides (OP metabolites, glufosinate, 2,4-D)

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UCSF Clinical Toxicology and Environmental Biomonitoring Laboratory

UCSF University of California San Francisco

Berkeley UCSF Joint Medical Program

Food Residues with Glyphosate

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

270.24 ppb

275.57 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

Glyphosate in Oatmeal, Pita Chips, California Wine

Oatmeal products from major food brand Quaker Oats were found to contain glyphosate, Food and Drug Administration (FDA) February 2016 FDA started testing for GLY. In November 2016 (Trumps'

10/36 (28%) Oatmeal products + glyphosate

812.53 ppb GLY

100% California Wines tested + GLYPHOSATE

Pepperidge Farm Crackers,
Little Debbie Oatmeal Crème Pies
Fritos, Lucy's Oatmeal Cookies, Back to Nature Crackers

Nabisco (continued)		
Double Oreo	Oreo Double Stuf Chocolate Sandwich Cookies	Glyphosate – 140.90* ppb
Double Oreo	Oreo Double Stuf Golden Sandwich Cookies	Glyphosate – 215.40* ppb
PepsiCo		
Stacy's	Stacy's Simply Naked Pita Chips (Frito-Lay)	Glyphosate – 812.53 ppb
Lay's	Lay's: Kettle Cooked Original	Glyphosate – 452.71* ppb
Doritos	Doritos: Cool Ranch	Glyphosate – 481.27* ppb
Fritos	Fritos (Original) (100% Whole Grain)	Glyphosate - 174.71* ppb
Campbell Soup Company		
Goldfish	Goldfish crackers original (Pepperidge Farm)	Glyphosate – 18.40 ppb
Goldfish	Goldfish crackers colors	Glyphosate – 8.02 ppb
Goldfish	Goldfish crackers Whole Grain	Glyphosate – 24.58 ppb
Little Debbie		
Little Debbie	Oatmeal Creme Pies	Glyphosate – 264.28* ppb
Lucy's		
lucy's	Oatmeal Cookies Gluten Free	Glyphosate – 452.44* ppb
Whole Foods		
365	365 Organic Golden Round Crackers**	Glyphosate – 119.12* ppb
Back to Nature		
back to NATURE	Crispy Cheddar Crackers	Glyphosate – 327.22* ppb

Organic Food Consumption