
Fetal Exposure to Glyphosate

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SCHOOL OF MEDICINE

Disclosure

The Investigators of this study have no financial relationships to disclose or Conflicts of Interest (COIs) to resolve.

Background

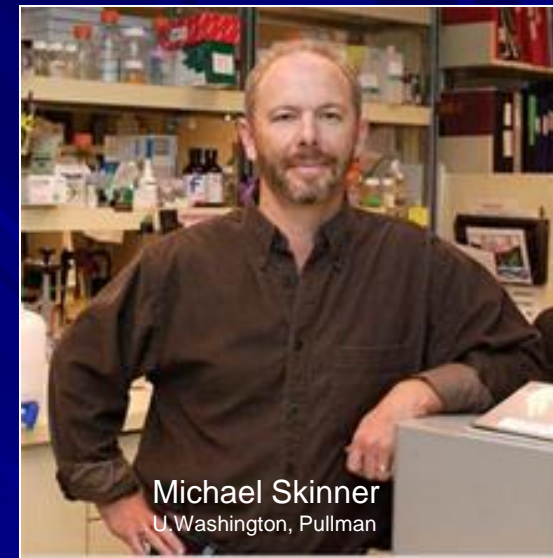
- 300 million pounds of glyphosate (GLY) (Roundup®) are applied each year in the US.
- Though glyphosate has been in use since 1974 no previous measures of GLY exposure in US pregnancies have been published.
- Rodent models of pesticide exposure in pregnancy correlate with adult and transgenerational disease through epigenetic mechanisms.
- We measured GLY in pregnant women to estimate fetal exposure and potential adverse effects on pregnancy outcomes.

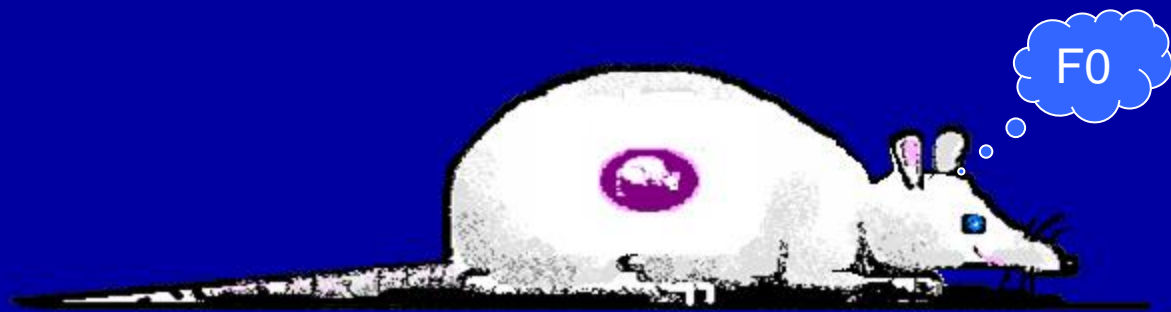
Environmentally Induced Epigenetic Transgenerational Disease

- 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

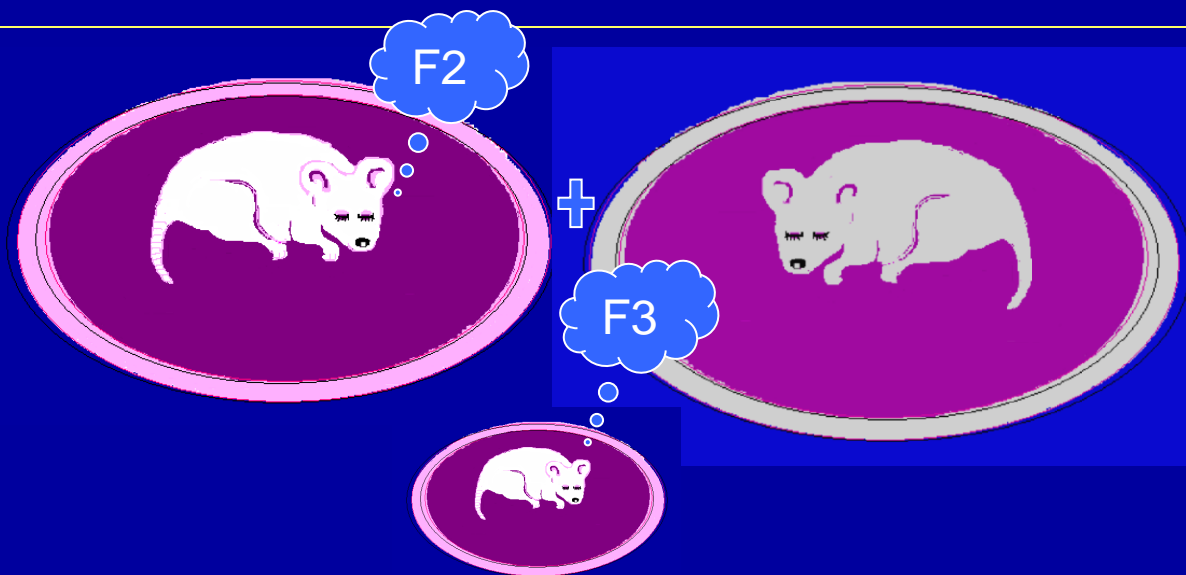
Is Vinclozolin Safe?

- 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!





Vinclozolin
Methoxychlor



Pregnant
Rat



Vinclozolin
Exposure
(PC days 8-14)



Offspring

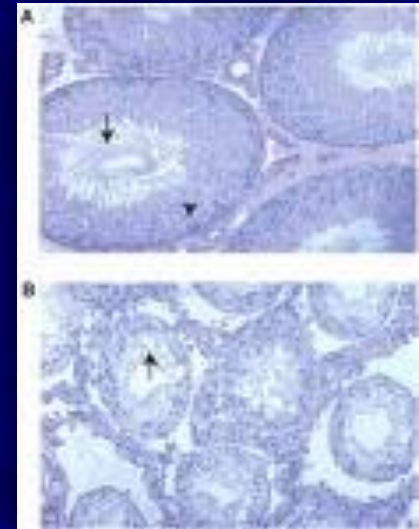
Failed Experiment ! The Baby Rats Were Normal



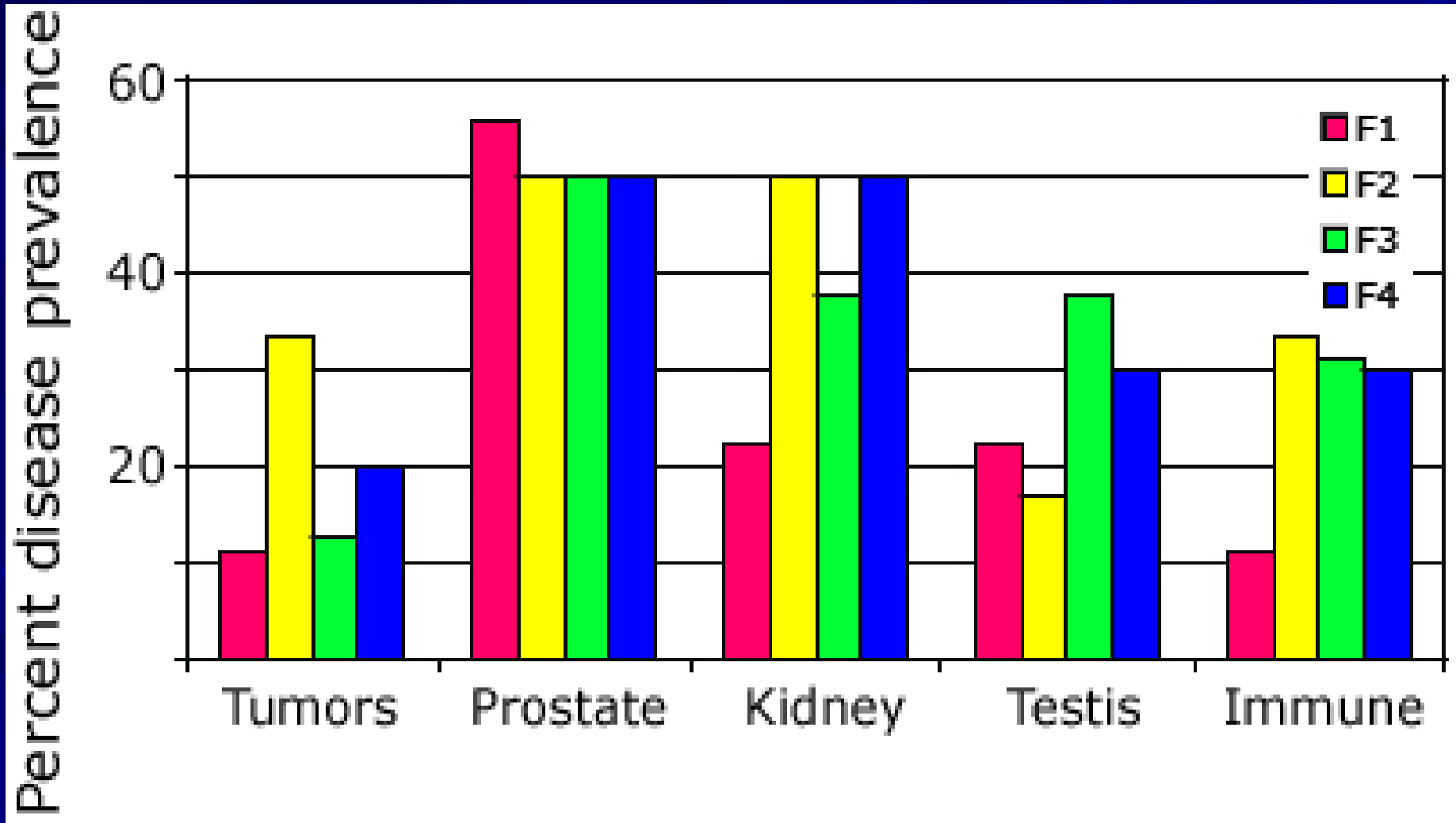
Adult Rats had Diseases

Fetal Exposure: Adult disease

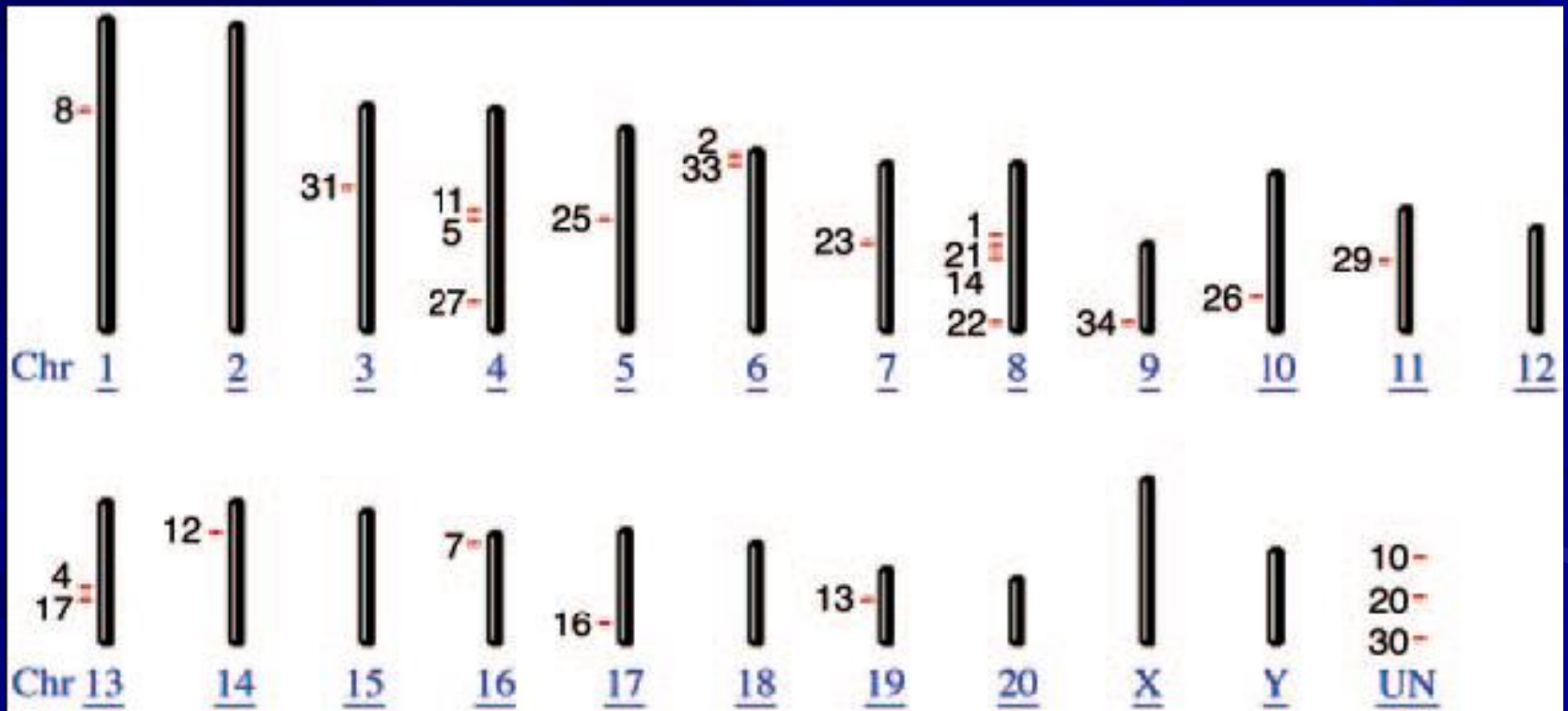
- Low sperm count
- Infertility
- Cancer
- Kidney
- Prostate
- Pregnancy abnormalities
- Immune dysfunction
- High cholesterol
- Accelerated aging
- Non “Sexy” Scent
- Anxiety Prone



Transgenerational Effects of Fetal Pesticide Exposure

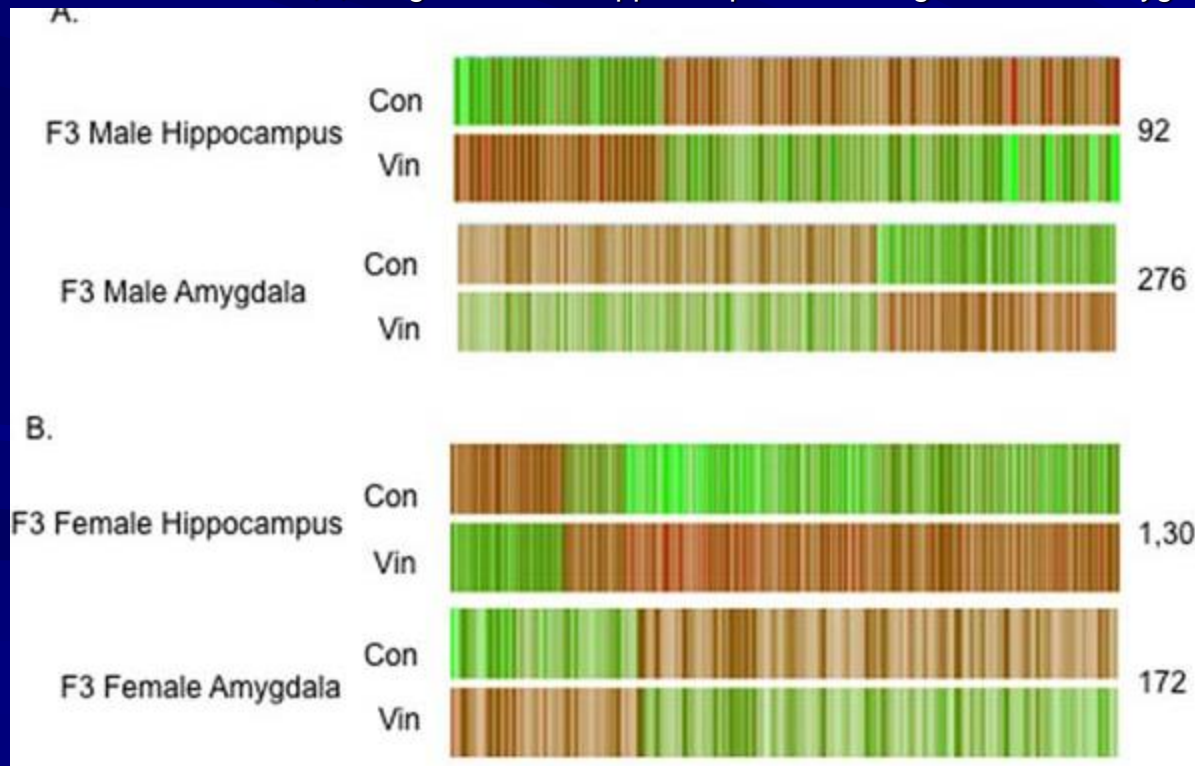


The chromosome location and physical mapping of each candidate (*numbers*) are indicated for each chromosome (Chr), with those unknown (UN) sequences not mapped indicated.



Vinclozolin ;Heritable Anxiety

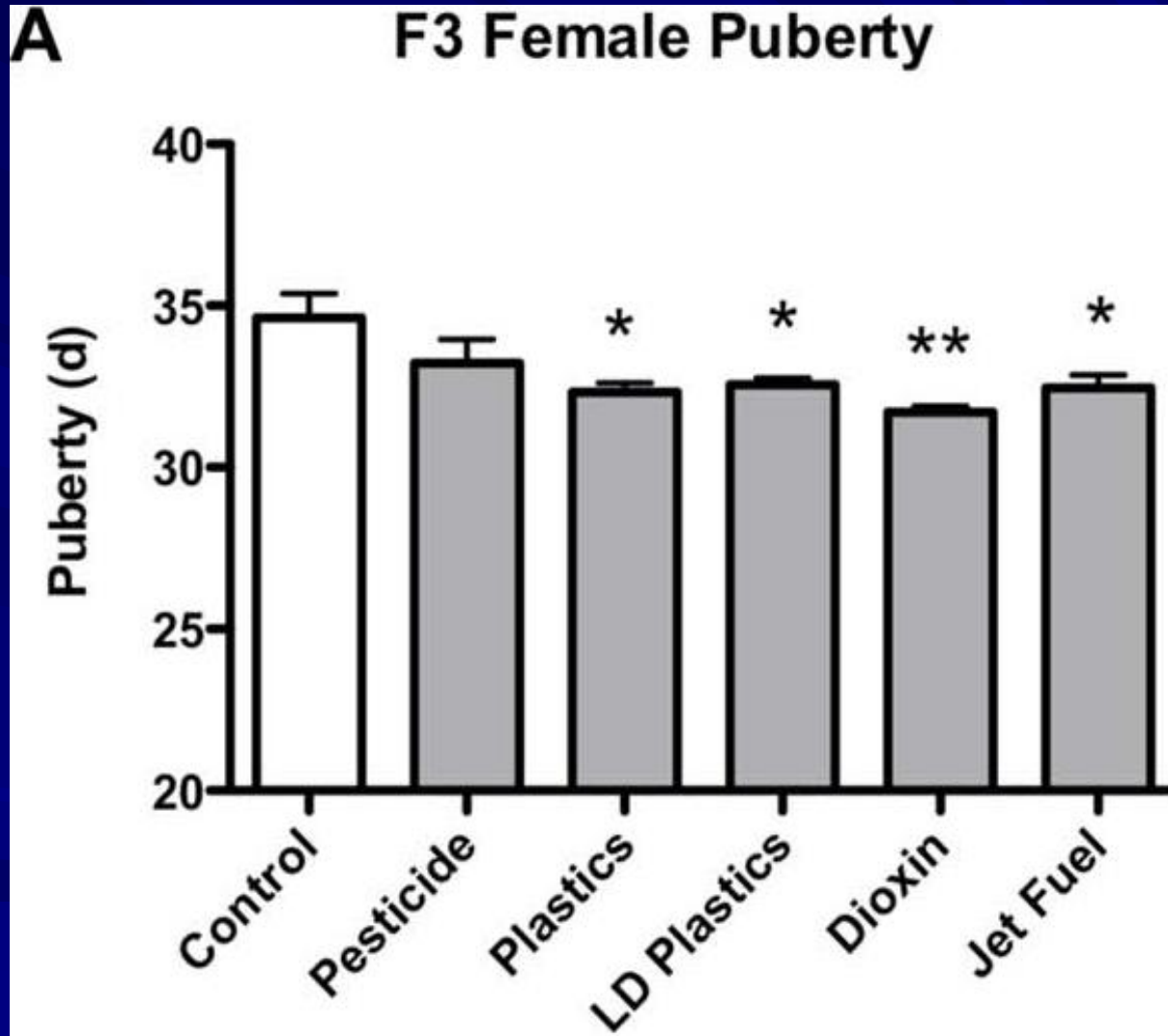
- F3 generation males had a decrease in anxiety-like behavior, 92 genes in the hippocampus and 276 genes in the amygdala
- Females had an increase in anxiety-like behavior, 1,301 genes in the hippocampus and 172 genes in the amygdala.



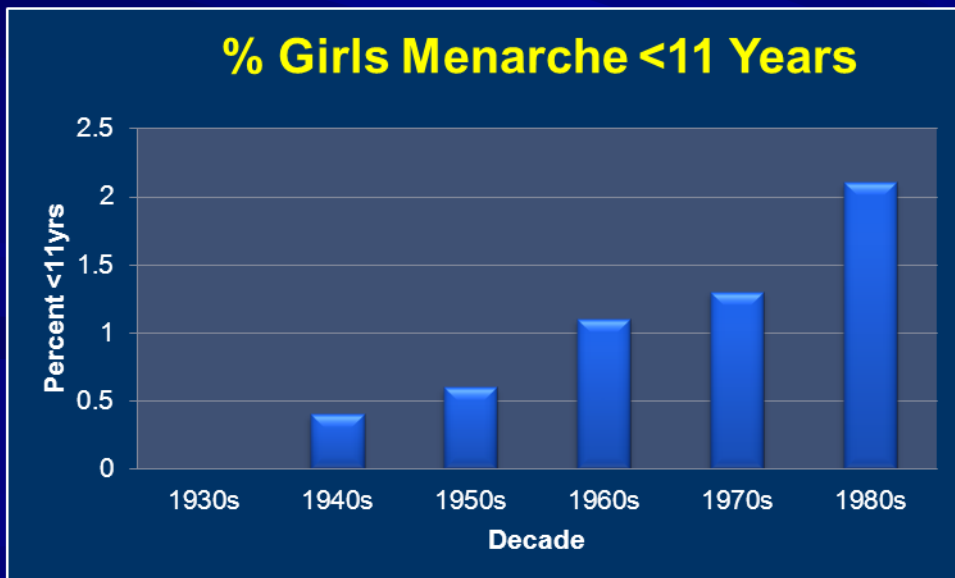
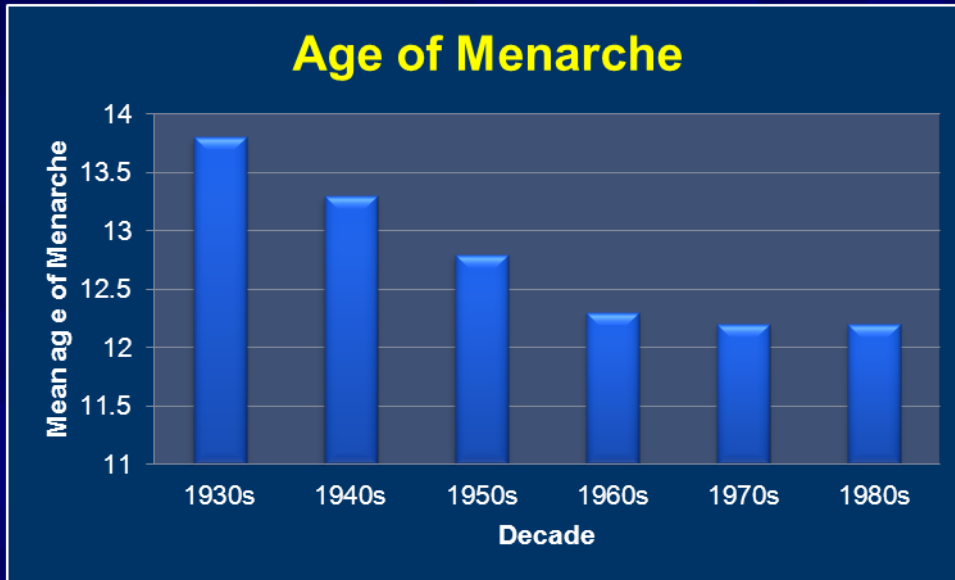
Brain transcriptome microarray analysis from F3 generation control (con) and vinclozolin (vin) animals.

Skinner MK, Anway MD, Savenkova MI, Gore AC, Crews D (2008) Transgenerational Epigenetic Programming of the Brain Transcriptome and Anxiety Behavior. PLoS ONE 3(11): e3745. doi:10.1371/journal.pone.0003745

Younger Age at Menarche



Declining Age of Menarche (Japan)



BMC Women's Health
2012 Jul 16;12(1):19

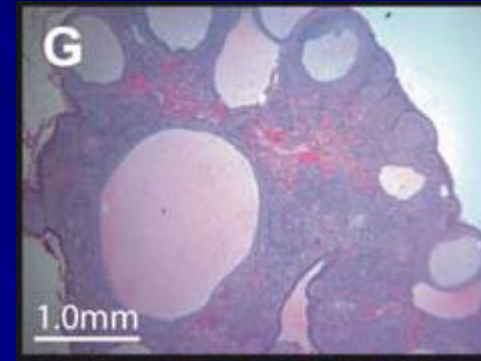
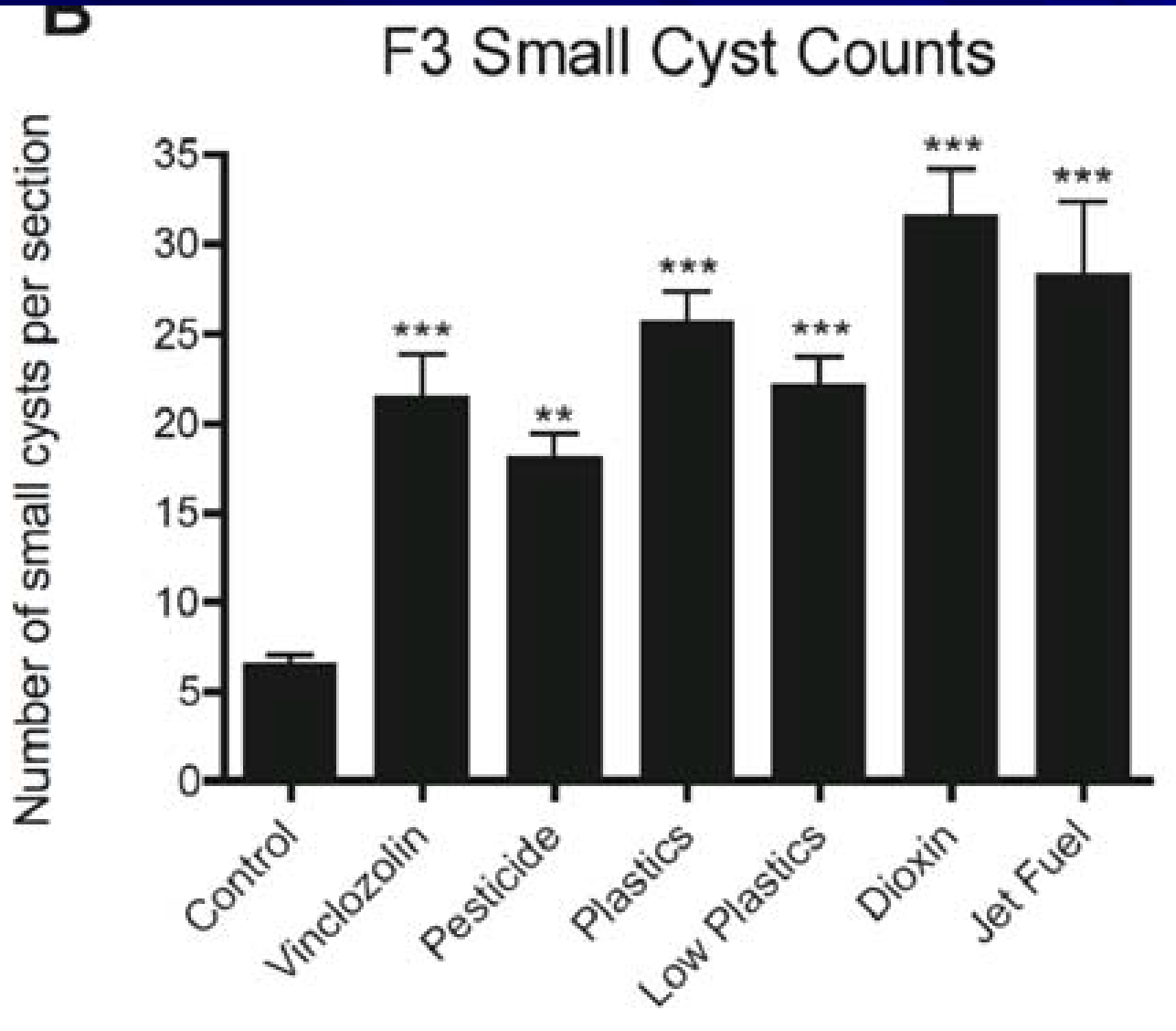
Hosokawa, Imazeki,
Mizunuma, Kubota,
Hayashi

Males Developing 2 years Earlier Too!

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

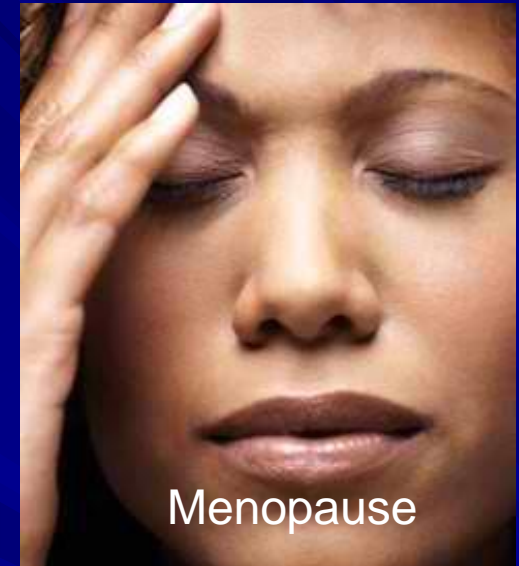
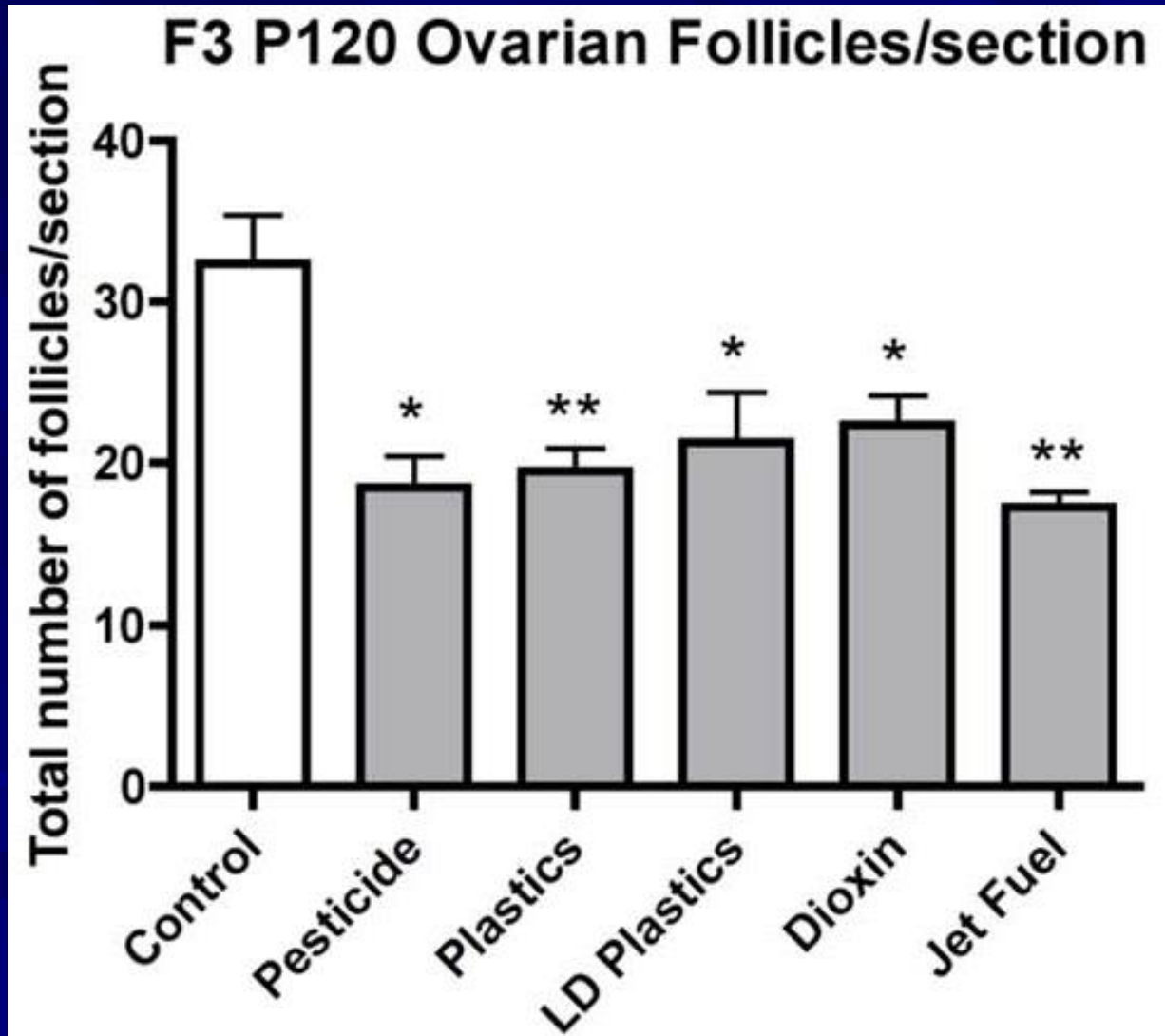


Polycystic Ovarian Disease



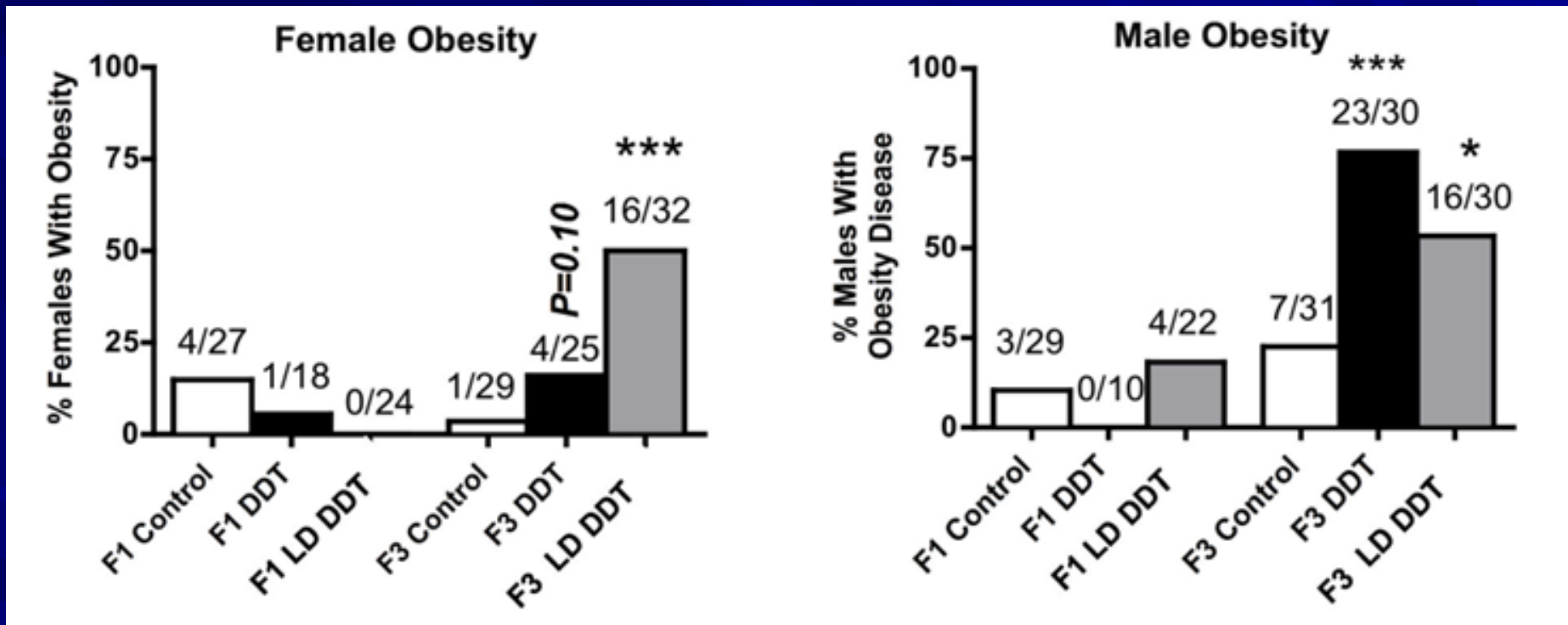
Nilsson E, Larsen G, Manikkam M, Guerrero-Bosagna C, Savenkova MI, Skinner M, et al. PLoS (2012)

Premature Ovarian Failure POF



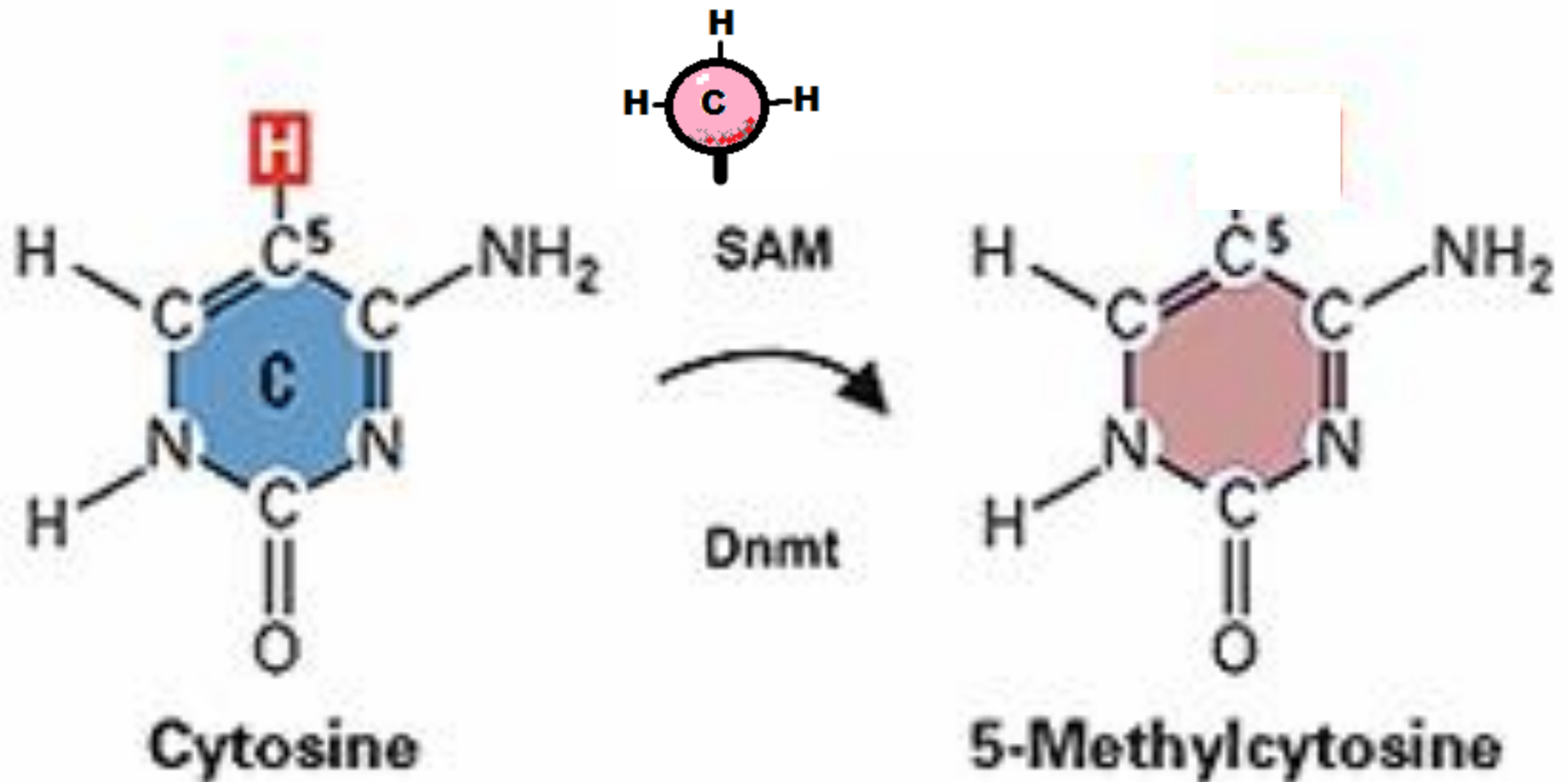
DDT; Transgenerational Obesity

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

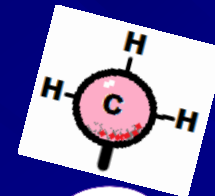


1. Michael K Skinner, Mohan Manikkam, Rebecca Tracey, Carlos Guerrero-Bosagna, Muksitul Haque, Eric E Nilsson. Ancestral dichlorodiphenyltrichloroethane (DDT) exposure promotes epigenetic transgenerational inheritance of obesity. BMC Medicine, 2013; 11 (1): 228 DOI: 10.1186/1741-7015-11-228

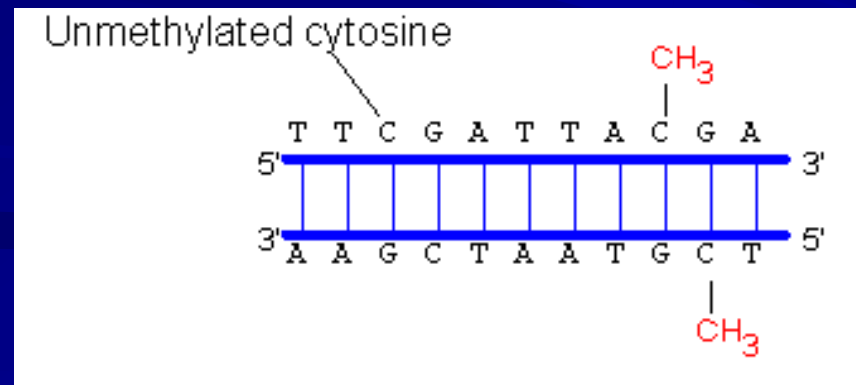
DNA Methylation



DNA Methylation at CpG Site

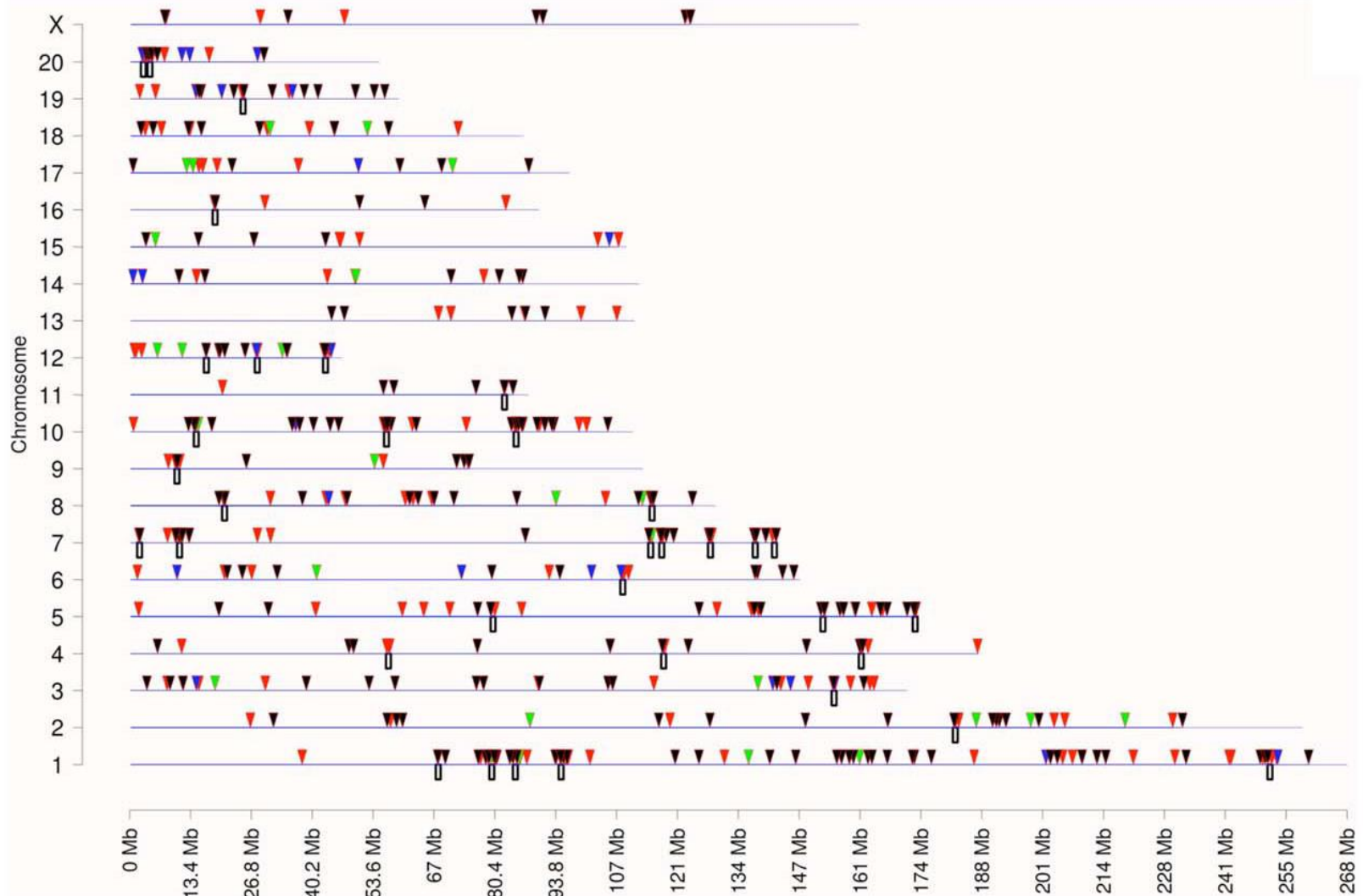


TTCGATTACGA



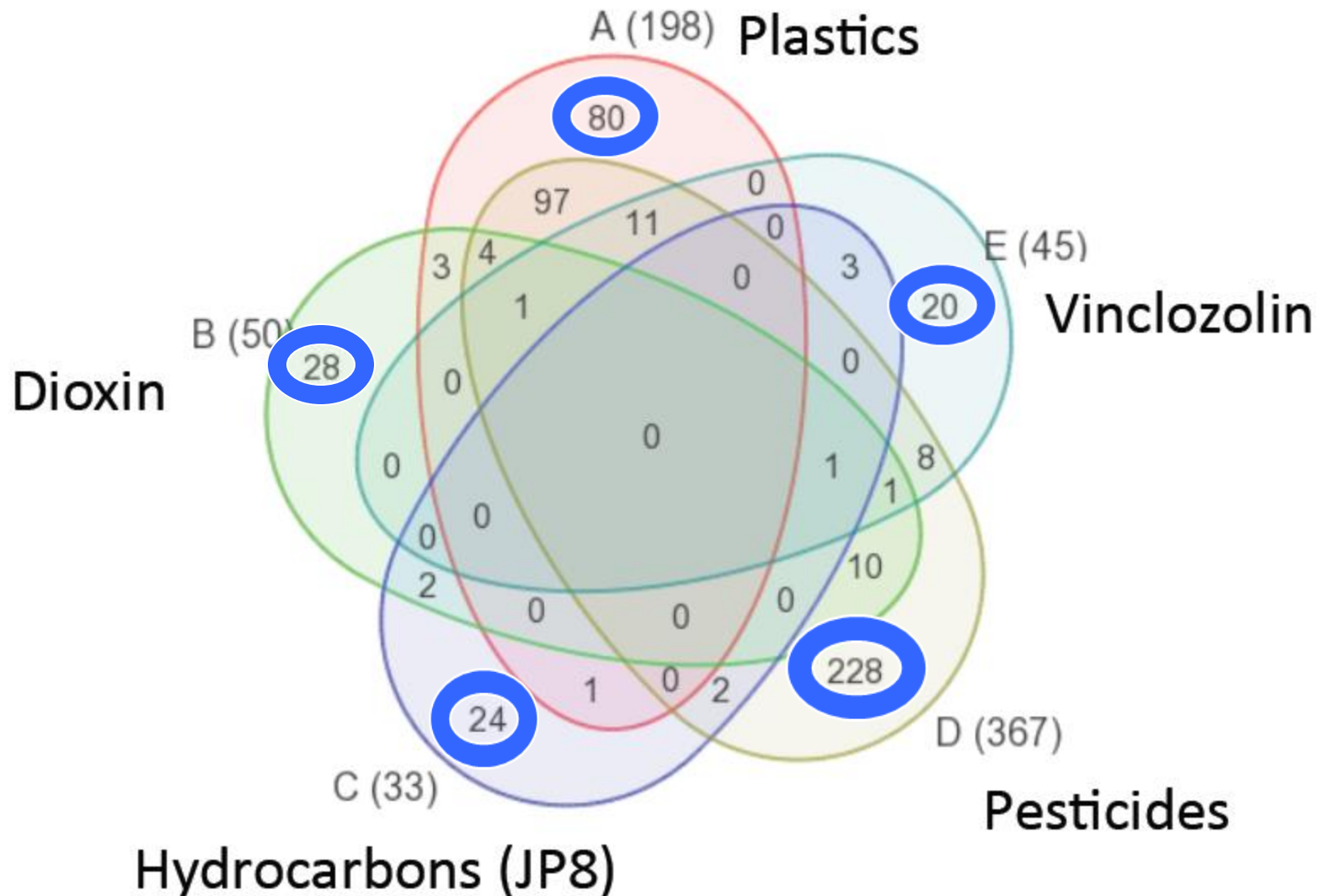
Contaminants Produced New DNA Methylation Sites

Differential DNA methylation regions (DMR) chromosomal locations



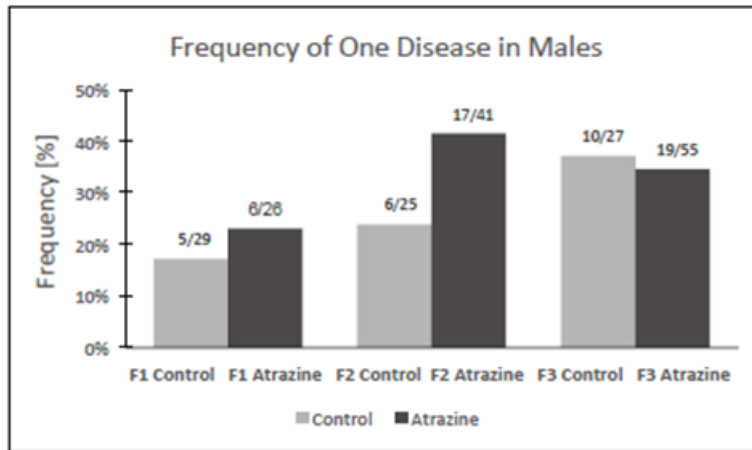
Transgenerational (F3) Sperm Epigenome Alterations

(Ancestral Exposure Specific Epimutation Biomarkers)

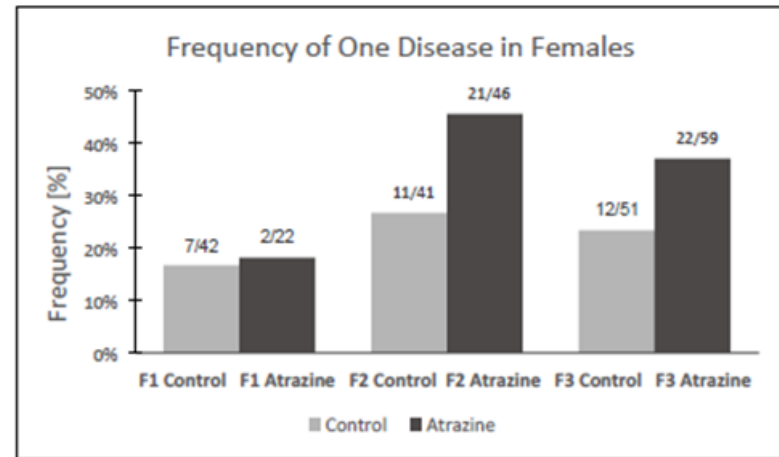


Prenatal Atrazine Causes Transgenerational Disease

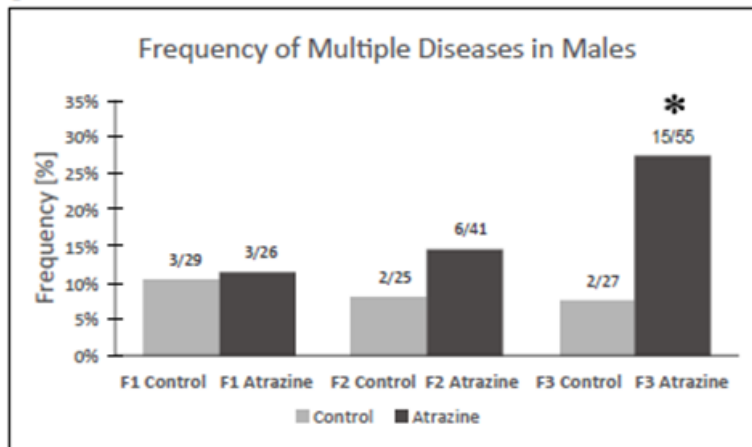
A



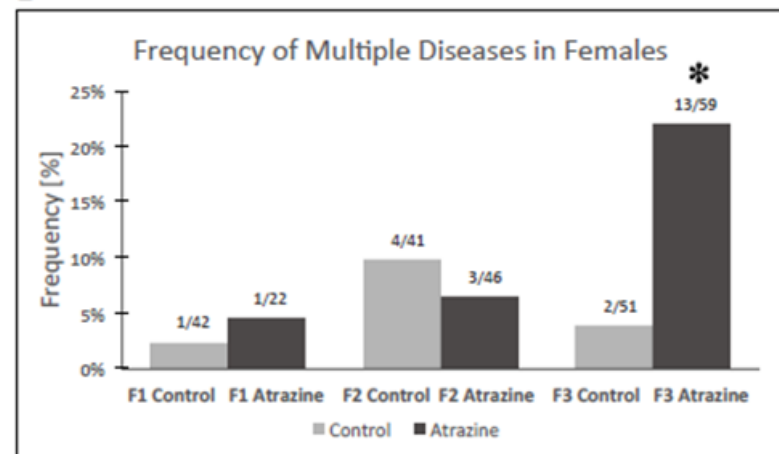
B



C



D



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

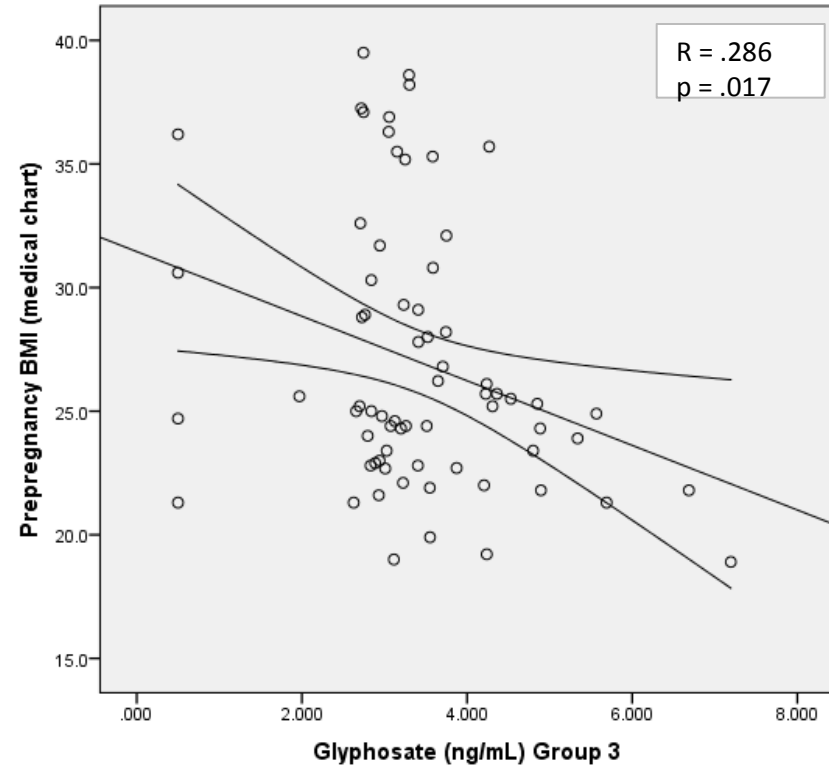
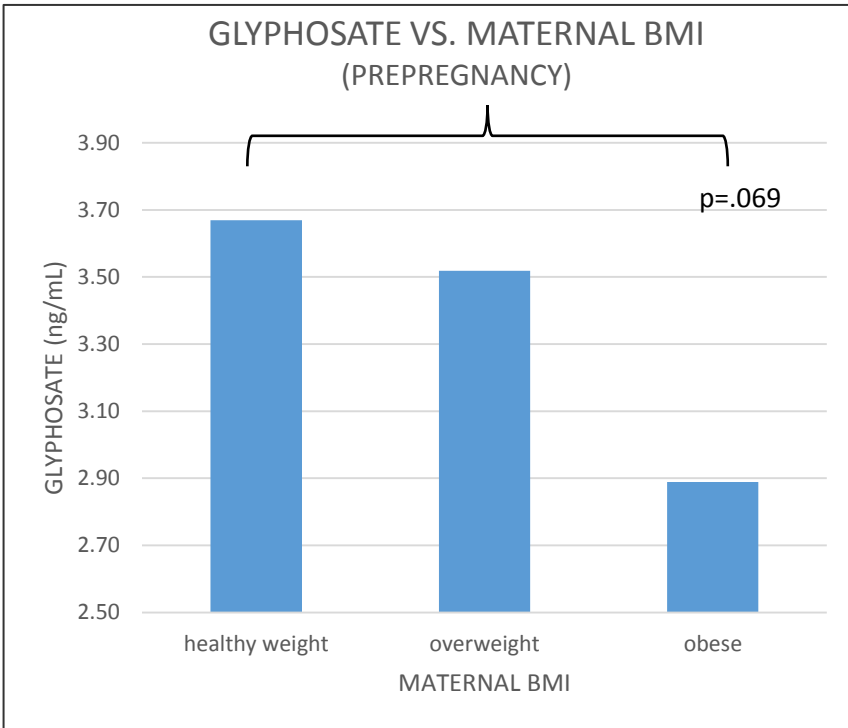
Variable	Value	N (%)
Residential Area	Rural	14 (20)
	Suburban	49 (71)
	Urban	6 (09)
Maternal Race	White	65 (94)
	Asian	4 (06)
Maternal Age	Less than 20 years	2 (03)
	20-35 years	63 (91)
	Greater than 35 years	4 (06)
Marital Status	Single	19 (28)
	Married	50 (72)
Primip	No	49 (71)
	Yes	20 (29)
Hypertension (CHTN & PIH)	No	51 (74)
	Yes	18 (26)
Diabetes (DM & GDM)	No	64 (93)
	Yes	5 (07)
Maternal Drug Use	No	68 (99)
	Yes	1 (01)
Maternal Tobacco Use	No	57 (83)
	Yes	12 (17)
Maternal Alcohol Use	No	66 (96)
	Yes	3 (04)
Route of Delivery	C-section	20 (29)
	Vaginal	49 (71)
Infant Sex	Male	36 (52)
	Female	33 (48)
NICU care	No	66 (96)
	Yes	3 (04)
Household Income	Less than \$25,000	6 (09)
	\$25,000 to \$34,999	10 (14)
	\$35,000 to \$49,999	8 (12)
	\$50,000 to \$74,999	11 (16)
	\$75,000 to \$99,999	14 (20)
	\$100,000 to \$200,000	14 (20)
	\$200,000 or more	1 (01)
	I don't know	5 (07)
County of Residence	Hamilton	1 (01)
	Hendricks	1 (01)
	Johnson	23 (33)
	Marion	42 (61)
	Morgan	2 (03)
Maternal Education	≤ High School Diploma or GED	14 (20)
	Some college or Associate Degree	23 (33)
	Bachelors degree	32 (46)

Results

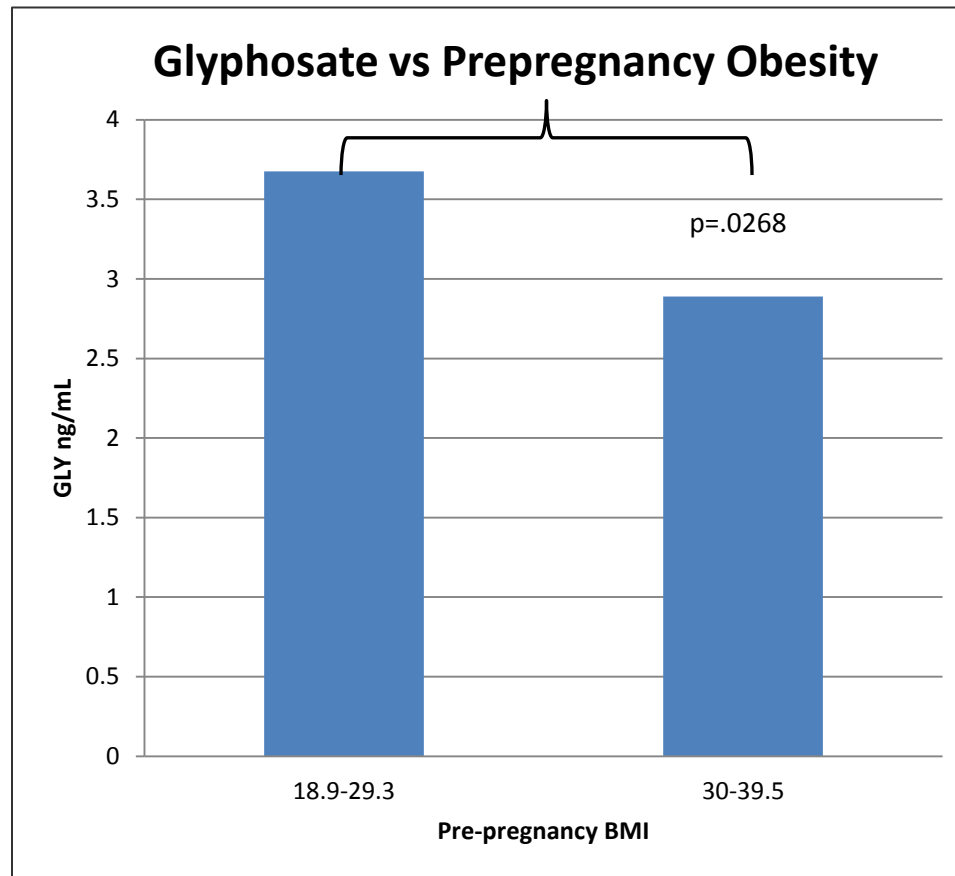
- 65 of 69 pregnant women (91%) tested + for GLY (>LLOD)
- Mean GLY concentration was 3.6 ± 0.12 ng/mL
- When GLY was compared with risk factors, it was found negatively correlated to women's BMI both @ beginning and end of pregnancy (r 's= -0.31, p 's=0.01)
- GLY levels were found higher in women who 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

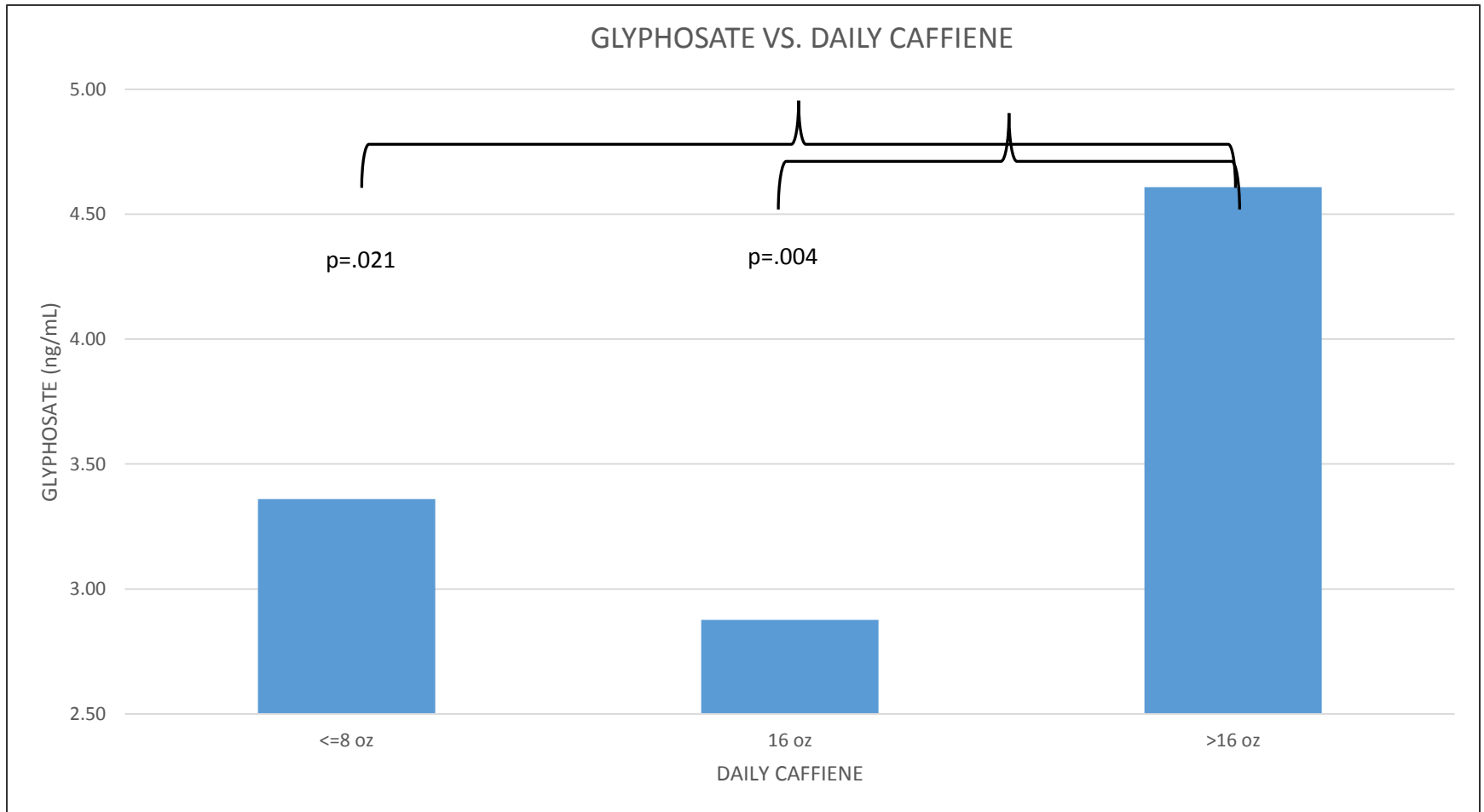


Pre-pregnancy Obesity (≥ 30 BMI) vs. GLY



Obese mothers had **lower** Glyphosate levels.

Caffeine vs. Glyphosate

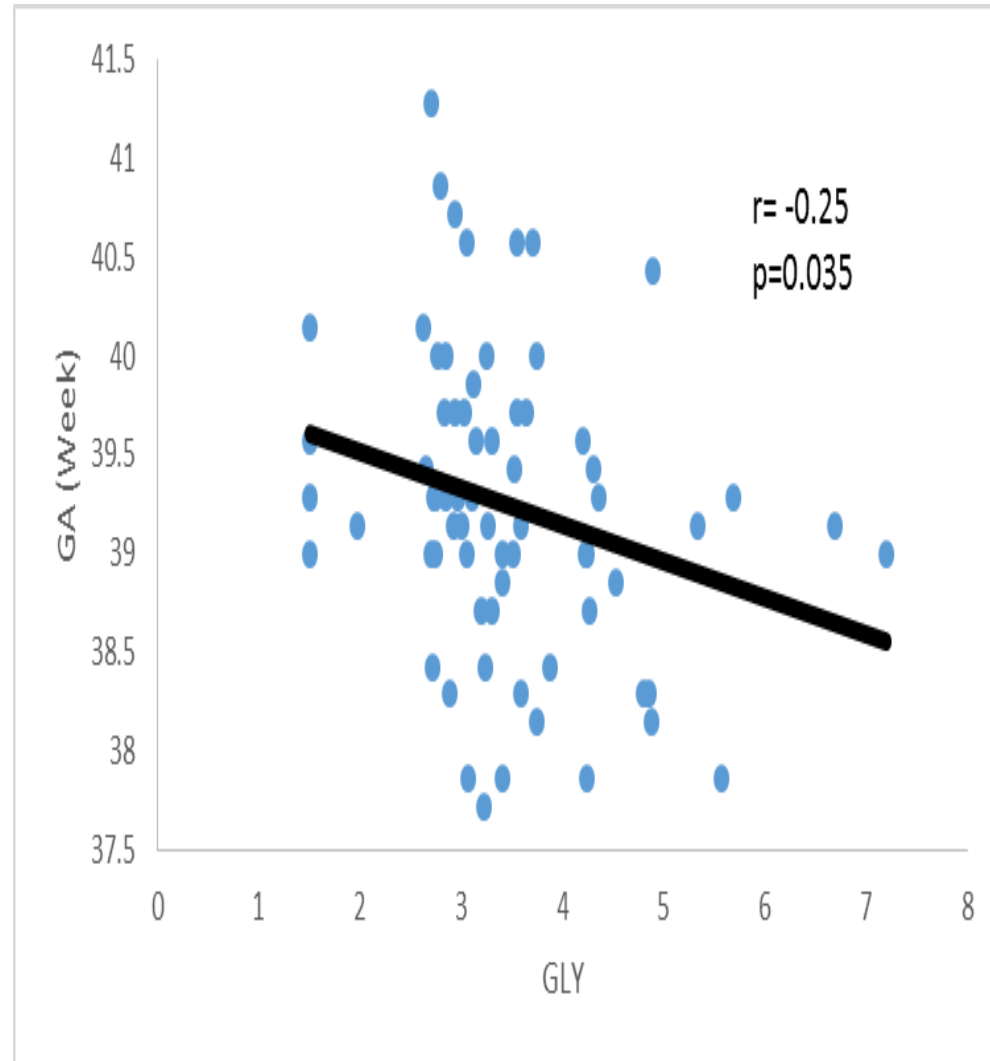
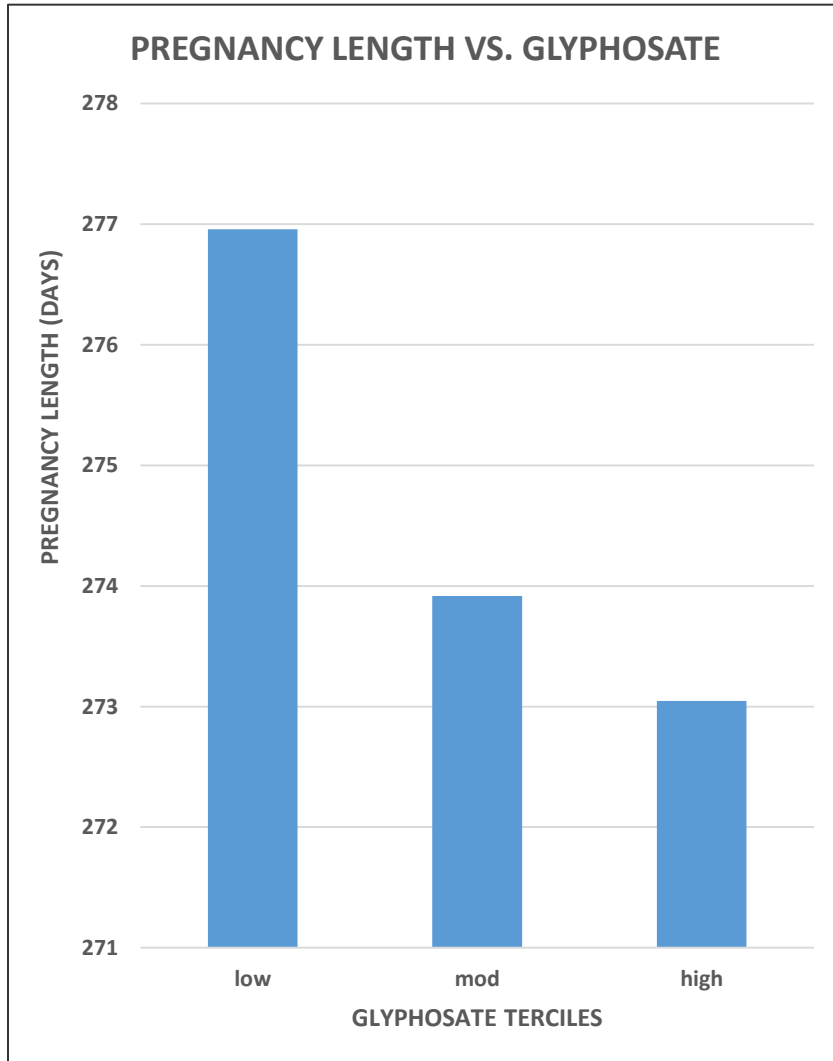


Do you currently consume beverages containing caffeine (coffee, caffeinated soda, tea, energy drinks)?

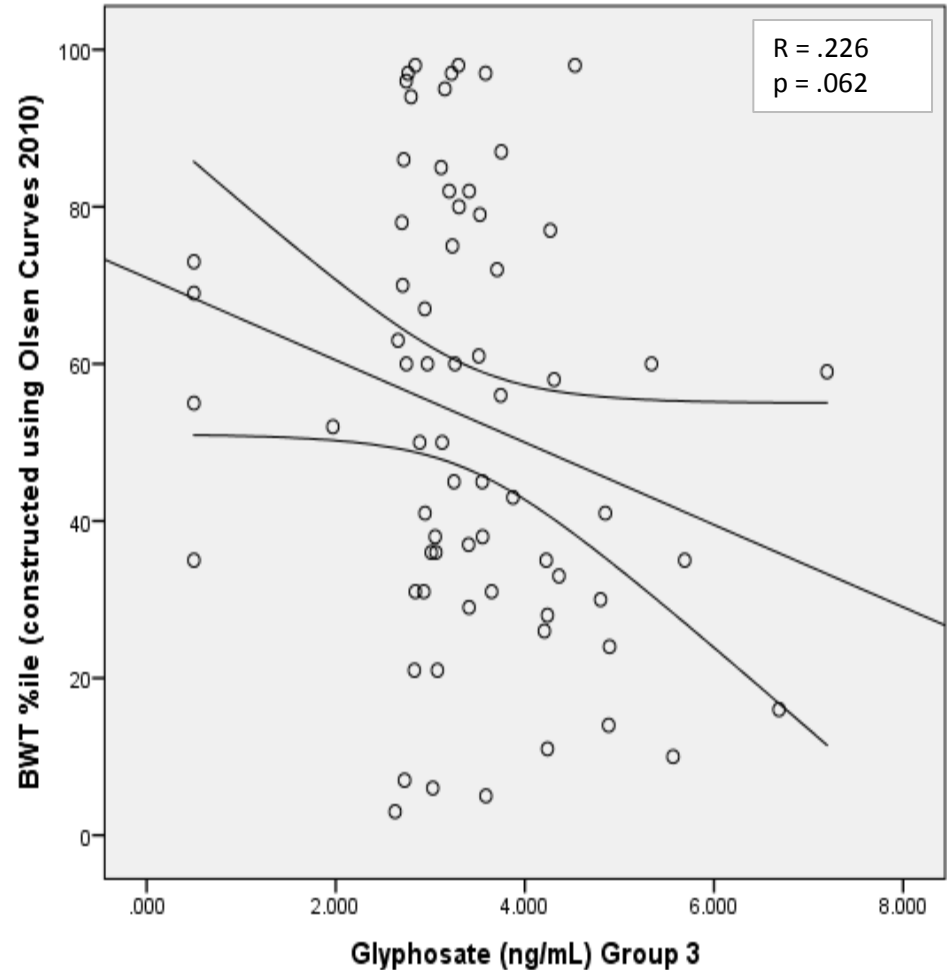
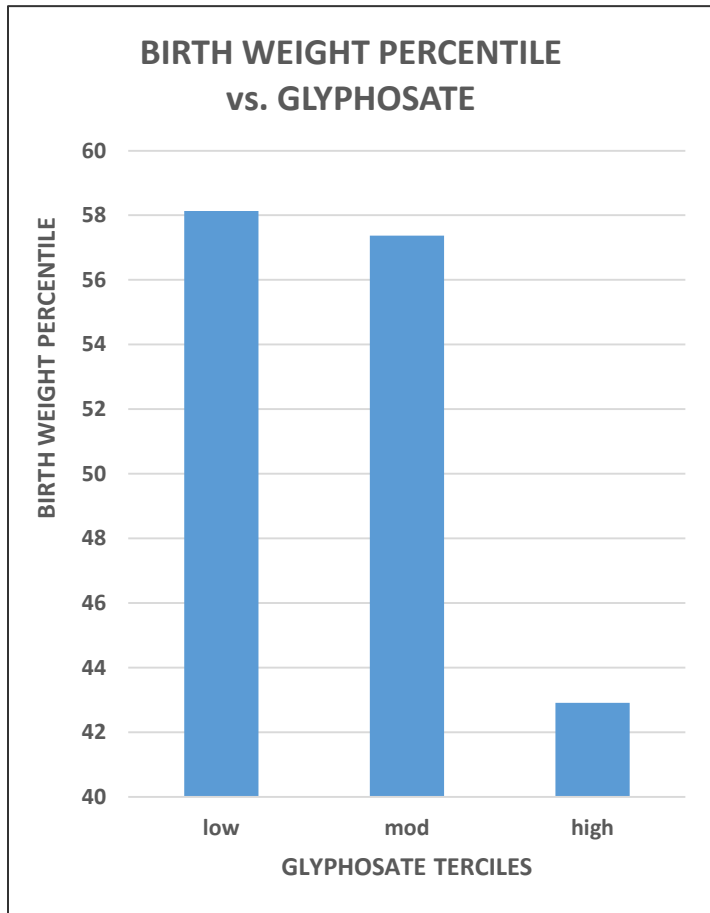
Results

- 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



Gestation-Corrected Birth Weight (Bwt %Ile) vs. Glyphosate



* 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

OB GYN of Indiana South

Office Manager

- Medical Assistants
- Physicians
- Study Population

Fairbanks Public Health

- Grant Support
- Study Design Collaboration
- Co-PI

UCSF Department of Laboratory

- Medicine

Indiana University

Neonatology Department

- Clinical Research Coordinator

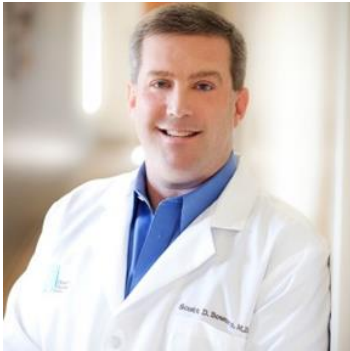


Riley Hospital for Children
Indiana University Health



SCHOOL OF MEDICINE
INDIANA UNIVERSITY

OB GYN of Indiana, Collaborators



Scott Bowers, MD



Jamie Boyce, DO



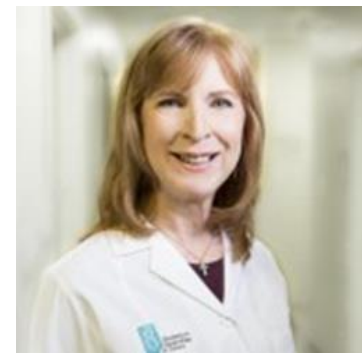
Tara Debikey, MD



Stephanie R. Young, MSN
AGPCNP-C



Thomas Wisler Jr., MD



Vicky Sherman, MD

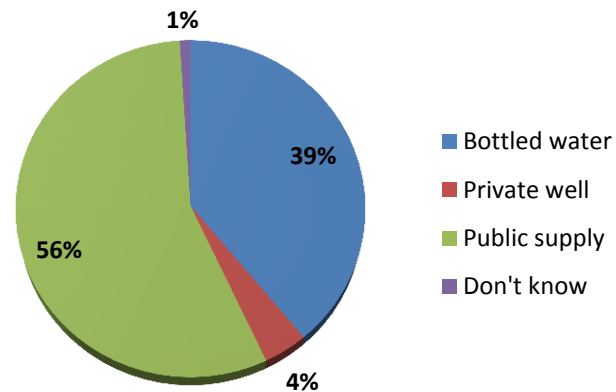
*Glyphosate Assay

- 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 ^{13}C -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

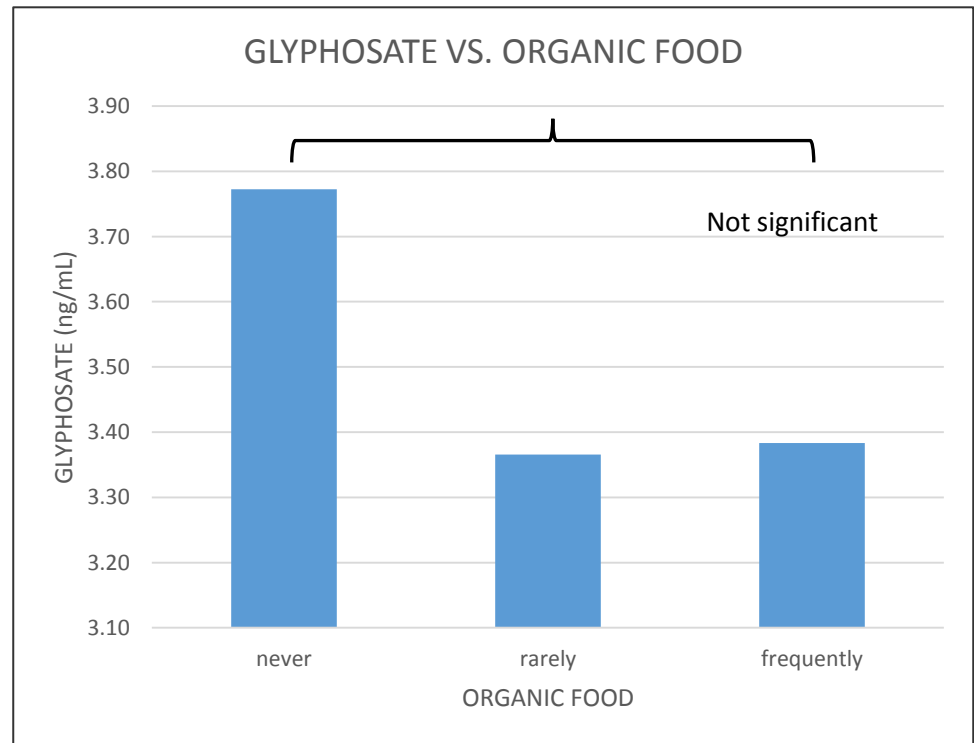
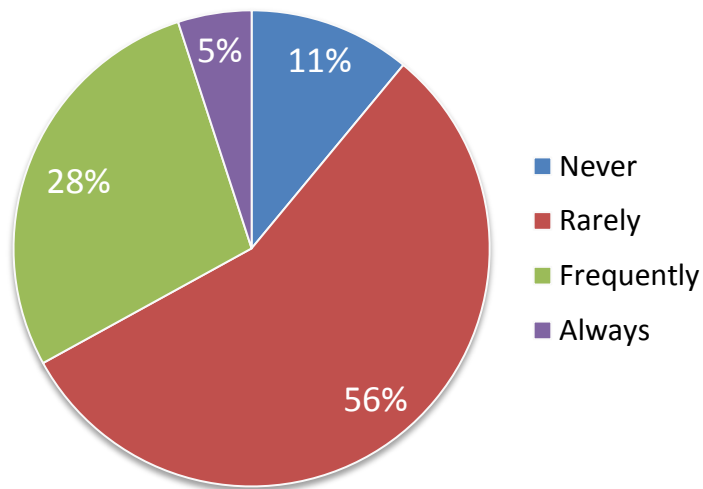
All water samples <LOD

**Sources of Drinking Water
(N=69)**



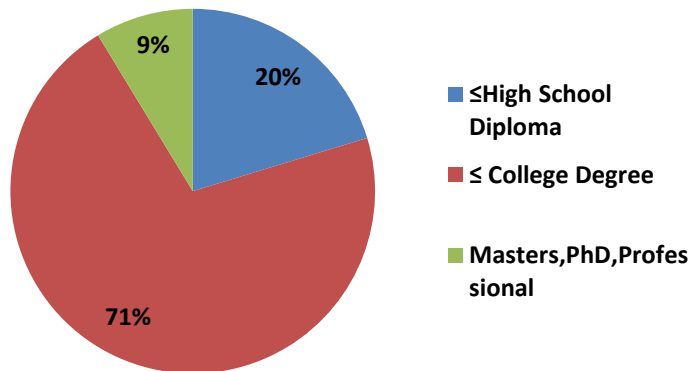
Organic Food Consumption

Organic Food Consumption

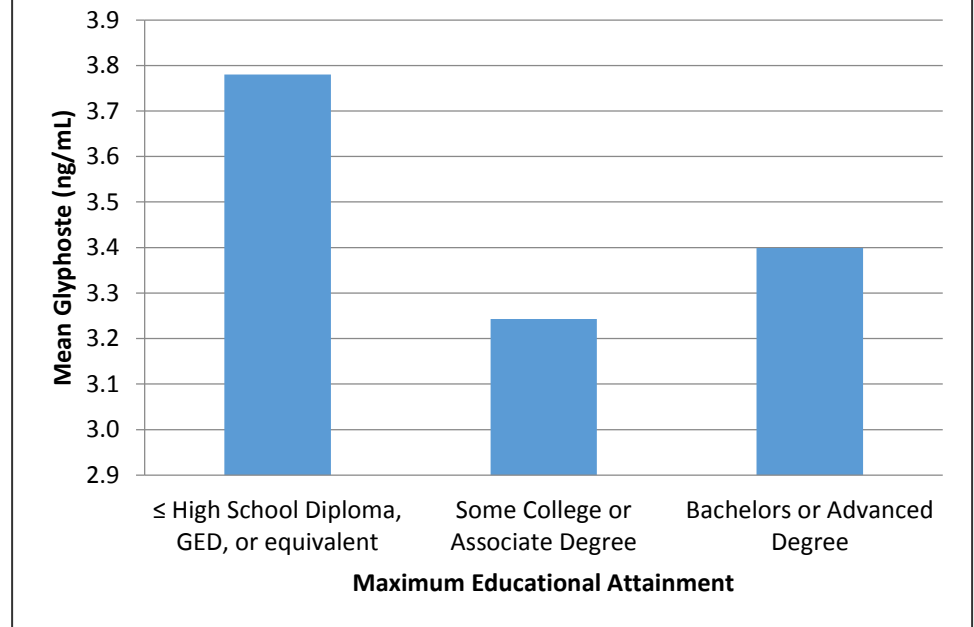


Education of GLY Study Participants

Maternal Education



Glyphosate vs. Maternal Education



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

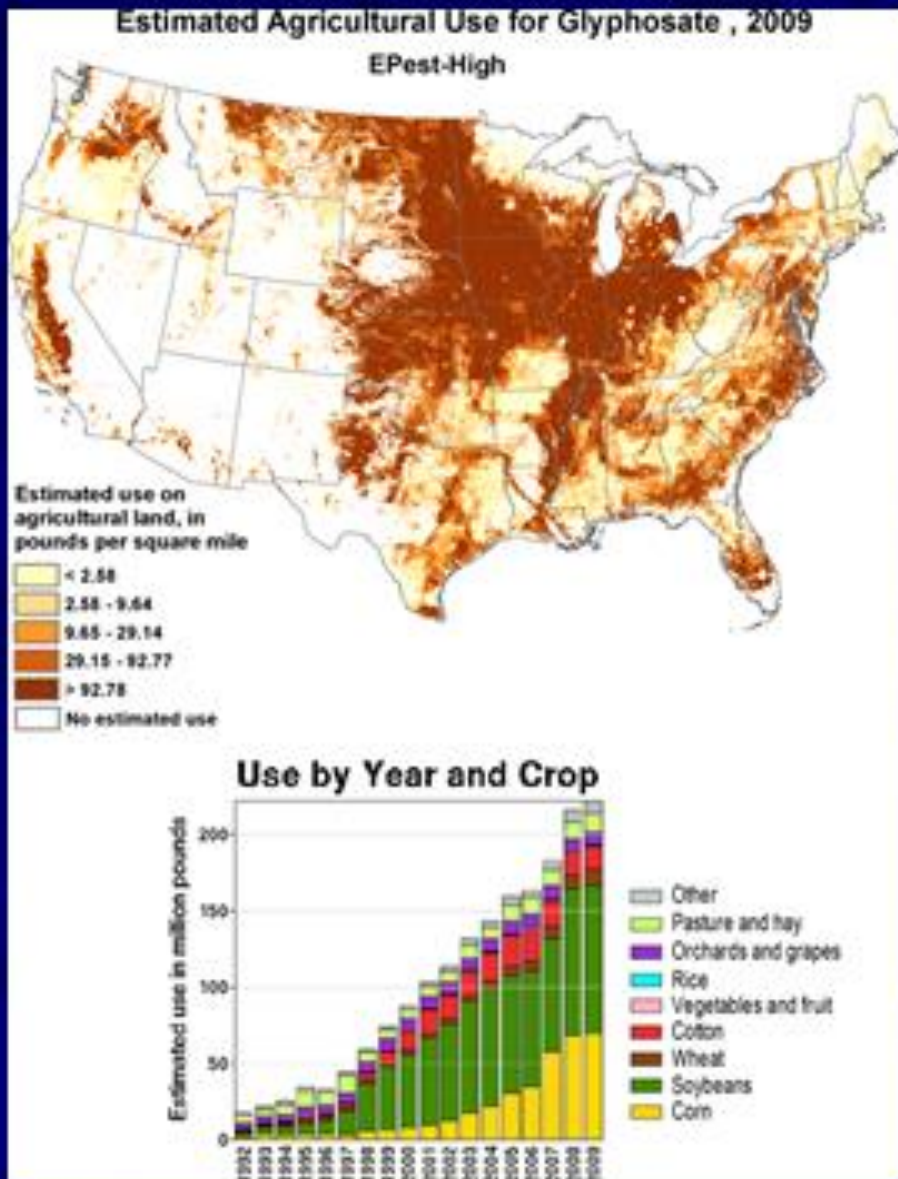
References	Analytical method, LOD/LOQ	Participants	Urine concentrations [$\mu\text{g/l}$]		Estimated exposure or systemic dose [$\mu\text{g/kg bw}$]		Percentage of ADI or AOEL [%]
			Mean	Maximum	Mean	Maximum	
Acquavella et al. (2004)	HPLC following ion exchange LOD 1 $\mu\text{g/L}$	48 male farmers from Minnesota and South Carolina (USA), their spouses and 79 children	3.2	233 (farmer) 29 (child)*	0.11 (systemic dose)	8.3 (systemic dose)	8.3 % of AOEL (maximum value), ca. 0.1 % of AOEL (mean value)
Curwin et al. (2007)	Immunoassay (fluorescent microbeads) LOD 0.9 $\mu\text{g/L}$	48 women, 47 men, 117 children from "farm" and "non-farm" households in Iowa	1.1–2.7 (in different groups)	18 ("farm child")*	0.5 (dietary exposure highest mean) 0.1 (systemic dose highest mean)		0.1 % of ADI
Mesnager et al. (2012)	HPLC–MS LOD 1 $\mu\text{g/L}$ LOQ 2 $\mu\text{g/L}$	1 farmer, his wife and 3 children, presumably Europe	n.a. (only single values available)	9.5 (farmer) 2 (child)*	0.33 (systemic dose)		<0.4 % of AOEL
Hoppe (2013)	GC–MS/MS following derivatisation LOQ 0.15 $\mu\text{g/L}$	182 citizens from 18 European countries	0.21	1.82		0.3–0.4 (dietary exposure)	<0.1 % of ADI
Markard (2014)	GC–MS/MS (presumably) LOQ 0.15 $\mu\text{g/L}$	40 male and female German students	n.a. (22 samples above LOQ)	0.65		0.13 (dietary exposure)	\ll 0.1 % of ADI
Krüger et al. (2014)	ELISA partly validated against GC–MS LOD/LOQ not given	>300 (mostly from Germany)	≤ 2	5		0.83 (dietary exposure)	<0.2 % of ADI
Honeycutt and Rowlands (2014)	ELISA LOQ 7.5 $\mu\text{g/L}$	35 women, men and children from USA	n.a. (13 samples above LOQ)	18.8		3.3 (dietary exposure) 0.66 (systemic dose)	<0.7 % of ADI, <0.7 % of AOEL

n.a. not applicable

* For children, comparisons to reference values were not performed since age, body weight and urine volume were not known

A critical review of glyphosate findings in human urine samples and comparison with the exposure of operators and consumers Lars Niemann • Christian Sieke • Rudolf Pfeil • Roland Solecki, Journal of Consumer Protection and Food Safety, J. Verbr. Lebensm. (2015) 10:3–12, DOI 10.1007/s00003-014-0927-3

Round Up Is UP

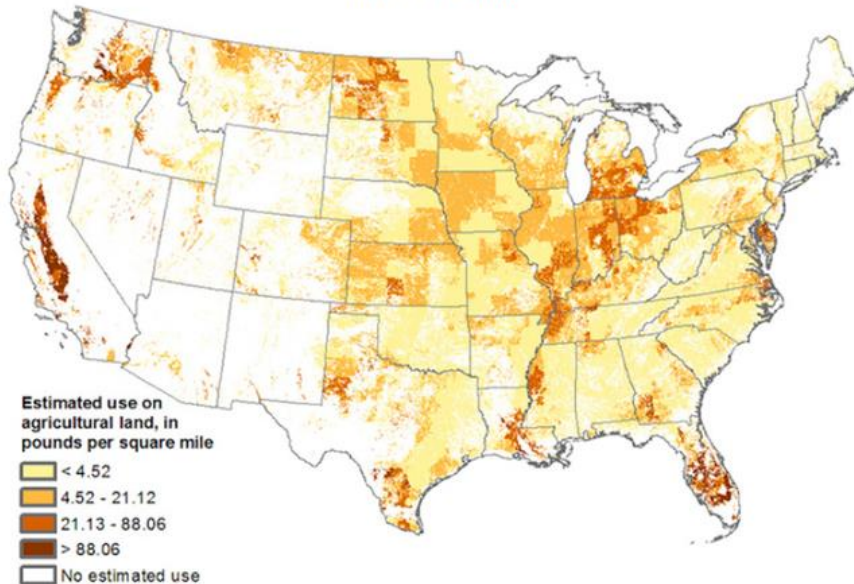


Roundup
Use up
11500%
Increase in
17 years.

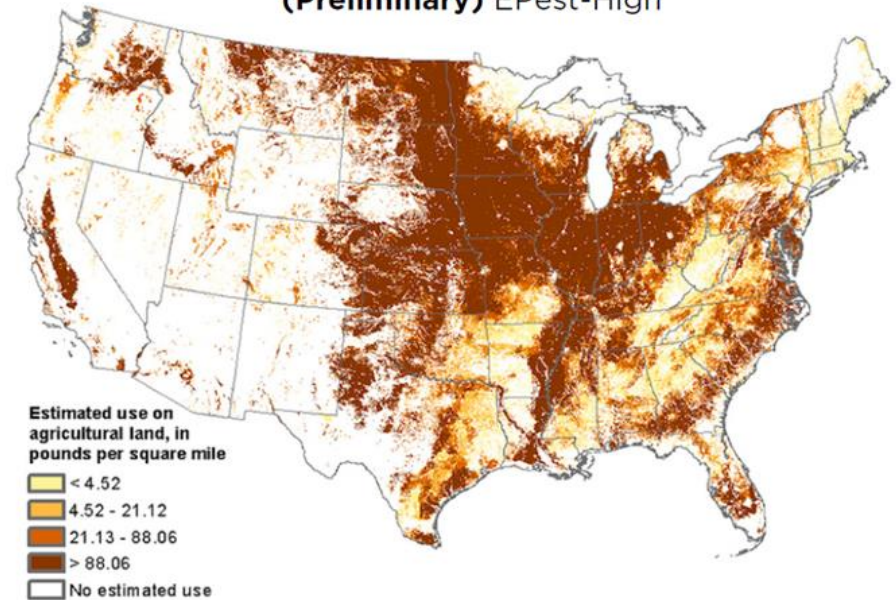
Glyphosate Use 1992-2014

20 X Increase in GLY Use

Estimated Agricultural Use for Glyphosate, 1992
EPest-Low



Estimated Agricultural Use for Glyphosate, 2014
(Preliminary) EPest-High



February 2016 FDA
started testing for GLY

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



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)

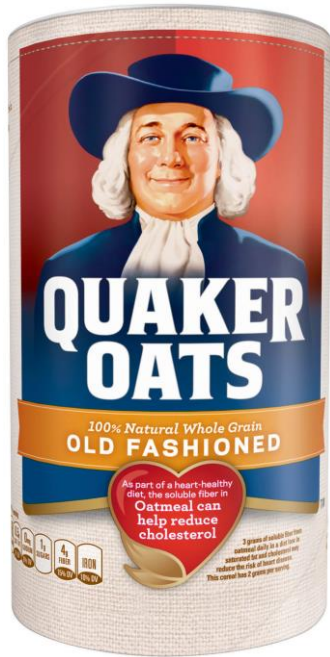
41 ppb
GLY



Glyphosate in Oatmeal, Pita Chips, California Wine

Quaker Oatmeal + glyphosate, FDA

Quaker Oats, owned by PepsiCo, has been sued over its "all natural" oats containing high levels of GLY.



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



812.53 ppb GLY



Glyphosate Found In 100% Of California Wines Tested
It's even in some labeled as organic

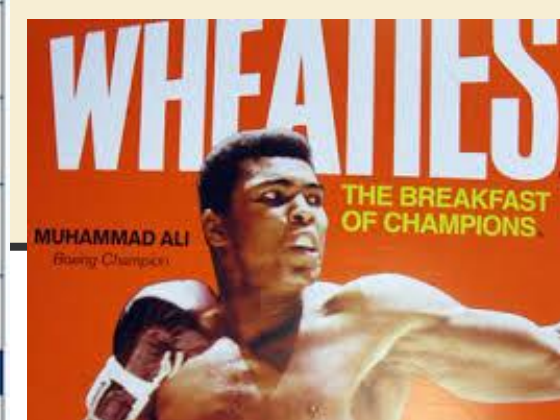




Oreos, Doritos,
Lays Chips,
Goldfish Pepperidge Farm
Crackers,
Little Debbie Oatmeal Crème
Pies
Fritos, Lucy's
Oatmeal Cookies,
Back to Nature
Crackers








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

	Honey Nut Cheerios	Glyphosate - 670.2 ppb AMPA - 14.5
	Wheaties	Glyphosate - 31.2 ppb
	Trix	Glyphosate - 9.9 ppb
	Gluten Free Bunny Cookies Cocoa & Vanilla	Glyphosate - 55.13* ppb
Kellogg's		
	Corn Flakes	Glyphosate - 78.9 ppb
	Raisin Bran	Glyphosate - 82.9 ppb
	Organic Promise**	Glyphosate - 24.9 ppb
	Special K	Glyphosate - 74.6 ppb
	Frosted Flakes	Glyphosate - 72.8 ppb
	Cheez-It (Original)	Glyphosate - 24.6 ppb
	Cheez-It (Whole Grain)	Glyphosate - 36.25* ppb
	Soft-Baked Cookies, Oatmeal Dark Chocolate	Glyphosate - 275.58* ppb
Nabisco		
	Ritz Crackers	Glyphosate - 270.24 ppb
	Triscuit	Glyphosate - 89.68 ppb
	Oreo Original	Glyphosate - 289.47* ppb



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

Purina Dog Chow Complete Dry – 98 ppb glyphosate.

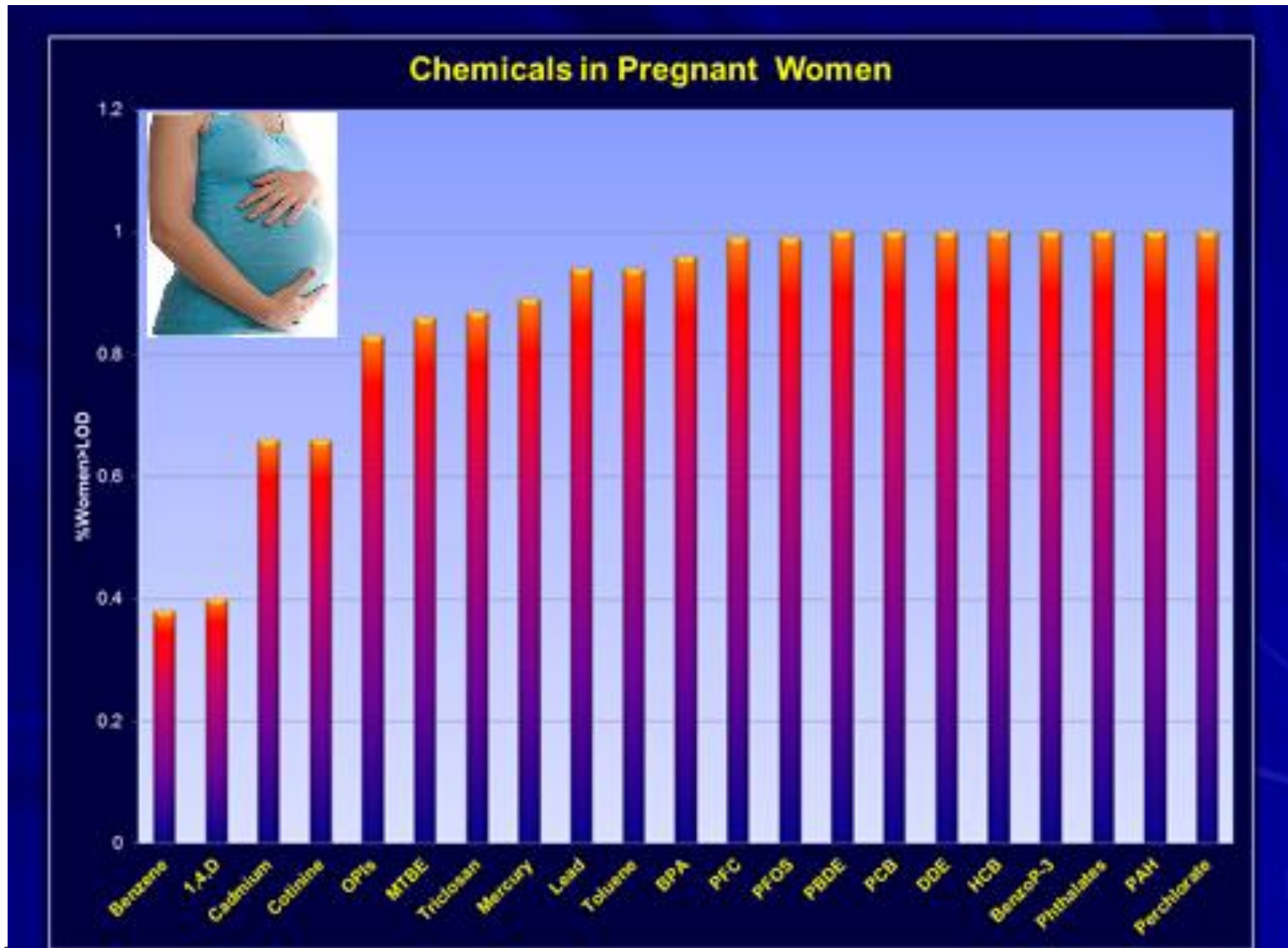
Kibble's 'n Bits Chefs Choice American Grill Dog Food Dry – 300 ppb glyphosate.

Iams Proactive Health Toy and Small Breed Dog Food Dry – 65 ppb glyphosate.

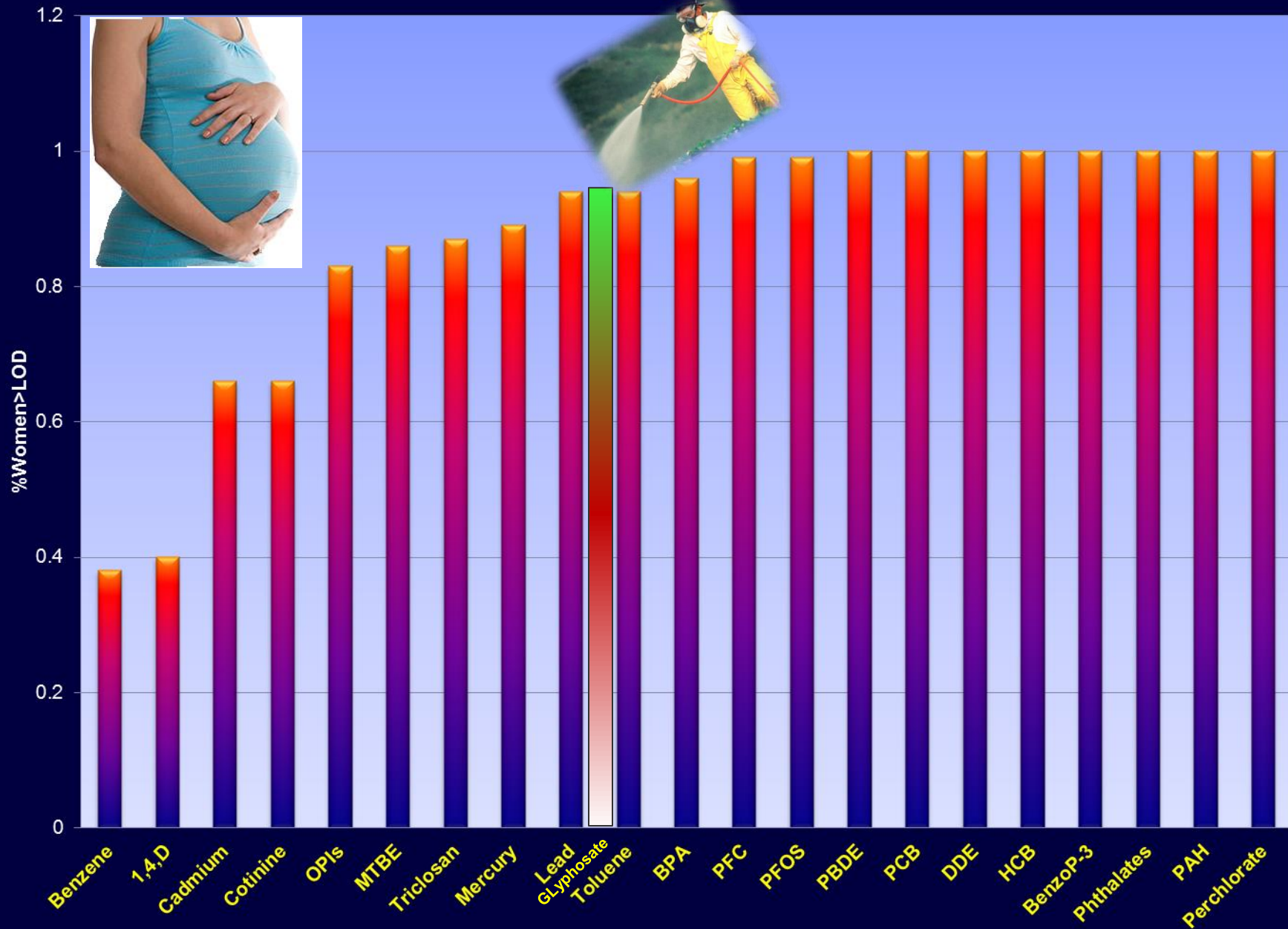
Rachael Ray Nutrish Real Beef and Rice Recipe Dog Food Dry – 140 ppb glyphosate.

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



Chemicals in Pregnant Women



FDA and EPA No Longer Tracking Glyphosate.

Myron Ebell

- 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’